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Poster – P1

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Interaction of prednisolone with β-lactoglobulin: UV-Vis study

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ABSTRACT

Protein drug transporters play an important role in the absorption, distribution, metabolism and excretion of drugs. The study of protein–drug interaction is a significant issue for drug development. Drug transporters are multi-specific transmembrane proteins that facilitate the membrane passage of a large number of drugs. Drug transporters have a distinct expression pattern in the human body lining pharmacological barrier tissues, most importantly the small intestinal epithelium, the endothelial cells in the blood–brain barrier, the epithelium of the proximal tubule cells in the kidney, and hepatocytes in the liver. However, it is both expensive and time-consuming to perform physical experiments to determine whether a drug and a protein are interacting with each other, while these studies are really useful in medical sciences. Bovine milk β-lactoglobulin (β-LG) demonstrates significant resistance against both gastric and simulated duodenal digestions. Therefore, it seems a realistic protein candidate for safe delivery and protection of particularly pH sensitive drugs in stomach. Prednisolone is a synthetic adrenal corticosteroid which is used to achieve prompt suppression of inflammation in many inflammatory and allergic conditions and to treat blood cell cancers (leukemia*)* and lymph gland cancers (lymphomas). In this study the intermolecular interaction of prednisolone with β-LG was investigated using UV-Vis spectroscopy. Moreover, the effect of Prednisolone complexation on the secondary structures of β-LG was studied and the results showed that the secondary structure of β-LG was preserved upon interaction of this drug. Based on the achieved results, this protein might be useful for delivery of Prednisolone.

Key words: β-Lactoglobulin (β-LG); Prednisolone; UV-Vis spectroscopy

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Kinetics of metal ions-induced aggregation of lysozyme studied by fluorescence spectroscopy and circular dichroism

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ABSTRACT

There are a group of diseases associated with protein misfolding and accumulation into amyloid fibers. While these disease-associated amyloidogenic proteins differ in their sequence, structure, and function, they all form similar, highly ordered amyloid fibrils exhibiting several morphological and histochemical staining properties in common. Alzheimer's disease (AD) has been identified as a protein-misfolding disease and it is found that metal ions (aluminium, copper and zinc) become highly concentrated within amyloid plaques and brain parenchyma. In this research, using hen egg-white lysozyme (HEWL) as a model system to induce aggregation, we set out to investigate how metal ions influence amyloid formation. We made use of thioflavin T fluorescence and circular dichroism to study the influence of Zn (II), Cu (II) and Al (III) ions on HEWL aggregation after incubation at 25 oC for 1, 2, 4, 8, 12, 16, 24, 48, 72 and 144 h, at stoichiometric concentrations. The kinetics of fiber formation typically follows a sigmoidal fiber growth curve, including a lag-phase (nucleation) and a growth rate (elongation). Our results show that both Cu (II) and Zn (II) reduce the lag period allowing the aggregation process to essentially "bypass" the critical nucleation phase, often considered the rate limiting step in aggregation in non-metallic solutions, at stoichiometric concentrations. Copper and zinc form amorphous aggregates. Al (III) has been shown to both accelerate and increased the rate at which HEWL formed β-sheets.

Key words: Alzheimer's disease (AD); Circular Dichroism; Egg-white lysozyme (HEWL); Thioflavin T fluorescence; Metal ions

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Purification and biochemical characterization of peroxidase from *Rosmarinus officinalis* leaves

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ABSTRACT

Peroxidases are a group of oxidoreductase enzymes that catalyze oxidation of a wide variety of phenolic compounds in the presence of hydrogen peroxide as electron acceptor. The purpose of this investigation was to purify peroxidase from leaves of *Rosmarinus officinalis* and to determine its biochemical properties. This peroxidase was purified 30 fold with 16.5 % yield by ammonium sulfate precipitation and ion-exchange chromatography. The enzyme biochemical properties, including the effect of pH, temperature and ionic strength were investigated with guaiacol as electron donor. For substrate specificity investigation of the enzyme, Michaelis-Menten constant (K_m) and maximum velocity of enzymatic reaction (V_{max}) values for substrates guaiacol and 3, 3′, 5, 5′-TetraMethyle-Benzidine (TMB) were calculated from the Lineweaver-Burk graphs. The Peroxidase optimum pH and temperature were 6.0 and 40 °C. The effect of ionic strength on peroxidase activity was determined by different concentrations of sodium phosphate buffer, pH 6.0 (0.05-0.3 M) and the highest activity was observed in 0.3 M of the same buffer concentration. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed for molecular weight (M_w) determination and molecular weight of the enzyme was found to be 33 kDa. To investigate the homogeneity of the peroxidase, native polyacrylamide gel electrophoresis was done and a single band observed. This peroxidase showed high stability at a wide range of pH and temperature. Thus it can be used as a new source of peroxidase for various applications in the medicinal, chemical and food industries.

Key words: *Rosmarinus officinalis*; Peroxidase purification; Ion-exchange chromatography; SDS-PAGE; Native-PAGE

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Association between genetic polymorphisms of *NQO1 C609T* and *CAT C-262T* with risk of liver acute rejection

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ABSTRACT

Liver transplantation is a therapeutic approach for patients with end stage of liver disease. Despite improvement of this therapeutic process, rejection is common and the main cause of failure of the procedure. Transplanted liver is prone to exposure to acute rejection for different reasons, including oxidative stress. Catalase and NQO1 are the important antioxidant enzymes in the body. The aim of this study was to investigate the relationship between genetic polymorphisms of *NQO1 C609T* (rs1800566) and *CAT C-262T* (rs1001179) with acute rejection of liver transplantation. The study included 217 liver transplant recipients, 47 of whom had acute rejection. Genotyping was performed by PCR-RFLP assay and data were analyzed by SPSS statistical software. After analysis the genotype and allele frequencies in *CAT C-262T* polymorphism between patients with and without liver acute rejection, no significant difference was observed. The C609T polymorphism in the *NQO1* showed that TT homozygous individuals against CC and CT were at higher risk of liver acute rejection (OR=3.92, CI=1.08-14.20, P=0.037).

Key words: Liver transplantation; Acute rejection; Oxidative stress; *NQO1*; *CAT*

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A combined spectroscopic and molecular docking study on the interaction of dopamine with apo-human serotransferrin as a new drug carrier

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ABSTRACT

Blood-brain barrier (BBB) is the most important obstacle against satisfactory effects in central nervous system (CNS) diseases treatment because, many therapeutic molecules are unable to cross BBB. Toxicity and low availability of drugs which are administered for CNS diseases make some problems in treatment. Dopamine is a neurotransmitter in the brain which its depletion is the cause of Parkinson and many other diseases. Human serum transferrin, (hTf) a natural iron binding protein, have already been one of the appropriate choices for passing drugs from luminal side of the BBB to the abluminal side via receptor-mediated trancytosis. Transferrin receptor is expressed on the epithelial of BBB in addition to neurons. It is therefore highly important to estimate dopamine-binding ability to this macromolecule in the early stages of drug discovery and in clinical practice. Thermodynamic parameters of interaction were determined by UV–Vis and FT-IR spectroscopy techniques. AutoDock 4.2 software was used to predict Gibbs free energy (ΔG) and dopamine binding site. Peak resolving method was used for investigation of changes in transferrin secondary structure after binding to dopamine. According to the spectroscopic data binding constant (3.0934E+5), ΔH (-20.736kcal), Δs (-44.818cal/°K) and ΔG (-7.482kcal/mol) were calculated in 25°C. The binding energy for the best flexible binding site was -7.75kcal/mol by AutoDock software. Peak resolving didn't show disruptive change in protein structure after ligand binding. This promising results show hTf as a good candidate for CNS diseases treatment through administering fewer amounts of drugs with more availability.

Key words: Transferrin; Dopamine; Blood brain barrier; Spectroscopy; AutoDock

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Determination of AaCel9A endoglucanase stability at different temperatures and in the presence of calcium and cobalt ions

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ABSTRACT

Cellulase enzymes are very common and expensive in the industry. Those applications in the food industry, textile, fuel and pharmaceutical industry have led many researchers to the importance of these enzymes. One application of these enzymes values used in the decomposition of the most abundant substance biodegradable in the nature, cellulose. By demonstrating the effect of any additives on stability of the enzymes, it will save a lot of abuse. In this study, the protein was achieved by *aaCel9A* gene expression in the bacterial expression *E.coli* strain BL21 and then purified by Ni-NTA affinity chromatography column. Enzyme stability at different temperatures was investigated and stability in the presence of calcium and cobalt ions was achieved, separately. Our results suggest that calcium ions stabilize enzyme but cobalt ions converts enzyme into a more instable mode. Half-life of the enzyme in the presence of cobalt was calculated at 23.814 minutes, in the presence of calcium 27.170 minutes and in the absence of these additives was calculated at 26.250 minutes. Enzyme inactivation constant (K_i) was figure out in the presence of calcium and cobalt ions, and in the absence of these additives -0.0250, -0.0291and -0.0264, respectively.

Key words: Additive; Half-Life; Inactivation Constant; Stability

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Design of Fe (III) and L-Cys mixed micelle system as an artificial catalase at pH 3.0

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ABSTRACT

Micellar system is genius structure and it makes them a powerful mixture in biological studies. Applying micelle as a hydrophobic pocket for catalytic center is considered as a rode toward more robust system. Micelles help the artificial catalyst to evaluate their function and stability. We have designed a mixed micelle body by means of sodium dodecyl sulfate (SDS), dodecyltrimethylammonium bromide (DTAB), Fe (III) and L-Cys that is stable in a wide range of pH. The aforementioned mixture catalase activity in the presence of suitable substrate at pH 3 which was studied by various physical methods such as dynamic light scattering (DLS), Zeta potential and ^{1}H-NMR. By lowering the pH, micelles increased in size and shape due to the SDS surfactant insertion. SDS plays a role as a mediator to decrease the repulsions between positive head of DTAB monomers.

Key words: Catalyst; DTAB; L-Cys, Micelle; SDS

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The evaluation of structural stability of KLF4 protein of stem cells by using three-dimensional fluorescence spectroscopy

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ABSTRACT

Krüppel-like factors are a family of transcription factors that play important roles in many fundamental biologic processes, including development, proliferation, differentiation and apoptosis. KLF4 as the 4th member of these transcription factors was one of the first KLF family members identified. In addition to regulating many important physiological processes, KLF4 has been shown to play a role in pathologic conditions such as cancer and inflammation. KLF4 has been implicated in both tumor suppression and progression. More recently, KLF4 was shown to play a crucial role in the reprogramming of somatic cells into induced pluripotent stem cells. Examination of KLF4 expression in mouse models of colorectal cancer has yielded similar results. In this study, the investigation of stability of KLF4 protein was done by using three-dimensional fluorescence spectroscopy. The three-dimensional spectra and contour maps of the KLF4 protein appears some peaks that each one shows some features of the protein structure; peak a indicates the Rayleigh scattering ($\lambda ex = \lambda em$), and peak b demonstrates the second-order scattering ($\lambda ex = 2\lambda em$), which is mainly caused by the $\pi \rightarrow \pi^*$ transition of the characteristic polypeptide backbone structure C=O of KLF4. The results shows that in all amounts of pH, the ranges of $\Delta\lambda$ in both peaks 1 and 2 are the same, indicates that transition of protein chromophors had no modification. The most measure of fluorescence intensity of peak 1 and 2 was related to ethanol-distilled water 50% solution that shows structural changes in protein that causes chromophor transitions to more polar environments and Tyr and Trp residues are involved to solvent. But the most reduction of fluorescence intensity of peaks 1 and 2 was related to ethanol-distilled water 100% that implicates chromophor transitions to more homophobic environments.

Key words: KLF4; Three-Dimentional Fluorescence Spectroscopy; Protein stability

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Molecular dynamics simulation study on the initial stages of amyloid formation in myoglobin and search for anti-amyloid compounds

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ABSTRACT

Aggregation of proteins results from a change in protein conformation. Special structured forms of aggregates known as amyloid are related to a wide range of diseases including Parkinson and Alzheimer’s disease. Amyloid fibrils of different proteins have similar morphological features, and in principle, the results obtained from the study of model proteins could be generalized. The current computational study deals with initial states of amyloid formation in myoglobin. Experimental studies have shown that the more unstable apo-myoglobin (lacking the heme group) may form amyloid fibrils, but adding molecules similar to heme (such as Nile red) could prevent or slow down the process. The present computational study aimed at selecting compounds similar to Nile red that could potentially prevent myoglobin aggregation. A pharmacophore search was first done and prominent features of Nile red were selected. A total of 105145 molecules were screened against this pharmacophore and 10 heterocyclic ligands were chosen for further studies. These molecules were first docked into myoglobin (3RGK.pdb) with the use of Swissdock server. Molecular dynamics runs of two nanoseconds were then performed on these structures as well as holo- and apo-myoglobin, with the use of MOE package at 500K, which leads to partial unfolding of the protein. Water accessible surface area, radius of gyration and secondary structure analyses of sampled structures were then compared. In conclusion, two of these ligands were able to stabilize the apo-structure. These ligands could be used as a base to design more effective myoglobin stabilizing compounds.

Key words: Amyloid; Aggregation; Myoglobin; Molecular dynamics; Stabilization

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Advances in proteomics analytical techniques

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ABSTRACT

Proteins are fundamental components of cells which mediate many essential biological processes. Proteomics is a rapidly growing field for the study of proteome, the protein complement expressed by the genome of an organism or cell type. The large-scale analysis of proteins leads to a more comprehensive view of molecular and cellular pathways that improves the overall understanding of the complex processes supporting the living systems. The analysis of proteome is significantly challenging due to high dynamic range and difficulties in assessment of low abundance proteins and the absence of efficient purification and identification techniques. The proteomic technology is a useful research tool in variety fields: food technology, drug discovery, clinical application (biomarker discovery), protein-protein interactions studies and it will continue to advance understanding of variety of molecular process in different disease. A variety of methods have been utilized for protein studies including gel-based techniques, protein microarrays, mass spectrometry-based approaches such as MALDI and SELDI, high and ultra-performance liquid chromatography and fourier transform infrared spectroscopy. NMR spectroscopy and X-Ray crystallography methods are also used for structural study of proteins this review aims to give a brief overview of the above techniques and their most recent advances. We also introduce Proteominer, a recent protein enrichment technology for the exploration of the entire proteome content.

Key words: Mass spectrometry; Electrophoresis; Analytical proteomics

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An investigation of inhibition potential and mechanism of some ligands on diheme cytochrome C of *Shewanella Baltica*

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ABSTRACT

Shewanella baltica is an important bacterium with capability of nitrate, sulfur, and Fe (III) bioreduction, and is very important in biodegradation/bioremediation. Existence of potential inhibitors in the environment can affect the effectiveness of bioremediation. Bisphenol-A (BPA) is an important pollutant in environment. Hence this ligand was selected as control ligand first to understand its inhibitory mechanism on diheme cytochrome C of *S. baltica;* and seconed as a control ligand to compare any other inhibitor with BPA.This study aimed to understand the detailed inhibitory mechanism of BPA, and to investigate whether Aminotriazole have any potential inhibitory effect on recombinant diheme cytochrome C of *S. baltica*. The 3D structure was obtained from protein data bank. Molegro Virtual Docker used for performing docking process with both MolDock and PLANTS scoring function. Discovery Studio, Ligandscout and MVD were used for visualizing the interaction between ligand and protein. Fifty docking poses were obtained for each method. The results revealed that both BPA and Aminotriazole interact with cytochrome C in active site and non-active site residues at heme groups. BPA as a control inhibitor interacts via hydrogen bonding, hydrophobic and electrostatic interactions, while Aminotriazole interact mostly via hydrogen bond. Moreover, MolDock and PLANTS score of Aminotriazol is much lower than the scores of BPA. Therefore, Aminotriazol may not be an inhibitor regarding the in-silico results and BPA can inhibit cytochrome C of *S. baltica*.

Key words: Aminotriazole; Bisphenol-A (BPA); Diheme Cytochrome-C; *Shewanella Baltica*; Virtual Docking

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Enhancement of resistance of *Saccharomyces cerevisiae* to cadmium by heterologous expression of rice metallothionein type (*osmti-1b*)

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ABSTRACT

Saccharomyces cerevisiae is the most important industrial yeast and the main microorganism employed in bioethanol production. Industrial yeast strains during fermentation are exposed to various stresses such as osmotic shocks and oxidative stress which lead to the loss of biological products. Toxic heavy metals like cadmium are prevalent non-essential, redox inactive that increase oxidative stress in *S. cerevisiae.* In this study, the gene encoding one of rice metallothionein isoform, *OsMTI-1b,* was cloned in GPD shuttle expression vector and transferred to *S. cerevisiae*. However, the engineered strain showed little resistance to cadmium stress. To enhance expression, solubility and stability of *OsMTI-1b*, the sequence encoding glutathione S-transferase (GST) was placed at the beginning of *OsMTI-1b*. Then the new construct, GPD-GST-OsMTI-1b, was transferred to *S. cerevisiae*. The heterologous expression of GST-OsMTI-1b was confirmed by western blotting. In order to test the accumulation of cadmium on new strains the engineered strain as well as control were grown in media supplemented with different concentrations of cadmium. The results revealed better growth and higher final biomass concentrations of yeast cells expressing *OsMTI-1b* fused with GST as compared to the control strain in the presence of 0.9 mM cadmium. Determination of cadmium concentration in the culture medium at T0 (immediately after the addition of cadmium to the medium) and T1 (five hours after the addition of cadmium) showed lower cadmium concentrations in the culture medium for the engineered strain which suggests cadmium accumulation in the yeast cells containing the GST-OsMTI-1b and the cadmium-binding ability of this protein.

Key words: *Saccharomyces cerevisiae*; Rice; Metallothionein; Cloning; Cadmium

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Optimization method of tyrosinase assay with using dihydroxyphenylalanine (L-DOPA) as enzyme substrates

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ABSTRACT

Tyrosinase (1.14.18.1) is an oxygen oxidoreductase enzyme that converts tyrosine to dihydroxyphenylalanine (L-DOPA) in the presence of oxygen, and subsequently, L-DOPA converts to dopaquinone. So far, tyrosine has been used for determination of tyrosinase catalytic activity. To optimize tyrosinase assay method, in this study instead of tyrosine, L-DOPA was utilized as substrate of the enzyme and results were compared with conventional method. In order to assay tyrosinase in the presence of tyrosine or L-DOPA, 20 μL of enzyme and 3 mL of phosphate buffer solution were added in test cuvette of spectrophotometer. Concentration of enzyme was exactly determined by Beer – Lambert equation, using 26 $mM^{-1}cm^{-1}$ as extinction coefficient at 344 nm. Then, 1 mM or 2.5 mM of tyrosine and 1 mM L-DOPA were separately added in test cuvette. Tyrosinase and each one of the substrates were immediately mixed by inversion of cuvette and rate of absorbance (dA/dt) was recorded at 280 nm for 10 minutes. Finally, tyrosinase catalytic activity was calculated based on Worthington manual book. Results showed that at low concentrations of tyrosine (less than 1mM), a lag time was observed before 3 min, after that; dA/dt was linearly increased. At higher concentrations of tyrosine (more than 2 mM) or when 1mM L-DOPA was utilized as substrate, such a delay was not observed. Slope of lines in presence of tyrosine and L-DOPA were 1.52×10^{-2}/min and 7×10^{-5}/min, respectively. These evidences clearly indicated that using of L-DOPA for tyrosinase assay is more sensitive than that of tyrosine.

Key words: Tyrosinase; Tyrosine; L-DPA; Assay Optimization; Catalytic activity

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The effect of sodium chloride on RADA16-I four chains secondary structure: molecular dynamics study

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ABSTRACT

RADA 16-I is a synthetic amphiphilic nanopeptide which is designed to self-assemble in a controlled way into fibrils and higher ordered structures, depending on solvent condition. This peptide has many applications in tissue engineering and wound healing. We have studied the interaction of four RADA 16-I chains into a cluster formation in the presence of 0.2 M sodium chloride, using all-atom molecular dynamics simulation. Several independent 20 nanosecond simulations were performed. They showed that the fastest first nucleation step occurred in the presence of 0.2 M sodium chloride, compared to water. It is also found that salt has significant effect on the compactness and secondary structure of this peptide. In fact sodium chloride helps each peptide to adopt transitional expanded α-helix conformation in order to induce better interaction sites for the self-assembly. The results give insight into the effect of ionic strength on self-assembly mechanism of RADA 16-I in the bulk solution.

Key words: Four chains cluster; Molecular dynamics simulation; RADA 16-I; Secondary structure; Sodium chloride

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Relation between G-quadruplex structure and catalytic activity in peroxidase DNAzymes

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ABSTRACT

DNAzymes are single stranded DNA oligonucleotides with catalytic activity. They can be obtained using chemical synthesis and the PCR technique. In recent years, development of DNAzymes has received increasing attention because of the many advantages of DNAzymes over conventional protein enzymes, such as thermal stability, simpler preparation and hydrolysis resistance. One of the most important DNAzymes is the G-quadruplex–hemin complex with peroxidase activity. Quadruplexes can exhibit many different topologies. The presence of cations is necessary for G-quadruplex formation and subsequently peroxsidase activity of DNAzyme. In this investigation, peroxidise activity of two G-quadruplex forming oligonucleotides were evaluated using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and hydrogen peroxide substrates by colorimetric method. The oligonucleotides were folded in presence of amonium, potassium and sodium cations at pH= 7.4 in order to evaluate the effect of different cations and the structure of quadruplex on activity of the enzyme. The result indicated that the catalytic activity of NH^{4+} forms of the both DNAzymes is more than the other forms. In addition, K^+ forms of the DNAzymes were found to be more catalytically active than the Na^+ forms. The result also demonstrated that concentration of the cations effect on the catalytic activity. In conclusion, there is relation between the catalytic activity of the peroxidise DNAzymes and conformation of the quadruplex DNA.

Key words: DNAzyme; Peroxidase; G-qudruplex

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Prediction of thermophilic proteins based on features of amino acid sequence

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ABSTRACT

Resolving the thermodynamical and structural characteristics of thermophilic proteins ultimately leads to understanding the mechanisms of protein's thermal adaptation. Usually, the experimental techniques that are required to understand the thermal adaptation mechanisms of proteins are costly and time consuming. In this study, we develop a method to predict thermophilic proteins based on the features of amino acid sequence via computational biophysics approaches. For the first time, by using the information content of protein sequences and the sequence-based prediction of structural and thermodynamical aspects of protein, we introduce a new precise metric that predicts the thermophilic proteins just by utilizing protein's sequence information. Current study depicts the role of hydration enthalpy and accessible surface area in thermal adaptation of protein. This method is useful for better identification of the stability and adaptation mechanisms of proteins. The new developed metric is also useful in biotechnology and drug design to produce enzymes with pre-determined stability temperature.

Key words: Computational biophysics; Sequence-based prediction; Biothermodynamical features; Thermophilic protein

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Immobilization of Paclitaxel drug on modified magnetite nanoparticles coated with poly (Ethylene glycol) for targeted drug delivery

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ABSTRACT

In the past few decades, magnetite nanoparticles (mNPs) have attracted growing research interest due to their many applications in medicine and drug delivery. Coated mNPs are very useful for delivering chemotherapeutic drugs. In the present study, mNPs were synthesized by co-precipitating Fe^{2+} and Fe^{3+} in an ammonia solution. Then, superparamagnetic mNPs were surface modified with poly (ethylene glycol) (PEG) to improve their intracellular uptake and their ability to target specific cells. After that paclitaxel was loaded on the surface of the modified mNPs.The structural, morphological and magnetic properties of the prepared samples were characterized, using X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectra and scanning electron microscopy/energy dispersive X-ray (SEM-EDAX) analysis. Furthermore magnetic measurements were done, using vibrating sample magnetometer (VSM). All results together confirm the production of stable nano drug. Their cytotoxic potential against MCF-7 cell line was evaluated, using the MTT assay. The results suggested strong cytotoxic activity for the nano drugs compared to paclitaxel. It could be concluded that prepared nanoparticles act as nanocarriers with effective dual targeting nanoplatform for delivery of anticancer agents and appropriate model for developing smart nano drug delivery system against cancerous cells.

Key words: Immobilization; Paclitaxel; Magnetite nanoparticles; Poly (ethylene glycol); Smart drug delivery

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Effects of heparin and hyaluronic acid on native and chemically modified lysozyme amyloid fibril formation

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ABSTRACT

Glycosaminoglycans (GAGs) such as heparin and heparan sulfate are frequently associated with extracellular and intracellular amyloid deposits in most amyloid diseases, and there is evidence to support their active role in amyloid fibril formation in some cases. However, it was not fully determined which mechanism, forces and moieties in GAG chains may be responsible for the observed effects and whether other non-sulfated GAGs like hyaluronic acid are also capable for a similar enhancement of fibril formation. In this work, the fibril formation of native and chemically modified lysozyme was investigated in the presence of a heparin and hyaluronic acid at pH 2 and 7 by using thioflavin T fluorometry to elucidate the role of electrostatic interactions and sulfate moiety in the fibrillogenesis. Lysozyme was modified by acetic anhydride and citraconic anhydride to convert lysine primary amine groups to the neutral and negative charged groups, respectively. Hyaluronic acid has no charge at pH 2 and suppresses lysozymes fibrillation, probably due to complex formation with undenatured states of proteins. At pH 7, both heparin and hyaluronic acid have negative charge and lysozymes normally do not show any tendency to aggregate. Moreover, the capability of hyaluronic acid in amyloid enhancement reveals that sulfate groups are not crucial for accelerating amyloidogenesis but the extent of acceleration is proportional to the number of negative charge. According to these findings, reconsideration in the development of future therapies based on glycosaminoglycans is proposed.

Key words: Amyloid; Heparin; Hyaluronic acid; Lysozyme; Modification

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Poster – P19

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Comparative studies on drug binding to the purified and pharmaceutical-grade human serum albumin

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ABSTRACT

Albumin, the most abundant protein in blood plasma, is a monomeric multidomain protein that possesses an extraordinary capacity for binding, so that serves as a circulating depot for endogenous and exogenous compounds. During the heat sterilization process, the structure of pharmaceutical-grade HSA may change and some of its activities may be lost, therefore, in this study, we investigated drug-binding and some physicochemical properties of purified albumin (PA) and pharmaceutical-grade albumin (PGA) using two known drugs (indomethacin and ibuprofen) and by using various spectroscopic methods such as intrinsic/extrinsic fluorescence, as well as UV-Visible spectrophotometry. PGA displayed significantly lower drug binding capacity compared to PA. Fluorescence data also indicated that binding of indomethacin and ibuprofen to both albumins are accompanied with quenching the intrinsic fluorescence signals. Analysis of the quenching and thermodynamic parameters indicated that intermolecular interactions between the drugs and the proteins are different from each other. Measuring PSH index of two proteins suggests that the surface hydrophobicity of PGA decreased compared to PA, also surface hydrophobicity of PA and PGA increased upon drugs binding. Analysis of the urea denaturation indicated that PGA stability is less than PA. Also, kinetic analysis of psudoesterase activities indicated that K_m and V_{max} parameters for PGA enzymatic activity are more and less than those of PA, respectively.

Key words: Drug binding; Purified albumin (PA); Pharmaceutical-grade albumin (PGA); Indomethacin; Ibuprofen

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Poster – P20

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Synthesis, characterization and thermodynamic studies of interaction between new water-soluble metal Schiff base complexes and DNA

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ABSTRACT

Schiff bases complexes have different applications including biological, clinical, analytical and industrial in addition to their important roles in catalysis and organic synthesis. Among the metals with established biological properties, great attention has been devoted to Zn complex that have been found to interact with DNA through different binding modes. Binding to ligand DNA is usually accompanied by marked absorbance changes in the UV-vis frequency range and, sometime fluorescence emission too, due to excitation of charge transfer transitions. In this project, we report green synthesis of Zn (II) Schiff base complex and spectrophotometric studies such as fluorescence, UV-vis spectroscopy and cyclic voltammetry of complex formation between water-soluble metal tetradentate Schiff base complexes and DNA. Also the synthesized water soluble Schiff base complex of Zn (II) was characterized by IR, ^{1}H-NMR, element analysis, fluorescence and UV-vis spectroscopy. Using UV-vis spectroscopy it was proved that the complex had strong intercalation interaction with DNA. Other techniques confirmed this conclusion.

Key words: Water-soluble; DNA; Schiff base complex

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Poster – P21 Open Access

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Calculations of non-covalent interactions in the active site of hydroxysteroid sulfotransferase enzyme using DFT method

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ABSTRACT

The human hydroxysteroid sulfotransferase enzyme (PDB codes: 1J99 and 1EFH which refer to with and without DHEA as substrate, respectively) catalyses the sulphonation of DHEA on the 3α-oxygen, with PAPS contributing the sulphate. The aim of this work is to study hydrogen bond interaction properties in active site of these two structures based on DFT-D methods. In order to further understand the nature of hydrogen bonding interactions at active site of mentioned structures, we studied them at the molecular level by nuclear magnetic resonance (NMR) and nuclear quadrupole resonance (NQR) spectroscopies. They are among the most efficient techniques for obtaining information about the properties of hydrogen bonding interactions. To accurately calculate the interactions energies as well as geometries, we used dispersion-augmented density functional theory technique (DFT-D), using B3LYP, M06, M06-L, M06-2X functionals, which was parameterized specifically for intermolecular interactions of biological interest, promises to be a useful tool for studying proteins, DNA and protein–ligand complexes. Our results show that O-3 atom of DHEA interacts with OH of Tyr-231 and Nε2 of His-99 residues respectively. Also its O-17 atom contributes in the hydrogen bonding interaction with Cβ of residue Met-137. H-bonding interactions have a significant effect on the calculated electric field gradient (EFG) and chemical shielding (CS) tensors of those nuclei which contribute in these interactions. Also, these results confirm the important role of Tyr-231, His-99 and Met-137 residues in human hydroxysteroid sulfotransferase enzymatic functions.

Key words: Enzyme hydroxysteroid sulfotransferase; DHEA; Hydrogen bonding; DFT; Electric field gradient; Chemical shielding; NMR and NQR

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Poster – P22

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Comparison of prion protein instability in different force fields of molecular dynamics simulations

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ABSTRACT

Transmissible spongiform encephalopathies or prion diseases are neurodegenerative disorders which ultimately kill their victims. These include kuru, Creutzfeldt-Jacob disease (CJD), Gerstmann-Stra¨ussler-Sheinker syndrome (GSS), and fatal familial insomnia (FFI) in humans and scrapie, bovine spongiform encephalopathy (BSE) and Chronic Wasting Disease (CWD) in animals. The prion diseases are caused by structural transformation of cellular prion proteins (PrP^C) to β-rich and anomalous isoforms (PrP^{Sc}) and accumulation of amyloid fibrillar deposits in the central nervous system. The precise mechanism underling this transformation is yet to be well understood. For a better understanding of the structural mechanism of PrP^C to PrP^{Sc} conversion, we have compared molecular dynamics (MD) simulations of a Prion Protein using 2 biomolecular force fields, GROMOS and AMBER, and trajectories at 37 ºC and 1 atmosphere pressure for 10 ns second period. The MD simulations were done in explicit solvent (SPC and SPC/E) for two wild-type human prion protein structures (huPrP 90-231) using Amber ff03, GROMOS96 43a1p force fields. Comparing the obtained results with that of crystal structures, we showed that simulations outcomes are influenced by different force fields and different water models. Our results indicated that among solvent models, SPC model in GROMOS forcefield provides more accurate model to preserve protein in destabilizing conditions.

Key words: Force field; Human prion protein; Molecular dynamic simulation; Secondary structure

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Comprehensive kinetic and structural studies shed a new light on the mechanisms of inhibition by which flavonoid derivatives inhibit tyrosinase activity

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ABSTRACT

The inhibitory effects of four flavonoids on the diphenolase activity of mushroom tyrosinase were investigated using spectroscopic approaches. Analysis of our kinetic data demonstrated that flavonoids led to a reversible inhibition on the enzyme activity. Further study showed that gallic acid acted as noncompetitive inhibitor, whereas chrysin, naringin and quercetin inhibited the enzyme activity in a competitive fashion. Comparison of the inhibition constants revealed that the strength with which the inhibitors restricted the enzyme activity was ranking as follows: chrysin (Ki: 7.9 mM) < quercetin (Ki: 7.44 mM) < naringin (Ki: 3.04 mM) < gallic acid (Ki: 1.5 mM). These data, therefore, suggest that gallic acid is the most potent inhibitor compared to the other flavonoids used. In line with the kinetic data, our structural analysis showed that these natural inhibitors exerted their inhibitory effects by changing the overall structure of the enzyme. Taken together, our data clearly demonstrated that the applied flavonoids played inhibitory effects on the mushroom tyrosinase enzyme activity.

Key words: Inhibition; Kinetics; Structure; Mushroom Tyrosinase

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The effect of the *Cyperus rotundus* terpen, alpha cyperone, on the polymerization of microtubules, *in vitro*

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ABSTRACT

The rhizomes of *Cyperus rotundus* (Cyperaceae) have been used in Asian traditional medicine for the treatment of several diseases. However, few studies have investigated the biological activity and molecular mechanism of action of a-cyperone, a major compound in the rhizomes of *Cyperus rotundus*, representing about 20% of the total essential oil. a-cyperone might interact with cellular proteins and modulate their functions, but the main target of this terpenoid and the other compounds of *Cyperus rotundus* have not been discovered yet. Microtubular proteins are one of the most important proteins inside the cells and have several functions in nearly all kinds of cellular processes. The aim of this study was to investigate whether a-cyperone effects on memory or learning process in brain due to polymerization of microtubule. The result of this investigation demonstrated that a-cyperone increased tubulin polymerization and microtubule nucleation rate. a-cyperone would be able to participate in cell signaling. So it would be suggested that alpha cyperone could improve memory and the rate of learning and may prevent and of Alzheimer's Disease improving.

Key words: *Cyperus rotundus*; Alpha cyperone; Tubulin; Microtubule; Memory

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Laccase activity, stability and kinetic parameters in aqueous surfactant solutions

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ABSTRACT

In the recent years, the use of enzymes for organic synthesis reactions has been extensively increased. Laccases are multi-copper oxidoreductases with wide potential applications in green chemistry and various industries. Surfactants could be used in enzymatic reactions for improving the solubility and availability of hydrophobic substrates. These compounds may also enhance enzyme activities in water/surfactant media as compared to those expressed in pure buffer solutions. The aim of this study was to investigate the activity and stability of a fungal laccase in the presence of anionic, cationic and nonionic surfactants, namely sodium di-2-ethylhexylsulfosuccinate (AOT), cetyltrimethylammonium bromide (CTAB) and Triton X-100 (TX-100), respectively. The effects of surfactants on kinetic parameters (i.e., K_m, V_{max}), optimum temperature, optimum pH and enzyme stability were then investigated. It was found that in the presence of low concentrations of AOT, the catalytic efficiency of the enzyme increased, whereas laccase inactivation was observed by CTAB and TX-100. CTAB shifted the optimum pH of laccase to acidic pH and the optimum temperature of the enzyme to lower degrees. Thermal stability studies at 50°, 60°, and 70°C revealed TX-100 stabilization and AOT and CTAB destabilization effects which may be attributed to the possible conformational changes of the enzyme.

Key words: Laccase; Enzyme activity; Stability; CTAB; AOT; TX-100

Poster – P26 Open Access

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Biocatalysis in water-in-oil microemulsion systems

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ABSTRACT

Water-in-oil (w/o) microemulsion is defined as dispersion of water droplets in a continuous oil phase, stabilized by the presence of surfactant and/or cosurfactant molecules. This system is thermodynamically stable, isotropic, and optically transparent. W/o microemulsion systems have found many potential biotechnological applications. They are considered as an alternative media to organic solvents for enzyme catalysis, due to their protective role against solvents detrimental effects through hosting enzymes in their water cores. Additionally, biocatalysis in w/o microemulsions offer more advantages including the ability to dissolve high concentrations of both hydrophilic and hydrophobic reactants simultaneously, the existence of a very large water-oil interfacial area through which catalytic reactions with water insoluble substrates can occur, enhancement of activity, stability, and stereoselectivity of enzymes, and the possibility of easily shifting the thermodynamic equilibrium of condensation/hydrolysis reactions by adjusting the water content. The activity and stability of the enzyme entrapped in microemulsion droplets have been found to be dependent upon various parameters such as water content, the type of the solvent, and the surfactant type and concentration. The efficacy of microemulsion systems for the activity of many types of enzymes, including hydrolases (lipases, esterases, and proteases), oxidoreductases (peroxidases, oxygenases, and dehydrogenases) and transferases (kinases) has been determined and a variety of reactions such as peptide synthesis, esterification and transesterification, oxidation, hydrolysis and reduction of steroids have been previously studied.

Key words: Microemulsion; Biocatalysis; Stability; Activity

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Immobilization of albumin on modified magnetite nanoparticles and characterization

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ABSTRACT

Magnetite particles (microspheres, nanospheres and ferrofluids) are widely used in purification, separation and immobilization of protein and enzymes. They are also used in the biomedical field as a solid support for immunoassays, DNA sequencing, and cell analysis and magnetically controlled transport of anti-cancer drugs. In this work, magnetic Fe_3O_4 nanoparticles were prepared by the co-precipitation of ferrous and ferric salts with NH_4OH. Magnetite NPs coated with 3-aminopropyl trimethoxysilane (APTMS) by silanization reaction. They were activated by the glutaraldehyde (GA) method for attachment to human serum albumin. The structural, morphological and magnetic properties of as-prepared sample were characterization by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectra, scanning electron microscopy/energy dispersive x-ray analysis (SEM-EDAX) and magnetic measurements were investigated using vibrating sample magnetometer (VSM). The binding of glutaraldehyde (GA) to the nanoparticles was confirmed by FT-IR analysis. Displaying immobilization of albumin to modified magnetic NPs, high saturation magnetization, the superparamagnetic albumin modified Fe_3O_4 NPs are of significance for magnetic applications in various bioprocesses, biomedical devices and biomedicine.

Key words: Immobilization; Albumin; Magnetite nanoparticles; Glutaraldehyde; characterization

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Effect of silver-albumin nanoparticles on breast cancer apoptosis

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ABSTRACT

Silver ions have been used in medical treatments for decades. But its medical consumption as anticancer agent is predominantly limited due to their toxic side effects. Albumin is a multipurpose protein carrier for drug delivery due to its nontoxic, non-immunogenic, biocompatible and biodegradable properties. Therefore, it is ideal material to synthesize nanoparticles for drug delivery. In the present study, silver ions were coated with bovine serum albumin to control apoptosis by a desolvation technique. The surface properties of the albumin-silver nanoparticles (AS-NPs) were characterized by dynamic light scattering. Human breast cancer cells (MCF7 cells) were then cultured in the presence of the nanoparticles to evaluate the cytotoxicity of AS-NPs by the MTT colorimetric technique. As a result we observed gradual change in the turbidity of solution as a sign of nanoparticle formation. The AS-NPs formation was confirmed by dynamic light scattering which showed nanoparticle size distribution from 91 to 148 nm and mean diameter of 111 nm. Zeta potential of AS-NPs was -96 mV in pH 6.05. The effect of AS-NPs on MCF7 exhibit a dose-dependent toxicity for the cells tested and the viability of MCF7 decreased to 50% by 40 µl/well. Further findings will be verified the cell compatibility of AS-NPs in comparison with non-bound silver ion. The strongly biocompatibility feature of the albumin nanoparticles are promising for use in medical fields for cancer therapy with less toxicity on normal cell.

Key words: Silver ions; Bovine serum albumin; Biocompatibility; MCF7

MBRC

Poster – P29

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Bioinformatic analysis of different antimicrobial peptides

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ABSTRACT

Antimicrobial peptides are a significance and varied group of peptides that are secreted by different tissues and cells in a wide variety of invertebrate, plant and animal species with widespread potency such as antibacterial, viral, fungal, parasitic, protist, cancer and antioxidant activities. Different bioinformatics approaches have been done for finding any reliable parameters between antimicrobial activities and their physicochemical properties. Analyzing about 2500 antimicrobial peptides show that more than 80% of antimicrobial peptides have antibacterial activity and only less than 6% are antiviral. It seems the disulfide bonds play an important role in antiviral activity because of having the highest disulfide population in antiviral peptides (~25%) in comparing the population in antibacterial (~10%) and antifungal peptides (~14%). The average length of antimicrobial peptides is 32.38 residues with average net charge of +3.19. It indicates that the basic charged residues including His, Lys and Arg dominated to acidic residues. Therefore most of antimicrobial peptides have basic properties. Also, the smallest residue of Gly participate more than 11% that resulted in highly flexible state of the antimicrobial peptides in solution. The least amount of residues in antimicrobial peptides population are Met and Trp with average compositions of 1.24%, 1.55% respectively. Moreover structural analysis indicates that the secondary helix conformation is dominated to beta strand and/or other combined structures in the antimicrobial peptides.

Key words: Antimicrobial peptides; Bioinformatics approaches; Average length; Basic charged residues

Poster – P30 Open Access

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Determination and analysis of chameleon protein sequences in the protein data bank

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ABSTRACT

A three-dimensional protein structure is determined by its amino acid sequence. Chameleon sequences are segments which can adopt alpha, Beta sheet or coil conformations in natural proteins. These sequences have been implicated in the pathogenesis of amyloid diseases and in the structural fold conservation and functional diversity of alternative splicing protein isoforms. In this study a program was developed to search peptide segments with identical sequences but significantly different structures. A set of 10133 non-redundant protein sequences were extracted in which had less than 25 percent identity from the protein data bank (PDB). The final transition lists were classified as helix to strand, helix to coil and strand to coil alterations respectively. In addition we analyzed the occurrence of single and paired amino acids and solvent accessibility in chameleon sequences. Based on the literature, this is the first report of an assay calculating the frequency of paired amino acid in chameleon sequences. Because of the recent explosive growth of the PDB, our work obviously facilitates updating the information providing important tools for secondary structure prediction methods (for example in neural network predictor) and also could be applied in identifying amyloid formation regions in neurodegenerative diseases.

Key words: Chameleon; Secondary Structure; PDB

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Poster – P31 Open Access

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Step-wise H atom donation capability of polyphenolic myricetin to scavenge ROS: quantum based DFT method studies

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ABSTRACT

Experimentally, complete reduction of five galvinoxyl radicals ($G^{\bullet}$) by one myricetin (Myc) molecule demonstrated that each Myc should donate at least 5 H atoms via two possible different mechanisms. First; concerted 5 H atoms donation from 5 different OH groups that directly results in $Myc^{5\bullet}$ radicals (Myc → $Myc^{5\bullet}$, having 5 unpaired valence electrons). Second possible mechanism is the step-wise radical formation in 5 different OH groups of Myc. But the first mechanism, is not likely to happen due to higher energy barriers of concerted mechanisms compared to step-wise mechanism. Hence, the only way to appear $Myc^{5\bullet}$ radical is the relative stability of $Myc^{\bullet}$, $Myc^{2\bullet}$, $Myc^{3\bullet}$ and $Myc^{4\bullet}$ radicals. Therefore, the step-wise picture for the radical formation mechanism in Myc has been broadly evaluated. Semi-empirical combined quantum chemical methods (AM1/DFT) and full DFT method (DFT/DFT) with B3LYP as well as the B3P86 functional level used to calculate such properties. Different basis sets including 6-31G (d), 6-311+G (d,p) and 6-311+G (2d,2p) were applied for this aim. Quantum based DFT method studies showed the different labile phenolic O-H sites with the order of 4'-OH > 3-OH > 7-OH that play an important role in the ROS scavenging activity of Myc. Consequently, three possibly evaluated sequential sites of Myc molecule for H atom elimination are consecutively 4'-OH → 3-OH → 7-OH. This excellent property should give an effective chain-breaking antioxidant activity for Myc in biological environment which is expected to have the least side effects.

Key words: Myricetin; DFT; BDE; ROS

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Poster – P32

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Quantitative structure–activity relationships in drug discovery: Pyridinone scaffolds as anti-HIV drugs

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ABSTRACT

Quantitative structure–activity relationships (QSAR) associate, within homogeneous series of compounds, biological activities with certain structural features or with atomic, group or molecular properties, such as polarizability, lipophilicity, steric and electronic properties. The improvement of powerful and effective antiviral drugs for the control of HIV-1 infection is one of the most important goals of recent medicinal chemistry. Reverse transcriptase (RT) is a key enzyme of the human immunodeficiency virus type (HIV-1) life cycle. Several pyridinone derivatives were evaluated for their anti HIV-1 activities based on the blocking of RT. The QSAR and molecular docking was used by computational approaches for evaluating the potent affinity of more than 40 compounds into the RT inhibitor binding pocket of HIV-1. Different computational QSAR procedures were used to estimate the relationship between the physicochemical and structural descriptors of 40 derivatives of pyridinone compounds with the biological activity. The QSAR models were obtained and validated by a test set acquiring r^2 values of >0.75. The obtained results provided useful information for a better understanding the effects of different groups of pyridinone scaffolds for the type of interactions that occur between RT inhibitor binding site and pyridinone derivatives. The QSAR models will be used to guide the rational design of potent pyridinone derivatives against HIV-1 RT activity.

Key words: QSAR; HIV; Reverse transcriptase; Pyridinone

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The effect of environmental factors on the binding of muscarinic antagonist to lipase of *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic Gram-negative non-fermenting *Bacillus* that belongs to the family *Pseudomonadaceae*. *P.aeruginosa* has minimal nutrition requirements, which contribute to its broad ecological adaptability and distribution. Therefore, the organism is common in the environment including soil, water, humans, animals, plants, sewage, and hospitals. Lipase is an enzyme that hydrolyzes lipids, the ester bonds in triglycerides, to form fatty acids and glycerol. Most lipases act at a specific position on the glycerol backbone of lipid substrate. The drug has little effect on glandular secretion or the cardiovascular system. It does have some local anesthetic properties and is used in gastrointestinal, biliary, and urinary tract spasms. In this research the effect of ionic strength, viscosity and different buffer system on the activity of the enzyme in the presence and absence of muscarinic antagonist was studied. Bacterium was cultured in minimal salt medium with 1% olive oil as a carbon source, the cells were harvested and the supernatant was used for enzyme assay. This study showed that increasing NaCl and sucrose concentrations could reduce the enzyme activity. Maximum activity was detected in phosphate buffer while the activity was minimum in Tris buffer. Our results showed that neither ionic strength nor viscosity changes could refuse binding of muscarinic antagonist to the enzyme but could decrease enzyme activity.

Key words: *P. aeruginosa*; Drug; Enzyme; Inhibition; Lipase

Poster – P34 Open Access

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The effects of hydroalcohol extract of zingiber officinale on the hepatic damages resulting from ferrous sulfate in male rats

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ABSTRACT

Liver plays a pivotal role in regulating various physiological processes in the body. Evidences have suggested that various forms of liver injuries may be caused by free radical formation and subsequent oxidative stress. Iron is an essential constituent of the body. At the same time, excess iron in the body is associated with toxic effects and poses health problems. Ginger (Zingiber officinale) has been used as a spice for over thousand years. Its roots contain polyphenol compounds which have a high antioxidant activity. Hepatic damage was induced by i.p. injection of ferrous sulfate (30 mg/Kg/day) for 14 day in male wistar rats (220-260g). In final blood samples collected for determination of the serum albumin, glucose, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) concentration. Then liver samples were removed and preserved for histological studies and estimation of lipid peroxidation. Hydroalcohol extract of Zingiber Officinale (400 mg/kg/day) was administered by gavage for 14 days. Ferrous sulfate caused a significant reduction in liver function, increased liver lipid peroxidation which correlated with elevated serum enzymes, ALP, ALT and LDH as well induction of hepatic histological damages. Extract treatment reversed the parameters of liver dysfunction, increased lipid peroxidation and liver enzymes levels as well reduced total hepatic histopathological scores. These data indicate that ginger has the ability to down regulate free radicals elevation, ameliorate hepatic marker enzymes, and normalize the hepatic tissue changes in rats.

Key words: Albumin; Ferrous sulfate; Glucose; Lactate dehydrogenase; Hepatic lipid peroxidation; Zingiber officinale

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Isolation and characterization of an alkaline protease producing *Pseudomonas aeruginosa* sp.byk *28* from kerman's dairy industry sewage

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ABSTRACT

Proteases account for about 60% of total of worldwide sale of industrial enzymes in the world market. Based upon their structure or active site, these enzymes classified to different groups such as: serine, metallo, aspartic, cysteine, acidic, neutral and alkaline protease. The sources of protease are plants, animal organs and microorganisms, but microbial source due to their rapid growth, the limited space required for their cultivation and their ability to genetically transformation for generation new enzymes with different properties is preferred. Usage of enzyme in industry is ecofriendly for example biological treatment in leather processing instead of chemical treatment prevent Environmental pollution. Water waste sample was collected from Kerman,s dairy industry sewage. For isolation of *Pseudomonas aeruginosa* sp.BYK28 from collected sample, skimmed milk agar medium was used. Amount of protease production was studied in different time period incubation. Isolate was identified by biochemical test then identification confirmed by molecular characterization of 16S rRNA gene. According to clear zone around colonies, protease production isolate was selected. The maximum amount of protease was found at 48 hour incubation. The obtained results showed isolate was motile, rod, Gram-negative, oxidase and catalase positive, could ferment Glucose and hydrolyze casein and was not able to hydrolyze gelatin. The result of molecular characterization showed that strain was *Pseudomonas aeruginosa* sp.BYK28, (AC: KM878675). The proteolytic activity of *Pseudomonas aeruginosa* sp.BYK28 able it to be used in different industry like detergent, food, pharmaceutical, leather, textile industry, etc.

Key words: Protease; Isolation; Characterization; *Pseudomonas aeruginosa*; Sewage

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Structural analysis and binding modes of benzodiazepines with modeled GABA$_A$ receptor subunit alpha-1

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ABSTRACT

Activation of chloride gated GABA receptors regulates the excitatory transmission in the epileptic brain. Positive allosteric modulation of these receptors via distinct recognition sites is the therapeutic mechanism of antiepileptic agents which prevents the hyperexcitability associated with epilepsy. These distinct sites are based on subunit composition which determines binding of various drugs like benzodiazepines. The binding of antiepileptic agents to this recognition site increases the affinity of GABA receptor for modulating the inhibitory effects of GABA-induced chloride ion flux. In the pentameric complex structure of these receptors, the α/γ interface forms the benzodiazepine (BZD) binding site on extracellular domain. Thus the α subunit is shown as highly required for functional modulation of the receptor channels by benzodiazepines. The extracellular domain of α subunit of human GABAA is modeled and docking studies are performed with clonazepam, clobazam, clorazepate, diazepam, midazolam, lorazepam. In order to accomplish this, the amino acid sequences human gamma-aminobutyric acid receptor subunit alpha-1 precursor was obtained from National Center for Biotechnology Information. Three-dimensional structure of protein sequences were received from phyre2 protein fold recognition server. *Molecular and structural properties of drugs were taken by drug bank server.* Autodock4.2 software was used for docking purpose. This study shows hydrogen bond interactions of GABRA1 with selected drugs and binding modes and the interacting amino acid residues involved in recognition of the compound. The results obtained from this study would be useful in understanding the modulatory mode of GABRA1 with benodiazipine drugs.

Key words: GABA; GABA$_A$ receptors; Epilepsy; Benzodiazepine drugs; Epilepsy

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The effect of DNA sequence on ACF remodeler mechanism

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ABSTRACT

The packaging of chromosomal DNA by nucleosomes condenses and organizes the genome, but occludes many regulatory DNA elements. However, this constraint also allows nucleosomes and other chromatin components to actively participate in the regulation of transcription, chromosome segregation, DNA replication, and DNA repair. To enable dynamic access to package DNA and to tailor nucleosome composition in chromosomal regions, cells have evolved a set of specialized chromatin remodeling complexes (remodelers). The chromatin remodeler ACF, as member of ISWI family, act as a dimer motor and produce equal spacing nucleosomes. Single-molecule experiments show that this remodeler move the nucleosome towards the longer flanking DNA. In this paper we propose a model to study the effect of different sequences of DNA on the remodeler function and nucleosome positioning, using monte-carlo simulation. In the proposed model, rates are dependent on the length of the flanking DNA.

Key words: ACF; Chromatin remodeling complex; Nucleosome; Remodeler

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The effect of charge repulsion on the structure and function of an anticancer peptide derived from endostatin

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ABSTRACT

Endostatin, a C-terminal fragment derived from collagen XVIII, is a potent inhibitor of angiogenesis and tumor growth. The crystal structure of endostatin exhibited a basic patch of the surface located Arg residues that are critical for heparin and integrin binding. A peptide fragment corresponding to the N-terminus of the endostatin was shown to be responsible for its antiangiogenic and antitumor activities. It was also shown that Arg^{27} is important in the binding of the mentioned peptide to the receptors. To investigate whether C-terminal moiety is involved in the anticancer properties of this peptide, we compared a native endostatin peptide with a variant having Ile26Arg mutation. The structure of the peptide variants was analyzed by far UV CD, FTIR and fluorescence spectroscopies. To compare the binding capacity of the peptides to the $\alpha_V\beta_3$ integrin, the resulting structures from MD simulations were docked to the receptor. Peptides were evaluated for anti-proliferation and capillary tube formation *in vitro*. In addition, the effect of peptides on breast tumor growth was examined in BALB/c mice. The results showed that the mutant peptide has different antiangiogenic activity *in vitro*, antitumor function *in vivo*, as well as secondary structure as compared to the wild type peptide. Theoretical studies revealed different RMSF and binding energy of the peptides to the $\alpha_V\beta_3$ integrin. We conclude that the repulsion between two positive charges at the C-terminal moiety of the mutant peptide results in structural alterations and, as a consequence, the functional differences in comparison to the wild type peptide.

Key words: Endostatin; Peptide engineering; Antitumor activity; Secondary structure; MD simulation

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Development of a thermostable and ionic liquid resistant laccase by saturation mutagenesis

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ABSTRACT

Laccases are particularly promising enzymes for biotechnology and bioremediation purposes. They are among the most effective enzymes capable of catalyzing the degradation of phenolic compounds with poor water solubility. The technological utility of lacasses can be enhanced greatly by their use in ionic liquids rather than in conventional organic solvents or in their natural aqueous reaction media. In the current study, a laccase from *Bacillus* HR03 has been engineered through a semi rational method. By screening a library of 450 clones, Glu188Tyr and Glu188Phe showed a distinct improvement in thermal stability and ionic liquid tolerance. In comparison with the wild type, selected mutants exhibited higher k_{cat}/K_m against ABTS in the imidazolium based ionic liquids, (1-ethyl-3-methyl imidazolium chloride [EMIm][Cl], butyl-3-methyl imidazolium chloride [BMIm][Cl] and hexyl-3-methyl imidazolium chloride [HMIm][Cl]). Glu188Tyr had a catalytic efficiency, two times greater when compared to the wild type in [HMIm][Cl]. Far-UV circular dichroism (CD) exhibited no significant changes in the secondary structure of the mutants and wild type. Glu188Tyr revealed a more compact structure using Near-UV CD and fluorescence spectroscopy that could account for its high thermal stability. According to bioinformatic analysis, π-π and anion- π interactions played the dominant role in stabilizing both variants.

Key words: Laccase; Ionic liquids; Semi-rational method; Anion-π; π-π interaction

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The fluorescence study on the interaction between β-lactoglobulin and resveratrol in the presence of electromagnetic field

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ABSTRACT

β-lactoglobulin (β-LG) is a globular protein and one of the major protein in the whey of ruminant milk. This protein is widely used in the food industry due to its high nutritional value. The interaction occur between β-LG and a great variety of hydrophobic ligands, such as retinal, fatty acids and carcinogenic hydrocarbons. Because of its stability under acidic pH conditions, β-LG is resistant to digestion in the stomach and therefore it is considered as one of the significant allergens in the human infant milk allergy. As a natural polyphenolic compound, resveratrol (3, 5, 4′-trihydroxystilbene/RES) is a produced in plants (e.g., grapes, peanuts) in response to injury and fungal attack. Due to its polyphenolic structure, RES possesses antioxidant activity. This compound has been shown to prevent or slowdown the progression of a wide variety of diseases, including cancer, cardiovascular disease and Alzheimer's disease. In this study, the interaction of RES and β-LG in the presence of electromagnetic fields (EMFs) (2.4GHz, 1.3GHz) was investigated, using fluorescence spectroscopy. The results obtained from this study indicated that RES has a strong ability to quench β-LG fluorescence emissions at the excitation wavelengths 280 and 295 nm, in the presence EMFs. The fluorescence intensity of β-LG decreased with increasing concentration of RES in the presence of EMFs, indicating that RES was bound to β-LG and β-LG-RES complex formed. In addition, a red shift occurred, which suggested that the microenvironment around the chromophore became more polar after addition of RES. In the presence of EMFs, the interaction between β-LG and RES was stronger than in its absence. Our results suggest that structure of the protein altered on exposure to the EMFs and affinity of the RES to β-LG was increased in the presence of EMFs.

Key words: Resveratrol (RES); β-lactoglobulin (β-LG); Electromagnetic field (EMF)

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Activity and stability analysis of immobilized chondroitinase on porous silicon nanoparticles

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ABSTRACT

Enzymes are valuable biocatalysts of nano-scale that regulate the critical activities in cells and organisms. Using enzyme as a drug in the treatments depends on several factors, such as physical and chemical stability, immunogenic and favorable pharmacokinetic properties. Different ways including immobilization, modification and engineering had been previously discussed to overcome protein drugs limitations and improve their stability. Chondroitinas ABC1 from *Proteus vulgaris* is a drug enzyme that can be used to treat spinal cord injuries. This enzyme breaks the glycosaminoglycan chondroitin 4 - sulfate, chondroitin 6 – sulfate, dermatan sulfate in chondroitin sulfate and proteoglycans. One of the main problems of chondroitinase is its low thermal stability. Therefore, it is necessary to inject the enzyme for several times to provide the active enzyme at the injury site. Repeated injection could increase the trauma and trigger an immune reaction in the patient. In order to resolve this problem, the enzyme was immobilized on porous silicon nanoparticles that were promising materials for drug delivery because of their biodegradability, biocompatibility instability and intrinsic photoluminescence. Biochemical characterization, structure and stability of the free and immobilized enzymes were compared and loading capacity and release behavior of nanoparticles were evaluated using UV and fluorescence spectroscopy.

Key words: Chondroitinas ABC1; Porous silicon; Immobilization; Stability

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Influence of histidine-histidine interaction on activity, stability and structure of uricase

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ABSTRACT

Enzymes as drugs have two important features that distinguish them from all other types of drugs. First, enzymes often bind and act on their targets with great affinity and specificity. Second, enzymes are catalytic and convert multiple target molecules to the desired products. These two features make enzymes specific and potent drugs that can accomplish therapeutic biochemistry in the body that small molecules cannot. These characteristics have resulted in the development of many enzyme drugs for a wide range of disorders. Urate oxidase or uricase catalyzes oxidation of uric acid to alantoin which can be produced as a recombinant enzyme drug for treatment of hyperuricemia. The inherent poor stability of this enzyme is known to limit its efficacy, So that, stabilization of this enzyme is considered.There are a lot of factors that influence proteins stability; recent studies have shown that His-His interactions could have be an important role in stability and activity of proteins. For this purpose, we used site-directed mutagenesis (SDM) to substitute Gly 267 with His, Ala and Pro. Purified variants were biochemically characterized and compared with the wild type. Results revealed considerable changes in the catalytic efficiency, thermal stability and tertiary structures.

Key words: Drug enzymes; Urate oxidase; Thermal stability; Catalytic efficiency

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Albumin nanoparticles containing copper complex as SOD mimetic enzyme

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ABSTRACT

Superoxide dismutase (SOD) is an antioxidant enzyme involved in scavenging of superoxide radical anion ($O_2^{\bullet-}$) and protects cells from oxidative damage. In cancer cells $O_2^{\bullet-}$ production and low SOD activity may cause the malignant cells highly dependent on SOD for survival. In this research, albumin nanoparticles containing copper-cysteine complex (ANPs-CuCys) have been designed and evaluated as SOD mimetic enzyme. The inhibition of pyrogallol autoxidation assay (IC_{50} value) method was used for assaying SOD mimetic activity. Dynamic light scattering (DLS) was used for investigating surface properties of the ANPs-CuCys. The IC_{50} value (0.5 µg/ml) showed that ANPs-CuCys is a good $O_2^{\bullet-}$ scavenger. The DLS results showed that the average diameter for diluted ANPs-CuCys was 28 nm. Zeta potential of ANPs-CuCys was -92 mV in pH 6.46. ANPs-CuCys is capable of removing the biologically important $O_2^{\bullet-}$ and it may also be useful for medical treatment of oxidative stress diseases.

Key words: Nano-albumin; Copper; SOD mimic enzyme; Superoxide radical anion

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Interaction of human serum albumin and fenvalerate; experimental and computational studies of albumin-fenvalerate interaction

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ABSTRACT

Fenvalerate is an insecticide toxin which has an application in agriculture. We investigated interaction of this toxin with human serum albumin (HSA) which is a carrier for small molecules such as drugs and toxins in the blood stream. In this study, four different methods including UV-VIS, FT-IR, fluorescence spectroscopy and molecular modeling were used to characterize the binding properties of the Fenvalerate to HSA at molecular level under physiological conditions. The experiment performed after incubating the protein and ligand for one day and 30 days. The binding constant which achieved with UV-VIS spectroscopy was 3/78 $\times 10^4$ M^{-1}, indicating a relatively good binding interaction between ligand and receptor. FT-IR results indicate a decrease in α-helixes and an increase in other structures such as beta-sheets and random coils upon ligand binding, particularly at high concentrations of the ligand. The Fluorescence intensity of HSA decreased regularly with the gradually increasing concentration of Fenvalerate in the mentioned incubation times. These results also were the evidence of ligand binding to the receptor; therefore they confirm the results of the UV-VIS. On the other hand, docking calculations illustrated a potential binding site in the region III-B of HSA. Overall, our results illustrated a binding site for Fenvalerate within HSA which probably suggest a chance for excretion of this toxin from human body with the help of this carrier protein. However, to confirm, the capability of HSA in Fenvalerate excretion from human body further experiments must be perfumed.

Key words: Human serum albumin protein; Fenvalerate toxin; Fluorescence; Molecular modeling

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Synthesis of novel water-soluble metal Schiff base complexes, spectrophotometric and thermodynamic studies of the interaction of these complexes with DNA

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ABSTRACT

Metal Schiff base complexes have been studied extensively because of their remarkable chemical and physical properties. Metal complexes of phenolate Schiff base with favorable cell membrane permeability have been exploited in cancer multidrug resistance and used as antimalarial agents. In this work a new water-soluble Schiff base complex of Ni (II) synthesized and characterized by IR, ^{1}H-NMR, element analysis, UV-vis spectroscopy. Spectrophotometric studies such as fluorescence, cyclic voltammetry and UV-vis spectroscopy of complex formation between water-soluble metal tetradentate Schiff base complex and DNA. In the present approach, H_2O was utilized as the environmentally green solvent throughout the preparation. Using UV-vis spectroscopy it was proved that the complex had strong intercalation interaction with DNA. Other techniques confirmed this conclusion.

Key words: Water-soluble, DNA, Schiff base complex

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A computational investigation on the molecular interaction of AzrC and FMN

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ABSTRACT

Bacterial azoreductases belong to the family of oxidoreductases which catalyze the reductive cleavage of the azo bonds, detoxification and decolorization of azo dyes. AzrC protein from *Bacillus* sp. B29 is a FMN-dependent azoreductase and has a homodymeric structure with two moles of FMN as a non-covalent prosthetic group. In this study, three MD simulations were performed in order to analyze molecular information about the effects of absence and presence of FMN in dynamic nature of active site residues. Pairwise decomposition and alanine scanning calculations provided important information about the FMN binding site. Furthermore, hydrogen bonds analyses determined the critical residues that have significant roles in making suitable hydrogen bond between AzrC and FMN. Finally, the MMPBSA/MMGBSA results of three MD simulations indicate two facts about FMN and ligand interactions with AzrC. First, suggest the VDW as the favorable contribution in FMN and AzrC protein and second verify the key role of FMN in ligand binding in addition to its catalytic function. In conclusion, our MD simulations reveal some molecular aspects of FMN and AzrC interactions, which may be useful for future experimental studies.

Key words: AzrC; Molecular dynamic; FMN; Alanine scanning; MMPBSA

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In silico study of disulfide bond in mammalian amylases

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ABSTRACT

Amylases are digestive enzymes which hydrolyze glycosidic bonds in starch to glucose, maltose, maltotriose and dextrin. They synthesized by bacteria, fungi, plants and animals. These enzymes have many applications in food and pharmaceutical industries. To improve the productivity of amylases, bioinformatic study of them can be useful. In order to investigate structural relationships among mammalian amylase and other amylase, 15 amylase with resolved three dimensional (3D) structure were retrieved as pdb and fasta format from Protein Data Bank server and were analyzed using various tools and softwares. The results showed that there is a remarkable similarity between mammalian alpha amylase such as number of disulfide bond, amino acids percentage, molecular weights (MW) and 3D structure. Due to their physiological conditions, disulfide bonds are available in mammalian amylases which give them more structural stability. Our results showed that most of mammalian amylases contain 5 disulfide bonds and their locations are conserved, as well. Moreover, evolutionary surveys by means of alignment studies were conducted. Evolutionary analysis represented that mammalian amylases exhibited same evolutionary course.

Key words: *In silico* analysis; Amylase; Disulfide bond; Protein stability

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Codon optimization and cytoplasmic expression of human epidermal growth factor (hegf) in SHuffle T7 *E. Coli* strain

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ABSTRACT

Epidermal growth factor (EGF) is a member of an extensive class of molecules with 53 amino acid residues in a single chain polypeptide and a molecular mass of 6200 Da. The EGF family member proteins have high similarity in characteristics of structural, functional, and biological effects and additionally contain one or more conserved amino acid sequence repeats. This conserved sequence contains 6 cysteine residues, forming three intramolecular disulfide bridges. Production of recombinant proteins, especially those having clinical applications are important in drug design strategy. Due to the use of this compound in wound healing and especially those wounds which caused by burns, carrying out essential research in this field is very important. The aim of this study was the construction of recombinant fusion/hEGF which will be used for expression of recombinant hEGF in the *SHuffle T7 E.coli* strain. This strain was engineered to produce proteins, containing disulfide bonds in the cytoplasm. Our result showed in favor of cytoplasmic expression system which may be suitable to produce the small eukaryotic disulfide-bonded proteins like hEGF.

Key words: Codon optimization; Epidermal growth factor; *SHuffle T7 E. coli* strain; Disulfide bonds

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Lack of the *DPY19L2* gene in patients with globozoospermia

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ABSTRACT

DPY19 like2 (*DPY19L2*) is a transmembrane protein located in the inner nuclear membrane of the sperm. Its absence leads to disruption of the layered structure present at the nuclear/acrosome junction during acrosome spreading and eventually to acroplaxome detachment from the nucleus. Lack of acrosome, decreases the capacity of the sperm fertilizing with the oocyte which consequently results in infertility. Globozoospermia is a rare (<0.1% in male infertile patients) and severe teratozoospermia characterized by round-headed spermatozoa lacking acrosomes. *DPY19L2* which is dominantly expressed in the testis is one of the genes that seem to be involved in this disease. In this study, 24 men with total globozospermia and 24 men with normal spermogram referring to Royan institute were selected and the deletion of their *DPY16L2* gene was examined using specific primers and PCR technique. The results showed a whole *DPY19L2* gene deletion in 10 cases of the patients group (41.7%), however, none of the individuals in the control group showed this deletion. According to our data and based on the critical role of *DPY19L2* protein in the attachment of acroplaxome to the nucleus, it can be concluded that absence of this protein can be one of the major causes for globozoospermia in Iranian infertile men.

Key words: *DPY19L2*; Globozoospermia; Male infertility

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Study on the resonance light scattering spectrum of cytochrome C in the presence of electromagnetic field

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ABSTRACT

Resonance light scattering (RLS) is an elastic scattering phenomenon that was first introduced and established by Pasternack et al. and its first application for analytical use was developed by Huang et al. It is often used to study the aggregation and assembly of biological macromolecules by means of an ordinary fluorescence spectrometer. RLS is an extremely sensitive and selective technique for monitoring molecular assemblies. In the recent years, the method has been developed for the determination of proteins. It can be combined with other techniques such as absorption, fluorescence and CD spectroscopies, and can compensate for the drawbacks of spectrophotometric and fluorometric measurements. The present study describes an investigation by RLS intensity of the Cytochrome C in the presence of electromagnetic fields (EMFs). The results obtained from this study indicated that EMFs had an ability to increase the RLS intensity.

Key words: Cytochrome C; Spectroscopy; Electromagnetic field; Resonance light scattering (RLS)

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Thermodynamic stability study of native and modified mushroom tyrosinase

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ABSTRACT

Mushroom Tyrosinase (MT) is a copper-containing enzyme, which is widely distributed in microorganisms, animals and plants. It is also a key enzyme in melanin biosynthesis, which plays a crucial role in determining the color of mammalian skin and hair. Nowadays melanoma is the one of the most terrified and lethal cancers. In this work, the modification of tyrosinase by Woodward's reagent k has been done and its thermodynamic stability was investigated. For the study of stability, thermodynamic parameters obtained from thermal and chemical denaturation of the native and modified enzyme. T_m values in thermal denaturation showed thermal instability for modified enzyme. T_m values for the native and modified enzyme with different concentrations of the modifier (0.5, 1, 5 and 10 mM) were determined 61.2, 60.1, 58.3, 53.9 and 45.5 (^{0}C) respectively. In chemical denaturation 8 M Guanidium Hydrochloride was used. The C_m (half of modifier's concentration) and ΔG_{H2O} (free energy) values for the native and modified enzyme were obtained. The values of ΔG_{H2O} for the native and modified enzymes were 17.22, 16.75, 15.0, 13.7 and 11.5 KJ/mol and the values of C_m are 8.0, 7.5, 6.7, 10.0 and 8.0 (M) respectively. Thus, decreasing in values of ΔG_{H2O} for the modified enzyme in comparison with its native form indicate the protein instability.

Key words: Tyrosinase; Woodward reagent k; Modification; Thermodynamic; Stability

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Lysozyme fibrillation inhibition by fisetin and diadzin small molecules

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ABSTRACT

Neurodegenerative disease such as Alzheimer, Parkinson and so many related diseases are associated with a form of protein conformational change known as amyloid fibrils product. It was showed that fibrils and protofibrils intermediates are cytotoxic, therefore numerous reports have been attempt to inhibit fibrillation process as a therapeutic methods. Peptides, surfactants and aromatic small molecules have been applied as fibrillation inhibitors. In this report, we examined the interaction of the two natural small molecules (fisetin and diadzin) with hen egg white lysozyme (HEWL) for inhibiting the fibril formation products with different kinds of methods such as fluorescence, dynamic light scattering, transmission electron microscopy and circular dichroism. The aim of this study was based on bringing information into possible mechanism of interaction of natural small molecules with amyloied formation products. This report showed that fisetin and diadzinconsiderably hindered nucleation, and therefore, fibrillation of lysozyme in a dose-dependent manner.

Key words: Hen egg white lysozyme; Fisetin; Diadzin; Amyloid; Fibril

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In silico inhibitory activity against aldose reductase by telmisartan

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ABSTRACT

Aldose reductase (ALR) enzyme plays a significant role in conversion of excess amount of glucose into sorbitol in diabetic condition, inhibitors of which are potential therapeutic candidates in the treatment and prevention of diabetic complications. Recent studies demonstrated that telmisartan provided a significant antidiabetic effect and ameliorated hyperglycemia. Therefore, the aim of this study was to investigate the ALR inhibitory activity of telmisartan using *in silico* docking studies. A known ALR inhibitor, Epalrestat, was used as the standard. The Autodock Vina program was used to estimate the conformation of the protein–ligand complex. The binding sites of the Epalrestat was found to be GLY 18, TRP 20, LYS 21, TYR 48, HIS 110, TRP 111, TYR 209, SER 210, SER 214, ILE 260. The potential binding site of telmisartan was, PRO 218, TYR 48, LEU 300, TRP 111, PHE 115, TRP79, LEU124, ALA299, PHE122, TRP219 and TRP20. Telmisartan showed better binding energy (−9.5 kcal/mol) when compared to standard Epalrestat (−8.5 kcal/mol). These molecular docking analyses might lead to the further development of potent ALR inhibitors based on telmisartan structure for the treatment of diabetes.

Key words: Aldose reductase (ALR); Telmisartan; Molecular docking

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Effect of copper oxide nanoparticles on liver enzymes activity in broilers

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ABSTRACT

Due to the widespread application of nanoparticles in our country and the lack of detailed documentation about the toxicity of the compound, as well as the location, structure, function and biochemistry of the liver tissue, which makes it highly vulnerable to toxic compounds, Therefore, the present study tries to investigate the effect of CuO nanoparticles on the pathological changes of liver tissue in broilers. In this experimental study, 60 one-day female broiler chickens of Ross 308, with an approximate weight of 40 g were examined. The chickens were randomly divided into three groups of, control, experimental groups 1 and 2. The control group received water and food, and no special experimental material were feed orally or by injection. The experimental group 1 was feed by 16 mg / kg and experimental group 2 received 32 mg /kg of body weight CuO nanoparticles. All medications were combined with feed and given to 5-days chickens for 30 days. At the end of the period, serum samples were obtained for determining ALT and AST enzymes as a liver injury index. This study showed that AST enzyme activity decreased significantly in experimental group 1 compared to control ($P<0.05$) and the enzyme activity of ALT and liver weight showed no significant differences. It can be concluded that CuO nanoparticles change no significant change in liver tissue and its enzymes activity.

Key words: Copper oxide nanoparticles; Liver; ALT and AST; Broiler chickens

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Predicting the effect of a single amino acid substitution on *Renilla* luciferase stability

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ABSTRACT

Renilla luciferase (RLuc) from the sea pansy *Renilla* reniformis is a 36-kDa monomeric enzyme that catalyzes the oxidation of coelenterazine to yield coelenteramide and blue light with a spectral maximum of 480 nm. In *Renilla* reniformis, RLuc is found in membrane-bound intracellular structures within specialized light emitting cells. In this study, the effect of proline 220 to alanine substitution on RLuc stability has been investigated by bioinformatic studies. The amino acid sequence of *Renilla* luciferase was obtained from NCBI data bank and analyzed by I-Mutant server. Proline 220, which is critical residue for active site geometry, was selected and tested by site-directed mutagenesis. The amino acid proline was replaced with amino acid alanine and ΔΔG between wild type and mutant form was calculated. The bioinformatic predictions showed that ΔΔG value is -1.05 kcal/mol. The negative value for ΔΔG indicates that the mutant enzyme is less stable than the wild type. Thus, in addition to the importance of proline 220 in formation of the active site geometry, this residue has an important role in protein stability. The result is consistent with the general function of proline, in which this amino acid increases the stability of the protein by reducing the total entropy.

Key words: *Renilla* luciferase; Protein Stability; Proline 220; I-Mutant server

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Isoleucine 163 may have a dual role both in activity and stability of *Renilla*-luciferin 2-monooxygenase, a bioinformatic study

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ABSTRACT

Renilla-luciferin 2-monooxygenase (EC1.13.12.5) is an enzyme that belongs to the family of oxidoreductases. This enzyme catalyzes the oxidative decarboxylation of coelenterazine in the presence of O2 and produce coelenteramide and blue light. One common protein engineering goal is increasing the enzyme stability by site-directed mutagenesis. An application of some bioinformatic servers is prediction of protein stability changes upon amino acid substitution. Isoleucine 163 was found as a critical residue in formation of substrate cavity in RLuc. In the present study the impact of the manipulation of Isoleucine 163 on *Renilla* luciferase stability has been investigated. For this purpose, the amino acid sequence of *Renilla*-luciferin 2-monooxygenase was taken from NCBI data bank and the three dimension structure was built using Swiss model. The stability of protein was predicted. Subsequently, isoleucine residue at position 163 in the wild type was manipulated by alanine-scanning method using bioinformatic server (folding.biofold.org), and $\Delta\Delta G$ was calculated. The Results revealed that the amount of $\Delta\Delta G$ was decreased after site-directed mutagenesis at Isoleucine 163. The amount of $\Delta\Delta G$ was obtained -2.62 by sever calculations. This result suggest that amino acid residue at position 163 may have importance in protein stability. Isoleucine 163 is located closely to the enzyme active site, thus probably this amino acid play a significant role in protein activity and stability.

Key words: *Renilla* luciferase; Enzyme cavity; Isoleucine 163; Protein stability

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Pathway and network analysis in primary open angle glaucoma

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ABSTRACT

Glaucoma is a group of multifactorial ocular diseases and the second leading cause of blindness worldwide. Primary open angle (POA) is the most common type of glaucoma, characterized by progressive optic nerve degeneration. Numerous genes and proteins have been revealed to be associated with POAG, but the pathologic mechanisms of the disease are still poorly understood. Proteomics, the collective study of proteins in an organism at a given condition, has extensively been used for the high-throughput identification of proteins related to POAG. A significant obstacle in proteomics studies is the data variability which makes it hard to interpret the results. Pathway analysis and network topological information can help address the challenge and provide a greater appreciation of the disease mechanism and progression. The purpose of this paper is to determine POAG biological and network information to further understand the mechanisms associated with POAG. PANTHER classification system is used, including classification with gene ontology, protein class and pathway. 474 gene/protein IDs were extracted from previous proteomic studies. Among pathways found by PANTHER, apoptosis signaling pathway was the most significant (with the p-value of 5.54E-12). Other PANTHER categories results demonstrated that developmental processes, receptor binding, extracellular region and extracellular matrix proteins were the most significant biological process, molecular function, cellular component and protein class respectively. A network analysis on proteins was also performed using STRING database and cytoscape software. The resulted POAG network, containing 312 nodes and 1348 edges was clustered into 9 subnetworks by CytoCluster application.

Key words: Glaucoma; Pathway analysis; Network; Proteomics

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Expression of neutral peroxidase from *Lepidium draba* in prokaryotic host

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ABSTRACT

Peroxidase is an enzyme which catalyzes oxido-reduction reaction between hydrogen peroxide and reductants. It has been extensively used in biotechnology and related sciences and can be considered as the most widely used enzyme. Thereby, it is necessary to find other new sources of the enzyme due to its importance. Up to now, horseradish peroxidase (HRP) that is one of neutral isoenzymes of *Armorica rusticana* (Horseradish) has been purified and because of the low level of production and high cost of purification, it is not a frugal enzyme. Thus, purification of the enzyme from other plants as well as obtaining of the recombinant protein from prokaryotic host can have scientific and economic benefits. For these reasons, here, the peroxidase gene from neutral isoenzymes of *L. draba* is studied and then cloned into the expression vector pET28a. Recombinant DNA is transformed into competent *E. coli* BL21 (DE3) cells. Analysis of clones is performed by two techniques: clones PCR and digestion of DNA by restriction enzyme. BL21 (DE3) cells harboring the expression constructs are grown in LB medium and then induced with IPTG. The cell pellet is broken in buffer in the presence and absence of reducing agent and then protein expression analyzed by SDS-PAGE. The SDS-PAGE analysis shows that the recombinant protein is successfully expressed. Also, based on the results, majority of the protein was expressed as insoluble inclusion body.

Key words: Expression; Inclusion body; *Lepidium draba*; Peroxidase; Recombinant protein

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Comparative view on the structure and cytotoxicity of the fibrils due to alzheimer's disease and diabetes

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ABSTRACT

In diabetic condition, the serum proteins become glycated in a non-enzymatic pathway, which produces cytotoxic fibrils. In Alzheimer's disease (AD), the oligomers and fibrils of the Aβ (a 39-43 amino acid peptide) are accumulated in brain, which cause synaptic loss and dysfunction. As AD and diabetes are linked together and have similar pathogenic and cellular pathways, in this study we compared the fibrils from glycated BSA and the Aβ (25-35) (the most important sequence in fibrillogenesis and toxicity) from the fibrils and cytotoxic point of view. To prepare the fibrils, BSA was glycated in separate solutions of glucose and fructose in Q-UV water in 37°C and Aβ (25-35) fibrils were also made in Q-UV water in 37 °C. To analyze the structure of the fibrils, intrinsic and tryptophan related fluorescence of the glycated samples, Thioflavin T, Congo red, surface hydrophobicity assays were used. In order to assess and compare the cytotoxicity of the fibrils, primary cultures of microglia were prepared. The rate of apoptosis and/or necrosis of treated microglia cells was detected by the results showed that although the glycated BSA (fructose and glucose) showed more fibril contents than the Aβ (25-35), but the Aβ (25-35) is more toxic for microglia cells than the glycated BSA and trigger more apoptosis in the stated cells. It can be deduced that in patients with diabetes who can acquire AD more than the prevalence rate of the normal people, the effect of AD fibrils on the cells is more deteriorating than the effect of diabetes.

Key words: AD; Diabetes; Aβ; fibril; Glycation

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Comparison of cadmium and zinc ion absorption by recombinant defensin

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ABSTRACT

Heavy metal pollutants such as Cd^{2+}, Hg^{2+}, Pb^{2+}, As^{2+} and Se^{2+} are global problem that are a growing threat to humanity. These metals and metalloids have toxic effect on plants and animals, which are strongly poisonous to metal-sensitive enzymes, resulting in growth inhibition and death of the organism. These metals also accumulating in plants reduce root growth rates, increase permeability of membranes, change processes of cytoplasm vacuolization, inhibit DNA synthesis and photosynthesis, disturb transfer of assimilates and mineral nutrition and etc. Higher organisms such as plants and animals generally respond to heavy metal stress by inducing metal-binding cysteine-rich peptides such as metallothioneins, phytochelatins and defensins. In this study, heavy metal bio-absorption potential of recombinant defensin protein expressed in bean was determined. Each mol of recombinant defensin is able to absorb 1.458 PPM zinc ions or 1.53 PPM of cadmium ions. Molecular dynamics simulations were carried out using a model of 6 Cd^{2+} or 6 Zn^{2+} and other ions enclosed in a fully hydrated simulation box with the defensin. Totally, 180 ns MD simulations were done in three phases. The experimental and molecular dynamics simulation results showed that recombinant defensin is not able to absorb zinc ions but it is able absorbing cadmium ions.

Key words: Absorption; Defensin; Cadmium; Zinc; Molecular dynamics simulation

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Calcium indicates significant destabilizing effect on peroxynitrite-modified lens crystallins

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ABSTRACT

Peroxynitrite (PON) is a highly potent reactive oxygen and nitrogen species which has been indicated in the pathogenesis of various ocular disorders. Calcium homeostasis has been indicated to possess fundamental importance in lens pathophysiology, while its level markedly increases in the cataractous lenses. Moreover, PON has been reported to induce calcium efflux from mammalian mitochondria. This study was performed with the aim to assess the impact of calcium ion on structure, protease stability and aggregation propensity of PON-modified lens crystallins, using different spectroscopies and gel mobility shift assay. The PON modification of lens proteins was accompanied with the increase in the contents of carbonyl group, dityrosine, nitrotyrosine and nitrotryptophan. PON-modified lens crystallins indicated an increased level of solvent exposed hydrophobic surfaces, high propensity for aggregation and increased level of proteolytic instability. The results of gel mobility shift assay demonstrated that calcium ion play an important function in the induction of disulfide and dityrosine covalent cross-linkings and induces formation of the protein oligmers with relatively bigger sizes. Overall this study suggests that the simultaneous rise of calcium and PON in eye ball are important risk factors for the development of cataract disorders.

Key words: Peroxynitrite; Calcium; Lens cryatallins; Oligomerization; Aggregation

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Effect of camel casein peptides on secondary structure of glycated human serum albumin

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ABSTRACT

The Maillard reaction or the non-enzymatic glycation after a complex series of reactions that involve reducing-sugars and proteins produce a large number of end-products that are known as advanced glycation end-products (AGEs). Today there is an increasing demand for natural AGE inhibitors and AGE breakers as potential therapeutics for curing diseases diabetes which is natural, less toxic, and cost-effective. Milk protein-derived bioactive peptides have been used frequently in food additives for the formulation of functional foods. The bioactive peptides derived from milk proteins display various functionalities such as antioxidant activities and anticancer activities. Camel milk proteins differ from bovine milk, which has a direct effect on its functional and biological properties. The object of this work was to investigate the effect of glycation on secondary structure of human sermon albumin (HSA) in absence and presence of camel casein peptides .The Far-UV circular dichroism (CD) measurements (190–260 nm) were carried out in a model 215 circular dichroism spectrometer (AVIV). The far UV spectrum indicates that there is a perturbation in the secondary structure of HSA after glycation, a decrease in HSA 222nm ellipticity upon glycation was observed which is an indicative of a lost in alpha helical content of protein. Presence of camel casein peptides declined the secondary structural change of HSA upon glycation. It can be concluded from this study that the camel casein bioactive peptides can be a promising natural source for prevention of glycation

Key words: Aloin; Camel milk peptides; Human serum albumin; Anti-glycation

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Inhibition of lysozyme amyloid fibril-induced reduction of brain hexokinase activity by resveratrol

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ABSTRACT

It is becoming increasingly recognized that biological membranes are primary targets for amyloid fibrils toxicity. In fact, interaction of amyloid fibrils with lipid bilayers alters the structure and properties of biomembrane and thereby interferes with membrane-bound proteins activity and their subcellular localization. In line with this concept, compounds that interfere with the amyloid fibrils to bind lipid bilayers may serve as therapeutic agents for the treatment of amyloid-associated disorders. Among the most studied are polyphenols, an important class of beneficial naturally-occurring antioxidative molecules that exert beneficial effects by their ability to interact with lipid bilayer and stabilize lipid membranes by decreasing membrane fluidity. In this study, we investigated the influence of mature Hen Egg White Lysozyme (HEWL) amyloid fibrils on the activity of the rat brain mitochondrial Hexokinase (HK). Moreover, the effect of naturally occurring polyphenol Resveratrol on the HEWL fibril-induced changes in HK activity was investigated. We found that the presence of fibrils gave rise to a reduction in HK activity in the both membrane-bound and solubilized forms, but the extent of reduction was much greater for membrane-bound enzyme than solubilized enzyme. However, pre-incubation of mitochondrial homogenates with 50 μM Resveratrol effectively hinder HK activity reduction induced by HEWL amyloid fibrils. Although the mechanisms by which Resveratrol inhibit HEWL fibril-induced reduction of HK are still unclear, we suggest that naturally occurring polyphenols could serve as scaffold for the design of more efficient inhibitors for amyloid fibril cytotoxicity.

Key words: Hen egg white lysozyme; Brain hexokinase; Amyloid fibril; Resveratrol

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Inhibition of lysozyme fibrillogenesis and cytotoxicity by resveratrol

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ABSTRACT

Misfolding and aggregation of various proteins and peptides is associated with a growing list of diseases, including neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases and peripheral disorders such as systemic amyloidosis and type-II diabetes. Consequently, inhibition of protein aggregation and amyloid fibril formation is viewed as one possible method to prevent the progression of these devastating disorders. A promising strategy for preventing of these diseases is to identify compounds that inhibit amyloid fibril formation. Using ThT fluorescence assay, transition electron microscopy and circular dichroism, here we examined the effects of the naturally occurring polyphenol resveratrol on the fibrillogenesis and cytotoxicity of Hen Egg White Lysozyme (HEWL) fibrils. Our results demonstrate that resveratrol effectively inhibit amyloid fibril formation in a concentration dependent manner. In addition, exciting fibrils of HEWL were disaggregated by resveratrol, as confirmed by ThT fluorescence assay. Furthermore, protective effects of resveratrol against cytotoxicity induced by HEWL fibrils were confirmed using cell viability MTT assay. Although the mechanisms by which resveratrol inhibit fibril formation and destabilize preformed HEWL fibrils are still unclear, we suggest that antioxidative properties as well as anti-amyloidogenic activities of resveratrol underlie its protective effects. It is concluded that naturally occurring polyphenols could serve as scaffold for the design of more efficient inhibitors for amyloid fibril formation *in vivo*.

Key words: Hen egg white lysozyme; Cytotoxicity; Polyphenol; Amyloid fibril; Resveratrol

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Prediction of antimicrobial peptides using sequence information

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ABSTRACT

During last decade, many natural antimicrobial peptides (AMP) are discovered. They are found in animal's body and plants. These amphipathic peptides are usually cationic molecules. Based on convenient model, they are attracted to membrane surface and accumulate there. Upon aggregation on lipid membrane they create hydrophilic pores to disrupt cell integrity. Herein we introduce new metric to predict AMPs by considering the thermodynamic characteristics of AMP sequences and their mechanism of action. Just the sequence of query peptide is required to predict its antimicrobial activity. The represented score opens new horizons to fight against antibiotics-resistant bacteria by suggesting new AMPs.Furthermore AMPs are able to interact to membrane so they are candidates for transport of molecules to cells.

Key words: Antimicrobial peptides; Sequence based method; Antibiotics-resistant; Computational approach

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Association between genetic polymorphisms of *GPX1 pro198lue* and *CAT A-21T* with risk of gastric cancer

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ABSTRACT

To protect cells and organs against reactive oxygen species, the body has evolved an antioxidant protection system. *CAT* and *GPX1* gene products act as an important defense barrier against free radicals. Thus, any defects or polymorphisms in these genes may be associated with the risk of many cancers, such as stomach cancer. The aim of this study was to investigate the relationship between genetic polymorphism of *GPX1 Pro198Lue* and *CAT A-21T* with the risk of gastric cancer. 159 patients with gastric cancer and 242 healthy controls participated in present study. Genotyping of *GPX1 Pro198Lue* and *CAT A-21T* were done by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. Differences in the frequency of the control group and patients were assessed using the χ^2 test and risk was calculated with an unconditional logistic regression was used to estimate odds ratios (OR) and 95 confidence intervals. As a result, there was significant association between CT genotype (OR=1.53, CI=1.01-2.32, P=0.042) and CT+TT genotype (OR=1.50, CI=1.00-2.26, P=0.046) of *GPX1 Pro198Lue* polymorphism and gastric cancer but there was no significant association between TT genotype compared with AA genotype of *CAT A-21T* polymorphism and gastric cancer (OR=1.45, CI=0.76-2.76, P=0.248).

Key words: Gastric cancer; Oxidative stress; Polymorphism; *GPX1*; *CAT*

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The effect of copper oxide nanoparticles as feed additive on some the blood proteins of broiler chickens

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ABSTRACT

Nanotechnology holds promise for both medication and nutrition, because materials at the nanometer dimension exhibit novel properties different from those of isolated atom and bulk material. The purpose of this study is the role of Copper Oxide (CuO) nanoparticles on broiler chickens blood proteins of Ross family. This experimental study was conducted on the 60 numbers of Ross family broiled chickens. Chickens randomly are divided into 3 groups of including control, experimental groups 1 and 2. Water and food were given to control group but no particular experimental material was injected or taken. In experimental group 1, 16 mg/kg/body weight CuO nanoparticles were taken by chickens, orally, for 35 days. In 25th day, 10 chickens randomly were chosen and blood sample was prepared. At the end of this period, in 35th day, blood sample was prepared in order to investigate the blood protein content by spectroscopic tools. This study indicated that HGB and Albumin concentrations in the experimental groups have significant decrease in comparison to control group. However significant increase was observed in the average of total protein. Furthermore, protein denaturation has occurred. Based on these results, it can be concluded that CuO nanoparticles, as feed additive, has toxic effect on the blood proteins due to protein denaturation.

Key words: Copper oxide Nanoparticle; Ross; Broiler chicken; Hemoglobin; Albumin

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Optimization of recombinant glutamate dehydrogenase expression in various strains of *Escherichia coli*

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ABSTRACT

Glutamate dehydrogenase (GDH) is an important mitochondrial matrix enzyme, integrating carbon and nitrogen metabolism. This enzyme catalyzes the reversible oxidative deamination of L-glutamate into free ammonia (NH_3) and α-ketoglutarate (α-KG), by using NAD^+ and $NADP^+$ as co-enzymes. In mammalian, GDH plays several roles including participation in urea synthesis pathway, involving in synthesis of some amino acids and contribution in the secretion of insulin by pancreatic ß-cells. In clinical diagnostics, increased circulating levels of GDH signal toxic or hypoxic liver damage. This enzyme is used to measure the concentration of ammonia, urea and glutamine, in various biological samples. In order to express and purify GDH, after optimization of cDNA sequence from human GDH for expression in *E. coli*, it was inserted in pET28a plasmid. To study the GDH protein expression, GDH-pET was transformed into *BL21 (DE3)* strain and expressed at different conditions of temperature, time and inducer concentration (IPTG and Lactose). Electrophoresis and western blotting of cell lysate showed that the overexpressed protein forms inclusion bodies and accumulates in sediment bacteria. Therefore, to optimize the soluble expression of this protein, several strains of *E. coli* such as *BL21 (DE3) pLysS*, *C41 (DE3)* and *JM109* were used. It was concluded that some of these hosts are more suitable compared to *BL21 (DE3)*. The best condition was *BL21 (DE3) pLysS* host in LB medium culture that incubated at 18 $^{\circ}$C for 20 hours by adding 1 mM Lactose.

Key words: Glutamate dehydrogenase; *E. coli*; Overexpression; Inclusion body

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An investigation on the mechanism of selective transport through the NPC using molecular dynamics simulation

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ABSTRACT

Nuclear pore complexes (NPCs) are selectively gated pathways between nucleoplasm and cytoplasm, while small molecules diffuse freely through the nucleus, large molecules pass when bound to transport receptors. Anchored on the inner surface of the NPC central channel many intrinsically unstructured FG-nups, proteins that contain phenylalanine-glycine (FG) repeating sequence, said to be responsible for selective mechanism of the NPC. In this article, mechanism of selective transport through the NPC is studied using coarse-grained molecular dynamics simulation. In order to investigate the mechanism of selective transport through these pores, we have studied individual and group behavior of Nsp1, one of the FG-nups fill central pore. Our results show that individual segments of Nsp1 form globular structures, whereas 5×5 array of Nsp1 segments tethered to a planar surface, form brush-like structures that seems to be necessary for the virtual gating mechanism for selective function of the NPC. In the following, we have studied formed brush interaction with NTF2 as a transport receptor. Adding NTF2 into the brush-like structures leads this brush-like structure to collapse and NTF2 can pass through this entropic barrier. Finally, a mutant Nsp1 array with all non-polar phenylalanines mutated to polar Serines was studied to explore the role of FG-repeats in forming brush-like structures. So we can conclude that the interaction between FG-repeats in nups and transport receptor, is responsible for the selectivity of the NPC.

Key words: Brush-like structure; Coarse-grained simulation; Entropic barrier; Virtual gating

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Novel targeting peptides for prostate cancer cells obtained by the screening of a phage display peptide library

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ABSTRACT

Phage display peptide libraries (PDPLs) harbor a large abundance of peptides displayed on the surface of filamentous phages, representing the potential to be used for a wide variety of applications. Over the recent years, PDPLs have been used for identifying targeting peptides to a variety of targets. Screening of PDPLs on human cells has proven to be a highly-valued venue for isolating peptides that recognize target cells with high affinity and specificity. Prostate cancer is one of the most common causes of cancer-associated death in men. Studies have indicated that targeting peptides to prostate cancer cells represent potential to be used as valuable diagnostic and therapeutic tools. In the current study, we aim to isolate peptides targeting to PC3 cells (human prostate cancer cells). A heptapeptide PDPL was used through biopanning to isolate peptides binding specifically to PC3 cells. The panning was performed on PC3 and control cells including 5637, Huh-7, SW480, AGS and human normal cells. Polyclonal-phage ELISA was exploited to evaluate the process of enrichment during biopanning. Subsequently, phage clones were randomly picked out from titer plates, amplified by using plaque-PCR and their genomic DNA was sequenced. Bioinformatic analysis was conducted for further characterization of isolated peptides. Several rounds of panning resulted in the enrichment of peptides. The peptide identified through biopanning can be considered as a potential specific binder to PC3 cells. Further analysis of this peptide is required to show its capacity for targeted delivery of various gene and drug delivery vehicles into prostate cancer cells.

Key words: Phage display peptide library (PDPL); Biopanning; Prostate cancer; Targeting peptide

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Protective effect of quercetin on ferrous sulfate –induced acute renal failure in rat

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ABSTRACT

Iron-induced oxidative stress is widely accepted as a critical mediator of diverse forms of glomerular and tubulointerstitial renal disease. Oxidative stress can also exacerbate systemic inflammation. This study was designed to investigate the effects of quercetin, a powerful antioxidant with radical scavenging ability, in ferrous sulfate-induced renal injury in rats. The protective effect of quercetin against the damage inflicted by reactive oxygen species (ROS) during renal injury was investigated in male Wistar rats using histopathological and biochemical parameters. Renal injury was induced by i.p. injection of ferrous sulfate (30 mg/Kg/day) for 14 days. Quercetin (50 mg/kg/day) was injected (i.p.) for 11 days. In final blood samples were collected for later assay of their creatinine and blood urea nitrogen concentration. Thiobarbituric acid reactive substances (TBARS) were determined in renal tissue, and the left kidney was removed and preserved for histological studies. Ferrous sulfate caused deterioration of renal function, renal morphology and a significant renal oxidative stress. Treatment of animals with quercetin markedly attenuated renal dysfunction, morphological alterations and reduced elevated TBARS levels. The findings imply that ROS play a causal role in ferrous sulfate-induced renal injury, and quercetin exerts renoprotective effects probably by the radical scavenging and antioxidant activities.

Key words: Quercetin; Blood urea nitrogen; Creatinine; Ferrous sulfate; Kidney

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Computational mutations for thermostabilizing α-amylase of *Bacillus amyloliquefaciens*

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ABSTRACT

The starch degrading enzyme α-amylase (EC 3.2.1.1) is widely distributed in nature. This extracellular enzyme hydrolyses α-1, 4 glycosidic linkages randomly throughout the starch molecule in an endo-fashion, producing oligosaccharides and monosaccharides, including maltose, glucose and alpha limit dextrin. α-amylases are widely used in industrial processing of starch-containing materials. The Bacillus α-amylases have found widespread use in industrial processes, and much attention has been devoted to optimizing these enzymes for the very harsh conditions. The majority of α-amylase reactions are conducted at high temperatures, resulting in high demand for the thermostable enzyme. In this study some amino acids from *Bacillus amyloliquefaciens* α-amylase were substituted with other amino acids, using *in silico* approaches to finally achieve mutations that can improve the thermostability of the enzyme. The I-Mutant2.0 web server results showed that two of selected mutations improve the thermostability of the enzyme (N190F and N265Y). Therefore, by the Swiss-PdbViewer software and I-Tasser, cph model and Swiss model web servers the mutations were exerted on the 3D structure of the enzyme and mutant 3D models from enzyme structure were achieved. The models were refined by 3Drefine, chiron and KoBaMIN web servers. The models quality assessment was performed by ModFOLD4 server. The best models were selected and then docked with partial amylose polymer to found the effect of mutations on the binding of enzyme with it substrate. Docking technique was performed by AutoDock software. The results showed that after exerting the mutations, the binding of enzyme and its substrate was not negatively affected. Thus, we suggest these two mutations for achieving α-amylases with high thermostability.

Key words: Alpha-amylase; Thermostability; Mutation; AutoDock

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Alpha-amylase production by some starch assimilating *Bacillus* species

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ABSTRACT

Alpha-amylases are starch-degrading enzymes which catalyze the hydrolysis of internal a-1, 4-O-glycosidic bonds in polysaccharides. These enzymes have many applications in food, paper, textile and pharmaceutical industries. About 30 % of world enzyme production market is provided by this enzyme. Alpha amylases are produced by plants, animals and microorganisms. However, microbial sources are the most preferred one for large scale production. Among microorganisms, alpha-amylase is mainly derived from Bacillus genus for commercial applications. Therefore, in this study, 13 different strains of Bacillus genus were tested for their ability to hydrolyze and consume starch. Comparative studies were performed on the strains which were capable to consume starch. The curve of starch consumption by applying logul's solution was depicted for strains. The enzymes activity was assayed by using DNS (3, 5-dinitrosalicylic acid) at various times and temperatures. The best strain according to the enzymatic features was selected. Enzymatic extract was achieved by centrifugation. As alpha-amylase was precipitated by ammonium sulphate, desalting process was performed by dialysis. Then zymography and determination of molecular weight for alpha-amylase were performed. The best strain regard to enzymatic features was Bacillus amyloliquefaciens. Maximum production of this enzyme was after 36 h. The enzyme activity was at the highest level at 70 °C. Molecular weight of the enzyme was about 55KD. In conclusion, the selected strain can be an appropriate candidate for industrial production of alpha-amylase.

Key words: Alpha-amylase; Starch; *Bacillus*; Zymography

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Studying the relationship between the prevalence of hepatitis B in patients with glucose-6-phosphate dehydrogenase deficiency

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ABSTRACT

G6PD deficiency, Favism, is an X-linked recessive hereditary disease caused by the deficiency of glucose-6-phosphate dehydrogenase. Fatigue, severe weakness, anemia, jaundice, and symptoms like hepatitis are the signs of a famous spring disease called Fava beans disease. Lack or deficiency of glucose-6-phosphat dehydrogenase in red blood cells prevents the regeneration of glutathione in the cell causing failure in the protection against oxidative stress like free radicals and infection. In the current research, we have studied the prevalence of hepatitis B in patients with Favism visiting SINA laboratory in Guilan. In this descriptive research 82 patients with Favism who had gone to the hospital during 2013-2014 were studied using ELISA test for HBS. Antigens, G6PD, CBC and other required information which collected as questionnaires. Finally all data analyzed using SPSS software. Also as control, amongst 7302 patients, 41 were positive in HBS.Ag and amongst these 41, (24.3) percent of the patients with Favism. Given that the prevalence of hepatitis is (0/56) percent, there is a Significant correlation among the patients with Favism and those with hepatitis. Therefore, there is a need for more investigation in the case of molecular mechanism of the patients with Favism susceptible to hepatitis.

Key words: Hepatitis; Favism; HBS (Antigen); G6PD

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Simulation of HIV-I integrase interaction with its inhibitors, using molecular dynamic methods

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ABSTRACT

Application of newly designed enzymatic inhibitors against the viral enzymes is one of the modern and effective approaches in viral diseases treatment. The increasing incidence of dangerous infections such as, AIDS syndrome, viral hepatitis and other such diseases that threaten human population clearly justify the importance of application of such inhibitors. One of the challenges to the application of the above mentioned inhibitors is the production of variant viral enzymes of different structures but of the same functions due to recombination, occurring during viral genome duplication. Such structural diversities create resistant mutants to inhibitors. Therefore, comprehensive researches are required to elucidate the molecular structure of mutants in order to help scientists to design more effective drugs. In this trend, the HIV-1 integrase is of special importance. So far, scientists have designed and introduced three integrase inhibitors namely Raltegravir, Elvitegravir, and Dolutegravir. Although, different inhibition mechanisms have been proposed, but none of them has been proven. In present work, in order to study inhibition mechanisms at molecular level, first each inhibitor was correctly stabilized on the enzymes by docking. Then, after selecting the most suitable position of inhibitor-enzyme attachment in the active site, the simulation of each system was ran separately for 10 nano second. The result showed that Dolutegravir was the most effective in inhibiting the enzyme. This inhibitor reduces the protein flexibility, specifically at its loop region that leads to decreased integrase affinity to DNA. Based on our data, it seems that Dolutegravir structure can serve as a suitable model for designing more effective drugs.

Key words: Molecular dynamic simulation; Integrase; Raltegravir; Elvitegravir; Dolutegravir

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Growth hormone and its mutants: a bioinformatics analysis

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ABSTRACT

Growth hormone (GH) is a protein that contains 191 amino acids with two disulfide bonds and four α-helixes. Its receptor belongs to class I cytokine receptor family. Two GH receptors bind to the hormone one after the other. Binding to the first site forms an inactive intermediate complex. The assembly becomes functionally active only when the second receptor molecule binds at the second binding site on the other side of the hormone. Any factor that disrupts this assembly can block the hormone's function. Changing a single glycine at position 120 to arginine will block receptor binding at the second site. By comparing the modified hormone with its wild type, it can be observed that the large side chain of arginine 120 cannot be accommodated when the second receptor binds. In this study, we considered the effects of mutagenesis on structure quality of the protein by bioinformatics. The 3D structure of growth hormone was obtained from PDB (http://www.rcsb.org/pdb/home). Modified growth hormone was modeled by M4T server. Then the analysis for structure was obtained with Qmean server: a server for quality estimation of protein structure models, http://swissmodel .expasy.org/qmean/cgi /index. The quality and stability of modeled structures were evaluated by Z-score value. This value for growth hormone and its mutant was -3.50 and -1.86, respectively. In the present study, we revealed that the mutant structure of growth hormone as an antagonist is a validate structure compared to GH and it would be very promising for using in cancer therapy.

Key words: Growth hormone; arginine 120; Cytokine; Disulfide bonds

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Prediction of growth hormone and growth hormone antagonist binding energy to their receptor

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ABSTRACT

Growth hormone (GH) is a single polypeptide hormone which is produced and secreted by cells of the anterior pituitary gland. This hormone exerts diverse growth promoting and metabolic effects. As GH circulates in the blood, it binds to GH receptors (GHR), a trans membrane protein expressed on the surface of liver, adipose, kidney, heart, intestine, lung and muscle cells. After receptor binding, GH induces GHR dimerization and JAK2 is activated after its association with a dimerized GHR. A growth hormone antagonist (GHA) was produced in *E.coli* by a mutation that would block GH by preventing the GHR dimerization. In this study, we evaluated the effect of mutagenesis on binding energy of GH and GHA to their related receptor. The growth hormone and its mutant 3D structures were obtained from PDB (http://www.rcsb.org/pdb/home) and M4T server, respectively. Likewise, GHR conformational structure, was found in NCBI (http://www.ncbi.nlm.nih.gov). GH and GHA affinity for binding to GHR was analyzed by HEX docking software and energy total (E total) was obtained. GH and GHA E total were -648.13 and -698.68 respectively. This data demonstrates that GHA affinity for binding to GHR is higher than GH affinity. So GHA in completion to GH will overcome GH in binding to GHR, it can act as an antagonist to prevent excessive growth and cure acromegaly, diabetes and cancer.

Key words: Growth hormone; Growth hormone antagonist; *E.coli*; Acromegaly

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DNA binding studies of biologically active nano water soluble Zinc (II) Schiff base complexes: effect of substitutional groups of ligand on the DNA-complex interaction

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ABSTRACT

Binding interaction of nano water soluble zinc(II) Schiff base complexes: [N,N′-bis{5-[(triphenylphosphoniumchloride)-methyl]salicylidine}-phenylenediaminato] zinc(II) perchlorate (Zn(L1)) and [N,N′-bis{5-[(triphenylphosphoniumchloride)-methyl] salicylicdine}-4-methyl-phenylenediaminato]zinc(II)perchlorate (Zn(L2)) with Herring Sperm DNA (HS-DNA) has been investigated by fluorescence, UV-vis spectroscopy, viscosity, and cyclic voltammetry techniques. Structural details of the intercalation site of the complexes were obtained by density functional theory (DFT) calculations. UV-vis titration data indicated hypochromic effect with addition of DNA to the complexes. The intrinsic binding constant was determined in water under physiological conditions and revealed significant binding of complexes with DNA *via* intercalation. Among two compounds comparatively better DNA binding was found for Zn (L2) complex. Free energies of complex-DNA interactions indicated spontaneity of their binding.

Key words: Schiff base complexes; Water soluble; DNA; Interaction

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Interaction of diazinon toxin with HSA protein; experimental and computational studies

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ABSTRACT

Human serum albumin (HSA) is a soluble blood protein which can bind to small molecules (such as drugs and toxins) and transfer them within the blood circulation. Fluorescence quenching, UV-Vis spectroscopy, FT-IRand Molecular docking were used to characterize the binding properties of Diazinon (The toxin of organophosphate) with HSA at molecular level under physiological condition, in two times of first day and thirty five days. UV-Vis spectroscopy illustrated increased absorption of samples at fixed concentration of the protein and increasing concentration Diazinon. The fluorescence intensity of HSA decreased regularly with the gradually increasing concentration of Diazinon. Based on binding constant (k_a=3.367× 10^{+4} M^{-1}), Diazinon has a relatively robust binding site with HSA. This decrease of fluorescence intensity and increased absorbance of samples in thirty five days were observed to a greater extent. In FT-IR method, the proportion of decrease in percentage of α-Helix for a day of incubation was 53.97% to 51.88%, other secondary structures increased, for example, turns increased from 8.49% to10.21%, ß-Sheet from 13.94% to 14.81%, β-anti from 8.2% to 8.25% and random coils from 15.4% to 17.24%. These changes for α-Helixes, Turns, ß-Sheet, anti-β and random coils after thirty five days were 56.7% to 47.11%, 25.3% to 29.75%, 6.93% to 10.94%, 2% to 2.83% and 9.08% to 10.86% respectively. Molecular docking revealed binding site of Trp-214 for Diazinon in HSA. Therefore, our results suggest that Diazinon binds to Trp-214 classic binding site of HSA and it could cause considerable alterations in various secondary structures of HSA.

Key words: Diazinon; UV-Vis spectroscopy; FT-IR; Fluorescence quenching; Molecular docking

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Study of chemical and heat-induced denaturation of wild-type and mutant mnemiopsin

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ABSTRACT

Previously; the only charged residue (Arg^{39}) in the cavity of Mnemiopsin was replaced by Lys as another charged residue (R39K mutant) and it was shown that the activity of this mutant increases 9 fold relative to the wild-type protein. Here; we studied thermodynamic stability of the wild-type and R39K mutant using Far-UV CD spectroscopy and differential scanning calorimetery (DSC). Far- UV CD spectroscopy was used for monitoring urea-induced unfolding of the secondary structure of mnemiopsin. The buffer used containing 50 mM NaH_2Po_4, 300 mM NaCl with different concentrations of urea (0-8 M); however, buffer in the thermal stability studies is out of urea. According to the CD-equilibrium studies, ΔG (H_2O) in the wild-type protein (16.15 $KJ.mol^{-1}$) is higher than that of R39K mutant (14.10 15 $KJ.mol^{-1}$). On the other hand, $[Urea]_{50\%}$ as the concentration of urea at which half of the protein is denatured, was 4.05 M for the wild-type and 2.94 M for the R39K mutant. These measurements suggest that R39K mutation destabilize secondary structure of the protein when compared with wild-type one. At the end, we analyzed results of DSC measurements. These data show higher ΔH (H_2O) in wild-type protein (372.4 $KJ.mol^{-1}$) than R39K mutant (267.7 $KJ.mol^{-1}$); indicating more stability in the wild-type protein relative to R39K mutant. We found that replacing of Arg by Lys results in destabilization of protein against both chemical and thermal agents; although the effect of this mutation in the activity of protein is inverse.

Key words: Mnemiopsin; Thermodynamic stability; Urea-induced unfolding; Far-UV CD spectroscopy; Differential scanning calorimeter

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Optimization of cholesterol oxidase protein expression in *E. coli*

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ABSTRACT

Cholesterol oxidase (CHO: 3β-hydroxystreoid oxidase; EC 1.1.3.6) is a Flavine Adenine Dinucleotide (FAD)-dependent bifunctional enzyme that catalyses both oxidation of cholesterol (5-cholesten-3-β-ol) to the temporary intermediates 5-cholesten-3-one with the reduction of oxygen to hydrogen peroxidase, and the isomerization of the steroid with a trans A: B ring junction to produce 4-cholesten-3-one. This enzyme is industrially important and useful for the clinical determination of serum cholesterol levels in combination with related enzymes. CHO has also been used as a molecular probe to elucidate cellular membrane structures. As this enzyme is unique to bacteria, it presents a potential target for a new class of antibiotics. In this study, expression and purification of a recombinant *streptomyces sp*. CHO is described. The recombinant CHO has been over-expressed in *E.coli* BL21 (DE3) by change of induction time, temperature and concentration of inducers (IPTG and Lactose). The optimum temperature for protein expression was 20˚C for 18 hours and the best inducer concentration was 0.5 mM IPTG. Expression result showed that the recombinant protein accumulate intracellularly in inclusion bodies form. To obtain soluble protein from inclusion bodies, purification was investigated under denaturing conditions using triton X-100 and different urea concentrations. Finally, this protein was purified by urea 2M and %1 Triton x-100 and refolded on column by washing buffers with urea gradient. In addition, %2 Triton X-100 without urea was a suitable condition to obtain some soluble protein.

Key words: Cholesterol oxidase; Expression; Inclusion body; Urea; Triton

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An association study of apolipoprotein A5 concentration with serum triglyceride levels as a risk factor of metabolic syndrome and coronary artery disease in Iranian population

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ABSTRACT

Apolipoprotein A5 (apoA5) found at very low concentrations in the bloodstream is associated with very low density lipoprotein (VLDL), high density lipoprotein (HDL), and chylomicrons. Evidence from genetic studies in humans and experimental models support the important role of ApoA5 in modulating serum triglyceride (TG) concentrations, as a risk factor of metabolic syndrome and coronary artery disease (CAD), through its direct interaction with lipoprotein lipase (LPL). However the associations between ApoA5 concentrations, TG and coronary artery disease (CAD) remain controversial. Therefore, we investigated these relations in the case-control study involving Iranian population. This study included 55 control subjects and 103 patients with CAD aged more than 50 years in Iranian population. Fasting blood sample was collected from all subjects. Plasma ApoA5 protein was measured by enzyme immunoassay (Human Apolipoprotein A5 ELISA kit, Millipore, MO). The lipoprotein parameters (TG, Total Cholesterol, HDL- Cholesterol, ApoAI, ApoB, Lipoprotein (a) (Lyp (a)) were measured enzymatically for each subject. Differences in lipid and ApoA5 protein values were evaluated by unpaired Student's *t*-test. Plasma ApoA5 concentration was significantly lower in CAD patients than controls (190.6±6.2 vs. 219.2±5.2ng/ml, P<0.001). ApoA5 was significantly higher in cases with TG <150 mg/dl compared to those with TG >150 mg/dl in control (228.2±6.1 vs 199.5±3. $P < 0.05$) and case population (195.5±7.2 vs 181.2±6.2, $P < 0.05$). Our data supports an inverse association between plasma ApoA5 concentrations and CAD risk, probably due to the observed negative relation of ApoA5 with TG that is a major risk factor of CAD and metabolic syndrome.

Key words: Apolipoprotein A5; Coronary artery disease; Triglycerides

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Prediction of anti-HER2 nanobody C7b antigenicity

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ABSTRACT

An epitope or antigenic determinant is the part of an antigen that is recognized by the immune response. After entering an antigen into the body, its antigenic determinants are presented by MHC molecule to T-cell which takes active part in host immunoreactions. HER2 is a tumor marker which is over expressed in tumor cells, especially ovarian and breast cancer cells and associated with decreased patient's survival. Stimulation of the immune response against HER2 can reduce tumor growth in cancer patients with over expression of HER2. In the early 90s, a series of monoclonal antibodies against the extracellular domain of the HER2 was designed to prevent its signaling performance. One of them is the Camelid antibody C7b. Camelid antibodies (nanobody) have no light chain. Since the human immune system recognizes murine mAbs as alloantigens and reacts by mounting a classical humoral, it might be occurred for the camelid nanobodies. In this study, the antigenic properties of the sequence from anti-HER2 nanobody C7b were analyzed and antigenic epitopes were determined, using Predicting Antigenic Peptides (http://imed.med.ucm.es/Tools/antigenic.pl). This web program predicts those segments within a protein sequence that are likely to be antigenic by eliciting an antibody response. The sequence of C7b nanobody (AFN61318.1) has 123 amino acids and shows 6 antigenic determinants. Average antigenic propensity of C7b nanobody was 1.0369 based on antigenic plot. Predicting and analyzing antigenic properties C7b nanobody has great importance due to the vast application of nanobodies in treatment of breast cancer. The results is useful for assess the nanobody immunogenicity.

Key words: Epitope; Nanobody; HER2; Antigenicity

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Effects of different levels of copper oxide nanoparticles supplementation as a growth factor on the antibody response of broilers to Newcastle disease

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ABSTRACT

Cu, as a C*eruloplasmin* cofactor, eliminates the free radicals produced in phagocytosis and it is one of the essential components in chronic immune system. The present study aimed to investigate the effect of CuO nanoparticles on the Antibody titer of Newcastle. In this study, 64 *Ross* broilers weighed 40 g were selected. After defining Antibody titer of 4 broilers, the other broilers were divided into 3 groups: control, experimental 1 and experimental 2. The control group received only food, water, vaccine and ordinary drugs in a period of time. After 5 days, the experimental group's 1 and 2 received 16 and 36 mg/kg body weight Nano CuO for 30 days, respectively. At day 9 age , Newcastle vaccines were injected to all. Two weeks after vaccination, 10 broilers were selected randomly from each group and the sera were assayed for antibody titer by both HI tests. Antibody showed no significant change. Body weight in the groups receiving Nano CuO significantly increased. There were no significant effects of treatment on antibody level ($p>0.05$). It is concluded that diet supplementation with Nano CuO cannot enhance the serological response of broilers.

Key words: Nano CuO; Broiler; Newcastle; Antibody; HI

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A study on the effects of Ca (II) binding on the structure and stability of calprotectin using molecular dynamics simulation

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ABSTRACT

Calprotectin (CP) is an antimicrobial protein produced and released by neutrophils that inhibits the growth of pathogenic microorganisms. CP is a tetramer made up of two heterodimer of S100A8 and S100A9. The two proteins belong to Ca (II)-binding proteins of the S100 family. S100 proteins comprise a family of proteins carrying two EF-hand domains for Ca (II)-binding, one in C-terminal and one in N-terminal of the proteins. In this work, for a better understanding of the role of Ca (II) on the dynamics of CP, the binding properties of Ca (II) to CP were studied using Molecular dynamics simulations. The simulations were carried out on Ca (II)-free and Ca (II)-bound CP models, using crystal structures obtained from the Protein Data Bank, and trajectories at 37 ºC and 1 atmosphere pressure for 10ns period. Our molecular dynamic analysis confirmed that Ca(II) binds to both seven-coordinate, "canonical" sites and five-coordinate, "non-canonical" sites. Also, the simulations demonstrated that Ca (II) ion can stabilize the conformation of their binding site, but it cannot function as an electrolyte interacting with negatively charged groups. During simulation, due to CP structure alteration, the RMSD of Ca (II)-binding to CP is slightly lower than its free state. Although the Ca (II)-binding to CP leads to decreased activity of the protein, the RMSF curve illustrated that Ca (II)-CP complex has lower flexibility and mobility. The results of this study showed that although Ca (II) in Hofmeister series of proteins known to be unstable, its natural presence to create lower flexibility in the protein structure CP is absolutely necessary.

Key words: Molecular dynamics simulation; Calprotectin; Ca (II)

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The supportive role of a Stem cell- derived protein (SCF) in recovery of a murine model from Acetaminophen nephrotoxicity

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ABSTRACT

Acetaminophen (APAP) is a drug which is safe at therapeutic doses, however in the case of overdose both liver and kidney injury observed in humans and laboratory animals. Renal damage and acute renal failure can occur even in the absence of liver injury. Stem cell factor (SCF) is a growth factor which is believed to be of supportive effect in liver recovery from APAP toxicity. This study was designed to examine the possible time dependent protective potency of SCF post treatment against APAP induced oxidative stress in kidneys of BALB/c mice. Groups of mice (n = 5/ group) were sacrificed at 1, 6 and 12 hour intervals after 450 mg/kg body weight of APAP. Parameters of oxidative stress evaluation containing; glutathione (GSH) level, lipid proxidation level and also protein carbonyl content (P.C.C), in kidney of APAP treated mice with or without SCF (40 µg/kg B.W) post treatment was compared to those of control groups (treated with normal saline) in every interval time after APAP injection. Results showed that, SCF caused a significant replenish in GSH level of APAP-induced GSH depletion and SCF did not conduct this effect through prevention from initiation of oxidative stress, because SCF post treatment did not prevent from lipid proxidation or protein carbonylation in kidney of mice under APAP challenge. Our data suggest that SCF treatment has a supportive effect in recovery from APAP- induced renal oxidative stress in kidney of mice through increasing GSH level in the tissue.

Key words: Acetaminophen; Glutathione; Stem cell factor (SCF); Oxidative stress

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Poster – P87

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Solubilization and refolding of recombinant BoNT/E light chain as expressed in the form of inclusion body in *E. coli*

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ABSTRACT

Botulism is a very dangerous syndrome in human, due to botulinum neurotoxins (BoNTs) A, B and E poisoning and serotype E. The main function and special toxicity of these neurotoxins are due to enzymatic activity of their light chains (LC). Since achiving of this subunit in isolated form is relatively hard, in this study, we decided to produce recombinant LC from, a construct containing BoNT/E LC synthetic gene. The construct with optimized codon usages was synthesized in pET28a. Afterwards, soluble and insoluble (inclusion body) expression of LC were evaluated in various mild conditions, such as different IPTG concentrations and temperatures as well as increased incubation times. Initial purification of expressed protein was performed, using Panda method and after solubilization in 2 M urea buffer with alkaline pH (non-chaotropic denaturant) and final purification by Ni-NTA column, refolding process was also accomplished by using dialysis with mild variations in refolding buffer. Despite the mild conditions of expression, the protein was totally produced in form of inclusion body. Initial solubilization was performed with more than 95% efficiency and during refolding process, despite the aggregation of great amounts of this protein. Finally the recovered protein was acquired in soluble form within about 25% efficiency. Over-expression of this protein and innate characteristics of it, resulted in producing the inclusion bodies. Final product, containing recovered and soluble proteins, obtained from dialysis and refolding process, in future studies, must be evaluated, using enzymatic activity assay.

Key words: Botulinum neurotoxin E (BoNT/E; Light chain (LC); Inclusion body; Mild solubilisation; Protein refolding

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Investigating the effect of macromolecular crowding on aggregation intermediat of β-Lactoglobulin

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ABSTRACT

The intracellular environment is highly crowded and it contains high concentrations of macromolecules. Molecular crowding decreases the effective volume available for the proteins and therefore has a significant effect on protein–protein interactions such as protein aggregation. The crowded effects may be mimicked *in vitro* by adding high concentrations of an inert molecule such as polyethylene glycol (PEG). In this investigation, the effect of different percentages of PEG on aggregation of β-lactoglobulin (BLG) and its intermediate state(s) was evaluated by spectroscopic techniques and SDS-PAGE. BLG has an intermediate with a non-native α-helical structure, although its native form is predominantly composed of β-structure. It has been known that trifluoroethanol (TFE) induces α-helical structure on BLG. In order to aggregate BLG, a solution containing 1mg/mL concentration of the protein was prepared in tris 20 mM, pH 7 containing 60 mM NaCl and incubated at 37 °C for 1 hour in absence and presence of TFE. The result of non-reducing SDS-PAGE shows formation of dimers, tetramers and oligomers upon aggregation of the protein. The result indicated that aggregation of the protein at 10% of TFE decreases in presence of PEG, but PEG has no remarkable effect in absence of TFE. Thus, it can be concluded that the molecular crowding inhibits aggregation of the intermediate state of the protein, while it has no meaningful effect on the aggregation of the native state under the mentioned condition.

Key words: Macromolecular crowding; β-lactoglobulin; Aggregation; Intermediate

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Resistance mutations in HIV-1 integrase

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ABSTRACT

The human immunodeficiency virus type1 is the causative agent of the immunodeficiency syndrome of human (AIDS). Several key enzymes in the replication cycle of HIV can be targeted for chemotherapeutic intervention. The treatment regimens of HIV-1 include reverse transcriptase and protease enzyme inhibitors to which resistance mutations have emerged that reduce their efficacy similar to other classes of antiretrovirals developed so far. Therefore, new antiretroviral agents targeting different viral essential enzymes are required. Integrase enzyme is an attractive target for antivirals development, because it is essential for HIV replication and unlike PR has no known counterparts in the host cell.

Key words: Human immunodeficiency virus; Strand transfer inhibitors; Integrase enzyme; Antiviral drugs

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Preparation of expression plasmid containing RGD/Mda-7 sequence for improved tumor gene therapy

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ABSTRACT

Gene therapy with Mda-7/IL-24, an apoptosis inducing cytokine seems to be an attractive method to overcome tumor challenge. Improving of mda-7 potency by tethering tumor targeting peptide is under developing nowadays. RGD peptides is one of the most known of such peptides. In our idea, a RGD motif in amino terminal of recombinant Mda-7 could target better this protein to integrin expressed tumor cells. In this research, we prepared a novel RGD-Mda-7 expressing plasmid and then evaluate its expression and secretion. For this goal, we amplified mda-7 sequence without signal peptide than separately prepared a fusion of novel signal peptide-RGD then ligate all together. Then the fusion of signal peptide-RGD-Mda-7 was inserted into pCDNA3.1 plasmid. The accuracy of methods, integrity of plasmids and sequence were evaluated by colony-PCR, digestions and sequencing sequentially. The expression of construct was first assayed by RT-PCR assay and Immunofluorescence in Huh-7 cells then the secretion potency also evaluated by ELISA assay. The results showed the integrity of construct backbone rather than sequence of signal peptide-RGD-Mda-7 as inside gene by different methods. Suitable expression of novel targeted Mda-7 protein and it's mRNA in Huh-7 cell confirmed by RT-PCR and Immune fluorescent assays. The ELISA assay also revealed the efficacious secretion of this novel protein into culture supernatant in compare to control untargeted mda-7. The plasmid construct express novel mda-7 protein and would be a probable more effective factor for further assessment.

Key words: Mda-7; RGD peptide; Tumor gene therapy

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Nanocomplexes arising from protein-polysaccharide associative phase separation as a promising vehicle for bioactive compounds

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ABSTRACT

Most of the nutraceuticals such as carotenoids, fat-soluble vitamins and phenolic compounds show instability against chemical or physical degradation and tend to degrade during processing or storage when incorporated into foods. Among different types of food products, incorporating bioactives into non-fat aqueous foods and beverages (especially clear ones) is challenging due to the hydrophobic nature of most bioactives and their instability. The main purpose of the current work was exploring the potential application of the protein–polysaccharide soluble nanocomplexes as delivery systems for nutraceuticals in liquid foods. In this study, the intrinsic transporting property of β-lactoglobulin (BLG) was utilized to develop nanoscale green delivery systems. The binding analysis using fluorescence spectroscopy suggested that the complexation between BLG and four nutraceutical models including β-carotene, folic acid, curcumin and ergocalciferol occurred under all conditions but varied as a function of pH and nutraceutical type. The ^{1}H-NMR study of hydrophilic ligands binding to BLG provided complementary information on the interactions between protein and water soluble ligands. These findings resulted in designing nanoscopic delivery systems for encapsulation of both hydrophobic and hydrophilic bioactives in clear liquid food products of acidic pH. The stability experiments demonstrated that the nutraceuticals of low solubility in water were successfully entrapped within electrostatically stable nanocomplexes arising from BLG-sodium alginate interactions. The electrophoretic mobility analysis showed that soluble nanocomplexes had good stability against aggregation.

Key words: Coacervation; Beta-lactoglobulin; Nanoscopic delivery system

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Screening, purification and partial characterization of a lipase from solvent-tolerant bacterium, *Stenotrophomonas* sp. Strain IE-93

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ABSTRACT

Organic solvent-tolerant bacteria are a novel and unique group of extremophilic microorganisms that can thrive in many harsh conditions. These bacteria are being explored for their potential in industrial applications, since their biocatalysis contents might be active in the presence of very high concentrations of organic solvents. In the present work, a screening programme was designed to isolate a novel methanol-tolerant bacterium. Among several different isolated bacteria, strain IE-93 was chosen as a best producer of lipase in the presence of 5% methanol. Phylogenetic characterization using 16S rDNA nucleotide sequence confirmed that the strain can be placed in *Stenotrophomonas* genus and tentatively named *Stenotrophomonas* sp. strain IE-93. The secreted lipase was purified by combination of amicon concentration, Q-Sepharose and Sephacryl S-200 column chromatography. The purified enzyme showed homogeneity on SDS-PAGE with apparent molecular mass of 60 kDa. The optimum pH and temperature for the enzyme were 8.0 and 35 °C respectively. The hydrolytic activity on various substrates (C4, C8, and C18 acyl chain) revealed that the purified enzyme has the highest priority for long acyl chains. Survey on lipolytic activity of the enzyme in the presence of various concentration of methanol (from 10% to 50%) showed its reasonable stability and more than 77% of initial activity was retained after 1 h incubation in 40% methanol. In overall, long alkyl chain substrate specificity and convenience stability in the presence of methanol suggest that the isolated lipase has biotechnological applications especially in low water content reactions and biodiesel production.

Key words: Solvent-tolerant lipase; *Stenotrophomonas*; Extremophile; Biodiesel

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Investigation of dopaminergic drug detection by surface plasmon resonance (SPR) sensor using laccase enzyme

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ABSTRACT

Dopamine (DA) with IUPAC nomenclature 4-(2-aminoethyl) benzene-1,2-diol, is one of the most important catecholamine neurotransmitters in the brain of mammalian central nervous system, where it acts as a chemical messenger to control a variety of functions. Abnormal levels of DA are related to neurological disorders, such as Schizophrenia and Parkinson's disease. Availability of dopaminergic drugs concentration in biological fluids of these patients is essential to investigate dose-response relationship, prevention of disease symptoms due to non-adherence to medication regimen and variation in pharmacodynamic and pharmacokinetic properties of a drug in patients. Surface plasmon resonance (SPR) is a powerful technique for detection of analytes in various fields such as medical diagnostics, environmental monitoring, and food safety and security. In order to construct a high sensitive dopamine diagnostic system, we have developed a new strategy based on surface plasmon resonance (SPR) by immobilization of laccase as a recognition element on the SPR sensor chip. As Laccase (EC 1.10.3.2) is used in biosensors for clinical diagnostics and environmental analysis of the compounds, and is able to oxidize phenolic compounds such as dopamine, it can be a potential candidate for our study. The immobilization was carried out via EDC/NHS coupling procedure on the CMD chip surface and the detection sensitivity was about nM.

Key words: Dopamine; Surface Plasmon Resonance; Laccase (EC 1.10.3.2); Schizophrenia; Parkinson's disease

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Antioxidative and anticancer peptides derived from Persian walnut proteins

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ABSTRACT

Walnuts are grown for commercial edible nuts. Generally plant proteins play a significant role in human nutrition because they are responsible for many functional properties of food product. Bioactive peptides from food with health promoting properties have been studied extensively in the recent years. In this work, walnut proteins are extracted and hydrolyzed with different digestive enzymes and obtained peptides were tested for antioxidant and anticancer properties. The proteolytic activity was determined using the *o*-phthaldialdehyde method. Our study demonstrated that peptides fraction resulted from hydrolysis with digestive enzymes can prevent the growth of HT-29 and MDA-MB231 cell lines by 60 percent utilize MMT test. Nearly in all cases, more antioxidant activity resulted in more anticancer property. Treatment of cancer is highly expensive involving drugs that have adverse side effects and toxicity complications. Cheaper alternative methods using bioactive peptides can have better prospects in the economics of cancer prevention and therapy. These bioactive peptides are acceptable due to their non-toxic nature so they may be considered as a potential treatment for cancer in future.

Key words: Persian walnut; Peptides; Antioxidant; Anticancer agent

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Study on unsaturated fatty acids as tau protein aggregation triggers *In vitro*

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ABSTRACT

Alzheimer ´disease is considered as one of the most important neurodegenerative disorders that are outbreak in the world wide. Tau protein belongs to microtubule associated protein ´s family (MAPS) which dramatic exists in central neuronal system and neuron ´s axons. Also Tau protein assists to microtubule ´s structure stability. There are a lot of factors that is affected on proteins' natural function which eventuates protein aggregation in neuronal system. Tau is included two domains with positive charge which is named proline rich region and Microtubule binding region (MTBR) that could interact with anionic fatty acids with negative net charge. Tau protein was expressed and purified through Stefan Barghorn protocol with some changes. Tau protein Purification was detected with SDS-PAGE gel chromatography. The data have been analyzed with ThT fluorescence emission spectrophotometry confirmed protein which interacted with poly unsaturated fatty acids, are aggregated and beget beta sheet amyloids. Arachidonic acid and oleic acid at the concentration about 10 mM have the potential to aggregate tau protein at the concentration about 20 μM. Zeta potential of the micelles have been measured through Dynamic light scattering (DLS). These data have been illustrated that anionic poly unsaturated fatty acids with more double bonds and zeta potential have the most effective factors in aggregation of tau protein. Hence oleic acid with the least double bonds and the most zeta potential has the maximum ability to aggregate tau protein.

Key words: Alzheimer ´s disease; Tau protein; Arachidonic acid; Oleic acid; Protein Aggregation

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Comparative study of sorbitol dehyrogenase kinetics in liver tissue

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ABSTRACT

Sorbitol dehydrogenase (SDH), an enzyme in carbohydrate metabolism, catalyzes production of fructose from glucose by using NAD^+ as cofactor. In this study, SDH kinetics was compared in four species including bovine, ovine, chicken and rabbit. To perform this study, liver tissue of four animals was homogenized with two fold volume of cold tris buffer (90 mM pH=7) and centrifuged. SDH activity was assayed using backward reaction which catalyzes reduction of D-fructose to sorbitol. Shortly varying concentration of fructose, as substrate and 250 µM/L of NADH were prepared in tubes for the test. 30 µL of tissue homogenate was added to each tube and change in concentration of NADH was monitored by spectrophotometer. Concentration of NADH was measured by reading light absorption in 340 nm. Plots of concentration versus time and reciprocal plot of 1/V versus 1/[s] was drawn. K_m and V_{max} was calculated for each species. Our study has shown that K_m for chicken, ovine, rabbit and bovine were 8×10^{-4}, 2.9×10^{-3}, 1.3×10^{-3}, and 5.1×10^{-3} mM, and V_{max} were 108.67, 51.64, 39.92 and 63.21 mM/s, respectively. In conclusion chicken has the highest K_m value and rabbit has the lowest one.

Key words: Sorbitol dehydrogenase; Liver; Kinetics; Comparative

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Comparative study of Rhodanese kinetics in liver tissue

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ABSTRACT

Rhodanese is an enzyme detoxifies cyanide by transfer of the reduced sulfur atom in a sulfane-containing compound to a thiophilic acceptor. This study was conducted to compare kinetics of rhodanese activity in sheep and chicken. To perform this study, liver tissues of both species were homogenized in sodium phosphate buffer and centrifuged in 400 g. The supernatant was precipitated with 70% ammonium sulfate and supernatant was used as the enzyme source. The enzyme activity was assayed in different concentration of thiosulfate and cyanide as substrate; then concentration of thiocyanate was determined by adding Golden stein's reagent. Potassium thiocyanate was used to draw Standard curve. For thiocyanate K_m values in sheep and chicken were 110.8 mM and 2285.7 mM respectively and V_{max} values were 0.5 and 0.07 mM/S for sheep and chickens respectively. K_m for potassium cyanate was 27.66 and 514.7 mM in sheep and chicken respectively. V_{max} was calculated 0.82 mM and 0.063 for sheep and chicken respectively. In conclusion rhodanese is less active in chicken than sheep.

Key words: Kinetics; Rhodanese; Comparative; Liver

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Change in photoprotein bioluminescence properties by conjugating to quantum dots (QD) nanoparticles

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ABSTRACT

Quantum dots (QDs) are semiconductor nanoparticles with very interesting optical properties, such ashigh molar extinction coefficients, broad absorption spectra, narrow and symmetric emission peaks (30–50 nm) and depending on thesize of thenanoparticles. Current applications of QDs are widespread, their use as fluorescence labels in bioassays. These nanoparticles are usually conjugated to biomolecules like antibodies, oligonucleotides, enzymes or protein to improve assay sensitivity. Researchers also use QDs as labels for bioluminescence resonance energy transfer (BRET) assays. In this study, by thermochemical method, water soluble Cdte nanoparticles with different sizes and thus different emission peaks were synthesized. BRET system was set up with these nanoparticles as an acceptor and photo protein (aequorin) as an energy donor. Aequorin is conjugated to the carboxyl groups on the QDs surface by EDC/NHS chemistry as cross linker. This protein has emission at 470 nm inthe presenceof calcium. When this protein is conjugated to nanoparticle this light is absorbed by QDs and emitted athigherwavelengths. This method makes it possible to obtain conjugated photoprotein with emission ranging from green to red. So far, the conjugation of quantum dots to aequorin by EDC/NHS is done and the bioluminescence properties of conjugated photoprotein is being studying.

Key words: Bioluminescence; Quantum dots; CdTe; Photoprotein; BRET

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Decreasing chaperone activity and change in the native structure of α-crystallin upon homocysteinylation

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ABSTRACT

The transparency and refractive power of eye lens depend on the native structure of crystallin proteins. As the main chaperone of eye lens, α-crystallin (α-Cry) plays a significant function in preventing protein aggregation and subsequently inhibiting the opacification of lenticular tissue. A number of chemical modifications have been already suggested to alter structure and to reduce chaperone activity of this protein. These modifications have been also implicated in the pathogenesis of cataract disorders. In this study α-Cry was modified in the presence of homocysteine thiolactone; then structure, aggregation propensity and chaperone activity of this protein were evaluated, using different spectroscopic techniques and gel mobility shift assay. The results indicate a substantial structural alteration of α-Cry which was accompanied with the significant reduction on its chaperoning ability and increased propensity of the protein for aggregation. Since cataract is known as essentially aggregation-based disease, the results of this study may suggest homocysteinylation of α-Cry as a new risk factor in the pathogenesis of cataract disorders.

Key words: Homocysteinylation; α-crystallin; Chaperone; Aggregation; Cataract

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Poster – P100 Open Access

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Investigating the interaction of antibacterial peptide, aurein 1.2, with the bacterial membrane using molecular dynamics simulation

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ABSTRACT

Antimicrobial peptides (AMPs) are produced by almost all living organisms as a component of their immune system representing the first line weapons against infective agents. They have common properties such as they are short (8-50 amino acids), amphipathic and have net positive charge. Even if intracellular targets of antimicrobial peptides (AMPs) are DNA, RNA and/or proteins, the primary target of AMPs peptides appears to be the cell membrane. Due to AMPs favorable characteristics such as rapid bactericidal and rare development of drug resistance, they become very attractive for scientists as good candidates for antibacterial treatment. Aurein1.2 a short peptide (13 residues) derived from skin of an Australian frog shows a broad spectrum of antibacterial and anticancer activity. The aim of this research is to use molecular dynamics simulations to examine the interaction of Aurein1.2 with bacterial membranes. To this end, in separate simulations dynamics of a single Aurein1.2 and groups of Aurein1.2, initially perpendicularly to the membrane bacterial, are studied. For modeling membrane of bacteria in these simulations, we used lipid compositions POPE/POPG (3:1 ratio). As a result of this simulation, the general aspects for the mechanism of action for this peptide as a bactericide are provided. The simulation results show that, in both simulation peptides tend to penetrate into the membrane in an inclined orientation and by their C-terminal face to the membrane. Also group of 5 Aurein1.2 penetrate easier into the membrane of bacteria than a single Aurein1.2.

Key words: Aurein1.2; Antibacterial peptides; Atomic-scale molecular dynamics; Antibacterial activity mechanism; Membrane bacteria

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Synthesis and labelling of [^{111} In-Dtpa°] Tyr^3-octreotide via solid phase method

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ABSTRACT

Over the years, radiolabeled receptor-binding peptides have become an important class of radiopharmaceuticals for tumor diagnosis and therapy. Peptide based radiopharmaceuticals are generally composed of a targeting vector (regulatory peptide), a metal or bifunctional chelator, a radiometal, and a linker. Octreotide, a metabolically stable somatostatin analog, inhibits the growth of tumor cells by binding to surface somatostatin, receptors. In this work, we have attempted the complete synthesis of *Tyr^3-Octreotide*. *Octreotide* was synthesized manually on 2-chlorotrityl chloride resin by standard Fmoc solid phase synthesis strategy. Fmoc-Thr(tBu)-OL/Fmoc-Thr-OL was treated with the swelled 2-CTC resin in DCM in the presence of DIEA, and substitution level was determined by weight gain measurements and UV method. After coupling of the first amino acid onto the resin, the unreacted linkers on the resin are protected, to avoid the undesired peptide chain formation, with a solution of DIEA and methanol in DCM. The complete synthesis was achieved by stepwise coupling of Fmoc-Amino acids to the growing peptide chain on the resin. The couplings were performed by dissolving the Fmoc-Amino acid and HOBt in DMF, and then DIC was added. The reaction mixture was added to the resin and stirred for 2 hrs. The crude peptide was conjugated with DTPA to afford *Tyr^3-Octreotide* (DTPA-TOC). The conjugated peptide could be efficiently labeled with ^{111}In, by addition of $^{111}InCl_3$ and ammonium acetate buffer pH=5 and heating (95°C, 20 min). The structures of the key intermediates and target molecule were confirmed by ESI-MS, IR and NMR.

Key words: Octreotide; Solid phase; DTPA; ^{111}In

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A noble real time PCR-based method for *Thermus thermophillus* DNA ligase activity assessment

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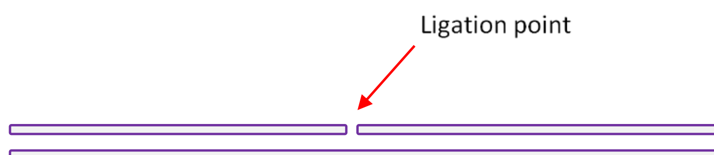
ABSTRACT

An important group of enzymes used in molecular biology are thermostable DNA ligases. Thermostable DNA ligases such as *Thermus thermophillus* (*Tth*) DNA ligases are used in variety of genetic disorders diagnostic methods including MLPA, so it can be useful to develop a quick method to measure their activity. Many different methods have been used to assay these enzymes activity quantitatively so far. However, in our project we are trying to establish a new real time PCR-based method to measure *Tth* DNA ligase activity. For this purpose, a large amount of three pieces of single stranded DNA fragments have been prepared in a way that two of them are semi complemented with the third larger fragment, thus after annealing completed, the substrate will be a double stranded DNA fragment with a nick in the middle.



Enzyme incubation will be done in different time periods and the products will be applied in quantitative real time PCR to evaluate concentration changes in time that it can ultimately give the kinetic properties of the enzyme.

Key words: Enzyme activity assessment; Thermostable; DNA ligase; Semi complemented; Quantitative real time PCR

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Theoretical chemistry and molecular dynamic analyses reveal the molecular interaction of Azrc Azoreductase and Azo dyes

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ABSTRACT

Aromatic azo dyes are the most widely used synthetic dyes which are characterized by one or more azo (-N=N-) group. These dyes are generally resistant to common physico-chemical wastewater treatment. Therefore, researchers were focused on biodegradation of azo dyes through biological treatment methods like using the bacterial systems. In the present study, the interaction of *Bacillus sp.* B29 AzrC azoredoctase with five common azo dyes was investigated. The stability of trajectory was confirmed by RMSD and RMSF analyses and the potent active site residues were indicated through hydrogen bond and Alanine scanning analyses. The results of molecular mechanics MMPBSA and MMGBSA analyses confirmed that the hydrophobic azo dyes like Acid red 88 bound more tightly to the AzrC protein. Furthermore, this study is the first survey using the MD analyses in order to compare the molecular interaction between five different azo dyes and AzrC protein. Our results will be helpful for future studies in order to make more efficient recombinant AzrC protein for azo dyes degradation. On the other hand, these comparative dynamic studies of the binding modes between AzrC and azo dyes results in several useful conclusions in order to improve our knowledge about biodegradation of azo dyes as a promising biological treatment method.

Key words: Azo dye; Molecular Dynamics; Alanine scanning AMBER; Azoreductase

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Role of lysine 69 on the binding and immunoreactivity properties of bovine β-lactoglobulin

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ABSTRACT

β-Lactoglobulin (β-Lg) is a lipocalin, which is the major whey protein of cow's milk and the milk of other mammals. However, it is absent from milk of primates. This globular protein of about 18 kDa is folded forming a β-barrel (or calyx) structure. The biological function of β-Lg is not clear, but its potential role in carrying fatty acids through the digestive tract has been suggested. β-Lg is also one of major allergens in milk. Lys69 has been found in an important allergic epitope of β-Lg. It has been also demonstrated that this amino acid has an important role in the binding of ligands to the hydrophobic pocket of β-Lg. In order to prove the dual function of Lys69 on the binding and allergenicity properties of β-Lg, a recombinant mutant of protein was investigated. The expression in the yeast *Pichia pastoris* of a mutant bovine β-Lg, in which Lys69 was changed into Asn (Lys69Asn) was accomplished during this study. Binding of IgE from patients with cow's milk allergy to native β-Lg, wild-type β-Lg and Lys69Asn mutant β-Lg was aslo measured by ELISA. Subsequently, the binding properties of recombinant Lys69Asn mutant β-Lg were compared with native one. The results showed that the substituted amino acid, Asn, reduced protein allergenicity due to degradation of its main epitope and enhanced its binding properties during interaction to some hydrophobic ligands.

Key words: β-lactoglobulin; Interaction; Allergy; Mutation; ELISA test

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Rational site selection for investigation of amino acids interaction by computational studies: streptokinase as a case study

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ABSTRACT

These days computational approaches of predicting protein-protein interaction (PPI), are considered among biologists due to the ability to predict protein-protein interactions and confirm the achieved experimental results. Because of therapeutic aspects of proteins, investigation of interaction between drugs and proteins is needed. This leads us to understand the mechanism of drug action at a molecular level. Although the using proteins as drugs have many problems such as low activity, physicochemical instability, short half-life and immunogenicity, there are several methods for improvement of the pharmacokinetics properties of proteins, such as amino acid substitution and conjugation. In the present study, we used bioinformatics tools in order to select the best position for amino acid substitution to increase activity of streptokinase (SK). Activation of the human fibrinolytic system by SK occurs during binding to plasminogen (Plg), the zymogen of plasmin (Plm). In detail, crystal structures of SK were retrieved from the protein data bank (PDB). Homology modeling was performed by the most recent version of program package MODELLER 9.13. SK-µPlm complex was employed as an excellent model for *in-silico* assessing intramolecular and intermolecular interaction of crucial exosites in SK and SK-µPlm complex respectively. After each amino acid substitution, importance of interactions such as aromatic–aromatic, aromatic–sulphur, cation–π, ionic interactions and hydrophobic interactions in the structure of protein was recognized. Finally the best amino acids were suggested as candidates for experimental studies.

Key words: Streptokinase; Mutagenesis; Amino acids interaction

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Purification of α-amylase from a strain of thermophilic anoxybacillus flavithermus

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ABSTRACT

Amylolytic enzymes are among the most important enzymes in industry and biotechnology. Alpha amylases (endo-1, 4- D-glucan glucanohydrolase, E.C. 3.2.1.1) are extracellular endo enzymes that randomly cleave the 1, 4-α linkage between adjacent glucose units in the linear amylose chain and ultimately generate glucose, maltose and maltotriose units. Amylases stand out as a class of enzymes, which are of useful applications in the food, textile, detergent and pharmaceutical industries. In the present study, a strain of amylolitic *Anoxybacillus flavithermus* was identified and 16S rRNA analysis was carried out on this thermophilic strain. Bacterial growth optimal production was investigated. Then, protein was partially purified by 85% ammonium sulfate and dialyzed. Besides, biochemical properties such as effect of temperature, pH and effect of denaturant such as SDS on the stability of enzyme were investigated. On the other hand, encode gene of enzyme was amplified and sequenced. Results indicate that the optimal conditions for bacterial growth are after 20 h, at 60 °C and pH 7.0. Also, maximum enzyme activity was obtained at 70 °C at pH 6.0 after 72 h of incubation and optimal temperature is more than other species and stability of the enzyme in the presence of SDS is the same as other species. Moreover, sequencing result confirms existence of the gene with the length of about 1500 bp.

Key words: Alpha-amylase; Anoxybacillus; Thermophile

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Berberine improves liver failure induced by ferrous sulfate in rat

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ABSTRACT

As liver is the major site of iron storage, it is susceptible to damage from iron toxicity. It has been demonstrated that ferrous sulfate induces hepatic toxicity in rats. Berberine, an isoquinoline alkaloid, has a wide range of pharmacological effects, including anti-inflammation. Thus, the effects berberine on liver injury induced by ferrous sulfate was investigated in rat. Liver failure was induced by i.p. injection of ferrous sulfate (30 mg/Kg/day) for 14 day. In final blood samples collected for determination of the plasma creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentration. Then liver samples were removed and preserved for histological studies and estimation of lipid peroxidation. Berberine (10 mg/kg/day) was administered 14 days by gavage. Ferrous sulfate caused a significant reduction in renal function, increase of lipid peroxidation, increase in liver inflammation, demonstrated by elevation in plasma levels of AST, ALT and ALP as well induction of hepatic histological damages. Berberine treatment reversed the parameters of liver injury, increase of lipid peroxidation and liver enzymes levels as well as reduced total hepatic histopathological scores. These data suggest the protective effect of Berberine against liver dysfunction induced by ferrous sulfate in rats.

Key words: Alanine aminotransferase; Aspartate aminotransferase; Berberine; Lipid Peroxidation; Liver; Ferrous sulfate

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Studies on the binding affinity of anticancer drug actinomycin D to core histones and H1 histone

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ABSTRACT

Actinomycin D is a polypeptide antibiotic and antitumor agent, widely used as a potent chemotherapeutic agent in the treatment of various cancers. This drug inhibits the transcriptions and replication of genes by intercalating between the DNA base pairs. We have previously shown that apart from DNA, chromatin components are a suitable target for this drug. Chromatin is a complex of DNA, RNA, histones and non-histone proteins. There are five main histones. Linker histones (H1 family) and core histones. We focused on the binding affinity of actinomycin D to core and H1 family. In the present study, we have investigated the interaction of Actinomycin D on core histones and H1 histone employing UV/Vis, fluorescence, CD spectroscopy. UV/Vis spectroscopic results obtain from the interaction of actinomycin D with the core histones and H1 histone showed that low concentrations of drugs induced hyperchromicity in the absorbance at 210 nm and at higher concentrations, hypochromicity was obtained. Fluorescence emission represented quenching of core histones and H1 histone with drug and decreased fluorescence emission at 307nm. CD spectroscopy showed drug induces structural changes in core and H1 histones and H1 histone was affected more than core histones. Result suggest that histone H1 and core histones can be considered as a target for Actinomycin D at the chromatin level and suggest that actinomycin D shows higher affinity to H1 histones compared to Core histones.

Key words: Actinomycin D; Core histones; H1 histone

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Structural comparison of amylin between species: molecular dynamics simulation

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ABSTRACT

Amylin is a 37 residue peptide with a disulfide bridge between the residues 2 and 7. This hormone co-secreted with insulin by pancreatic islet β-cells. It has been reported that amylin is able to form amyloid fibrils. Although these fibrils are not related with the manifestation of type 2 diabetes, they have a significant role in the aggravation of diabetic condition. In this study, the potential of amyloid formation and stability of amylin has been studied in 10 species with high sequence similarity (80%), with the use of molecular dynamics simulation. Based on the structure of human amylin (2L86.pdb from Protein Data Bank) homology modeling technique was used to create 10 different species amylin. Simulation was performed using GROMACS version 4.6.1, with a 10 ns duration and 500K. The systems were solvated with SPC water molecules. Secondary structure content was calculated using DSSP. Other analyses such as energy, solvent accessible surface, radius of gyration, RMSD and RMSF were performed, using GROMACS. Human and Rat amylin were selected as non-aggregating peptides respectively. During simulation amylin showed remarkable structural changes, and in most cases α-helical structures were lost. In some species with aggregating amylin, β-sheets were formed at the end of the simulation. Between the studied species, *Pongoabelii* peptide, one of the two species of orangutans used in this study, showed more stability alongside with more similarity to human amylin. Based on these results, this amylin specie could be proposed as a potential therapeutic peptide, as an alternative to rat amylin.

Key words: Amylin; Amyloid formation; Molecular dynamics simulation

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Small peptides are natural antibiotics

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ABSTRACT

Antimicrobial peptides (AMPs) have recently gained much attention because of their ability to overcome drug resistance and have emerged as a potential new class of antibacterial agents. These peptides are part of the innate immune system of all living organisms, including vertebrates, insects, bacteria and plants. AMPs encompass a number of different classes, including those that are rich in a particular amino acid. Important subsets are peptides rich in Arg and Trp residues that have broad and potent antimicrobial activity. These residues possess some crucial chemical properties that make them important components of antimicrobial peptides. Trp side chains are also implicated in peptide and protein folding in aqueous solution, where they contribute by maintaining native and nonnative hydrophobic contacts. This has been observed for the antimicrobial peptide from human lactoferrin, possibly restraining the peptide structure in a suitable conformation to interact with the bacterial membrane. These unique properties make the Arg- and Trp-rich antimicrobial peptides highly active even at very short peptide lengths. The long peptides interact very weakly with the lipids membrane. The antivirus mechanism of the biologically active peptides (peptaibols) sheds light on the potential use of peptaibols in plant viral disease control. The serious problems caused by drug resistant bacteria have created an urgent need for the development of alternative therapeutics. In this respect, AMPs are considered as promising antimicrobial agents for producing new generation antibiotics and also for improving plant resistance against bacterial and virus lesion. So it is reasonable to focus on small peptides.

Key words: Antimicrobial peptides; Small peptides; Lactoferrin; Drug resistance

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The Interaction between oleuropin and bovine serum albumin

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ABSTRACT

Oleuropein is the active and main compound in the olive leaf and oil. Oleuropein scavenges superoxide anions and free radicals and saves the cell from oxidative stress. The interaction between oleuropin and Bovine serum albumin (BSA) has been investigated by several kinds of spectral methods. With the addition of oleuropein's concentration to albumin solution, the absorption is increased while there were no changes in peak location. Fluorimetry was applied to study the mechanism of oleuropein attachment with serum albumin. Our results showed that Oleuropin and BSA can form a 1:1 complex. The Ksv values increased with increasing temperature, this shows dynamic quenching mechanism. The fluorescence emission and the synchronous fluorescence spectra primarily indicated oleuropin binding results in an increased hydrophobicity around tryptophan residues.

Key words: Oleuropein; Albumin; Fluorimetry; Binding

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Probing of possible olanzapine binding site on human serum albumin: combination of spectroscopic methods and molecular dynamics simulation

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ABSTRACT

Human serum albumin (HSA)-drug binding affinity is one of the major factors that determine the pharmacokinetics, halftime and bioavailability of drugs in various tissues. In the present study, the interaction of olanzapine (OLZ), a thienobenzodiazepine drug, administered for the treatment of schizophrenia and bipolar disorder, with HSA has been studied using spectroscopic methods such as ultraviolet absorbance, fluorescence and FTIR combined with computational procedures. Analyzing of the Stern–Volmer quenching data showed only one primary binding site on HSA with a binding constant of 4.12×10^{4} M^{-1} at 298 K. Thermodynamic analyses showed enthalpy change ($\Delta H°$) and entropy change ($\Delta S°$) were 28.03±3.42 $kJmol^{-1}$ and -25.52±11.52 $J.mol^{-1}.K^{-1}$, respectively. Molecular docking results suggested the hydrophobic residues such as Val_{216}, Leu_{327}, Ala_{350} and polar residues such as Glu_{354} play an important role in the drug binding. Decrement in α-helix content of the protein upon OLZ binding was also confirmed by evidences provided by molecular dynamics simulation as well as FTIR spectroscopy.

Key words: Human serum albumin (HSA); Olanzapine; Fluorescence quenching; Molecular dynamics simulation

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Activity, stability and structure of bacterial laccase in deep eutectic solvents

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ABSTRACT

A special group of ionic liquids, deep eutectic solvents (DESs) are opening up a new field of nonaqueous enzymology and hold potential as green solvents because of their lack of vapour pressure. Deep eutectic solvent (DES) is a mixture of two or more compounds that are capable of self-association, often through hydrogen bond interactions, to form a eutectic mixture which has a melting point lower than either of its components. These solvents have to meet different criteria such as high biodegradability, low toxicity, high solubilization ability, low cost and simple synthesis. Since laccases are of prime interest in industrial field and has been extensively utilized as the catalyst for removing phenolic compounds in industrial wastewaters, therefore, in this study, we discuss the effects of betaine based DESs on the performance, stability and structure of laccase from *Bacillus* sp. HR03. Laccase was more stable in these solvents compared to the aqueous buffer. As the concentration of DES increased, K_m increased. Associated conformational changes caused by solvents were monitored using fluorescence technique. Finally, the effects of DESs on the enzyme activity and stability were discussed.

Key words: Ionic liquids; DESs; Green solvents; Biodegradable; Fluorescent

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The role of disulfide bridge covalent cross-linking on the structure, chaperone activity and aggregation propensity of wild-type and R12C mutant αA-crystallins

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ABSTRACT

By the mean of chaperone-like activity, alpha-crystallin (αA and αB) has been indicated to play an important function in the maintenance of eye lens transparency. The R12C substitution has been shown to have important association with development of congenital cataract. In the current study, under oxidizing condition, the formation of disulfide bridge covalent cross-linking was induced in mutant αA-crystallin (R12C) and its wild-type protein counterpart. The structure, aggregation propensity and chaperone activity of these proteins were assessed before and after cross-linking, using different spectroscopic analysis and gel mobility shift assay. As a result of protein cross-linking, the significant structural alteration and slight variation in their chaperone-like activity were observed. Both structural integrity and chaperone function of alpha-crsyatllin are significant for its critical role in maintaining the transparency of lenticular tissues during the life span. Therefore, under oxidative condition the enhancement in the rate of crystallin covalent cross-linking can be considered as an important parameter in the pathogenesis of cataract disorders.

Key words: αA-crystallin; Disulfide crosslinking; Chaperone activity; Cataract diseases

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Minimutate: minimization on mutated protein structure

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ABSTRACT

There is an increasing trend towards the engineering characteristics and utility of proteins in order to the industrial, biotechnological and biopharmaceutical applications. Lots of protein manipulations can be achieved through site directed mutagenesis. It is expensive and time consuming to analyze the effect of mutation by getting an X-ray structure of mutant proteins. The use of predictive computational methods has become more prevalent in recent years leading to decrease the cost of experiments. Therefore, it is needed and common to predict the influence of engineering on protein structure with molecular dynamics (MD) simulation before going to the experiment. In this work, the software "*MiniMutate*" has been developed which can do a single mutation in a protein structure. Mutant coordinate file which is generated after the local and the global minimization with molecular dynamics program NAMD will be returned as the output. This file can be used as an input of MD simulation packages. In comparison to similar software at WHATIF server, MiniMutate can do mutation in a specific chain that user is selected, the important option which is lacking on WHAT IF, and with minimizing mutant structure it can be more precision than the WHAT IF output. In addition, this software can be used to construct missing side chains from PDB file. The software is freely available at: http://bioinf.modares.ac.ir/software/minimutate/.

Key words: Site Directed Mutagenesis; Protein Engineering; Minimization; Missing Atoms

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Production and characterization of a novel halophilic protease produced by the isolate *Salicola* sp

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ABSTRACT

Halophilic enzymes are active and stable at low water conditions and can be used in the presence of organic solvents, extreme salt content, and high temperature and pH values. They can also be used for biotechnological and biopolymer-degredation applications. Proteases have extensive applied in industries such as detergent, leather, wool, diary and food, and pharmaceutical. In 2013, 40-60% of enzymes marketing were for proteases. In the present work, the production of a serine halophilic protease from an isolated halophilic bacterium was optimized through statistical design methods. 16S rRNA analysis, morphological and biochemical studies were performed for identification of the isolate obtained from hypersaline lake of Golestan. The enzyme showed the maximal activity at 40 ºC and pH 7 in 3.5 M NaCl. To select considerable factors out of seven media components, fractional factorial design was used as an initial screening design and significant factors which affect protease production were chosen to be optimized by Box-Behnken design. The maximum proteolytic activity was attained at the defined concentration of peptone, K_2SO_4, and KNO_3. The enzyme was stable in the presence of different organic solvent and heavy metals. Furthermore, the enzyme was completely stable in front of chemical detergents such as EDTA, 2-Mercaptoethanol and Cetyl trimethylammonium bromide. Biodegradation of feather was observed by the strain, which is valuable to poultry feather waste management industry. The keratin released by keratinolytic protease activity from 1 g of pretreated feather was 86 µg/ml.

Key words: Protease; Halophile; Optimization; Production; *Salicola* sp

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Adenosine deaminase activity in cattle with theileriosis

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ABSTRACT

To study adenosine deaminase activity in bovine tropical theileriosis, blood and serum sample were collected from 49 infected and 15 uninfected cattle. Haematological parameters were measured by standard methods in both groups. The results showed significant decrease in red blood cells (RBCs) count, hemoglobin and packed cell volume (PCV) which all indicating the occurance of anemia in infected animals. Furthermore, the infected cattle had higher lymphocyte, monocyte and eosinophil. Compared to uninfected, the infected animals showed higher adenosine deaminase activity. These findings along with lymphocytosis in peripheral blood circulation are showing an active immune response to infection and its related inflammatory reactions in different tissues. High levels of adenosine deaminase activity in infected cattle maybe regarded as an important index which needs more studies to elucidate its role in pathogenesis or immunopathogenesis in bovine tropical theileriosis.

Key words: Bovine tropical theileriosis; Adenosine deaminase

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Protein-protein interaction: computational prediction methods

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ABSTRACT

Proteins are key players in cellular pathways and interactions between them play main role in wide range of process in cells such as cellular motion, signal transduction and transport. Furthermore, information and knowledge about protein-protein interactions and constructing of protein interaction graph has very important role in understanding of biological process. Since protein-protein interaction information can define function of a protein through position of that protein in a protein web, further such information have key role in supporting of biological researches. Akin to the complete sequencing of genomes, complete descriptions of interactomes is a fundamental step towards a deeper understanding of biological process. Experimental detection of protein-protein interactions include CO-IP, TAP-MS, Y2H but these methods limited by time consuming, high cost and have more false positives. Computational analysis of PPI networks is increasingly becoming a mandatory tool to understand the functions of unexplored proteins. Thus *in silico* methods which include sequence-based approaches, structure-based approaches, chromosome proximity, phylogenetic tree, phylogenetic profile and gene fusion approaches were developed. Also a variety of web server have been developed to prediction the interactions that have been detected by experimental approach such as: STRING, PRISM, Mirror tree, InterpreTS and struct2net. Recent developments have also led to the construction of networks having all the protein-protein interactions using computational methods for signaling pathways and protein complex identification in specific diseases. In this review, we discuss about *in silico* methods in prediction of PPI that is important field in protein research.

Key words: Protein-protein interaction; Prediction server; Protein network; Experimental methods; Computational analysis

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Conserved proline causing a supportive interaction with the substrate cavity in Renilla luciferase, a bioinformatic study

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ABSTRACT

The Renilla luciferase (Rule) is a 36kDa protein produced by a luciferase gene from *Renillareniformis*. Evolutionary, Rluc and bacterial haloalkane have a similar ancestor. Interestingly, although the activity between Rluc and bacterial haloalkane are absolutely different and these two belong to the oxiduredoctase and hydrolases respectively, the structure shows high similarity. Both has a conserved catalytic triad including aspartic acid, glutamic acid, and Histidine and a substrate gateway that was formed from gathering surface secondary structures during folding. Several residues are conserved in the gateway and the following cavity (active center) in Rluc. Among them proline 224 (P224) has been reported as a key residue for interaction with substrate cavity. Thus we have predicted the Rluc stability upon replacing P224 by Alanine. The modeling was carried out using CPHmodels 3.2 Server (http://www.cbs.dtu.dk/services/CPHmodels/) and the tertiary structure was evaluated using bioinformatic servers. The Result was subject to calculation the free energy changes using I-Mutant2 server and consider for stability. The data show that the changing of P224 into Alanine decreases the protein stability. In conclusion, the results from bioinformatic analysis show that P224 might be an important residue to preserve the three dimensional structure of the cavity site in Rluc. The data are in agreement with the sequence similarity between Rluc and bacterial haloalkane whereby P224 is conserved residue during evolution.

Key words: Renilla luciferase; Free energy; Proline 224; Substrate cavity; Bioinformatics

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New evidence shows link between evolutionary trend and the structural stability of a conserved isoleucine in the Renilla luciferase tunnel site, a bioinformatic study

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ABSTRACT

Renilla luciferase (Rluc) is a bioluminescent enzyme which shows high homology (34-56% similar) to a number of bacterial halo alkanede halogenases. Rluc has a conserved catalytic triad consisting of D120, E144, and H258 and a group of amino acids that forms the enzyme tunnel site. The tunnel site is important for substrate access to the catalytic triad, but there is a question that what role the amino acids in the tunnel site may have in structural stability. In order to answer this question, we have considered the role of Isoleucine 150 (I150) in Rluc, which is reported as a conserved amino acid in the Rluc tunnel site. The 3D structure of Rlu was modeled based on 2PSH model (www.PDB.org) using M4T server (manaslu.aecom.yu.edu/M4T/). The model was evaluated using bioinformatic servers and analyzed for changing the DDG using a web-based server (http://mordred.bioc.cam.ac.uk). The mutation caused a negative value of $\Delta\Delta G$ (-2.58 Kcal/mol) which means manipulation of I150 has an unstable effect on Rluc structure. Therefore, I150 not only is important for the formation of the enzyme tunnel site, but also might be an important residue in Rlu stability. The result is consistent with the evolutionary trend in sequence of Rlu, during which I150 is a conserved residue during evolution.

Key words: Renilla luciferase; Protein Stability; Isoleucine 150; Bioinformatics

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Poster – P121

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Interaction of anthracycline antibiotic daunomycin with HMGB1 as non-histone protein in solution

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ABSTRACT

High mobility group (HMG) proteins are the most abundant group of chromatin non-histone proteins. This family is composed of four major variants, including HMGB1, HMGB2, HMGB3 and HMGB4. As a highly evolutionary conserved protein, HMGB1 is the most studied and important member of this group. Daunomycin is a potent chromatin-binding antitumor drug belonging to the family of anthracycline antitumor drugs. In this work, HMGB1 was purified from rat liver and incubated with various concentrations of daunomycin and then its binding to daunomycin was investigated, using equilibrium dialysis and circular dichroism spectroscopy techniques. The Scatchard plot obtained from the equilibrium dialysis study exhibited positive cooperative binding behavior and the occurrence of a negative Gibbs free energy ($\Delta G^{\circ} = -6.57$ Kcal/M), suggesting that the interaction process was exergonic. Association (K_a) and dissociation (K_d) constants were also determined (K_a: 7.71×10^4 M^{-1}, K_d: $1/3 \times 10^5$ M). According to the circular dichroism data, upon addition of various concentrations of the drug, secondary structure of HMGB1 was altered in a dose dependent manner. Prediction of secondary structure of HMGB1 using relevant software showed that upon binding of daunomycin to HMGB1 the alpha-helical content of the protein was increased. Summing up, the results suggest that daunomycin binds to HMGB1 protein and this binding may influence HMGB1-DNA interaction followed by DNA-dependent activities of the protein in the cell.

Key words: Chromatin; DNA; HMGB1 protein; Antitumor drugs; Daunomycin

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Interaction of human serum albumin with linolenic acid: stability and structural analysis

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ABSTRACT

The interaction between Human Serum Albumin (HSA) and linolenic acid (LA) as unsaturated fatty acid have been investigated by the methods of UV–VIS spectrophotometer, fluorescence, circular dichroism (CD) spectroscopy and ELISA test. The thermodynamic parameters were assessed from thermal and chemical denaturation of the HSA with and without presence of LA. In thermal denaturation the magnitudes of T_m and $\Delta G^0_{(298K)}$ were obtained 327.7 K and 88 kJ/mol for sole HSA and 313.23 K and 86 kJ/mol for its treatment by 10 µM LA. The same manner of reduction in Gibbs free energy as the criterion of protein stability was achieved in chemical denaturation by urea in the presence of LA. The interaction of LA with HSA was emphasized after its competition with L-thyroxin through ELISA assay. Although the regular secondary structure of HSA by CD showed a minor change after incubation with LA, its tertiary structure revealed an observable fluctuation. Thus, it seems, that the interaction of LA with HSA needs a minor instability and partially change of its structure.

Key words: Human serum albumin; Linolenic acid; Structure; Thermodynamic; L-Thyroxin

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Enzymatic xanthan degradation by isolated bacteria from soil farms in Tehran suberb

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ABSTRACT

Polysaccharide-degrading enzymes are widespread in nature. They can be found in every type of living organisms. Xanthan is an extracellular hetropolysaccharide produced by phytopathogenic bacterium *xanthomonas campestris*. It has considerable industrial significance and numerous applications. There are two types of xanthan-degrading enzymes that have some applications such as enhanced oil recovery (EOR). One type of the enzymes is xanthanase, catalyzing the hydrolysis of the β (1→4) glucan backbone of xanthan. Xanthan lyase is another one which can be used for making novel modified structures with physicochemical functions. In this study to isolate the bacteria which produced xanthan-degrading enzyme, soil samples were collected from cabbage farms at south of Tehran, They were enriched in a medium containing 0.3% xanthan as sole carbon source, plus mineral component. The enriched media were incubated for 4 days until viscosity decreased below 10 cp. They were spread on agar medium. Xanthan-degrading strain colonies with clear zone were selected and then incubated at 30 °C. Then degradation has been searched in liquid media. Endoxanthanase activity was assessed using xanthan as the substrate. Strain with the most enzyme activity was chosen for the most parts of study, Partial purification of xanthanase was done and some of properties of this enzyme, including optimum pH and temperature for enzyme activity were investigated. The substrate specificity of xanthanase was estimated by using some carbohydrates such as cellulose, sucrose, lactose, and maltose. Protein content of the enzyme containing solutions determined by Bradford assay as serum albumin was the standard protein.

Key words: Xanthanase; Xanthan lyase; Enzyme activity; Xanthomonascampestris; Xantha

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Poster – P124

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Investigation of structure, thermodynamics and oligomerization of geobacillus maltogenic amylase

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ABSTRACT

In present study the effects of osmolytes (betaine, sorbitol, glycerol and sucrose) and chaotropic agents (urea and guanidine hydrochloride) on the behavior of the maltogenic amylase (MAase; EC 3.2.1.133), isolated from a thermophilic *Geobacillus* strain were investigated by heat and stability analysis. MAase as a member of subfamily 20 of glycoside hydrolase family 13 has a molecular weight of 65 kDa and has its maximum activity at temperature around 55-65 ° C with the optimum pH of 6.0. Dimeric MAases prefer CDs to starch or pullulan as substrate. High stability and efficacy in accessibility and specifity of active sites are the most important and major advantages of oligomerization. The analysis of enzyme stability was carried out by thermal denaturation and kinetic experiments. The addition of betaine, sorbitol and glycerol increased enzyme activity. Thus by using these additives, the thermal stability of MAase was studied. As it has been observed, kinetic and thermodynamic stability of MAase were enhanced by betaine, sorbitol and glycerol osmolytes. We are currently studying the effect of the presence of stabilizer additives and role of dimerization in enzyme stability. As well as, we are determining the thermodynamic parameters of transition process from monomer to dimer state and structural analysis by nano DSC and spectroscopy techniques respectively.

Key words: Cyclodextrin; Kinetics stability; Thermodynamic stability; Dimerization

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Engineering and stabilization of *A. Variabilis* phenylalanine ammonia lyase

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ABSTRACT

Several complications exist in using low-phenylalanine diets in phenylketonuria (PKU). Enzyme replacement therapy (ERT) of PKU has been offered during the last decade to decrease serum phenylalanine levels. *Anabaena variabilis* phenylalanine ammonia lyase (*Av*PAL) has recently been introduced in clinical trials of phenylketonuria for its outstanding kinetic properties. In the present study, the possibility of rational engineering of this therapeutically valuable enzyme was investigated. The goal was to reach mutants with more stability properties. Site directed mutagenesis was performed using a quick-change PCR method. Recombinant wild-type and mutated enzymes were expressed in *E. coli* and his-tagged proteins were purified by Ni-NTA affinity column. Formation of disulfide bond was confirmed by Ellman's method, and chemical unfolding, kinetic behavior, and thermal inactivation of mutated enzymes were compared to the wild-type enzyme. Based upon our results the Q292C mutant differs from the wild-type enzyme in chemical unfolding and kinetic stability properties significantly but not considering kinetic parameters and enzyme pH profile. In contrast, L127C/S226C mutant showed the same stability properties as the wild-type enzyme. Due to the constant need of PKU patients to replacement therapy during lifetime, higher stability and longer half-life will be of high interest. We proposed here Q292C mutant as the next potential candidate for the enzyme replacement therapy of phenylketonuria. The results of the present study provided an insight towards designing phenylalanine ammonia lyases with higher stability.

Key words: Phenylketonuria; Phenylalanine ammonia lyase; Disulfide bond; rational engineering

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Independence of c-terminal Cys-rich region of a rice metallothionein type 1 isoform in metal binding

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ABSTRACT

The members of plant metallothionein (MT) subfamily p1 are characterized with the presence of six Cys at each end of N- and C-terminal of their amino acid sequences which are arranged in a CXCXXXCXCXXXCXC and CXCXXXCXCXXCXC structure, respectively. In this study we evaluated the independence of C-terminal Cys-rich region of a type 1 MT isoform from rice (OsMTI-1b) in forming metal-thiolate cluster. To this end the C-terminal of OsMTI-1b (N-OsMTI-1b) was heterologously expressed in E. coli as fusion protein with glutathione-S-transferase (GST). The E.coli cells expressing GST-C-OsMTI-1b were able to remove Ni2+ from culture medium. The recombinant GST-C-OsMTI-1b was purified, using affinity chromatography. The UV absorption spectra recorded after the reconstitution of the apo-protein with Cd2+ and Ni2+ confirmed that GST-N-OsMTI-1b was able to form complexes with Cd2+ and Ni2+. These results demonstrate the formation of independent metal-thiolate cluster at C-terminal Cys-rich region of GST-N-OsMTI-1b without participation of C-terminal Cys-rich region.

Key words: Metallothionin; C-Terminal; Metal binding; Rice

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Immobilization of laccase enzyme on biocathode for use in enzymatic fuel cell

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ABSTRACT

Biofuel cells are kind of fuel cells that are known as a small and flexible energy source to produce continuous electrical current. Enzyme-based fuel cells can use enzymatic reactions to convert chemical energy into electrical energy. Enzymatic fuel cells are designed in two types, mediated (MET) and direct (DET). As mediators cause over potential and also removing the mediator increases the miniaturization potential of biofuel cell, direct method is used more. Enzymes require specific condition to carry out chemical reactions; therefore the immobilization process is needed. Immobilized enzyme in comparison with free enzyme had advantages such as increased stability, reuse and purification of enzyme. In this study, the direct transfer of electron (DET) of laccase enzyme has been done to build an efficient biocathode. To achieve direct electron transfer, binding needle-like carbon nanotubes to electrode surface has been studied. the results of cyclic voltammetry of laccase showed a pair of redox peak that deals to laccase immobilization on needle-like assembled CNTs. Redox peak is achieved at 200 µA in 5 µM o-dianisidin as substrate. Cycle voltammetry results showed that immobilized laccase on needle-like carbon nanotubes has high performance by DET. Therefore, the presented method can be used to fabricate biosensor or biocathode of enzyme -based biofuel cells.

Key words: Needle-like assembly; Biofuel cell; Enzyme immobilization; Carbon nanotubes

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Fabrication of enzymatic bioanode using MWCNT and Nafion polymer

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ABSTRACT

Today, energy generation using enzymes with the aim of constructing implantable and portable device is a significant issue because catalyzing specific substrate by enzyme leads to high efficiency. Since, the main factor to fabricate an enzymatic bioanode is appropriate electrical contact between the enzyme and the electrode, so many materials and methods have been studied to improve the electrical connection. So, nanotechnology and conductive polymers has become a unique tool to achieve efficient interaction between the enzyme and electrical circuits. In this study, fabricating bioanode using MWCNT electrode and nafion-MWCNT have been done to investigate the direct transfer of electron. Therefore, the glucose oxidase enzyme was dissolved in potassium phosphate buffer. Secondly mixture of MWCNT and enzyme solution was dropped on the electrode. Nafion was then dropped on the electrode then allowed to fully dry. Finally, electrochemical measurements were performed in the presence of β-D-glucose. The results of cycle voltammetry showed a pair of redox picks that deals to the direct transfer electron of immobilized enzyme using MWCNT and nafion polymer. The redox peak was obtained in the specific range of enzyme and maximum redox peak was achieved at 80 µA in 0.4 M β-D-glucose solution as substrate. The cycle voltammetry results indicated that the immobilization of glucose oxidase enzyme using introduced method has higher efficiency for direct electron transfer when compared to the other published studies.

Key words: Biofuel cell; Enzyme immobilization; Glucose oxidase; Nafion; MWCNT

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Glucose oxidase: a catalyst for enzymatic biofuel cell

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ABSTRACT

Glucose oxidase (β-D-glucose: oxygen 1-oxidoreductase; EC 1.1.3.4) is a flavoprotein which catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone and H_2O_2 using molecular oxygen as an electron acceptor. Glucose oxidase (GOx) has important applications in several different industries. One of its new applications is in biofuel cell (BFC) industry. Biofuel cell is a bio-electronic device which convert biochemical energy into electrical energy using a biocatalyst. Enzymatic biofuel cell uses enzymes, for example glucose oxidase as a biocatalyst to produce electrical energy. In this study, we report the fabrication and characterization of a carbone nanotube (CNT)-based sugar/oxygen BFC operating in phosphate buffer. GOx as anodic bioelement, was immobilized on carbon fiber electrode modified with CNT and nafion. For this purpose, first, 2 mg/ml GOx enzyme solution was prepared in 200 µl potassium phosphate buffer pH=7.4. Second 100 µl of MWCNT solution was mixed with 100 µl glucose oxidase enzyme and was dropped on carbon fiber. Then 100 µl nafion was added as a thin layer on the electrode. The GOx/CNT-based bioanode and one air-cathode were combined into a functional BFC and the function of BFC was carefully investigated. After 150 min of injection of glucose solution in cell, the current was 700 µA at 490 mV. Results of fabricated cell show that presented enzymatic BFC can be used as a power source to generate electricity for implantable and portable miniature devices.

Key words: Biocatalyst; Glucose oxidase; Enzymatic fuel cell

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Effects of some toxins on fibrillation processes of proteins

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ABSTRACT

There are a group of diseases associated with protein misfolding and accumulation into amyloid fibers. Many of these diseases have a major impact on the human health. The focus of this research is to highlight how some contaminants and toxins influence on amyloid formation in a number of proteins. The effect of some heavy metal ions and pesticides on fibril formation for amyloidogenic proteins is investigated. These results were supported by a thioflavine-T (ThT) fluorometric assay, determination of reactive oxygen species (ROS) and atomic force microscopy experiments. Metal ions and pesticides are capable of affecting amorphous aggregation, oligomerization and fibrillization of a number of amyloidogenic proteins associated with protein misfolding. The synergistic effects observed between pesticide and metal suggest that the total brain load of pesticides and metals, rather than individual levels, is a very important contributor to the potential effect on protein fibrillation. The results of this research indicate the environmental contaminants affect substantially the rate of protein fibrillation.

Key words: Pesticides; Metals; Amyloid fibrils; Oxidative damage; ROS

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β-lactoglobulin; a new transport vehicle for anti-diabetic drugs

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ABSTRACT

Since the prevalence of diabetes has an increasing pace, in the current century the demands for new and effective anti-diabetic drugs have been bolded. The inhibition of α-glucosidase (α-Gls) is a thirty year old approach that can significantly reduce the postprandial hyperglycemia which in turn lowers the complications of diabetes. Since this enzyme is active in the small intestine, its inhibitors should be successfully delivered in the gut. For this reason, we investigated the interaction of novel anti-diabetic compounds termed pyrimidine fused heterocycles (PFHs) with the carrier protein, β-lactoglobulin (β-LG). Since β-LG is not affected by stomach digestion, the complexation between this protein and drugs results in safe delivery of the desired agents to the small intestine. In this study, Fluorescence, circular dichroism and UV-visible spectroscopic studies were applied to investigate the interaction of PFHs and β-LG. The results of this study indicate that these ligands mostly interact with β-LG through the hydrophobic interactions and the complexation was spontaneous. Moreover, the structure of β-LG was not disturbed significantly upon the interaction with these ligands. Additionally, β-LG and PFHs were proved to form static equimolar complexes. The significant binding of these potentially therapeutic compounds to β-LG proves that these compounds can successfully be delivered to small intestine for possible inhibition of α-Gls which in turn reduces the blood glucose levels in diabetic patients.

Key words: β-lactoglobulin (β-LG), Pyrimidine fused heterocycles (PFHs), Fluorescence, Circular dichroism

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Poster – P132

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Partial purification and characterization of an extra cellular α-amylase from *Bacillus licheniformis* BR1390

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ABSTRACT

In the present study, secretion and biochemical characterization of an alpha amylase from newly isolated bacterium, *Bacillus licheniformis* BR1390 was investigated. Strain BR1390 was cultured in a medium containing (g/L): yeast extract (5), peptone (5) and NaCl (5) along with 5% (v/v) tap water at 30 ºC (pH 7.0). Amylase secretion induction was performed by addition of 0.1% starch solution to 24 hours bacterial culture. Results showed that the maximum secretion of the enzyme is after 18 h incubation time. The extracellular α-amylase was purified by a combination of acetone 70% (v/v) precipitation and DEAE-Cellulose ion exchange chromatography. The partially purified enzyme was showed optimum activity at 70 ºC and pH 6.0, when used 0.5% starch as substrate. The enzyme was stable in broad pH ranges from 5-12 and it remains more than 75% of its initial activity after 60 min incubation at 85 ºC. Kinetic parameters of V_{max} and K_m were 0.42 µM/s and 1.02 mg/ml, respectively. Although amylase activity was enhanced to 105 and 107% in the presence of 5 mM Mg^{2+} and Ca^{2+} respectively, it was completely inhibited when incubated by 5 mM of Fe^{3+} ions. Incubation of the enzyme in the presence of 5 mM EDTA decreased only 27% of its initial activity. Taken together, the wide range pH and temperature stability and reasonable Ca^{2+} independency of the enzyme activity introduced the BR1390 amylase as a suitable candidate to be replaced some important industrial counterparts.

Key words: α-Amylase; *B. Licheniformis;* Starch industry; Thermostable

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Consideration the antioxidant activity of yeast protein hydrolysate in *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*

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ABSTRACT

The purpose of this study was to compare the antioxidant activity of yeast extracts obtained after sonication and autolysis process and investigation the effect of yeast protein enzymatic hydrolysis (using trypsin and chymotrypsin). The Saccharomyces cerevisiae (S.cerevisiae) and Kluyveromyces marxianus cells were used as protein sources. Peptides in each hydrolysate were fractionated with ultrafiltration membranes. The antioxidant activity was determined using a Trolox equivalent antioxidant capacity (TEAC) scale using DPPH and ABTS radicals scavenging assay. The 96 hrs Autolysis, in the presence of ethyl acetate, produced hydrolysates with the highest Degree of hydrolysis (DH) in two yeast strains (48.75% and 39.51% for S. cerevisiae and K. marxianus, respectively). Although there is no significant (PV>0.05) difference between antioxidant activity of sonication and autolysis products of K. marxianus, but autolysis process results in significantly (PV<0.05) lower DPPH radical scavenging activity in S.cerevisiae. The trypsin and chymotrypsin hydrolysis didn't cause significant differences (PV>0.05) in DPPH and ABTS scavenging activity in K.marxianus but significantly (PV<0.05) increased antioxidant activity in the S.cerevisiae yeast extract. Peptides fraction with molecular mass smaller than 3 kDa obtained from trypsin hydrolysis of S. cerevisiae showed the most antioxidant activity against DPPH and ABTS radicals, respectively equal to 7718.31 and 455.43 TEAC for 1 mg/ml protein solution.

Key words: *S. cerevisiae*; *K. marxianus*; Yeast extract; Antioxidant activity

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Production of recombinant protein MAP30 in *Nicotiana tobacum* hairy roots

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ABSTRACT

In recent decades, the resistance of bacteria to antibiotics has extremely increased, while modern medicine sometimes unable to control bacterial infections. The World Health Organization (WHO) has verified this problem as one of the three greatest dangers to public health. Recently, antimicrobial proteins as alternative antibiotics have considered as promising candidates for control of bacterial infections. Unlike antibiotics, the antimicrobial proteins not only do not increase the resistance of bacteria but also control them as well as antibiotics. MAP30, an attractive protein has been previously found to have the anti-HIV and antitumor activities. MAP30 is one of the most important type1 ribosome inactivating proteins (RIPs). RIPs hold broad interest because of their potential use as plant defense factors against pathogens and cancer cells. In this study, for the first time, *MAP30* was cloned and expressed in *Nicotiana tabacum* hairy roots and the effects of the recombinant protein on several important plant and animal pathogenic bacteria were investigated. The results showed that the total protein extracted from transgenic hairy roots has significantly antibacterial effect. Antibacterial effect of MAP30 was observed in dose-dependent manners at the different concentrations. The possibility to express functional proteins in hairy roots makes this plant material attractive for molecular farming. Proteins such as MAP30 have great clinical significance and may provide useful insights in designing and developing antibiotic, antiviral and antitumor agents with specific therapeutic targets toward infected or tumor cells with minimum cytotoxicity.

Key words: MAP30; Antimicrobial proteins; Hairy roots

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A comparative study of purification procedure of His-tagged tau protein (1N4R) using Ni-NTA agarose and SP- sepharose chromatography

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ABSTRACT

Tau is a major microtubule-associated protein in brain. The aggregation of the natively disordered protein, tau, to form lesions called neurofibrillary tangles is a characteristic feature of several neurodegenerative tauopathy such as Alzheimer's disease. Tau fibrils form *in vitro* from recombinant protein closely resemble those isolated from AD-diseased brain. In order to *in vitro* studying, the high purified tau is most critical for its polymerization to PHF (Paired Helical Filaments) using the poly anions such as heparin, poly-glutamic acid, RNA and fatty acids (arachidonic acid) as an inducing agent are common. As, it was suggested that tau as an DNA-binding protein has a higher affinity for DNA than for microtubules using purification method which omit all contaminants such as DNA, RNA and other proteins are critical to kinetic investigation of tau to form PHF. So, in current study, we compared two kinds of chromatography, Ni-NTA agarose column and SP-sepharose column (using FPLC) for purification of recombinant tau in parallel. Then, it was used SDS-PAGE electrophoresis in addition to western blot assay for detecting tau protein and other proteins, and for DNA and RNA detection, acrylamide electrophoresis using ethidium bromide and silver nitrate stains. Consequently, circular dichroism was carried out to check their secondary structures. Finally, after comparing the data, we found out using just one kind of chromatography is not sufficient for tau purification, so we suggest using both of them for preparing purified tau is essential.

Key words: Tau protein; DNA-binding protein; Purification; Cation exchange chromatography; Affinity chromatography

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Study on the proteolytic activity of lactic acid bacteria for decreasing cow's milk α_{s1}-casein allergenicity

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ABSTRACT

Milk provides the essential nutrients of newborn needs, but many children can't benefit from it because milk allergy is the most common food allergy in young children and ranking in third place of allergenic food based on some reports. Cow's milk proteins are the first source of antigens encountered in large quantities in infancy, and due to it affecting their growth, it is the most serious allergy in children with an incidence of about 2% to 7.5% in the population. Casein proteins, which consists of 4 different proteins (α_{s1}-, α_{s2}-, β-, and κ-casein), have been considered as the major allergens of milk proteins. Most patients are more than 90% sensitive to α_{s1}-casein. Previous studies have shown that the proteolytic systems of lactic acid bacteria are able to hydrolyze antigenic epitopes with the consequent decrease of milk allergenicity. In this study, Lactic acid bacteria were isolated from Iranian traditional dairy products and consequently the bacteria with the most proteolytic activity on α_{s1}-Casein were selected based on the SDS-PAGE and RP-HPLC results. Then the immunoglobulin E (IgE) binding to native and hydrolysated α_{s1}-casein (in the presence of isolated bacteria) was assayed by competitive ELISA test using sera from cow's milk allergy patients. Results of this study demonstrated that the hydrolysated α_{s1}-Casein was less recognized than native one by IgE from cow's milk allergy patient's sera which indicating reduces in the allergenicity properties of this protein. These bacteria could be used as probiotic material for developing hypoallergenic dairy products.

Key words: Casein; Cow's milk allergy; Lactic acid bacteria; Proteolytic activity

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Sustained release of chondroitinase ABC from enzymatically crosslinked tragacanth biopolymer

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ABSTRACT

Following damage to spinal cord the chondroitin sulfate proteoglycans (CSPGs) are up-regulated and inhibit axonal regeneration. One of the potential therapeutics for spinal cord injury is chondroitinase ABC (chABC) that can degrade CSPGs and promote regrowth and repair. However, sustained delivery of bioactive chABC to spinal cord is difficult because rapid clearance of drugs from the injury. Furthermore, chABC is thermal sensitive and short halflife at 37°C. Hydrogels are good candidates for sustained protein delivery and generally regarded as biocompatible materials as well as their structure and water content make them suitable to load active proteins and peptides. Recently, insitu forming gels, injectable hydrogels have started to dominate in biomedical applications over traditional preformed hydrogels as they can be inserted into the target region by straight injection with minimal invasive damage. Enzymatic crosslinking is viewed as a very convenient method for fabrication of insitu forming chemically crosslinked hydrogels that can be used in biological systems. In this work a phenolic hydroxyl group (Tyramin) was incorporated into tragacanth (Tg), using aqueous-phase carbodiimide activation chemistry, to obtain insitu gellable and injectable biopolymer for drug delivery. By this means, Tg derivatives that were gellable via a peroxidase-catalyzed reaction were obtained. The gelation time decreased by increasing content of the phenolic (Ph) groups, peroxidase concentration and decreasing H_2O_2 concentration. ChABC was expressed and purified by using a nickel affinity column and SEC. Experimental results indicate that ChABC activity can be preserved during release by encapsulating chABC in Tg hydrogels.

Key words: Spinal cord injury; Chondroitinase ABC; Controlled release; Tragacanth; Enzyme

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The comparison of the interaction between α-casein and β-casein with ACE peptide by spectroscopy and molecular modeling techniques

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ABSTRACT

In this study we report interaction between milk alpha and beta casein with (ACE) peptide by fluorescence spectroscopy method. Caseins are the major phosphoproteins of mammalian milk and exist as micelles made of polypeptides known as α-, β and κ-caseins. Another unique feature of caseins is the large amount of propyle residues, specially in β-casein, which greatly effect the structure of caseins because the proline residues disrupt the information of α-helical and β-sheet. Angiotensin converting enzyme (ACE) is an important enzyme in rennin-angiotensin system which increase blood pressure by catalyzing the conversion of the inactive decaptide angiotensin-I to the strong vasoconstrictor angiotensin-II. Addition of ACE peptide caused a dramatic change in the emission spectra of α and β casein. The fluorescence intensity decreased gradually with the increase in concentration of ACE peptide, indeacating the formation of the drug-protein complex. The synchronous spectra provide information about the molecular environment in the vicinity of the choromosphere molecule [3]. This is carried out by measuring the shift in the emission maximum, λmax, which reflects the corresponding changes of the polarity around the chromophore molecule. The effect of ACE peptide on the β casein showed that emission maximum at Δλmax of β-casein produced a blue shift with ACE peptide and a red shift with α-casein-peptide complex in the experimental concentration range and with Δλ=60nm. The blue shift effect suggested that the polarity around the tryptophan residues decreased and the red shift effect demonstrates that the polarity around the tryptophan residues increased.

Key words: Spectroscopy; Casein; ACE peptide

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Effect of peptide functionalized gold nanorod on cellular response

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ABSTRACT

Gold nanorods (GNRs) are of interest in many biological applications, including photothermal therapy and gene/drug delivery. They are absorbing at near-infrared (NIR) frequencies strongly and can be employed as multifunctional agents for biological imaging and theragnostics. GNRs stabilize with cationic agent cetyltrimethylammonium bromide (CTAB). GNR-CTAB not only have poor stability in biological solutions but also exhibit significant cytotoxicity. Several surface coatings have been proposed in order to reduce the apparent toxic effect of the GNRs such as by "cloaking" the CTAB-GNRs with polyelectrolytes, phospholipids or exchanging the CTAB with another molecule such as thiolated PEG. These different GNR ligands can affect cell uptake differently either increasing or hindering it. In this study, we synthesized CTAB-GNRs in special size and then functionalized CTAB-GNRs via a ligand exchange method, using a positive charge cysteine terminated peptide and investigated their stability in biological media and subsequent toxicological effects to Hella cells with MTT assay. It was found that peptide functionalized GNRs could have a proper cell uptake and less cytotoxicity compared to CTAB-GNRs. This kind of functionalization is suitable for gene delivery.

Key words: Peptide; Gold nanorods (Gnrs) Functionalization; Cytotoxicity

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Identification of recombinant matrix metalloproteinase-9 with diagnostic purpose by using SPR technique

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ABSTRACT

Matrix metalloproteinase (MMP) comprises a family of zinc-dependent endopeptidases that degrade various components of the extracellular matrix. MMP-9 has been associated with malignant tumor progression and metastasis by degradation of the matrix and to facilitate tumor invasion into other tissues. Human gelatinase (MMP-9) is a member of the matrix metalloproteinases family (MMPs). Measurement of MMP-9 in human plasma or serum has been reported to be useful for early detection of cancer dissemination. Identification and evaluation of MMP-9 is important for understanding the mechanism of basement membrane remodeling and aberrant collagen catabolism in pathological conditions such as periodontitis, atherosclerosis, cancer, and rheumatoid arthritis. Therefore, using MMP-9 as a biomarker could be useful for early detection of mentioned diseases. Surface plasmon resonance (SPR) provides excellent technique for a label-free and real-time monitoring of biomolecular interactions such as antigen-antibody. In this study the expression vector pET28a containing the cDNA fragment encoding the active form of MMP-9 was transformed into *E.coli* BL21. The recombinant protein was expressed in the form of inclusion bodies and extracted as a 50 kDa species. The inclusion bodies were solubilized in a buffer containing 8 M urea and loaded on a Ni-NTA affinity column. The recombinant protein was refolded in the presence of Ca^{2+} and Zn^{2+}. Subsequently, anti-MMP-9 was immobilized via amine coupling through EDC/NHS on CMD50 D and different concentrations of MMP-9 were detected.

Key words: Matrix metalloproteinases; MMP-9; Biomarker; Surface plasmon resonance (SPR); Amine coupling

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The investigation of localization of 2-hydroperoxy coelenterazine in cetenophore photoproteins: a molecular dynamics simulation

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ABSTRACT

Berovin is a Ca^{2+}- binding photoprotein from Beroe abyssicola that belongs to the Ctenophores group. A comparison of the two most famous groups of calcium-regulated photoproteins, Coelentrates and Ctenophores, showed high degree of structural similarity regardless of their low sequence identity. Up to now, the crystal structures of several photoproteins such as aequorin, obelin have been solved. Based on these crystal structures, a mechanism have been proposed to explain how Ca^{2+}binding might trigger the bioluminescence reaction in Coelentrates group. The side chains of His175 and Tyr190 along with the Trp179 in obelin are in close vicinity to the peroxy group of 2-hydroperoxy coelenterazine implying strong hydrogen bonds between them. It was suggested that the His–Trp–Tyr triad participates in stabilization of the 2-hydroperoxy derivative of coelenterazine and might be involved in some steps of the bioluminescence reaction. Despite the precise investigation of Ctenophore binding site, sequence alignment between Coelenterates and Ctenophores group and mutagenesis studies on berovin and mnemiopsin, we could not obtain its catalytic triad. This result refers to this point that another light emission mechanism can be existed in Ctenophores group. In this study, the localization of coelentrazine in photoprotein of berovin was investigate by docking study. We optimized the full structure of berovin resulting from docking simulation by molecular dynamics simulation. At the optimized conformation, the interactions between amino acids of cavity and the ligand were examined. The results showed that the coelentrazine was located in protein structure with different orientation compared with other Coelentrates photoproteins.

Key words: Photoprotein; Light emission mechanism; Docking study; Molecular dynamics simulation

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New inhibitor based on 5-alpha reductase modeling

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ABSTRACT

Malfunction of 5-alpha reductase enzyme, which converts testosterone to dihydrotestosterone, can lead to disorders like male pattern hair loss (MPHL) in men. Due to the lack of three-dimensional structure of this enzyme and being membranous, it is difficult to design new inhibitors. It can build an efficient model of the enzyme to vindicate the aim of designing new inhibitors. By produced FASTA format of both isoenzymes sequence, single-model and multiple-template homology modeling were done by Modeller 10.1. The best models in terms of energy were selected and evaluated by verify3D and Procheck programs. By GROMACS program 30 nanosecond molecular dynamics simulations were performed on the models. Kinetic and potential energy changes are negligible in the 15 nanosecond; therefore, it was revealed that the system had reached thermal equilibrium. By Autodock 4.2 Both optimized models docked to some of their specific inhibitors. By replacing a -COOH group in some of these inhibitors, the new inhibitor was built. Due to sequence similarity less than 33%, homology modeling weren't optimal. Only 1.4% and 0.4% Residues respectively in model I and II isoenzymes were in disallowable Ramachandan plot. By simulating, most of errors in the model were fixed. all in all the overall quality of the models is improved. In some case, docking new inhibitors significantly had higher bond strength and energies were more negative.

Key words: Homology modeling; Molecular dynamics simulation; Docking; 5-alpha reductase new inhibitor

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Hsp104 domains affect the loss of the yeast [*PSI*$^+$] prion distinctively

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ABSTRACT

Prion is usually associated with neurodegenerative diseases; however the budding yeast can harbour prion without any damage on cellular health. Considering this and the fact that yeast has proved a useful model system to study human diseases, it can be used to determine the factors affecting prion propagation and elimination. Prion is an unusual conformer of an otherwise physiologic protein. The prion conformer is prone to form oligomers which can act as seeds to promote conversion of the physiological conformer to the prion leading to the formation of large β-amyloid aggregates. In yeast [*PSI*$^+$] is the prion conformer of Sup35p; a translation termination factor. Propagation of [*PSI*$^+$] requires physiological levels of Hsp104 which is a protein disaggregase typically involved in dismantling protein aggregates generated post-stress conditions. It is now believed that Hsp104 at physiological levels acts on prion aggregates and generates shorter oligomers which can act as seeds which are essential for perpetuation of the prion phenotype. This severing activity of Hsp104 is similar to its function posed on protein aggregates formed post-stress conditions. Hsp104 overexpression on the hand leads to the loss of [*PSI*$^+$], however various observations suggest that this is most likely not through the higher severing activity of Hsp104. To understand the mechanism by which excess Hsp104 eliminates [*PSI*$^+$], the significance of its domains in this process was studied. Our findings support the hypothesis that Hsp104 overexpression leads to a non-functional interaction between Hsp104 and the prion aggregates and thereby a defect in the generation of seeds.

Key words: Yeast; β-amyloid; Prion; [*PSI*$^+$]; Molecular chaperone; Hsp104

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Studying the side effects of deferasirox on the structure and function of liver catalase

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ABSTRACT

Transfusional iron overload is a major cause of mortality in thalassemia, sickle-cell disease and other chronic anemias. To chelate iron overload, orally bio available iron chelator, deferasirox, is used for the treatment of patients suffering from thalassemia. Using this drug leads to some complications such as fancony syndrome and acute kidney insufficiency. In this article we investigate the effect of drug on the activity and structure of liver catalase enzyme using UV-Visible and fluorescence spectroscopic methods at physiological condition. UV-Visible results have shown that increasing the drug concentrations leads to significant decreasing in the enzyme activity and consequently inhibition of catalase enzymatic reaction. Also intrinsic emission of enzyme decreased in the presence of drug and significant changes have been done at three dimensional environment around the chromophores of enzyme. The liver catalase enzyme has two binding sites for the drug deferasirox. Results indicate that deferasirox binds to enzyme and chelates the active site iron. Therefore it causes some side effects in liver by inhibiting catalase enzyme.

Key words: Deferasirox; Catalase; Structural studies; Enzyme function

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Analysis of genes and proteins of HCV basis on rare codon clusters

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ABSTRACT

Coding nucleotide sequences carry an integral message containing several different types of information for the various molecular mechanisms. Recent studies also suggest beyond the amino-acid sequence lies an additional layer of information, hidden within the codon sequence, able to mediate local kinetics of translation. Here, we analyzed and reported rare codon clusters (RCC) in HCV genome and then identified location of these rare codon clusters in the structure of HCV protein. By use of Sherlocc program, we identified the rare codon cluster in three regions of HCV genome; NS5A, NS2 and NS3 coding sequence of HCV genome. We also studied location of these rare codon clustersin structure of NS2, NS3 and NS5A proteins. We identified some of critical residues near or within rare codon clusters. As we know these residues are critical in enzymes and proper position of these residues should adjusted accurately.These data show that rare codon cluster may play a critical role in proper folding and action of protease activity. These data indicates that in HCV life cycle, rare codon clusters play an important role that must investigated. However, the other rare codons clusters may exist that not identified by Sherlocc program and require further studying. Due to the most frequency of rare codon clusters in NS3, it appears that these clusters may play more significant role than other HCV proteins. This information is helpful in development of new avenues for vaccine and treatment protocols.

Key words: HCV; RCC; *Sherlocc* program

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Toxicological and biological aspects of transition metal complexes

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ABSTRACT

Binding studies of small molecules with proteins are important in the design of new and more efficient drugs. Serum albumins; the most abundant proteins in the circulatory system; act as transporter and disposer of many endogenous and exogenous compounds. The interaction of bovine and human serum albumin (BSA and HSA) with Lanthanide Schiff base complexes was investigated by spectroscopic techniques like absorption and fluorescence spectroscopy. Absorption spectroscopy proved adducts formation between protein and the complex. Distance between the fluorophore in the protein and the complex was evaluated. Binding constant and number of binding sites were determined for complex-protein adduct in phosphate buffer (pH 7.40). The values of the thermodynamic parameters suggested that the mode of interaction was hydrophobic. The mentioned complexes were also screened for their cytotoxic activities as Jurkat leukemia cell line was the target. According to IC_{50} values, it can be concluded that these complexes intrinsically possesses low toxicity.

Key words: Lanthanide Schiff base complexes; Human serum albumin; Bovine serum albumin; Toxicity

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Cloning, expression and purification of cholesterol ester hydrolase from Streptomyces

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ABSTRACT

Cholesterol esterase (CHE; EC 3.1.1.13) is a serine protease that belongs to the family of hydrolase. CHE plays an important role in the absorption and metabolism of cholesterol in many organisms. The genes encoding CHE from micro-organisms such as Pseudornonas fluorescens and Streptomyces lavendulae is useful for a diagnostic measurement of total cholesterol that result in different problems such as cardiovascular disease and atherosclerosis . In this study, Genomic DNA was extracted from Streptomyces sp. After the detection of the Streptomyces bacterial species by PCR amplification of 16S rDNA gene, genomic DNA as a template for producing the CHE enzyme by PCR reaction was used. PCR products and PET28a plasmid with BamHI and SalI restriction enzymes were digested. The recombinant plasmids were generated by T4-DNA ligase and then transferred to competent proliferative DH5α strain. After sequencing to study the protein expression, CHE-pET was transformed to *E.coli* strain *BL21 (DE3)* and was expressed at different conditions of temperature, time and inducer concentration. Expression analysis determined by SDS-PAGE.. The results showed that the major protein was expressed as inclusion body and accumulate in sediment bacteria. The reason for the separation of proteins in solution of different concentrations of urea were used and purified by Ni-sepharose affinity chromatography.

Key words: Cloning; Expression; Purification; Cholesterol esterase; Streptomyces

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Structural study of CD & FG helices of bacteriorhodopsin under electrical field by molecular dynamics simulation

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ABSTRACT

Recent advances in the field of Molecular dynamics (MD) can help us look into these systems at the molecular level. However, molecular dynamics simulation (MD) is the most systemic approach to study the molecules tentatively. MD simulation is widely used to study the structural properties of proteins, pure bilayers, composite bilayers, lipid rafts, cellular processes such as signaling and transport. With MD, we aimed to simplify access to dynamic information extrapolated from structures and provide molecular modeling tool that can be used productively by a widegroup of biomedical researchers, including particularly experimentalists. In the present project, the molecular dynamics program NAMD together with its sister molecular graphics programVMD were used in order to mine structure information about Bacteriorhodopsin (bR) helices. Bacteriorhodopsin (bR) is a purple membrane protein that acts as a light-driven proton pump in *Halobacterium salinarum.* This protein contains seven transmembrane α-helical subunits, helices A–G. Experimental studies show that CD & FG helices of bR are more important in absorbing electrical energy so electric field was applied in the MD simulation in order to study the effect of electric field on these helices. Based on the obtained results, it is considered that helices oriented without being denatured at field strengths between 0.5 V/nm and 1.5 V/nm but start to be denature when exposed to too strong electrical fields, above 1.5 V/nm so it is concluded that at strong values of electrical field can unpack the bR protein completely.

Key words: Bacteriorhodopsin; Electrical fields; Molecular dynamics; NAMD; VMD

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Prediction of protein flexibility based on information theory

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ABSTRACT

Proteins as flexible macromolecular systems can alter their structure from special folded conformation to another for doing a variety of functional biological activates such as catalysis, macromolecular recognition, single transduction, macromolecular assembly, allosteric regulation and thermal enzyme adaptation. Thus, structural flexibility is an essential attribute of protein because of the link between structure and function. In addition, knowledge about flexibility of protein may provide insights into the protein folding process and improve prediction of 3D structure and even is helpful in drug design. In this research, we utilize information theory approach to predict flexibility of proteins from its sequence according to B-factors of protein crystal structure reflect the fluctuation of atoms about their average position. Prediction is done by applying self-information and pair information for a window seventeen. Results demonstrate that information obtained by pairwise amino acid values is better than single amino acid. This improvement indicates that in folded proteins; amino acids flexibility not only depends on physicochemical characteristics of the single amino acid, but also affects adjacent amino acids characteristics.

Key words: Protein Flexibility; Protein Structure Prediction; Information Theory

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Inhibition study on lysozyme fibrillation by safranal and crocin small molecules

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ABSTRACT

Recent investigations show that fibrillation products and intermediates bring neurotoxicity. Inhibition of protein aggregation and amyloid fibril formation are viewed as one possible method to prevent the progression of these devastating disorders. This toxicity is predominantly associated to their intermediate oligomeric state. This has been demonstrated to be induced by enhancing membrane permeability and perturbation, eventually leading to cell death. In the present report, we study interaction of the two small molecules (safranal and crocin) with hen egg white lysozyme (HEWL) for inhibiting the fibril formation with different kind of methods such as fluorescence, dynamic light scattering, transmission electron microscopy and circular dichroism. The aim of this report was based on gaining insight into possible mechanism of interaction of small molecules with amyloied formation products. These studies demonstrated that safranal and crocin considerably hindered nucleation, and therefore, fibrillation of lysozyme in a dose-dependent manner.

Key words: Hen egg white lysozyme; Safranal; Crocin; Amyloid fibril

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Cloning, optimization and catalytic activity evaluation of recombinant lysostaphin as a new anti-staphylococcal enzybiotic in *Escherichia Coli*

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ABSTRACT

The emergence of antibiotic resistance in medically significant pathogens has promoted the scientific efforts to synthesize or find a novel therapeutic. Lysostaphin a zinc metalloprotease with the molecular weight of 27 kDa and specific lytic activity against *Staphylococcus aureus* degrades the *S. aureus* by hydrolyzing the pentaglycine crosslinks present in its cell wall. Due to such potential, lysostaphin will be a therapeutic for the treatment of antibiotic-resistant staphylococcal infections. The mature lysostaphin gene was cloned and expressed in *E.coli* with the carboxyl terminal hexa-histidine fusion tag under the transcriptional control of T7/lac promoter/operator. The expression of desired protein was investigated by IPTG induction at RNA and protein levels by RT-PCR and SDS-PAGE. Immunoblotting with anti His-tag antibody confirmed the identity of expressed recombinant protein. Ultimately, the lysis activity of recombinant lysostaphin was evaluated by disk diffusion. The transformed *E. coli* BL21 (DE3) cells produced catalytically active lysostaphin and the expressed recombinant protein was positively and effectively functional against *S. aureus* MRSA. This study shows that the *E. coli* expression system is suitable for overexpression of recombinant lysostaphin and the expressed protein can be considered as a highly effective therapeutic agent.

Key words: Enzybiotics; Genetic engineering; Recombinant lysostaphin; Therapeutics

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Computational molecular dynamics simulation of functionalized fullerene [C60] derivatives as HIV protease inhibitors

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ABSTRACT

Although the excessive advances achieved in the AIDS therapy, the rapid mutation of HIV that results in resistance toward the AIDS therapies and also the high toxicant of currently used drugs strongly support the discovery of new drugs. HIV protease is a fundamental enzyme for the virus survival. The HIV protease active site is a semi-opened hydrophobic ellipsoid. The diameter of this cavity is around 10 A° close to the diameter of the fullerene [C60] sphere. Already it was performed and found out that HIV protease could be complexed and inhibited by the introduction of a C60 molecule into the catalytic cavity. Herein, a new functionalized fullerene C60 derivatives bearing solubilizing chains have been evaluated for their activity against HIV via Gromacs software. We perform geometry optimization for C60 and its derivatives using DFT quantum mechanical method. The complexed structures of C60/HIV protease and two C60 derivatives/HIV protease have been simulated using molecular mechanics approach. The C60 derivatives showed to fit vigorously in the active site of the viral protease with strong interactions on the enzyme hollow surface. The proper position of substituents able to bring about electrostatic interactions with the active site's charged residues, could raise the C60 derivatives binding.

Key words: Gromacs; MD; HIV; C60; Fullerene

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Developmental exposure to organophosphates alters the expression of nitric oxide synthase in the rat supraoptic and paraventricular nuclei

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ABSTRACT

Nitric oxide synthase (NOS) is highly expressed in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus. These nuclei are involved in the neuroendocrine and autonomic functions and nitric oxide critically contributes to the regulation of these functions. Organophosphate (OP) insecticides are conventional acetylcholinesterase inhibitors but can induce several long-lasting neurochemical, endocrine and cardiovascular alterations through mechanisms unrelated to their primary action. We studied the late-arising effects of developmental exposure to low dose of organophosphate chlorpyrifos (CPF) and diazinon (DZN) on the expression of NOS in the SON and PVN. A daily dose of 1mg/kg of either CPF or DZN was administered by subcutaneous injection to developing rats during gestational days 15–18 or postnatal days (PND) 1–4. Brain sections from PND 60 rats were processed using NADPH-diaphorase and neuronal NOS immunohistochemistry and the optical density (OD) were assessed in the SON and PVN. Prenatal exposure to CPF and DZN significantly increased the OD only in the SON. On the other hand, neonatal exposure to CPF and DZN increased OD in both subnuclei, supporting enhanced expression of NOS. These findings suggest that overexpression of NOS in the SON and PVN may contribute to the mechanisms inducing or compensating for endocrine and autonomic abnormalities after developmental exposure to OPs.

Key words: Nitric oxide synthase; Rat; Organophosphate; Supraoptic nucleus; Paraventricular nucleus

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Assessment of the interaction between human serum albumin and curcumin by fluorescence method

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ABSTRACT

Human serum albumin (HSA) plays a significant role in the transport and disposition of endogenous and exogenous ligands, presenting in the blood stream. The operative quenching mechanism of HSA by curcumin can be explained based on the dependence of the fluorescence intensity as a function of the quencher concentration. When the excitation wavelength is 295 nm, only Trp residue has a fluorescence emission whereas at the excitation wavelength of 280 nm, both Trp and Tyr residues demonstrate fluorescence emission. Comparison of the fluorescence quenching of protein which excited at 280 nm and 295 nm allows estimating the participation of Trp and Tyr residues in the complex. The quenching take place when the ligand (quencher) is sufficiently close to the either Trp or Tyr residues. Then the energy transfer between a ligand and fluorophore is possible. The fluorescence quenching spectra of human serum albumin were obtained as the protein sample excited at 280 and 295 nm in the presence of curcumin. The addition of varying concentrations of curcumin caused a noticeable decrease in the fluorescence intensity of HSA. Also, the maximum emission wavelength produced a blue shift. The strong quenching of the Trp 214 indicated that HSA conformation can be changed and an intermolecular energy transfer can be occurred between curcumin and this protein.

Key words: Spectroscopy; Curcumin; Human serum albumin (HSA)

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Lysozyme fibrillation inhibition by phloridzin small molecules

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ABSTRACT

Amyloidogenic proteins undergo an alternative folding pathway under stressful conditions leading to formation of fibril products, presenting cross β-sheet structure which is the hallmark of many neurodegenerative diseases. The current work demonstrates the effectiveness of phloridzin small molecule on the inhibition of hen egg white lysozyme (HEWL) amyloid formation. The inhibitory effects were analyzed by thioflavin T-induced fluorescence, circular dichroism, and atomic force microscopy. This report demonstrated that naturally occurring small molecules may serve a function that is typically done by protein chaperones, and it provides a hint for designing inhibitors against amyloid formation products associated with neurodegenerative diseases. This report showed that phloridzin considerably hindered nucleation, and therefore, fibrillation of lysozyme in a dose-dependent manner.

Key words: Hen egg white lysozyme; Amyloid; Fibril

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Fusion proteins in pharmaceutical sciences: today and tomorrow

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ABSTRACT

Proteins play significant roles in our lives. Any even very negligible change in one or more proteins can somehow lead to a very life-threatening result. Because of this great range of functions and effects in our bodies, protein is one of the most interesting fields of research.In the recent years, genetic engineering developments by joining two or more proteins together, regardless of their origin, aled to a novel field of protein studies, called Fusion Proteins. Chimeric monoclonal antibodies and polyvalent vaccines have been the fruits of this technology. Etanercept is the first commercialized Fc-fusion protein approved by the Food and Drug Administration (FDA) for rheumatoid arthritis therapy. Many metabolic and hereditary diseases unresponsive to usual drugs can be cured by fusion proteins. These engineered polypeptides are successful in lessening cost and procedure time in medical diagnostic kits, especiallywhere detection of more than one antigen is needed. Despite these benefits and a good outlook, many issues still make serious barriers for these products to be entered intothe market. In this article s pharmaceutical fusion proteins, their production challenges and promising future will be discussed.

Key words: Fusion Proteins; Pharmaceutical; Production; Application; Barriers

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Investigation on the interaction between an antioxidant peptide derived from ostrich egg with human serum albumin by circular dichorism spectroscopy

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ABSTRACT

The aim of present study was to investigate the interaction between an antioxidant peptide derived from ostrich egg with human serum albumin (HSA). The effect of antioxidant peptide on HSA was analyzed using circular dichorism (CD) spectroscopy, According to CD results the formation of the antioxidant peptide- serum albumin complex caused changes of the secondary structure of HSA. In the presence of antioxidant peptide, alpha helix content of HSA was reduced.

Key words: Human serum albumin; Antioxidant peptide; Interaction; Circular dichorim

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Study on the interaction between an antioxidant peptide derived from ostrich egg with human transferrin by circular dichorism spectroscopy

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ABSTRACT

This paper describes the interaction between an antioxidant peptide derived from ostrich egg with human transferrin (hTF). The effect of antioxidant peptide on conformation of hTF was analyzed using circular dichorism (CD) Spectroscopy. Circular dichroism data revealed that the presence of antioxidant peptide caused a decrease of the α-helical content of hTF, and induced a remarkable mild denaturation of hTF.

Key words: Human transferrin (hTF); Antioxidant peptide; Interaction; Circular dichorim

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Co-expression of human endostatin with artemin as chaperone

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ABSTRACT

Inhibition of angiogenesis is one of the most attractive strategies applied for cancer therapy. Endostatin, the C-terminal globular domain of collagen XVIII, is an endogenous inhibitor of angiogenesis and numerously investigated as an anti-cancer therapeutic agent. Recombinant endostatin tend strongly to form the inclusion body and therefore eligible refolding strategy is a limitation for basic and applied purposes. In the present study, the coexpression of human endostatin gene with a chaperone protein Artemin derived from *Artemia urmiana* is investigated in *E. coli*. The codons was optimized to optimize the expression of eukaryotic protein in *E.coli*. The synthesized cDNA encoding endostatin's and artemin, separately cloned into pET28a+, was cotransfomed to *E. coli*, and the expressed was optimized. Purification of soluble protein performed using Ni-NTA nickel agarose affinity chromatography and pufrification of proteins analysed by SDS-PAGE gel electrophoresis.

Key words: Endostatin; Artemin; Protein expression; Co-transformation

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Development of non rejectable allogeneic skin grafts by induction of local immune tolerance

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ABSTRACT

Autotransplantation of skin is a common method of managing soft-tissue injuries, such as extensive traumas, infections or oncologic defects. Despite the effectiveness of skin autotransplantation, the high degree of immunogenicity of skin precludes the use of allogeneic skin grafts. Systemic immunosuppression is generally felt to be inappropriate for isolated skin grafts. This study examines the potential to create an allogeneic skin transplant that avoids rejection by inducing localized immonosupression. Specifically, IDO (indoleamine 2 3-dioxygenase)-expressing fibroblasts are introduced into the dermis of donor subjects to provide a tryptophan depleted local environment in the recipient. High level of IDO expression as an immunesuppressive enzyme within the graft might lead to development of an immune privileged area and therefore local immune tolerance toward the graft. Regular (control) and IDO-fibroblast were injected intra dermally in allogeneic recipients (n=5/group). Cells were injected at different time point (days 0, 5, 10) to the same area and a 6mm graft was then harvested from that region and transplanted to the allogeneic subjects. Preliminary results from skin transplantation studies demonstrate that IDO expressing grafts remain viable for significantly longer than control allogeneic grafts (p=0.01). These data suggest that local immunosuppression can be provided by the delivery of IDO expressing fibroblasts in allogeneic skin transplantation. The potential of this research goes far beyond the promising role for skin transplantation. This "cell-based" approach to localized immunosupression not only provides potential opportunities to skin transplantation but also to autoimmune skin disorders such as Alopecia Areata.

Key words: Transplantation, Indoleamine 2 3-dioxygenase, Skin grafting, Immune tolerance.

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Study and identification of cutinase-producing bacterial strains

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ABSTRACT

Cutinase is a carboxylic ester hydrolase that degrades cutin polymers. This enzyme has many applications in biotechnology, medical and industrial sciences including environmental pollution elimination, plastic decomposition and important chemical activities such as transesterification and esterification. For these reasons, identification of new cutinase from native strain is of highly importance. In this study we aimed to screen the bacteria and fungi producing cutinase. For this reason, the sampling of peel and garden soil, leaf surface or fruit suspected, mass of bacteria, and vermicompost collected from Kerman province and cultured in specific medium supplemented by cutin. Screening of samples on solid medium done after samples primary growth. In the next step, the strains screened and cutinase activity assayed with specific substrate paranitrophenol butirate at 405 nm. Results showed that six screened fungi and bacteria strains have a very good activity and were able to produce cutinase. At the moment, identification of these strains is in progress. In the next step, the process of cloning of the enzyme gene will be performed. According to the importance of cutinase in biotechnology and industrial science, the finding of this study has key role in identification of novel cutinase enzyme and introduction of this enzyme to medical and industrial sciences.

Key words: Screening; Cutinase; Cutinase assay

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Modelling of new neurotrophic factor protein by computational techniques and calculating interactin of protein-protein

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ABSTRACT

Neurotrophic factor is a member of the neurotrophin family of growth factors. The neurotrophic factor proteins is present in high concentration in cerebral cortex.This protein have 247 amino acids .The mature neurotrophic factor is secreted to extracellular and bind to TrkB. Interaction to TrkB can lead to a variety of intracellular signaling cascades such as Ras-MAP kinase cascade. These cascades help to support the survival of existing neurons.computers techniques or bioinformatic techniques were applied to investigate the interaction of mutate neurotrophic factor to TrkB compairing to Native neurotrophic factor. Amino acids of neurotrophic factor that were involved in interaction were identified .one of the amino acids was Gln84. This amino acid was mutated to Lys84 amino acid,by swiss pdb viewer software. This software produced pdb file format and the pdb file uploaded to 3Drefine server. This server consistent protein structure refinement by optimizing hydrogen bonding network and atomic-level energy minimization and modelled 5 structure. RAMPAGE server and Qmean server was used to select best structure. Finally binding energy was calculated by Hex.8.0.0 software. Etotale of. Native neurotrophic factor is -675 and mutate BDNF is -678. The results showed the mutant structure interaction with TrkB is better than native protein. Neurotrophic factor shows pharmaceutical properties and modelling of new proteins and produce them can help in the development of the drug industry and mutant forms a good choice for treatment of Diseases of the nervous system.

Key words: Neurotrophic factor; Computers techniques; Modelling of protein

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Cloning of neurotrophic factor protein in prokariotic system and expression of this protein to treatment of metabolic neurological diseases

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ABSTRACT

Nerve growth factor (NGF) was discovered in the early 1950. Neurotrophic factor is a member of the neurotrophin family of growth factors. Prohormone convertases cleave the pro neurotrophic factor (M.W. ~30kDa) to the mature neurotrophic factor (M.W. ~14kDa). The neurotrophic factor protein is present in high concentration in cerebral cortex of brian.The mature neurotrophic factor protein bind to tropomyosin-related kinase B receptor. Interaction of neurotrophic factor protein to this receptor can leads to a variety of intracellular signaling cascades such as Ras-MAP kinase cascade and phosphorylation of cyclic AMP-response element binding protein (CREB). These cascades help to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses. So neurotrophic factor is a powerful therapeutic protein for the treatment of various metabolic neurological diseases. In this research cloned the functional neurotrophic factor coding sequence in a prokariottic vector to produce neurotrophic factor recombinant protein in E.coli (BL21).this fragment, was synthesised into PUC57 plasmid.This plasmid transformed in E.coli (DH5) for amplifying purposes.Then extracted plasmids were treated by restriction endonucleases. The fragments were inserted into the pET26b vector.At the next step, the recombinant vector was transformed into Ecoli (TOP10) cells for amplifying purposes.The recombinant plasmid was extracted and transformed into Ecoli (BL21) cells for expression of the protein. The expression of the recombinant protein was confirmed by SDS-PAGE technique.

Key words: Neurotrophic factor; Computers techniques; Modelling of protein

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The National Conference on Protein & Peptide Sciences: From Basic to Medical & Industrial Applications

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Analysis of host cell proteins, the uninvited guests

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ABSTRACT

Since 1990s, biopharmaceuticals have held great shares of the pharmaceutical market. Among these products, recombinant proteins are of the most important. One of the dialectic concerns about mentioned proteins is the presence of host cell proteins (HCPs) contaminating the final product causing immunogenicity in patients. HCPs are proteins except for the intended one, expressed in the eukaryotic or prokaryotic host cells. Although all biopharmaceuticals pass several purification processes, there are always some residues left. Hence there must be methods to analyze these marginal contaminations. In spite of being neonates in pharmaceutical sciences, the foresaid methods for recombinant proteins are required by pharmacopoeia. The most regulatory organizations such as FDA and EMEA, ask drug-developing companies to present robust assay procedures to quantify HCPs before entering the clinical trials and as an obligatory test for each batch qualification assessment. For frequently used cell lines including CHO, *E. coli*, etc. ELISA kits have been prepared by various companies to assay HCPs, but for many other cell lines there are no commercial reagents. In the current review we are going to summarize both commercial and in-house non-commercial methods for evaluating HCPs in different cell cultures in order to give an overview to researchers seeking for an appropriate method of analysis.

Key words: Host cell protein; HCP; Assay; Analysis; Methods

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Spectroscopic evidence for the formation of insulin-cationic gemini surfactant complex

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ABSTRACT

Gemini, or dimeric surfactants are amphiphilic molecules composed of two hydrocarbon tails and two polar head groups joined together by a spacer. They represent unique properties as compared with single chain conventional surfactants, such as lower CMC (Critical Micelle Concentration) values, better wetting properties and more effectiveness in lowering the surface tension of water. In this research, a novel cationic Gemini surfactant is used to prepare nanoparticles as drug delivery vehicles. The interaction of human insulin, as a model peptide drug, with the surfactant is studied by several methods, such as fluorescence and UV-Vis spectroscopy. The fluorescence of insulin at different surfactant concentrations was recorded. Results show a gradual decrease in the fluorescence intensity of insulin, while there is no shift in the maximum emission wavelength (λ_{max}=303 nm). The Stern–volmer plot exhibits a downward curvature indicating both the dynamic and static quenching of flours. Furthermore, UV-Vis. spectroscopy of insulin titrated by surfactant solution show a new absorption peak for the mixture which is quite different from the protein and the surfactant solutions. Altogether, these observations confirm the formation of complex between protein and the surfactant.

Key words: Gemini surfactant; Insulin; UV-visible spectroscopy; Fluorescence spectroscopy

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Fibroblast growth factor receptor 2b protein structural changes due to the interaction with naringin

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ABSTRACT

FGFR2b plays a significant role in cell signaling pathway. Genetic alterations of the tyrosine kinase domain of FGFR2b, point mutations for example, occur in many cancers such as breast cancer, ovarian cancer. Several epidemiological, *in vitro* and animal studies have demonstrated that flavonoids can influence the growth and proliferation of many different human tumor types. Naringin significantly regulates phosphorylation of two members of the PI3K/AKT and RAS/MAPK signal transduction pathways decreases. The present study was performed to analyze its tertiary structure changes upon interaction with naringin. Expression of recombinant protein was induced with 1mM IPTG at 37 ºC and analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). The protein was purified by Ni^{2+}-NTA affinity chromatography. The protein sample was dialyzed and then used to analyze its interaction with both wild type and mutant SH2 domains of PLC, using polyacrylamide gel electrophoresis (PAGE). Chemical denaturation and intrinsic fluorescence spectra of the purified proteins were carried out by adding different concentrations of as naringin. The fluorescence emission spectra of recombinant kinase domain of FGFR2b (38 kDa) in the presence of naringin shows an increase in fluorescence maximum emission wavelength (nm) and FGFR2b protein was unstable. Regarding to the results, the tertiary structural change of kinase domain reflects a conformational alteration within the protein that is important for the biological function of FGFR2b.

Key words: Fibroblast growth factor receptor; Kinase domain; Naringin; Fluorescence spectroscopy

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The expression of the GNBP in human brain tumor discovery by neuroproteomics analysis

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ABSTRACT

Clinical neuroproteomics aims to advance our understanding of diseases and injuries affecting the central and peripheral nervous systems through the study of protein expression and discovery of protein biomarkers to facilitate diagnosis and treatment. We extracted proteins of tumor and normal brain tissues and then evaluated the protein purity by Bradford test. In this study, we separated proteins by Two-Dimensional Gel Electrophoresis method and the spots were then analyzed and compared using statistical data and specific software, Spots were identified by pH isoelectric, molecular weights and data banks. We have determined their protein profiles using a 2D gel electrophoresis and MALDI-TOF-TOF Mass Spectrometry approach. The 2D gel showed totally 1328 spots among which 157 spots were under expressed and 276 spots were over expressed. Our present proteomic analysis revealed changes in expression of Guanine Nucleotide-Binding Protein (GNBP) $G_I/G_S/G_T$ subunit beta-1 in human brain tumor. Comparison of brain tumor and healthy subjects revealed differentially expressed and statically significant ($P < 0.05$) proteins in brain tumor. Among the identified proteins GNBP-beta-1 is very significant due to their functional consequences in tumor growth and migration. GNBP mediated heterotrimeric G protein signaling is known to regulate cellular motility, growth and differentiation, and gene transcription, three factors central to the biology of cancer. In addition to GNBP activation a variety of stimuli including cellular stresses such as UV irradiation or calcium dependent responses or activation of the Ras/MAPK pathway.

Key words: Brain tumor; Neuroproteomics; MALDI-TOF-TOF; 2DG Electrophoresis

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Protein extraction from human brain fresh-frozen tissue for separation by electrophoresis

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ABSTRACT

Proteins from frozen tissue samples need to be extracted efficiently and without degradation to make the best use of a limited resource and to ensure, as much as possible, that an accurate representation of the proteins in the living tissue is obtained. The emergence of powerful proteomic techniques that make possible studies of many proteins simultaneously and the possibility of automated dispensations from biobank facilities have refocused attention on optimized. Tissues were obtained, with informed consent and institutional review board approval, from patients undergoing tumor resection. For this study, all individuals filled a written informed consent form tissue were surgically removed at hospital. Tissue samples of both tumoral and normal brain tissue were snap-frozen immediately after operation in liquid nitrogen and stored at -80ºC until used for proteomic analysis. We extracted proteins of tumor and normal brain tissues and then evaluated the protein purity by spectrophotometry method. The first-dimensional electrophoresis was performed using 18 cm, pH 3–10 IPG strips. The total number of protein features was matched and analyzed between gels in the control group and tumor group; 343 spots (around 49% of the entire detected spots) were matched across all the gels. In software analysis, a total of 343 differentially expressed spots satisfied the statistical parameters (p <0.05). Efficient extraction is in fact, in part, dependent on breaking protein interactions to release proteins bound in macromolecular assemblies. Disruptive methods of solubilization can, and should, therefore be applied, short of hydrolyzing the protein amino-acid chain or posttranslational modifications.

Key words: Brain; Protein extraction; Frozen tissue

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Probing the interaction between β-lactoglobulin and peptide antioxidant extracted from ostrich egg white by zeta potential and dynamic light scattering

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ABSTRACT

The Purpose of this study was to assess the interaction of β-lactoglobulin (β-LG) and peptide antioxidant extracted from ostrich egg white by zeta potential and dynamic light scattering. β-LG is one of the major milk protein and it is a source of bioactive peptides, indicating important effects on human health such as lowering cholesterol, sedative properties and anticancer effect. The peptide fragments which extracted from ostrich egg white demonstrate antioxidant and anticancer activity. These peptides have a phenolic ring with the molecular weight of about 1582.74 Da. The results of zeta potential study represents the interaction of β-LG with these peptides which was accompanied with the changes in the native conformation of the protein. Dynamic light scattering results show that due to addition of the peptides, the protein will fold. β-LG plays an important role as a carrier protein transfers. The results of this study suggest that antioxidant peptides placed in the active site of the protein. Consequently β-LG can be considered as important carrier for the antioxidant peptides.

Key words: β-lactoglobulin (β-LG); Dynamic light scattering; Compound antioxidant; Ostrich egg white; Zeta potential.

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Poster – P170 Open Access

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A thermodynamics study of the interaction between human serum albumin and polymyxin B as A group of peptide antibiotics

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ABSTRACT

Polymyxins, is a group of cyclic polypeptide antibiotics that compose of a positively charged cyclic peptide ring and a long hydrophobic fatty acid tail. From this group, polymyxin B and polymyxin E (Colistin) are frequently used in the clinical practice to treat infections caused by multidrug-resistant (MDR) Gram-negative bacteria. As an antibiotic binding to drug-transport plasma; e.g., human serum albumin (HSA) may play a critical role in determining its pharmacological and pharmacokinetic profile. Accordingly, we planned to study the binding properties of polymyxin B to HSA under simulative physiological conditions *via* combination of biophysics techniques such as fluorescence spectroscopy and circular dichroism (CD). Our results showed that there is a considerable quenching of the intrinsic fluorescence of HSA on binding the drug. The binding constants (K_a) for these complexes and the corresponding numbers of binding sites (n) were 131.61×10^6 (M^{-1}), and 2, respectively. In addition, near-UV CD spectra studies revealed that the interaction of polymyxin B with HSA modified the tertiary structure of protein. These findings provide a molecular-level understanding of the energetics of polymyxin- HSA binding interactions, which can be used as a useful guideline for further drug design.

Key words: Polymyxins; Human serum albumin; Fluorescence; Thermodynamics studies

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Synthesis of a peptide-drug conjugate with RGD peptide through linkages containing carbon-nitrogen bonds as an integrin-mediated targeting of drug delivery

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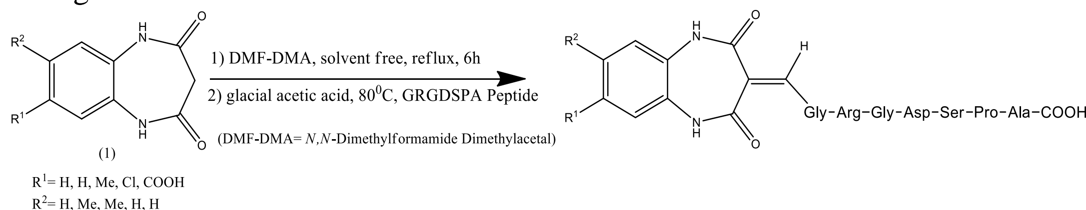
ABSTRACT

Cell adhesion molecules (CAMs) such as integrins have important roles in different disease states such as cancer, angiogenesis, thrombosis, inflammation, osteoporosis and autoimmune diseases. Integrins are involved in a wide range of cell-to-extracellular matrix (ECM) and cell-to-cell interactions. Integrin can undergo endocytosis and exocytosis during cell locomotion and migration. Moreover, integrins modulate programmed cell death (PCD) or apoptosis. Integrin αvβ3 is an ideal target of specific delivery of chemotherapeutic agents, because integrin αvβ3 can be internalized by cells on activation with anchoring ligands, and also integrin αvβ3 is overexpressed in angiogenetic endothelial cells as well as it is absent in pre-existing endothelial cells and normal tissues. Thus, many cell adhesion peptides (i.e., RGD peptide) were used to target drugs and diagnostic agents for a specific cell that has increased expression of cell adhesion receptors such as αvβ3 and αvβ5 integrin. Benzodiazepines and their polycyclic derivatives are used in pharmaceutical and biological chemistry. They also are screened for *in vitro* cytotoxicity against a number of cancer cell lines, such as colon cancer, breast cancer, lung cancer, and bladder cancer. In order to delivering drugs to targeted cells by using peptide-drug conjugate, compound 1,5-benzodiazepine-2,4-Dione (1) reacts with DMF-DMA and then linkages containing carbon-nitrogen bonds formed through reaction intermediate enaminone with GRGDSPA peptide. The FT-IR, ^{1}H NMR and ^{13}C NMR spectra confirms the structure of the products. The effects of drug-peptide conjugate with biological tests will have studied.



Key words: Cell adhesion; αvβ3 Integrin receptor; Bioconjugation; RGD Peptide; Benzodiazepine

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Poster – P172

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The study of mechanical properties of colon cancer cell line (Ht29) after treatment with albendazole by micropipette aspiration and atomic force microscopy

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ABSTRACT

The ranking of colon cancer reported in the world is third in the number of patients and second in the number of deaths. An important issue in cancer studies is the changes in the mechanical properties of the cells and their effects on the cellular responses to biochemical and biophysical signals and determine injuries factors. In this study, the viscoelastic properties of grade II Ht29 colon cancer cell line were investigated with and without treatment by albendazole by Atomic Force Microscopy and Micropipette Aspiration methods. Through these two methods, the amounts of microtubules as the major factors in creating the cell mechanical properties were assessed and compared in treated and untreated cancer cells. The overall results showed the significant decreasing in the amounts of tubuline in cancer cell lines after treatment by albendazole is in agreement with its cytotoxicity. So in line with other studies, the cancer cells are face with reduction in the cell mechanical properties by reducing the amount of microtubules relative to normal cells and the albendazole strengths this property.

Key words: Colon cancer cell line; Albendazole; Mechanical Properties; Atomic Force Microscope; Micropipette Aspiration

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Poster – P173 Open Access

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The effect of choline-based deep eutectic solvent on wild type and an arginine rich mutant luciferase

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ABSTRACT

According to versatile applications of luciferase in different scopes, optimization of physicochemical properties of this enzyme is very important. As a mesophile enzyme, luciferase has low stability that this feature leads to limit luciferase application and decreases its sensitivity and precision in its analytical uses. In regard to the effects of solvents to stabilize enzymes, in this study, a member of deep eutectic solvents (choline chloride: glycerol) as a new generation of ionic liquids with optimized characteristics such as diminished toxicity and enough biodegradability has been used. In the presence of deep eutectic solvent, thermal stability and relative remaining activity of wild type and Arg-rich mutant (E354 R/R-Q35 R-T232 R-I 182 R) were improved. In addition, optimum temperature of activity for the Arg-rich mutant was increased to 40 °C (5 °C higher than in the deep eutectic-free reaction medium). In the presence of deep eutectic solvent, optimum pH of activity was decreased 0.5 unit for the wild type and increased 0.5 unit for the Arg-rich mutant. Besides, other kinetic parameters, for both enzymes such as Km for luciferin and ATP were also examined in the presence of this solvent.

Key words: Luciferase; Deep eutectic solvent; Thermal stability

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The study of calcium ion absorption by the aspartame via molecular dynamics simulation

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ABSTRACT

Aspartame (N- (L-Aspartyl) -L-phenylalanine, 1-methyl ester) is an artificial sweetener and because of high sweetness and low calorie has wide use in food industries. Experimental studies show that aspartame affects the amount of calcium in the body and cause osteoporosis in long time. In this study interaction of aspartame with calcium ions was investigated via molecular dynamics simulation.MD simulation was carried out using a model of Ca^{2+} and other ions enclosed in a fully hydrated simulation box with aspartame. The results showed that aspartame was capable to sequestering calcium ions for a long time. The theoretical results are consistent with experimental results.

Key words: Aspartame; Calcium; Absorption; Molecular dynamics simulation

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The National Conference on Protein & Peptide Sciences: From Basic to Medical & Industrial Applications

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Microbial production of silver and selenium nanoparticles and their influence on amyloid aggregation process

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ABSTRACT

The novel properties of nanoparticles have been exploited in a wide range of potential applications in medicine. It has been suggested that some nanoparticles are capable to retard fibrillation of the Alzheimer's disease-associated amyloid β protein (Aβ). Currently, many methods have been reported for the synthesis of the particles by using chemical, physical, photochemical and biological routes. In this present work, effect of microbial production (*Bacillus* sp. GFCr-4) of silver and selenium nanoparticles was investigated on hen egg white lysozyme (HEWL) aggregation. The particles were screened via ThT, AFM and Congo red assay. We found that these nanoparticles effect on the nucleation step of HEWL fibrillation. Pre-incubation of the protein in the amyloidogenic condition in the presence of both nanoparticles lead to decrease in the nucleation phase. Elongation phase also in the presence of both nanoparticles was increased. The results showed that nano silver is capable to dissociation of formed mature fibrils but nano selenium was unaffected in the period. The likely role of the nanoparticles in this process is binding to lysozyme, which increases the local concentration and the likelihood of formation of a critical nucleus for fibrillation. Depending on the relative affinity of nanoparticles for protein monomers, unfolded monomers, oligomers, critical nuclei, and other prefibrillar states, the influence of nanoparticles on protein fibrillation kinetics are completely different.

Key words: Lysozyme; Amyloid aggregation; Nano silver; Nano selenium

Poster – P176 Open Access

The National Conference on Protein & Peptide Sciences: From Basic to Medical & Industrial Applications

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Inhibition of amyloid formation and cytotoxicity by *Satureja hortensis* extract

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ABSTRACT

Amyloid fibrils have long been associated with dozens of diseases including Alzheimer (AD), Parkinson and prion diseases. Pharmacological and biological investigations justified the traditional application of *Satureja hortensis* L extract (SHE) in treatments of age-related diseases. The research reported here is aimed at exploring possible effects of the extract on amyloid fibrillation inhibition of hen egg white lysozyme (HEWL) and possible its role in treatment of amyloidosis diseases. The acidic pH and high temperatures were used to drive the protein towards amyloid formation. Effect of the extract was screened via various techniques including ThT fluorescence, Congo red analysis and atomic force microscopy. MTT reduction analyses were used to examine cell viability. In the absence of SHE, soluble oligomers became evident after 24 h of incubation, followed by subsequent appearance of mature fibrils after 48 h. Upon incubation with various extract concentration in range of 0.1-1 mg/ml, formation of soluble oligomers and following fibrillar assemblies dose-dependently were inhibited. The results showed that the extract is also capable to dissociation of formed mature fibrils after 48 h incubation. Our results also demonstrated that the cytotoxicity effects fibrillar HEWL species on MCF7 cells after incubation with the extract significantly ($p<0.05$) decreased. Our observations at the study, taken together suggest that aromatic compounds in the extract may directly insert into amyloidogenic core of early aggregates and inhibit amyloid fibril formation.

Key words: Lysozyme; Amyloid aggregation; *Satureja hortensis* extract; Cytotoxicity

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Association between genetic polymorphisms of *XRCC5* VNTR and *ACE* intron 16 I/D with risk of colorectal cancer

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ABSTRACT

DNA double-strand break are repaired by the homologous recombination (HR) and non-homologous end joining (NHEJ) pathways. *XRCC5* gene has important role in NHEJ pathway. Polymorphism of *XRCC5* gene is associated with changes in DNA repair capacity and may be involved in colorectal cancer susceptibility. The *ACE* Intron 16 I/D polymorphism have recently been linked to the pathogenesis and progression of human cancers. Association between genetic polymorphisms of *XRCC5* VNTR and *ACE* Intron 16 I/D in 203 colorectal cancer patients and 237 controls subjects were performed. Genomic DNA was extracted from peripheral blood and genotyping of *XRCC5* VNTR and *ACE* I/D polymorphisms were performed by polymerase chain reaction. Statistical analysis was done using the binary logistic regression. A p value < 0.05 was considered statistically significant. After statistical analysis, there was no significant association between *XRCC5* VNTR polymorphism (OR= 1.24, 95%CI= 0.07-20.32, P= 0.880) and *ACE* Intron 16 I/D polymorphism (OR= 1.19, 95%CI= 0.80-1.78, P= 0.376) and risk of colorectal cancer.

Key words: Colorectal cancer, *XRCC5*, *ACE*, Genetic polymorphism

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The National Conference on Protein & Peptide Sciences: From Basic to Medical & Industrial Applications

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Prediction of protein subunits number by using primary structure

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ABSTRACT

The biologically active form of many proteins is a complex of two or more polypeptide chains. Quaternary structure of proteins is required for proteins functionality. It was mentioned that the subunit interfaces are composed of hydrophobic and hydrophilic patches. The studies for predicting the multi-subunit proteins have been accomplished heretofore by using 3D structure of proteins. Herein we use only the information of protein sequences to predict number of protein subunits. The structural futures and physicochemical parameters of proteins are computed based on information of protein sequence. Accessible surface area and depth of residues are examples of the important considered parameters. Aberrant subunit assembly could prepare conditions for anomalies associated with protein aggregation in cells and therefore knowledge about protein subunit assembly help us to manage protein aggregation effects. The results of current study define important parameters in protein subunit assembly.

Key words: Computational approach; Sequence information; Protein interface; Protein subunits

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Interaction of gallium water soluble complex with bovine serum albumin

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ABSTRACT

A new water-soluble Schiff base complex of [Ga (L)] NO_3; where L denotes a N_2O_2 Schiff base ligand; was synthesized and characterized by IR, ^{1}H-NMR, elemental analysis and UV-Vis spectroscopy. The mechanism of binding of this complex with bovine serum albumin (BSA) was studied by fluorescence spectroscopic technique. The results of this investigation showed that the intrinsic fluorescence of BSA was quenched by the complex which was rationalized in terms of the static quenching mechanism. Type of quenching, binding constants, Stern-Volmer constants, number of binding sites, and binding stoichiometries were determined by fluorescence quenching method. Additionally thermodynamic parameters were calculated by van't Hoff equation, indicating that the binding was entropy driven and enthalpically disfavored. Also distance between complex and protein were calculated by forest equation.

Key words: Water-soluble Schiff base complex; Human Serum albumin; Fluorescence; Binding

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Study on the interaction of prodigiosin with human serum albumin

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ABSTRACT

Prodigiosin is a red three pyrrolic pigment produced by several bacteria, especially *Serratia marcescens,* with various biological profiles including antimicrobial, immunosuppressive and anti-cancer activity. In the present investigation, an attempt has been made to study the interaction of prodigiosin (PG) from a newly isolated *Serratia* sp. strain S2B with the transport protein, human serum albumin (HSA), employing column chromatography, fluorometric spectroscopy and circular dichroism (CD) techniques. The gel filtration column chromatography revealed a significant interaction of PG with HSA showing overlapped absorbtion peaks of drug and the protein. The spectroscopic results indicated that binding of PG to HSA caused fluorescence quenching of the protein through static quenching mechanism with binding constant of 0.2552 x 10^4 M^{-1} at 27 °C. Moreover, hydrophobic interactions play a major role in binding and stability of protein-PG complex and the process of the binding was driven by enthalpy. The results of CD experiment showed that the binding of this drug to HSA induced conformational changes in HSA and increased its structural stability. All these results revealed a strong binding event between PG and HAS, changing the conformation and microenvironment of the protein.

Key words: Prodigiosin; Human Serum Albumin; Interaction; Column chromatography; Spectroscopic methods

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Association between genetic polymorphisms of *GPX1* pro198leu and *SOD1* A251G with risk of kidney acute rejection

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ABSTRACT

Kidney transplantation is a therapeutic approach for patients with end-stage renal disease. Despite the increase and improvement of this therapeutic process, yet because of recipient immune response against the donor tissue, rejection is common and the main cause of failure of the procedure. Transplanted kidney is prone to exposure to acute rejection for different reasons. Oxidative stress with negative impact on the survival and function of transplanted tissue can cause graft rejection. GPX1 and SOD1 are antioxidant enzymes. The aim of this study was to investigate the relationship between polymorphisms of *GPX1*pro198leu (rs1050450) and *SOD1*A251G (rs2070424) with acute rejection of kidney transplantation. In this study 262 kidney transplant recipients, 46 of whom had acute rejection, were enrolled. Genotyping performed by PCR-RFLP method and data were analyzed by SPSS statistical software. Analysis the genotype and allele frequencies in *GPX1*pro198leu polymorphism between patients with and without renal acute rejection, showed no significant difference. The A251G polymorphism in the *SOD1* gene also showed no significant differences between patients with and without acute rejection. In conclusion, the *GPX1* and *SOD1* mentioned gene polymorphisms genotype and allele frequencies have no role in the incidence of renal acute rejection.

Key words: Kidney transplantation; Acute rejection; Oxidative stress; *GPX1*; *SOD1*

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Isoniazid-induced hemolytic anemia with hemoglobin degradation

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ABSTRACT

Exposure to many chemical agents such as drugs can cause hemolysis of red blood cells and can cause hemolytic anemia. The aim of this study is to investigate the hemolytic effect of Isoniazid. This experimental study was done on 40 adult *Wistar* rats. Animals were divided randomly into 3 groups. Control group: This group received only enough food and water. Reference group: This group received Solvent isoniazid (physiology serum). The Experimental group received isoniazid with 50 mg/kg dose of the animal weight for 14 days. Blood samples were applied to measure various parameters such as hematocrit level, hemoglobin level, red blood cells number, the red blood cell fragility. For evaluating the osmotic fragility sensitivity, red blood cells were incubated at 37 ° C at different concentrations of NaCl for 30 min and the extent of hemolysis was measured by colorimetric solution. Hemolysis was expressed based on the percentage of hemolysis of red blood cells in the presence of distilled water (100% hemolysis). The results showed a significant decrease in hemoglobin and hematocrit levels in the isoniazid receiving group. However, Red blood cell count didn't show any significant change. Furthermore, osmotic fragility test showed increased fragility of red blood in experimental group. In conclusion, Isoniazid cause to Hemoglobin degradation and prevent hemoglobin synthesis. But based on osmotic fragility test, Isoniazid can induce oxidative stress and RBC lysis at higher dose.

Key words: Osmotic Fragility Test; Red Blood Cells; Isoniazid; Hemoglobin; Hemolysis

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An extremely halophilic laccase from the saline water isolate *Chromohalobacter* sp.

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ABSTRACT

Since hypersaline environments are on the increase, halophilic organisms will prove even more valuable and provide significant opportunities for industrial biotechnology. Halophilic microorganisms produce stable enzymes capable of functioning under conditions such as high ionic concentration, presence of organic solvents, alkaline pH values, low oxygen availability, high or low temperatures, presence of heavy metals, and other toxic compounds. Laccases are blue copper-containing oxidases that catalyze the oxidation of a wide variety of organic and inorganic compounds to the corresponding reactive radicals, with the reduction of molecular oxygen to water. Laccases have been found in a wide range of organisms with different biological roles. Due to various applications in industrial scale, laccases from extremophile bacteria can be very interesting attractive. In the present study, an extracellular laccase producing halophilic bacterium was isolated from the Ajigol salty wetland and identified as *Chromohalobacter* sp. After inoculation fully grown purified strain, the isolate was cultivated in Luria-Bertani broth medium containing 3 M NaCl. After 3 days, the supernatant was precipitated. The precipitant was dialyzed and purified to an electrophoretically homogenous state by the affinity chromatography. The molecular mass of the purified enzyme was estimated to be about 60 KDa and demonstrated optimal activity at NaCl 3 M, pH 8.0 and 45ºC. $CuSO_4$ was the most effective inducer for laccase production. The enzyme was active up to 65ºC and at pH range of 4−10 and was highly stable in the presence of various concentrations of LiCl and KCl.

Key words: Laccase; Halophile; Purification; Characterization; *Chromohalobacter* sp

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Structure-based design of inhibitory peptides for LRP6 inhibition

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ABSTRACT

Wnt signaling pathway plays a major role in regulation of cell proliferation, migration, tissue homeostasis and tumor progression. This pathway can be antagonized by DKK proteins, which disrupt the intiationary complex (Frizzled-LRP5/6 complex). Nowadays, use of peptides in order to target tumor cells is attracting much attention. In this study, a computational protocol is presented to structural design of inhibitory peptides against Low-density lipoprotein receptor-related protein 6 (LRP6) as receptor. Firstly, natural ligand of LRP6 divided to 12 fragments as peptide derivatives. Then, molecular dynamics simulations used for structural energy minimization by means of Gromacs 4.5.4. The binding affinities of designed peptides were investigated via molecular docking using ClusPro webTool. Finally, stability and binding free energy of peptides were calculated by FoldX software. The results showed that four of designed peptides had the highest affinity to interact with the receptor and can be considered as candidates for inhibition of wnt signaling pathway through LRP5/6 receptor.

Key words: Wnt/b-catenin pathway; Peptide design; Docking; Energy minimization

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The effect the proteolytic activity of lactic acid bacteria in cow's milk β-lactoglobulin allergenicity

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ABSTRACT

Although cow's milk is a very popular drink and has a high nutrition value containing all the essential amino acids, some reports declare that it is the third most allergenic food. It is more prevalent in infants and children, affecting 2 to 7.5% of them. Milk proteins are the main cause of milk allergenicity. β-Lactoglobulin (β-LG), a relatively small protein with 162 residues and 18.3 kDa molecular weight, is one of the major allergens of milk. Lactic acid bacteria, a group of probiotic bacteria, can produce proteolytic enzymes to hydrolyze β-LG structure and consequently decrease its allergenicity properties. In the present study, traditional yogurts have been collected from different regions of Iran and the proteolytic activity of these bacteria were assayed using SDS-PAGE and RP-HPLC techniques. The immune-reactivity of the protein hydrosate was investigated using competitive ELISA experiments, to compare the amount of antibody attachment to native β-LG and its hydrolysates. In this experiment, a pool sera of patients under 5 years of age with cow's milk allergy were used to find IgE attachment on β-LG and its hydrolysates. The results of this study demonstrated that the proteolytic effect of these bacteria can significantly reduce the allergenicity of bovine β-lactoglobulin.

Key words: β-Lactoglobulin; Lactic acid bacteria; Cow's milk allergy; Proteolytic activity; Competitive ELISA

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The National Conference on Protein & Peptide Sciences: From Basic to Medical & Industrial Applications

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Microglial cell death induced by glycated insulin

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ABSTRACT

Diabetes mellitus, a common metabolic disorder resulting from defects in insulin secretion or action or both, causing the elevated level of blood sugar (hyperglycemia) which accompanies acceleration of the glycation of proteins as a nonenzymatic process. It has been approved that glycation makes toxic aggregates known as soluble amyloid protofibrils. Moreover, during the glycation and fibrillation process, damaging free radicals are produced as reactive oxygen species (ROS). Protofibriles and free radicals initiate membrane damage, so induce apoptotic responses and cell death. It has been studied that these toxic agents cause microglia apoptosis and development of Alzheimer's disease (AD) in diabetics. Microglia are macrophage-like immune cells in the brain that play critical roles in the inflammatory aspects of the AD. In order to show the stated toxic phenomenon, insulin was incubated in the presence of glucose or fructose (in diabetic condition concentration), and formation of related advance glycated end products (insulin-AGE) was monitored using spectroflourimetric methods. Insulin glycation not only can impair insulin action but also make this peptide to be toxic on microglia cells. The product of insulin glycation is suggested to exert specific effects on glial cells that impact their viability and causes microglia apoptosis that has been observed using MTT test and flow cytometery, respectively. The insulin glycation-induced liposomal membrane peroxidation was approved by monitoring the formation of malondialdehyde (MDA). As a result, insulin aggregation products activate microglia cells and induce apoptosis, which progressively brings about more neural cell death and AD.

Key words: Diabetes; Glycation; Lipid peroxidation; Microglia; Apoptosis

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Protein/hydroxyapatite constructs for bone tissue engineering applications

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ABSTRACT

Extracellular matrix (ECM) is a complex mixture of proteins and sugars beyond cell. The success of bone tissue engineering relies on our ability to generate three-dimensional (3D) scaffolds resembling that of the natural ECM. Protein matrices with their beneficial characteristics such as high permeability for transport of metabolites have been used for generating 3D ECM-like scaffolds. Among proteins, collagen which is the most abundant ECM protein has great potential to be used as the structural scaffolds. However, collagen usually suffers from poor mechanical properties and inability to induce mineralization. Accordingly, this study represented a successful attempt to render collagen-based matrix suitable for hard tissue regeneration. For this, gelatin molecules, as a less expensive form of collagen, were treated by addition of methacrylate to their amine-containing side groups. The resulting methacrylate-treated gelatin was then combined with rod-like hydroxyapatite (HAp) nanoparticles followed by photopolymerization. According to the results, rod-like HAp significantly improves the stiffness of protein matrix, while maintains its porosity and swelling ratio. It was also found that while the bare protein was inert in terms of bioactivity, a homogeneous biomineralization occurs throughout the protein/HAp constructs after incubation in simulated body fluid. This demonstrated that the nano-HAp incorporated into the protein not only acts as reinforcing filler, but also increases the bioactivity of the matrix. On the basis of these results, the protein/HAp constructs developed in this study may be promising candidates for preparing tissue-like bioactive scaffold for engineering of calcified tissues.

Key words: Bone scaffold; Protein matrix; Hydroxyapatite; Collagen; Mineralization

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Poster – P188 Open Access

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The effect of gallic acid on the structure and stability human serum albumin

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ABSTRACT

The stability and structure of human serum albumin (HSA) with Gallic acid as an endogenous plant polyphenol have been investigated by of fluorescence, UV–VIS and circular dichroism (CD) spectroscopy. The melting point (T_m) of protein and $\Delta G^O{}_{(298K)}$ as two thermodynamic parameters obtained from thermal denaturation of the HSA with and without the presence of Gallic acid. The magnitudes obtained 332.5 and 329.2 K for T_m, and 97.4 and 95.4 kJ mol^{-1} for $\Delta G^O{}_{(298K)}$ for the sole HSA and its incubation with gallic acid, respectively. In the protein chemical denaturation the magnitudes of $\Delta G^O{}_{(H2O)}$ and C_m for sole HSA and its treatment by gallic acid obtained: $\Delta G^O{}_{(H2O)}$ = 12.5 and 9 kJ mol^{-1}; Cm = 0.22 and 0.17M, respectively. The stern-volmer analysis of fluorescence quenching spectra of HSA with different concentrations of gallic acid a static quenching mechanism have been revealed and the thermodynamic parameters indicates that the hydrophobic interactions played a major role in destabilizing of the HSA-Gallic acid complex. According to above mentioned thermodynamic parameters and structural assessment of HSA with fluorescence and CD, the interaction of gallic acid induced protein instability.

Key words: HAS; Gallic Acid; UV-Visible; Fluorescence; Circular dichroism Thermodynamic parameters

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Poster – P189 Open Access

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Inhibition of advanced glycation end-product formation on eye lens proteins by *Salvia officinalis* extract

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ABSTRACT

Glycation is a spontaneous reaction occurring non-enzymaticly between reducing sugars and amino groups of proteins, nucleic acids and lipids. This reaction has an important impact on the physical and functional properties of proteins. These alterations have consequences in several pathological conditions such as cataract, arteriosclerosis and Alzheimer's disease. Most of the glycation effects are due to advanced glycation end-products (AGE) adducts on long-lived proteins such as lens proteins. The correlation between oxidation and protein glycation is clearly demonstrated by *in vitro* experiments in which promotion of diabetic complications occur. Therefore, natural compounds which possess both antioxidant and antiglycation activities might have great therapeutic concern in treatment of diabetic complications. The main purpose of this study was to evaluate the anti-glycation and antioxidant properties of *Salvia officinalis* extract. Hydroalcoholic extract of *salvia officinalis* was tested for its antiglycating ability against fructose-induced glycation of goat lens total soluble proteins (TSPs). Glycation of lens proteins in the absence and presence of *salvia officinalis* extract (0.001-1mg/ml) were evaluated by monitoring protein cross-linking on SDS-PAGE, brown staining method and Congo red assay. Antioxidant activity of *salvia* was determined by DPPH assay and total phenol content. It was observed that *salvia officinalis* has antioxidant properties and AGEs inhibitory effects on phosphate-buffered fructose and TSP reaction. All these features introduce an outstanding natural resource with dual antioxidant and anti-AGE functions, considerable protection against fructose-induced cellular damage, remarkable prospect for treatment of complications such as diabetes, ageing and a broad range of other conformational disorders.

Key words: Eye lens proteins; Antiglycation; *Salvia officinalis*; AGEs

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Antiglycation properties of *Salvia Officinalis* against the glucose-induced glycation of bovine serum albumin

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ABSTRACT

Non-enzymatic glycation of proteins which occurs by reducing sugars such as glucose leads to the formation of advanced glycation end products (AGEs). Among the reducing sugars, the role of glucose has widely been studied and implicated in diabetes. Glycation, brings about alterations in the biological function of proteins and their degradation processes. Among the proteins, glycation of serum albumin is of special interest. Abumin is of the most abundant protein in plasma, and is highly sensitive to glycation in this study; we investigated the antiglycative potential of *salvia officinalis* extract using serum albumin under *in vitro* condition. D-glucose and bovine serum albumin (BSA) were incubated for 8 weeks with and without *salvia officinalis* extract (0.001-1mg/ml) and the observed changes were evaluated by SDS-PAGE, brown staining method and Congo red assay to monitor the protein glycation process. It was observed that *salvia officinalis*, especially at concentration of 1mg/ml has significantly antiglycative properties and has been inhibited the aggregation of BSA. The obtained results clearly showed that *salvia officinalis* has anti-AGEs properties, and in particular protect albumin against glucose-induced alterations. Accordingly, the herb could be consider as a remarkable source for treatment of diabetes and other conformational disorders.

Key words: BSA; Antiglycation; *Salvia officinalis*; AGEs

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Prediction of protein amino acids' retardation factor in different solvent mixtures of NP-TLC

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ABSTRACT

Amino acids, which are famous as basic tiles of peptides and protein, are important issues in protein chemistry. However studies on both protein and non-protein amino acids are remarkable for clinical, biochemical, and medical reasons. Different chromatographic methods, including gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been used for separation of AAs. But these techniques beside of their known benefits, are usually time-consuming and require expensive equipment. On the other hand thin-layer chromatography (TLC) could be a good replacement of complex separation methods because of its simplicity, cost-effectiveness and convenience for separation of small molecules e.g. amino acids [1]. A quantitative predictive/descriptive model was proposed for retardation factor (R_F) of protein amino acids (AAs) in normal phase thin layer chromatography (NP TLC). Experimental R_F of 126 chromatographic mixtures (21 protein Amino acids in different mobile phase) were used as the independent variable. The matrix of dependent variables of model was build using structural descriptors of AAs and empirical parameters of solvents of applied mobile phases. After variable selection, a five parametric model was proposed for R_F of amino acids which covered about 84% and 79% variance of data in training and cross validation respectively. The correlation coefficient of the external test set was obtained equal to 0.88 which shows the prediction potential of proposed model as well as its good applicability domain that was checked using standardized residual-leverage plot.

Key words: Amino Acid; Thin Layer chromatography; QSPR; Solvent; Retardation factor

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A bioinformatic study of the effect of Phe180 on the sability of *Renilla* luciferase

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ABSTRACT

Renilla luciferase is an enzyme that catalyzes the chemical reaction which is responsible for the green bioluminescence of *Renilla reniformis*, a soft coral also known as sea pansy. In vitro, it catalyzes the oxidative decarboxylation of coelenterazine *(Renilla* luciferin) in the presence of O_2, resulting in the emission of blue light (with an emission maxima of 480nm). The enzyme exists as a single polypeptide chain of 36 kDa in the active form that contains 311 amino acid residues. The amino acid sequence of *Renilla reniformis* Luciferase was taken from NCBI (accession number: AAA29804.1) protein database in FASTA format. To change enzyme stability alanine scanning technique was performed. To this aim, Phe180 residue in active site of the enzyme was replaced with Ala residue. Then, the amino acid sequence was submitted to" I-Mutant" server. Finally the change in protein stability was measured and reported. The result shows that replacement of Phe180 with Ala decrease the *Renilla* Luciferase stability at 25 ˚C and pH7. Phe180 is one of the residues in the enzyme active site pocket which has a bulky hydrophobic side chain. In spite of having role in substrate binding, Phe180 may be responsible for enzyme stability.

Key words: Renilla luciferase; I-Mutant; Phe180

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The effect of a single amino acid mutation of Met-185 to Ala in *Renilla* luciferase stability, a bioinformatic study

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ABSTRACT

Renilla luciferase from the sea pansy *Renilla reniformis* is a 36-kDa monomeric enzyme that catalyzes the oxidation of coelenterazine to yield coelenteramide and blue light with a spectral maximum of 480 nm. The protein contains 311 amino acid residues and immediately is activated following translation without requiring posttranslational modifications. It is widely employed in molecular biology as a reporter gene in both cell culture experiments and small animal imaging. The amino acid sequence of Renilla Reniformis Luciferase was taken from NCBI database protein and then using Alanine scanning Technique, Met-185 was replaced by Ala and considered for changing in free energies using bioinformatic server. In the present study, it was shown that amino acid mutation of Met-185 to Ala may decrease Renilla luciferase stability in 25°C and pH 7.0. Met-185 which is one of the subset responsible for binding to substrate has a potential role in Renilla Luciferase stability and probably appropriate conformation of active site.

Key words: Met-185; Renilla Luciferase; Alanine scanning Technique

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Immobilization of lactoperoxidase onto copper nanoparticle

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ABSTRACT

Lactoperoxidase (EC.1.11.1.7), an enzyme of peroxidase family, is one of the most abundant bovine milk enzymes in which enter the whey in the process of cheese production. The lactoperoxidase enzyme has been of great interest due to its valuable properties, which can be widely used in medical field as well as in food fields. Recently, some studies have revealed that thermostability of the enzyme is not appropriate to use the enzyme in industry. The purpose of the present study was to immobilization of lactoperoxidase onto copper nanoparticles, and compare the thermostability of the native and the immobilized enzyme. Lactoperoxidase was purified from whey, a by-product in cheese industry by cation exchange and gel filtration chromatography .Then, purified enzyme was immobilized on the surface of copper nanoparticles by the method of Link et al. The immobilized enzyme displayed a similar pH- dependence behavior and an enhanced thermostability up to 56% compared to the native enzyme. Copper nanoparticles can be considered as an appropriate support for improvement of thermostability of the peroxidase enzymes family.

Key words: Immobilization; Copper nanoparticle; Lactoperoxidase; Thermostability; Peroxidase

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Effect of quercetin on ferrous sulfate-induced liver failure in rat

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ABSTRACT

Iron in excessive amounts may be toxic particularly to the liver since it is the major site of iron storage. The mechanisms by which excess iron exerts its cytotoxic effects include enhanced formation of free radicals and lipid peroxidation in organelle membranes. Since quercetin is a powerful antioxidant with radical scavenging ability, its effects on hepatic functional disorders and histological damages induced by ferrous sulfate was investigated in rat. Hepatic damage was induced by i.p. injection of ferrous sulfate (30 mg/Kg/day) for 14 day inmale wistar rats (210-250g). In final blood samples collected for determination of the serum protein, urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) concentration. Then liver samples were removed and preserved for histological studies and estimation of lipid peroxidation. Quercetin (50 mg/kg/day) was injected (i.p.) for 10 days. Ferrous sulfate caused a significant reduction in liver function, increased liver lipid peroxidation which correlated with elevated serum enzymes,AST, ALT and LDH as well induction of hepatic histological damages. Quercetin treatment reversed the parameters of liver dysfunction, increased lipid peroxidation and liver enzymes levels as well reduced total hepatic histopathological scores. These data indicate that quercetin reduces the ferrous sulfate-induced hepatic damage by lowering lipid peroxidation in rats.

Key words: Ferrous sulfate; Lactate dehydrogenase; Lipid peroxidation; liver; Quercetin; Urea nitrogen

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A bioinformatics study for deciphering the impact of aspartic acid 120 on free energy changes in *Renilla* luciferase

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ABSTRACT

Renilla luciferase (RLuc) commonly is used as a reporter protein. Despite their interesting aspects in enzymology, native luciferases are not necessarily optimal as protein reporters. Therefore searching for stabilizing network in protein has great importance in Rluc. Rluc has a expand gateway that continued by a deep cavity. Both of the catalytic and substrate-binding residues are located in this centre closely. The catalytic residues were predicted as Aspartic acid 120, Glutamic acid 144, and Histidine 285 and forming a H-boding network, and it may be assumed that their local interactions are important for enzyme stability. In this report the impact of Aspartic acid 120 on the structural stability of RLuc was analysed by studying the free energy changes. The sequence of RLuc was obtained from GenBank (www.ncbi.nlm.nih.gov) and was subjected to Alanine scanning. Finally, several bioinformatics servers that have been supported by Expasy (www.expasy.org) have been used for modelling, structure evaluation and free energy calculation. Measurement of unfolding free energy between wide type and manipulated RLuc showed that the mutation of Asp to Ala causes negative amount in the free energy changes. Therefore, Asp 120 not only has importance on catalytic activity in Rluc but also may play a key role in structural stability. The results might show that the Aspartic acid 120 is involved in a stabilizing network in Rluc.

Key words: *Renilla* luciferase; Coelenterazine; Protein reporter; Aspartic acid 120; Ala scanning

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In Silico mutagenesis of *Renilla* luciferase may yield enhanced stability, a bioinformatic study

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ABSTRACT

Renilla Luciferases (RLuc) have been developed as reporter protein in biological research and small animal imaging. As a protein reporter, the bioluminescence activity of commonly used luciferases is too labile to inactivating factors, especially thermal inactivation. In the hopes of further enhancing the resistance to inactivation of RLuc, a search for finding the suitable residues in Rluc has been carried out using *in silico* analysis. The protein sequence of RLuc was obtained from protein information resource (pir.georgetown.edu). The sequence was modeled using HHpred server (http://toolkit.tuebingen.mpg.de/hhpred). The mutation on structure was carried out using Deep View. v4.1 (spdbv.vital-it.ch), and finally the free energies of mutants were assayed using bioinformatics. Interestingly, changing Glutamic acid 144 to Alanine generates a more stable mutant form of Renilla luciferase. The quantity of altered stability was obtained by calculation of free energies between folding/unfolding states of wide type and mutant. Results from *in silico* mutagenesis analysis may used to generate a mutant optimized for use as an enhanced reporter protein and may allow enhanced structural stability in Rluc in pre-existing luciferase-based kits.

Key words: *In silico* mutagenesis; *Renilla* luciferase; Enzyme stability; Reporter Protein

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Analysis of aggregation behavior of alpha interferon during agitation and freeze-thawing stresses

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ABSTRACT

Therapeutic proteins are susceptible to aggregation in response to a wide variety of stresses encountered during their manufacture, storage and delivery. Stresses that frequently provoke protein aggregation, such as agitation or freezing are commonly induced in the manufacturing and shipping of therapeutic proteins. Agitation can result in aggregation during manufacturing, shipping and handling of the product. Likewise, a protein drug substance is commonly frozen as a storage step in the manufacturing process. In this study, the effect of agitation and freeze-thawing on the aggregation of recombinant human interferon α2b (rhIFNα2b) was assessed using different analytical methods. Agitation was performed by continuous shaking of rhIFNα2b at 250 rpm (for 24, 48, 168 and 240 hr) and 1000 rpm (for 72 hr) on an orbital shaker at 25°C. Each protein sample at a concentration of 100 µg/mL in 100 mM phosphate buffer (pH 7.0) was frozen in liquid nitrogen and transferred to a water bath maintained at 37°C in order to thaw the samples. The results obtained from samples shaken at 250 rpm for up to 240 h showed no significant difference with unstressed rhIFNα2b. Optical density at 350 nm also increased and the formation of dimer/higher molecular weight aggregates formation was observed following shaking at 1000 rpm for 72 h. Data obtained from freeze-thawed samples after 15 cycles revealed significant aggregation. In conclusion, data obtained could be used to improve the stability of rhIFNα2b and therefore develop a stable therapeutic formulation.

Key words: rhIFNα2b; Aggregation; Agitation; Freeze-thaw; Stability

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Optimization of culture medium for production of a halophilic lipase from the saline water isolate *Alkalibacillus* sp.

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ABSTRACT

Halophilic enzymes, which tolerate critical conditions such as wide ranges of temperature, pH, salt, low water content, and organic solvents, have been widely used in most harsh industrial processes in recent decades. Halophilic lipases with hydrolysis ability on long-chain acylglycerols at oil-water interface, have been found to apply in numerous biotechnological purposes such as biosensors, fats and oil modification, leather manufacture, pulp and paper industry, medical biotechnology, bioconversions in organic media, oleochemical industry, biodiesel production, flavor and aroma industry, agrochemicals, and resolution of racemic acids and alcohols. In the present study, production of an extracellular lipase from *Alkalibacillus* sp. was optimized via the statistical design method. During a screening program, the microorganism was isolated from water saline, on the basis of primary screening on Luria- Bertani agar containing Rhodamine B. It was identified using 16S rDNA sequencing, morphological and biochemical studies. The optimization study, using Plackett-Burman design and response surface methodology showed that a suitable ratio of olive oil to Tween 80, glucose, NaCl, and KH_2PO_4 were most effective factors on the lipase production. Moreover the effects of different factors such as pH, temperature, salt, metal ions, detergents, substrates with different acyl chain lengths, and organic solvents on the lipase activity and stability were investigated. The enzyme displayed maximum activity at pH 8, 35 °C, 3 M NaCl, when using *p*-nitrophenylpalmitate as substrate. Lipase activity had notable stability in almost all organic solvents tested.

Key words: Lipase; Production; Halophilic bacteria; Optimization; *Alkalibacillus* sp

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Investigation on the kinetic parameters of human ceruloplasmin upon interaction with lead

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ABSTRACT

Ceruloplasmin (Cp) alteration is assumed as a mechanism underlying the development of neurological disorders. Kinetic investigations of the Cp upon interaction with a ligand can help to better understanding Cp Mechanism. For achieving this, kinetic behavior of the enzyme in the presence of lead (Pb) was studied. The enzyme activity was determined using o-dianisidin dihydrochloride as a chromogenic substrate. After preincubation of serum with 0.1 – 0.8 mM concentrations of Pb for 10 min, oxidase activity of the enzyme compared with control levels, and showed to be decreased with increasing Pb^{2+} concentrations. Maximum inhibitory effect of Pb^{2+} was shown at 0.6 mM where about 80% of the enzyme activity was lost. Vitamin C (ascorbate) *in vitro* had no significant effect on Pb-induced inhibition. Lineweaver-Burk reciprocal plot of Cp activity in the presence of Pb^{2+} showed that the mode of inhibition was noncompetitive. Fluorescence studies also were carried out on the native and Pb-inhibited enzyme. Maximum emission spectrum of the inhibited enzyme showed an increased level of about 70% intensity with regard to the native enzyme. Pb-induced enzyme inhibition was prevented by thiol group containing compounds such as glutathione (1.2, 12 mM), and β-mercaptoethanol (1.2 mM). Data suggested that a conformational change in the Cp due to Pb^{2+} binding to some functional groups of the enzyme caused enzyme inactivation, and thiol groups in the enzyme which are available to interaction with lead ions, may be involved in this enzyme inactivation.

Key words: Kinetic parameters; Lead; Ceruloplasmin; Inhibition; Oxidase activity

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The effect of valproic acid on viability and histone proteins of bone marrow stem cells

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ABSTRACT

Valproic acid (VPA) is a branched short-chain fatty acid which widely used as an antiepileptic drug and for treatment of various cancers. VPA is a histone deacetylase inhibitor that acts on chromatin remodeling. In addition, it has been recently suggested that chromatin can be a novel target of VPA. In this study, the effect of VPA on non-adherent bone marrow stem cells was investigated, employing gel electrophoresis, Trypan Blue and MTT assays. The cells were incubated in the absence and presence of various concentrations of VPA (0-400 mg/ml) for 12 h and viability analyzed. Also histone proteins (H1 and core histones) were acid-extracted and analyzed on SDS-PAGE. The time-course study showed that incubation of non-adherent bone marrow cells in the presence of VPA reduced the viability as drug concentration increased. Analysis of histone proteins revealed that the content of histone H1 was decreased in the presence of VPA but the amount of core histones remained unchanged. From the results, it is concluded that binding of VPA to histone H1 may affect H1-DNA interaction in chromatin, whereas core histone which are covered with DNA in chromatin structure are not accessible to the drug and cannot be considered as a potent target for VPA.

Key words: Chromatin; Valproic acid (VPA); Histone protein

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The studies of interaction between β-lactoglobulin and alprazolam with UV-Vis

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ABSTRACT

In recent years quick development is fared in drug discovery methods due to increase the production of new drugs. The most important issue in drug delivery system is discussion about controlling of drug delivery to the body. With common methods for use, such as: oral and injectable of drug consumption, the drug will be distributed throughout the body and the body will be under its effects and side effects. Proteins can be used as drug carriers. The interactions of proteins with different ligands play an important role in many cellular processes, and also pay special attention to the interaction of the drug design to achieve a particular effect needs to consume large amounts of drugs. Bovine milk β-lactoglobulin (β-LG) demonstrates significant resistance against both gastric- and simulated duodenal digestions. Therefore, it seems a realistic protein candidate for safe delivery and protection of particularly pH sensitive drugs in stomach. Alprazolam is a short-acting anxiolytic of the benzodiazepine class of psychoactive drugs. Alprazolam, binds to specific sites on the GABA receptor. In this study the intermolecular interaction of alprazolam with β-lactoglobulin was investigated using UV-vis spectroscopy. Moreover, the effect of Alprazolam complexation on the secondary structures of β-LG were studied and the results showed that the secondary structure of β-LG were preserved upon interaction of this drug. Based on the achieved results, this protein might be useful for delivery of Alprazolam.

Key words: β-Lactoglobulin; Alprazolam; UV-vis spectroscopy

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Molecular docking study of gallium complex with bovine serum albumin

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ABSTRACT

Molecular docking theory is a strong technique for understand the ligand – protein interaction which can corroborate our experimental results. It also can be used after experimental screening for help in better understanding of bioactivity mechanism. At this work a new Gallium water soluble schiff base complex was synthesized and characterized by different spectroscopy technique and then its interaction with bovine serum albumin was studied. MGL tools with autogride4 and autodock4 were used to set up and exert blind docking calculation between complex and BSA. The result showed that the BSA interacts with complex by hydrophobic force. The binding constant which calculated by the free energy obtained from the docked drug – BSA model was 4.3×10^{2} $Lmol^{-1}$.

Key words: Molecular docking; Bovine serum albumin; Schiff base complex

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Substrate binding of maltogenic amylase based on molecular docking simulation

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ABSTRACT

Maltogenic amylases (MAase) are a group of cyclodextrin (CD) hydrolyzing enzymes which recently attract increasing amount of attention, due to its industrial applications in baking and confectionary industries and also its use in pharmaceuticals. We decided to investigate affinity of the *Geobacillus* sp. Gh6 MAase to various substrates by molecular docking simulation and compared with kinetic parameters. Molecular docking is a well-established computational technique which predicts the interaction energy between two molecules. Previous studies have shown a noticeable preference for α- , β- and γ-CD compared to polymeric substrates (amylose and amylopectin) possibly due to steric interference. The K_m for CD substrates increased in the order of α-CD >> β-CD > γ-CD, and k_{cat}/K_m increased as α -CD > β-CD > γ-CD, implying that increased substrate specificities are mainly attribute to $k_{cat.}$ In this study, binding of the enzyme to α- , β- , γ-CD, amylose and amylopectin was studied using molecular docking and free energy of binding and dissociation constant was estimated. Interestingly, dissociation constant is highest for polymeric substrates compared to CDs and also α-CD >> β-CD > γ-CD and order of the free energy of binding is amylopectin > amylose >> α-CD > β-CD > γ-CD. All of the results showed that the affinity for substrates increased in the order of γ-CD > β-CD > α-CD >> amylose > amylopectin.

Key words: Molecular docking; Kinetic parameters; Maltogenic amylase; Cyclodextrins

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Expression and purification of the recombinant kinase domain of FGFR2b and study of its structural changes due to the interaction with gallic acid

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ABSTRACT

FGFR2b plays a significant role in cell signaling pathway, regulating several key biological processes including cellular differentiation and proliferation. Genetic alterations of the tyrosine kinase domain of FGFR2b occur in many tumor cells. Several epidemiological and animal studies have demonstrated that flavonoids such as gallic acid (GA) can reduce the growth and proliferation of many different human tumor types.The aim of this study was to express and purify the recombinant human FGFR2b kinase domain and analysis of its structural changes upon interaction with GA. Recombinant pLEICS-01 vectors containing the target gene were transformed into E coli BL21. Expression of recombinant protein was analyzed by SDS-PAGE. The protein was purified by affinity chromatography and the protein sample was dialyzed and then used to assess its interaction with both wild type and mutant SH2 domains of PLC, using PAGE. Chemical denaturation and intrinsic fluorescence spectra of the purified proteins were carried out by adding different concentrations of GA. Results, using the PAGE approach, confirmed that the purified protein was in active state. The intrinsic fluorescence assessment of kinase domain in the presence of gallic acid shows an increase in the intensity and maximum emission wavelength. According to the obtained results, the recombinant kinase domain of FGFR2b (38 kDa) was expressed, solubilized, purified and confirmed that it was in active state. The tertiary structural change of kinase domain reflects a conformational alteration within the protein that is important for the biological function of FGFR2b.

Key words: Fibroblast growth factor receptor; Protein purification; Kinase domain; Gallic acid; Fluorescence spectroscopy

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Novel protein based bionanocomposites on the basis of egg white and nanoclay

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ABSTRACT

Exfoliated egg white /nanoclay bionanocomposites were prepared with different loadings of Na-montmorillonite (Na-MMT) i.e. 0, 5, 10, 15 and 20 wt % by mixing known value of egg white with Na-MMT suspension. Some important properties of the prepared bionanocomposites such as morphology, gel fraction, swelling behavior and thermal properties were investigated. The X-ray diffraction method showed that prepared bionanocomposites have exfoliated structure. This structure showed that protein chains have been diffused into Na-MMT layers and the layers disarranged. The swelling measurement showed that swelling ratio has inverse dependence on the nanoclay loading level in the bionanocomposites. It was also shown that the nanoclay acts as crosslinker in the bionanocomposites so the gel fraction values are increased by increasing the nanoclay loading level. The DSC results showed that the denaturation and degradation transition temperatures of egg white are increased by increasing the nanoclay loading level because nanoclay acts as a crosslinker in the bionanocomposites.

Key words: Bionanocomposite; Egg white; Nanoclay; Na-Montmorillonite

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Modeling and theoretical studying of the DnaB helicase in the transcription process

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ABSTRACT

In most living organisms, the preparation of the genomic DNA for replication requires the unwinding of the parent double-stranded DNA (dsDNA) into single-stranded DNA (ssDNA) templates for the replication machinery, a job that is performed by helicases. Most replicative helicases are ring-shaped enzymes that couple the energy release of nucleotide triphosphate hydrolysis into ssDNA translocation and, by extension, dsDNA unwinding. The objective of this study was to perform modeling and theoretical evaluation of the DnaB Helicase in the transcription process. Considering the diversity of the helicases, we focused on the DnaB helicase. The DnaB hexamer (DnaB6) is the bacterial replicative DNA helicase that unwinds doublestranded DNA (dsDNA) ahead of the replication fork and provides single-stranded DNA (ssDNA) templates for the DNA polymerase III holoenzyme. This helicase has 5' to 3' polarity. The subunits of DnaB6 are arranged in a right-handed spiral staircase conformation and are bound to two nucleotides of an A-form ssDNA per subunit. DnaB6 and other SF4 helicases may utilize a hand-over-hand translocation mechanism in which sequential hydrolysis of NTP is coupled to 5’ to 3’ translocation of the subunits with a step size of two nucleotides. In this paper the efficacy of these mechanisms are studied by using modeling and Monte-Carlo simulation, to moves DnaB along the DNA and to open its screws. Comparing the data from simulation and experiment, we are tried to obtain important simulation parameters. In addition the effects of the DNA sequence on the helicase activity are studied.

Key words: ssDNA; DnaB6; NTP; Hand-over-hand mechanism; Monte-Carlo simulation

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A study on magnetic core-bilayer shell nanoparticle: a novel vehicle for entrapment of drugs

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ABSTRACT

Magnetic nanoparticles can potentially be used in drug delivery systems and for hyperthermia therapy. Nanoparticles have been widely used for a variety of biomedical applications and there is a growing need for their highly specific and efficient uptake into target cells. In this work, Fe_3O_4 nanoparticles having a relatively uniform size distribution were synthesized by co-precipitation method. Then, surface modifications have been carried out by Maleic anhydride-methyl acrylate (MAN-MA). The Doxorubicin (DOX) drug was then loaded to the modified magnetic nanoparticles. The structural, morphological and magnetic properties of the prepared sample were characterized by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectra and scanning electron microscopy/energy dispersive X-ray analysis (SEM-EDAX). Also, magnetic measurements were investigated using vibrating sample magnetometer (VSM). We demonstrate that the DOX is attached to the nanoparticles surface and the binding of DOX to the nanoparticles was confirmed by FT-IR analysis. This novel magnetic nano complex might be suitable for use as an efficient drug delivery vehicle with tunable drug released properties.

Key words: Magnetic core; Bilayer shell; Nanoparticle; Novel vehicle; Entrapment of drugs

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Doxorubicin-loaded bilayer-surface magnetite nanoparticle: a novel vehicle for entrapment of drugs

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ABSTRACT

In the past few decades, Magnetite (Fe_3O_4) nanoparticles have attracted growing research interest as these materials have many applications in medicine and drug delivery. Coated magnetic particles, called carriers are very useful for delivering chemotherapeutic drugs. We are herein reporting a synthesis of doxorubicin-loaded bilayer-surface magnetite nanoparticles. The particles were first stabilized with Stearic acid as a primary surfactant, followed by Maleic anhydride-methyl acrylate (MAN-MA) copolymer as a secondary surfactant to form nanoparticles with hydrophobic inner shell and hydrophilic corona. The Doxorubicin (DOX) drug was then loaded to modified magnetic nanoparticles. The structural, morphological and magnetic properties of as-prepared sample were characterization by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectra and scanning electron microscopy/energy dispersive X-ray analysis (SEM-EDAX). The magnetic measurements were investigated, using vibrating sample magnetometer (VSM).The particles were exhibited superparamagnetic behavior at room temperature with saturation magnetization (Ms) about 50 emu/g magnetite. We demonstrate that the drug DOX is attached to the nanoparticles surface and the binding of DOX to the nanoparticles was confirmed by FT-IR analysis. The present finding show that DOX loaded nanoparticles coated by copolymer are promising for magnetically targeted drug delivery.

Key words: Doxorubicin; Loaded bilayer; Magnetite nanoparticle; Novel vehicle; Entrapment of drugs

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In silico study of the interaction between four newly synthesized platinum complexes and human serum albumin

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ABSTRACT

Molecular docking which provides useful information on drug-receptor interactions has been frequently used to predict the binding orientation of small molecules to their protein targets. In the current study molecular docking was performed to understand the binding site and the mode of interactions between human serum albumin (HSA) and four newly synthesis Pt complexes, including two platinum (II) complexes with non-leaving lipophilic ligands; deprotonated 2-phenylpyridine (ppy): $\mathbf{C_1}$ and deprotonated benzo [h]quinolone (bhq): $\mathbf{C_2}$ and two Pt (IV) complexes with the general formula [Pt(X)2Me2 (tbu2bpy)], where tbu2bpy = 4,4′-ditert-butyl-2,2′-bipyridine, with two leaving groups of X = Cl : $\mathbf{C_3}$ or Br : $\mathbf{C_4}$. The crystal structure of HSA was taken from protein data bank (PDB). The molecular docking of the Pt complexes with HSA was calculated by Molegro Virtual Docker (MVD) software. The potent binding sites with expanded Van der Waals surfaces which known as cavities were nominated to extend the grids over the probable binding sites. At a grid resolution of 0.30 Å, the MolDock scoring functions were adjusted as to give 30 final poses. Each pose suggests the best binding conformation, energy and binding site of the drug into HSA in a cycle of runs. The results of *in silico* molecular docking study suggest the involvement of π-π stacking and hydrophobic interactions between HSA and Pt (II) complexes. Also, these results confirm the existence of steric effects and hydrogen bonding interaction between albumin and Pt (IV) complexes. Moreover, the interaction of synthetic Pt complexes occurs in the area between sub-domains IIA and IB.

Key words: Human serum albumin; Pt complexes; Molecular docking; Molegro Virtual Docker (MVD)

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Antidiabetic pyrimidine-fused heterocyclic compounds as low risk inhibitors for α-amylase and α-glucosidase

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ABSTRACT

Diabetes Mellitus (DM) is a global health crisis which characterized by chronic elevation of blood glucose level. Pancreatic α-amylase (α-Amy) and intestinal α-glucosidase (α-Gls) play a significant role in the digestion of complex carbohydrate into absorbable monosaccharides. The inhibition of α-Amy and α-Gls has been already indicated to play an important function in prevention of postprandial hyperglycemia in diabetic patients. The currently in use inhibitors of these enzymes are associated with development of important gastrointestinal complications which arise from their strong inhibitory action against α-Amy. To avoid such complications, low-to-moderate inhibition of α-Amy and strong inhibition of α-Gls are desirable. In continuation of our previous works, in the current study, numbers of pyrimidine-fused heterocycle (PFH) compounds were synthesized and their inhibitory actions against Yeast/Mouse α-Gls and pancreatic α-Amy were evaluated, spectroscopically. Our studies suggest PFH ring as an important molecular scaffold in designing of antidiabetic compounds with desirable inhibitory action against α-Amy and α-Gls. The PFH containing inhibitor molecules may open a new window to the anti-diabetic drugs with low potential for development of gastrointestinal side effects.

Key words: α-Glucosidase; α-Amylase; Inhibition; Pyrimidine fused heterocycle (PFH); Gastrointestinal side effects

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Determination of serum albumin by time-insensitive colorimetric sensor

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ABSTRACT

Serum albumins, as the major soluble protein constituents of the circulatory system, have many physiological functions. Quantification of albumin is clinically important, especially in low concentration is of great significance and is routinely measured by UV spectroscopy. However, these methods tends to overestimation, and more critically, depends highly on the timing, protein variation and suffer from interference in the measurements. Here, to overcome these difficulties, we report a simple, time-insensitive and superior sensitive method based on a binding of a new cationic cyanine dye with BSA under a spectrophotometric and image analysis technique that can be used as a sensor to quantify BSA. This method is more convenient because it allows for UV spectroscopy measurements during a >25 min period. Optimum conditions for the determination of BSA were also investigated by experimental design method. The linear ranges for BSA are 22.00 -112.00 nM and 0.43-4.10 μM and limits of detection is 6.25 nM. The relative standard deviation of six replicate measurements was 2.33% for 2.50 μM BSA. This method is simple, practical, and relatively free interference from coexisting substances and can be successfully applied to determination of HSA in urine sample.

Key words: Serum albumin; Cationic cyanine dye; Spectroscopy

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Study of 2-mercaptobenzoic acid and 2-pyridinethiol as inhibitors on the cresolase and catecholase reactions of mushroom tyrosinase

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ABSTRACT

Catecholase and cresolase activities of mushroom tyrosinase (MT) were studied in the presence of 2-mercaptobenzoic acid (thiosalicylasaure) and 2-pyridinethiol inhibitions. Caffeic acid and p-coumaric acid were used as natural substrate for the enzyme for the catecolase and cresolase reactions, respectively. The catecholase and cresolase activities of MT in the presence of 2-pyridinethiol as inhibitor achieved in the concentrations of (1.5, 3, 4.5 and 6 μM). In addition, the cresolase and catecholase activities of MT in the presence of 2-mercaptobenzoic acid used in presence of (2.5, 5, 10 and 15 μM). The inhibition constant (Ki) values of 2-pyridinethiol obtained 0.84 and 5.37 μM for catecholase and cresolase reactions, respectively, with noncompetitive pattern. But for the 2-mercaptobenzoic acid revealed a competitive mode of inhibition with the inhibition constants of 5.45 and 9.35 μM, for catecholase and cresolase reactions, respectively. Thus, the results showed that the carboxyl and sulfydryl functional group of these organosulfur compounds play a crucial role in the inhibition of MT. Their Ki values showed that they are among the good inhibitors of enzyme.

Key words: Mushroom tyrosinase; 2-mercaptobenzoic acid; 2-Pyridinethiol; Inhibition; Sulfur

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Contact finder: a web server for identifying amino acids involved in the connection of subunits in multi-subunit proteins

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ABSTRACT

Subunit interaction is an important phenomenon for protein optimally functions. Their final structures are mostly the result of clustering of several individual peptide chains. A variety of interactions including hydrogen bonds, salt bridges and disulfide bonds hold the various chains into a particular geometry. In fact, these bonds between the amino acids of polypeptide chains have significant influence on the topography of protein quaternary structure. Thus, the nature of these amino acids is considerable in protein engineering. Thereby manipulating them can help the biologists to improve the stability of the final structure. *CFinder* is web based software that has been designed to facilitate the processes of identifying amino acids which are in contact with a specified polypeptide chain. *CFinder* as an interactive interface assists users by determining the amino acids which are associated to generate a complex multi-subunit shape.User can enter the input information by either uploading PDB file or entering PDB code and the desired chain name. *CFinder* calculates the accessible surface area of each amino acid in two states: the complete protein and the desired chain alone. The amino acids which show different accessible surface area in these two states are the output results. *CFinder* is freely available at URL:http://bioinf.modares.ac.ir/software/cfinder

Key words: Multi-subunit Proteins; Contact amino acids; Accessible surface area

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The effect of ethephon on the release of proteins from the cells of barley aleurone layer

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ABSTRACT

The aleurone layer of cereal seeds plays a key role in germination, responding to a gibberellic acid (GA) signal by synthesizing hydrolytic enzymes that are released to the endosperm. The barley (*Hordeum vulgare*) aleurone layer can be separated from the other seed tissues and maintained in culture, allowing the study hormones and other signals in an isolated system. In the present study in order to study the effects of ethephon on the release of α-amylase and limit dextrinase (LD), the isolated aleurone layers were treated in gibberellic acid (GA) for 24 h and then incubated in different amounts of ethphon for 12 h. After extraction of total soluble proteins from these treated aleurone layer as well as control, the spatio-temporal of α-amylase and limit dextrinase (LD) in aleurone layer and medium were analyzed using western blotting. The results showed that the amount of total released proteins in the medium was increased with the addition of more ethephon. Moreover the western blotting showed that ethephon can induce the release of amylase and LD from aleurone layer.

Key words: Aleuron layer; Ethephon; Protein release

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Study on the interaction of vitamin D_3 with insulin

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ABSTRACT

Insulin is a peptide hormone that exists in the blood and it regulates the metabolism of carbohydrates by promoting of glucose absorption from blood to skeletal and fat tissues. Human insulin is a peptide containing 51 amino acids in two chains and three disulfide bonds. Cholecalciferol is a form of vitamin D, also called vitamin D3 that it exists in blood stream as well. It is structurally similar to steroids such as testosterone, cholesterol, and cortisol. Both of insulin and cholecalciferol have hydrophobic core in their structure. Researchers believe that vitamins are key factors for treatment of some disease such as diabetes. There are several methods for analysis of protein and ligand interaction, such as fluorescence and UV-Visible spectroscopy, circular dichroism and calorimetric instruments. Fluorescence intensity measurements were carried out on Varian spectrofluorimeter, with an excitation wavelength of 276 nm. A strong fluorescence quenching interaction of vitamin D_3 and human insulin was observed. The quenching constant was determined, using the modified Stern-Volmer equation. The calculated binding constant of vitamin D_3 and insulin was 3×10^5 M^{-1} and the corresponding average number of binding sites was 1.28. This study shows that vitamin D_3 can be bound to human insulin and quenches the fluorescence spectra of this protein. The structural change of insulin due to the binding of vitamin D_3 was also investigated by circular dichroism.

Key words: Insulin; Cholecalciferol; Vitamin D; Diabetes

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An investigation on the effect of ionic liquids on stability and hydrolytic activity of the *Thermoanaerobacter thermohydrosulfuricus* lipase

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ABSTRACT

Various investigations on ionic liquids (ILs), as green alternatives to organic solvents, have shown positive effects on activity and stability of biocatalysors in biotransformations, especially in the case of lipases. *Thermoanaerobacter thermohydrosulfuricus* lipase (Ttl) is a thermotolerant enzyme which is highly enantioselective for secondary alcohols in resolution of racemic mixtures. In the present work, the effect of various alkyl chain lengths of C_nMIM Br (n= 2, 4, 6) on the lipase activity was examined and the optimum concentrations were determined. The results revealed that Ttl had maximum activity when using 0.3, 1, and 0.3 M of C_2MIM Br, C_4MIM Br, and C_6MIM Br, respectively. Furthermore, thermostability of the lipase in the presence of C_4 alkyl chain of ILs having Br and PF_6 as anions at 82, 85, and 90 °C showed an elevated structural stability of the enzyme and more than 50% of its initial activity retained after 45 min incubation. According to present findings, it could be concluded that the Ttl enzyme can be used for several biotransformations using ionic liquids as solvent.

Key words: Ionic liquids; Lipase; Enzyme activity; Thermostability

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Investigation on the formulation and stability of recombinant human granulocyte-colony-stimulating factor

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ABSTRACT

Granulocyte-colony-stimulating factor (G-CSF) is a glycoprotein consisting of 174 amino acid residues with a molecular weight of 19.6 kDa. G-CSF, produced mainly by macrophages, induces proliferation of neutrophil colonies and differentiation of precursor cells to neutrophils, and stimulates the activity of mature neutrophils. Recombinant human granulocyte-colony stimulating factor (rh-G-CSF) is a therapeutic unglycolysated protein is produced in E.coli in our institute and used primarily to reduce incidence and duration of severe neutropenia and its associated. The stability of recombinant proteins has become an increasingly important consideration as more protein therapeutics is developed. In this study, the purified rh-G-CSF was characterized by following the changes on the structure, purity, dimerization and aggregations of protein in time of 0-6 months and two temperatures (4 and 25 °C) by using biochemical techniques including reverse-phase chromatography (RPC), size-exclusion chromatography (SEC), electrophoresis and circular dichroism. The results were compared with Neupogen filgrastim as a reference standard. According to the inspection chromatogram, obtained peak conforms to molecular weight of rh-G-CSF without any aggregation forms in the protein structure (less than 1%) and disulfide bonds are in correct position. RPC results showed the similar hydrophobicity for rh-G-CSF and reference standard. CD results showed the same secondary and tertiary structure of G-CSF and reference standard in addition to the fact that the G-CSF secondary structure is predominantly helical.

Key words: Rh-G-CSF; Stability; SDS-PAGE Electrophoresis; Circular dichroism; Chromatography

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Differential expression of proteins in resistant and susceptible wheat cultivar during early stage of infection by *Fusarium graminearum*

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ABSTRACT

Fusarium head blight caused by *Fusarium graminearum* is one of the most destructive disease of wheat (*Triticum aestivum*) in the world. Importance of this disease is due to yield reduction and contamination of grains to mycotoxins which is harmful for both human and animal. The mechanisms underlying resistance to Fusarium – caused disease are still unknown. In the present study, we employed proteomic approach to investigate differential profile of protein responding to *F. graminearum* infection in two genotypes of wheat; Falat as a highly susceptible and Sumai3 as a resistant cultivar. Proteins extracted from spike 4 days after inoculation were separated by 2-DE. About 800 protein spots were displayed on 2-D gels stained with Coomassie Brilliant Blue (CBB). Induced protein spots were detected and analyzed with TOF-MS/MS. Many of identified proteins were related to carbon metabolism, photosynthesis and stress defense of plants. Our results illustrated that expression of proteins related to defense response such as superoxide dismutase, catalase and glutathione s-transferase were significantly up regulated in the resistant, whereas their expression decreased in the susceptible cultivar. These results showed that the ability of wheat plant in management of metabolic pathways and induction of defense proteins is crucial for offering resistance to FHB.

Key words: Fusarium head blight; Wheat; Resistance; Metabolism; Defense proteins

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Innovative position of aptamer in the protein world

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ABSTRACT

Aptamers are single stranded oligonucleotides (ssDNA/RNA) or peptide molecules that can specifically and with a high affinity bind to their targets due to their particular 3D structures. Nowadays, Aptamers found their paths as bio-affinity ligands in numerous investigations such as diagnostic and therapeutic tools, biosensors, development of new drug candidate or drug delivery systems, and also, in basic research areas such as target validation. Aptamers are widely known as potential substitutes for antibodies because of their higher stability, low immunogenicity, and more diversity in targets, ease of production and chemical modification and also conjugation with fluorescence or radio labels. Proteins are the most applicable and promising targets for oligonucleotide aptramers selected by SELEX or non-SELEX technology and various specific aptamers have been designed for plasma proteins like coagulation factors, hormones, or specific surface receptors or antigens, etc. Protein specific aptamers could cover almost all applications of protein specific monoclonal antibodies. Up to now, these aptamers have been applied in various analytical researches for designing of biosensors specially label free electro-sensors, also, in clinical application such as treatment of different cancers or inflammatory diseases or as a targeting drug delivery system. Aptamers have particular potentials for in-vivo imaging due to the ease of fluorescent or radio labeling and diverse systematic distribution. In addition, they could be used in basic research for the discovery of novel drug targets or biomarkers. Aptamers, during two decays, have opened a new promising window in various fields of researches and could be integrated into a variety of assays, basic research and clinical applications.

Key words: Protein; Aptamer; Clinical; Analytical; Basic research

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Antimicrobial properties of bioactive peptides derived from trypsin and ficin treated ovotransferrin

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ABSTRACT

In recent years, increased interest is seen among scientist and pharmaceutical industries to replace the synthetic antibiotics with antimicrobial agents that are perceived as "natural". Currently, peptides with known sequences have been identified which exhibit antimicrobial effects. These peptides are produced *in vitro* and *in vivo* by proteolysis of naturally occurring parent proteins. Ovotransferrin (OT) is an egg white protein with iron binding properties. The purpose of this research was to isolate, purify and characterize novel antimicrobial peptides from ficin-and trypsin-treated OT. OT was purified from chicken egg white by ammonium sulfate precipitation followed by DEAE-Sephadex ion exchange chromatography and subjected to treatment with different concentrations of ficin and trypsin. The produced peptides were separated by Sephadex G-50 gel filtration chromatography. The antimicrobial effects of selected peptides against *Staphylococcus aureus* (G^+) and *Salmonella typhimurium* (G^-) was evaluated. Results showed that purified peptides decreased the number of *S. aureus* and *S. typhimurium* by 97% and 28% respectively. Taken together, the results of this study suggest that it is possible to substitute the antimicrobial agents of chemical origin with the peptides which are obtained from OT.

Key words: Ovotransferrin; Bioactive peptides; Antimicrobial; *S. aureus*; *S. typhimurium*

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Poster – P222 Open Access

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Stimulatory effect of potassium sorbate on human serum albumin glycation products: carbonyl contents detection

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ABSTRACT

The glycation process of protein as a cascade reaction is usually triggered by protein oxidation via glucose. The results of this destructive process are the formation of main oxidative toxins which have stimulatory effects on the process in turn, interact with other proteins and intensification of devastating products. The main products also are following by the production of active oxidative ingredients which stimulate this process more. Dicarbonyl contents as one of the most important toxins are produced in the middle of glycation process. These kinds of compounds have higher chemical activity than glucose and can improve protein glycation process even at low concentrations. The detection of carbonyl compounds amounts resulting from human serum albumin oxidation via oxidative preservative in present and absent of glucose is our aim in this study. For this purpose, the effect of potassium sorbate as an industrial preservative on human serum albumin after 21 days of treatment in the presence and absence of glucose was studied using absorption spectroscopy. The results indicated that potassium sorbate has an obvious role in the production of carbonyl contents as a glycation products intermediates. Also it should be noted that there were the highest amount of carbonyl contents in human serum albumin samples which are treated simultaneously with potassium sorbate and glucose and the synergistic effect between these two oxidative matters.

Key words: Human serum albumin; Glycation; Oxidative toxins; Potassium sorbate; Carbonyl contents

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Design and construction of gene encoding fusion functional domain of activin A: a model bond study

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ABSTRACT

Nowadays there is a growing interest in the general field of peptide growth factors, their importance in embryo culture media and the role of these factors in the control of preimplantation embryo development process. Activin is one of the growth factors that its subunits and receptor mRNAs are expressed throughout embryo development. It plays important roles not only for embryonic development but also for implantation. Activin-A is a member of the transforming growth factor-β (TGFβ) super family, formed by disulfide linkage of two β-subunits, resulting in either activin-A (βAβA). Subunits βA is product of some processing stages from larger precursor protein that consists of a 290 amino acid pro-region and 116 amino acid C-terminus and consensus furin motif (RRRR). This motif of the β-subunit is cleaved by furin and created functional domain of activin-A. In addition activin has strictly conserved Cys residues in the mature region, all of the nine conserved Cys residues in the mature part of the activin βA-subunit are essential for either the biosynthesis or biological activity of activin-A. In this study, we have designed and constructed a gene encoding the functional domain of activin-A which is linked to a collagen like fragment sequences.Three-dimensional structure of fusion gene is predicted by phyre2 and raptorx servers. The disulfide bonding state of Cys residues is predicted by DIANA, DBCP servers. These gene segments are connected by a SOEing PCR for future applications.

Key words: Fusion protein; Activin A-collagen

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Inhibitory effects of quercetin and kaempferol as two propolis derived flavonoids on tyrosinase

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ABSTRACT

Tyrosinase is a copper-containing enzyme, which is widely distributed in microorganisms, animals and plants. It is also a key enzyme in melanin biosynthesis, which plays a crucial role in determining the color of mammalian skin and hair. In this work, the effects of quercetin and kaempferol as propolis-derived compounds on activity of mushroom tyrosinase (MT) were studied. These flavonoids induced inhibition on the catecholase and cresolase reactions of the enzyme with using of caffeic acid and p-comaric acid as substrates, respectively. The inhibition mode of quercetin and kaempferol were competitive towards both catecholase and cresolase activities of the enzyme. The inhibition constants (Ki) of quercetin and kaempferol were determined as 0.072 and 0.112 mM for catecholase, and 0.016 and 0.06 mM for cresolase activities, respectively. In general, quercetin and kaempferol can be used as good candidates in melanogenesis inhibition. Moreover, they should be considered as good blockers of enzyme activity in hyperpigmentation and clinical applications.

Key words: Mushroom tyrosinase; Inhibition; Quercetin; Kaempferol

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Poster – P225 Open Access

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Co-expression of artemin with firefly luciferase in *E. coli* for real-time monitoring of preventing cold induced aggregation by artemin and its chaperone activity *in vivo*

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ABSTRACT

Artemin is an abundant thermostable protein in *Artemia* encysted embryos under severe environmental stresses. Our previous studies on transformed *E. coli* cells by artemin isolated from *Artemia urmiana* showed a thermotolerance enhancement. Based on this, it has been predicted that artemin can also act in other conditions such as oxidative, salinity and cold stresses. In this study, the possible role of artemin in cold protection of bacterial cells was confirmed by using the growth and survival rate determination of the cells and measuring luciferase activity as a reporter. Luciferase activity during cold stress showed that the presence of artemin can increase the activity of this enzyme as a model protein which can be due to the artemin role in preventing cold denaturation. It was shown that about 35% of the luciferase activity can be remained after 6 days but this amount decreased to 20% in the 8th day, whereas in the non-producing artemin cells, the activity is about 5%. Also the effects of low temperature on the bacterial cells viability were evaluated by measuring their growth rate during 10 days. Cell viability decreased rapidly under cold stress (0 °C) conditions within 5 days, but *E. coli* expressing artemin exhibited higher survival rate in comparison with the non-producing ones. These results showed that artemin could enhance *E. coli* transformed cells' viability under cold stress. These results suggest that artemin co-expression can suppress luciferase aggregation formation, thus it can be utilized for long-term storage of variety of proteins prone to cold aggregation in *E. coli*.

Key words: Artemin; *In vivo*; Chaperone activity; Luciferase activity; Co-expression

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Essential amino acids for the stability of *Bacillus licheniformis* alpha amylae as predicted by CUPSAT Server

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ABSTRACT

Amylases are enzymes which hydrolyze starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units. Alpha amylase are available from different sources and they have extensive commercial applications in starch processing, brewing and sugar production. An online server CUPSAT (http://cupsat.tu-bs.de/) is employed in the present study. The protein (PDB ID: 1BLI) used for the current stability prediction has been taken from the PDB database (http://www.rcsb.org/pdb/). The server calculates the overall stability change using the atom and torsion angle potentials and returns comprehensive stability and predicted ΔΔG (kcal/mol) information for all the 3 possible substitutions (N190F, Q264S, N265Y). Multiple sequence alignment of *Bacillus licheniformis* alpha-amylase and its homologous 1HVX (*Bacillus Stearothermophilus*), 3BH4 (*Bacillus amyloliquefaciens*), 2GUY (*Aspergillus niger*), and 1UA7 (*Bacillus Subtilis* was performed using the Clustal Omega programme available at European Bioinformatics Institute (http://www 2. Ebi .ac .uk /clustalOmega/). Overall stability is calculated from atom potentials and torsion angle potentials. In case of unfavourable torsion angles, the atom potentials may have higher impact on stability which results in a stabilising mutation. The results showed that mutations in N190F with 77.68% solvent accessibility and -78.70, -26.90 torsion angles (φ, ψ) does not show instability, while Q264S with 13.65% solvent accessibility and -151.7o, 138.8o torsion angles (φ, ψ) is highly unstable. This study bodes well as a forerunner for mutation and protein engineering studies of *Bacillus licheniformis* alpha amylase.

Key words: Alpha amylases; CUPSAT server; Stability; ΔΔG

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Prediction of silver nanoparticles interaction with L- lactate dehydrogenase (LDH1) in *Toxoplasma gondii*

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ABSTRACT

The aim of present study was to study bioinformatic interaction of silver nanoparticles (Ag NPs) and L- Lactate Dehydrogenase enzyme *in Toxoplasma gondii* to determine harmful effect of silver nanoparticle on the enzyme. In this study we used Metal Detector Predicts v2.0 software, DiANNA 1.1 web server, Molecular docking web server and 3D Ligand Site server, to analyze enzyme structure and Ag NPs inhibitory effect on its function. The results obtained from docking showed that free energy of binding from docking score for L- Lactate Dehydrogenase was -3.83 kcal/mol and inhibition constant (Ki) equal to 1.56 mM. Ag NPs may facilitate the interaction with Cysteine in 10 positions of enzyme. Ag NPs naturally interact with the membrane of pathogens and disrupt the membrane integrity, and silver ions bind to sulfur, oxygen, and nitrogen of essential biological molecules and inhibit pathogens growth.

Key words: Silver nanoparticles (Ag NPs); Lactate dehydrogenase; *Toxoplasma gondii;* Molecular mechanism

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Design, construction and characterization of A BTEX biosensore

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ABSTRACT

Now a day Water pollution is one of the most important problems for human beings. Among the pollutants BTEX (Benzene, Toluene, Ethylbenzene and Xylenes) have vast application in industry and their carcinogenic effect on human body has approved also by health care organizations. Hence these part of water pollutants (water-soluble aromatic components) have more importance. Monitoring systems that can detect presence of BTEX in water supplies are much expensive such as gas chromatography and HPLC so we need simple systems for infield sampling to reduce the number of samples that we are suspected to them for more analysis by much more expensive systems. Bioreporters are a subgroup of biosensors which are using for sensing and monitoring some signals or reagents. A bioreporter is an organism like a bacteria or a plant that is genetically manipulated to have a promoter which is sensitive to a chemical or physical signal. In the presence of the signal the promoter would be activated and there is a reporter gene downstream of promoter. Activation of the promoter leads to product of the reporter gene which can be sensed or calculated by our laboratory supplies. We have used a green fluorescent protein gene as a reporter gene downstream of *PtbuA1* as a BTEX sensitive promoter in Escherichia coli bl21 strain and its response to BTEX has been investigated. Our results show our bioreporter can sense BTEX in a range of BTEX concentration and the optimum time and temperature for the bioreporter is also defined. Our data shows a mixture of the BTEX has an additive effect on the bioreporter response.

Key words: Bioreporter; Biosensor; BTEX

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In silico prediction of exposed amino acid sequences of *H. Pylori* outer membrane proteins

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ABSTRACT

There are several outer membrane proteins (OMPs) of *H. pylori* which attach to corresponding receptors on the host gastric epithelium. Exposed amino acid sequences of outer membrane proteins have important role in interaction with stomach epithelial cell and developing of gastritis, peptic ulcer and gastric adenocarcinoma. Therefore, detection of these sequences is very useful for locating of corresponding receptors on the surface of gastric epithelial cell. We have systematically analyzed exposed amino acid sequences for detection of adhesion motifs and possible receptors. Secondary and tertiary structures of membrane proteins including *OipA* , *BabA, BabB, AlpA* , *AlpB, vacA, SabA* and *SabB* predicted by PHD and GOR 4 servers. The presence and location of signal peptide cleavage sites in amino acids sequences were predicted by SignalP 4.1 Server. PHYRE server was applied for finding of homology modeling. VADAR server was applied for prediction of accessible surface area amino acids among sequences of external loops. Based on our analysis, exposed amino acid sequences which detected among various outer membrane proteins are including *oipa*, *BabA*, *BabB*, *Alpa, AlpB, VacA*, *SabA* and *SabB* with 6, 4, 2, 5, 4, 7, 5 and 6 sequences of amino acids, respectively. Structural similarity between proteins is a very good predictor of functional similarity. Higher similarity was found between two pair of *AlpA-AlpB* and *SabA-SabB* proteins. This structural similarity may be helpful to design new drugs or vaccines with simultaneous interaction against such target sites. Taken together, based on our findings detection of these sequences will be very useful for specific targeting of cell surface receptors.

Key words: *Helicobacter pylori*; Outer membrane proteins; *In silico*

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Molecular docking study of the interaction between poly-hydroxyl functionalized acridine derivatives and β-Lactoglobulin

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ABSTRACT

Development of successful medication is a complicated process. Recently, poly-hydroxyl functionalize acridine (PHFAs) revealed inhibitory properties against α-glucosidase which is an exoenzyme present in the brush-border of the small intestine. This enzyme catalyzes the final steps in the digestive process of carbohydrates and hence its inhibitors blunt postprandial hyperglycemia which is a prominent and early defect in diabetes. Regarding to the needs for secure delivery and protection against acidic environment of stomach, it is essential for α-Gls inhibitors to be delivered safely to the small intestine where they exert their pharmaceutical activities. Beta-lactoglobulin (β-LG) seems a realistic protein candidate for safe delivery and protection of particularly pH sensitive drugs in stomach. In the present study, the interaction between whey carrier protein β-LG and PHFAs (**L_1** and **L_2**) was investigated by molecular docking simulation, using Autodock-vina. The results showed that binding of these compounds to β-LG was spontaneous. Collectively, hydrophobic interactions and hydrogen bonding most likely play major roles in β-LG-PHFAs binding. Our funding revealed that the binding sites for **L_1** on β-LG mainly located within the protein's hydrophobic cavity and **L_2** bounded to the surface cleft located in a groove between the helix and the barrel. Based on the achieved results, these potentially therapeutic agents can significantly bind to β-LG. Consequently, this protein might be useful for delivery of PHFA ligands to small intestine where representing their potential ability to inhibit α-Gls.

Key Words: Beta-lactoglobulin; Poly-hydroxyl functionalized acridine; Molecular docking simulation

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Expression and localization of septin 14 protein in testis tissue of azoospermic men

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ABSTRACT

Septins belong to a family of GTP binding proteins that are recognized as novel components of cytoskeleton. Disruption of septin functions has been implicated in the pathology of many diseases, including male infertility. The aim of present study was to assess the expression pattern of septin14 which is specially expressed in the testis. Testicular tissues (N=30) were obtained from biopsies of azoospermic men and subdivided into three groups (complete spermatogenesis, maturation arrest and sertoli cell only) according to the pathology report. Protein expression was evaluated by immunohistochemistry using secondary antibody conjugated with HRP and Real-time PCR. Septin 14 expression became evident in spermatogonia, spermatocytes, spermatid and leydig cells in control and maturation arrest tissues but it was not detected in sertoli cells. After normalizing the relative amount of septin 14 by the amount of β-actin transcript in the same sample, it was shown that protein level in samples with complete spermatogenesis was significantly higher than two other gorups. This is the first report on the localization of septin 14 in human testis. It was indicated that septin 14 is expressed in all germ cells and leydig cells and deficiency of this protein could be related with spermatogenic failure.

Key words: Septin14; Expression; Immunohistochemistry; Male infertility

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Enhancement of total protein content and amino acid accumulation of two *Salvia* species under moderate salinity

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ABSTRACT

Salvia mirzayanii and *Salvia macrosiphon* are endemic medicinal plants in Iran, belonging to Lamiaceae family, which has many pharmaceutical properties. In order to meet the ever increasing demand of medicinal plants, for pharmaceutical industry, some medicinal plants such as *Salvia* species need to be cultivated commercially. However, soil salinity and other forms of pollutants pose serious threats to medicinal plant production. There is therefore a need to determine the underlying biochemical mechanisms of salinity tolerance so as to provide plant breeders with appropriate indicators. Because of the plants ability to tolerate salts is determined by multiple biochemical pathways include those that lead to synthesis of osmotically active metabolites and specific proteins, therefore, in the present study, the effects of different concentrations (0, 25, 50, 75 mM) of sodium chloride (NaCl) on protein contents and proline accumulation of these valuable plants were investigated. Our results showed an increase in leaves and roots proline accumulation and protein contents with the increase in salt concentration. However, the increase in protein contents were more pronounced under moderate salinity. Finally, we concluded that some proteins and amino acids which are well-defined indicators could be used to facilitate the improvement of salinity tolerance in these valuable medicinal plants through breeding strategies.

Key words: Salt stress; Proline; Protein; Medicinal plants

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Conformational changes of human insulin upon interaction with DEHP as A plasticizer

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ABSTRACT

Phthalates are synthetic plasticizers widely used in plastics and other common consumer products, such as food packaging, toys, cosmetics, clothing, and biomedical devices. Di (2-ethylhexyl) phthalate (DEHP) is one of the most common phthalates. DEHP is not covalently bound to polymers in plastic. Thus, it leaches out of products and gets into the environment, making it a wide spread environmental contaminant. Humans are exposed to DEHP predominantly via contaminated food or beverages. The human toxicity of DEHP continues to be a subject of intense debate between public health advocates, researchers and the industry because there are essentially no studies on the health effects of DEHP in humans. Once DEHP gets into the gastrointestinal tract, it is quickly absorbed to blood. In this study fluorescence spectroscopic method is used to examine the effect of different concentrations of DEHP on insulin. Results show that the tertiary structure of insulin was changed by increasing the DEHP concentration. It can be concluded that DEHP may increase the risk of diabetes mellitus due to inducing conformational changes in insulin and may increase the advanced glycation end products (AGEs) generation.

Key words: DEHP; Insulin; Phthalates; Plasticizer

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Improvement of the organic solvent stability of engineered thermolysin

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ABSTRACT

Proteases are an important class of enzymes with diverse applications in peptide synthesis, protein processing, food, pharmaceutical and detergent industries. Thermolysin-like proteases (TLPs) are neutral proteases and their catalytic activity depend on a zinc ion located in the active site. They bind to different number of calcium ions which are essential for stability of the enzyme. They hydrolyze peptide bonds in aqueous and synthesize them in non-aqueous environments. For peptide synthesis, proteases need to be stable in the presence of organic solvents. Thermolysin is a thermostable metalloprotease generated by gram-positive bacteria *Bacillus thermoproteolyticus*. Considerable efforts have been devoted to the engineering of thermolysin in order to improve the stability and activity of enzyme for industrial applications. In the present study, in order to achieve an aqueous and organic solvent stable thermolysin variant, a chimeric enzyme from thermolysin and *Pseudomonas aeruginosa* elastase were designed and constructed. Then, biochemical properties like thermodynamic parameters and organic solvent stabilities were compared to the native enzyme. Based on the results, $t_{1/2}$ remarkably improved for chimeric enzyme. Besides, significant improvement was observed in the stability of the enzyme in organic media.

Key words: Protein engineering; Organic solvents; Stability; Chimer

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Determination of kinetics parameters of binding of cimetidine to alkaline phosphatase of *Escherichia coli*

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ABSTRACT

Alkaline phosphatase (ALP) is a hydrolytic enzyme responsible for elimination of phosphate group from various substrates such as phospholipids, nucleotides, proteins, and alkaloids. The enzyme needs Zn^{2+} and Mg^{2+} for its reaction. In bacteria, this enzyme does not well generate in the phosphate rich culture medium, while it will be produced by the cell in poor phosphate medium. *Escherichia coli* is a Gram-negative *bacillus*, facultative anaerobic, rod-shaped bacterium. Cimetidine (trade name Tagamet) is an antagonist of histamine H_2 receptor that inhibits the production of gastric acid. *E. coli* was cultured in nutrient broth containing 1% glucose. The cells were harvested and broken by ultrasonic equipment and the supernatant was used for enzyme assay. Lineweaver-Burk plot was used to calculate the kinetics parameters. Our results showed that cimetidine could inhibit ALP by un-competitive manner. Both K_m and V_{max} of the enzyme were changed with increasing the drug concentration. The IC_{50} and K_i of the drug were determined to be about 1.3 mM and 1.81 mM which revealed that cimetidine bound to the enzyme with medium affinity. Maximum activity of enzyme was observed at 40 °C and pH 8.0 in the presence and absence of the drug. No activity was observed at 60 °C. The results showed that change of temperature could not affect the binding of the drug to the enzyme. In conclusion, cimetidine could inhibit ALP of *E. coli* and changed its activity. The change of temperature could not refuse the drug to bind the enzyme.

Key words: Alkaline phosphatase; Enzyme; Inhibition; Cimetidine; *E.coli*

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Binding properties of curcumin-based pyrano [2, 3.D] pyrimidine derivatives to β-lactoglobulin as a carrier protein

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ABSTRACT

β-lactoglobulin (β-Lg) is the major whey protein in many mammalians species which composed of 162 amino acids. This globular protein is a member of lipocalin family, demonstrating high affinity for small hydrophobic molecules. Due to its significant resistance at low pH of stomach, β-Lg is considered as an efficient carrier for safe delivery of drugs through the stomach. In this study, the β-Lg binding properties of four curcumin-based synthetic inhibitors of intestinal alpha-glucosidase were evaluated, using UV-Vis and florescence spectroscopy, as well as, docking simulation. The results of spectroscopic study suggest range of binding affinities for the synthetic inhibitors against β-Lg and hydrophobic interactions were proved to play a significant role in the protein-ligand binding. The structural and thermodynamic analysis demonstrate that all of the synthetic inhibitors have a relatively high affinity for β-Lg. Molecular docking analysis revealed that these ligands bind to the protein calyx (which is a hydrophobic cavity in β-Lg structure) with high affinity. Overall, β-Lg can be considered as a good carrier for possible safe delivery of these potentially anti diabetic compounds through the stomach, as their primary site of action is small intestine.

Key words: Curcumin derivatives; β-lactoglobulin; Binding affinity; Molecular docking

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Study on the role of calcium ion on structure and aggregation properties of eye lens crystallins

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ABSTRACT

The eye lens comprised of three main types of different proteins so called α-, β- and γ-crystallins. These proteins include ninety percent of the total soluble lens proteins (TSPs) which play an important role in the lens transparency. Both native structure and fine short-range ordered packing among these proteins are highly important for their appropriate functions in the lenticular tissues. On the other hand, the homeostasis of calcium ion is important for the lens transparency. The increased calcium level has been indicated in both diabetic- and cataractous eye lenses. In this study, the impact of calcium ion on structure and fibrillation of lens crystallin proteins were studied, using different spectroscopic techniques and gel electrophoresis. The spectroscopic assessments of TSPs in the presence of calcium ion indicated significant protein structural alteration which followed by their aggregation/fibrillation. The formation of large protein aggregates and amyloid like entities play a significant role in the lens opacification and cataract development. The results of this study may highlight the importance of the above mentioned molecular events in the pathomechanism of calcium-induced cataract formation.

Key words: Lens crystallins; Calcium ion; Aggregation; Cataract diseases

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Preparation of supplement for cell culture medium from bovine serum

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ABSTRACT

Cell culture is a complex process which was carried out for the first time by William Rooks and published by G Harrison in 1910. It is generally referred to a culture of eukaryotic cells (especially animal cells). The common cell culture media are RPMI and DMEM which comprise of nutritional media and minerals but are different in pH , glucose concentration, and nutrients. Some of the cells are fastidious and difficult to culture like the Plasmodium parasite or Raji cells when they are first rejuvenated from liquid nitrogen. A supplement prepared from bovine serum can be used to help these cells attain better growth. This supplement is prepared in the biochemistry department of Pasteur Institute of Iran from bovine serum. Two ammonium sulfate precipitations of different concentrations of the bovine serum result in only its purified lipoproteins. This supplement is tested in different cell cultures with satisfactory results.

Key words: RPMI medium; DMEM medium; Supplement; Malaria parasite; Raji cells

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The effect of electro magnetic field on total proteins of Zea Mays seedling

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ABSTRACT

Studies were planned to analyze the response of Zea Mays 704 to Electro Magnetic Field (EFM) stress (high frequency 940 MHZ) mobile phone of Iran. The rate of Proline, soluble sugars and the activities of Guaiacol peroxidase and catalase antioxidant enzymes have been determined by the spectrophotometer. As the proline EMF stress increased the sample group was faced with a meaningful increase in soluble sugars rate which the increase rate in the resistant plants was more than that of the sensitive plants in all cases. The consideration of Guaiacol peroxidase and Catalase enzymes activity rate in the sensitive and resistant plants roots and stem indicated that these enzyme in resistant plants was more active than the sensitive one. As the EMF stresses increased the activity of reactive oxygen species, this also decreased the plants growing process as well. We can therefore suggest the increasing of antioxidant enzymes activity and also the soluble smolites as the mechanisms of resistance to the EMF stress for the resistant plants.

Key words: Proline amino acid; EMF stress; soluble sugars; Zea mays

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Affinity to bovine serum albumin and anticancer activity of some new water-soluble metal Schiff base complexes

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ABSTRACT

The binding of the Schiff base metal complexes to the most abundant carrier proteins (serum albumins) has been a subject of interest as such drug–protein binding greatly influences absorption, drug transport, storage, metabolism and excretion properties of typical drugs in vertebrates. Serum albumins are most plentiful proteins in the circulatory system of a wide variety of organisms. Among the serum albumins, bovine serum albumin (BSA) is a striking macromolecule often used in biophysical and biochemical studies because of its structural homology with human serum albumin (HSA), its accessibility, low cost and unusual ligand binding properties. The interaction of some complexes with bovine serum albumin (BSA) was studied by fluorescence spectroscopy. Type of quenching, binding constants, number of binding sites and binding stoichiometries were determined by fluorescence quenching method. The results showed that the mentioned complexes strongly bound to BSA. Thermodynamic parameters indicated that hydrophobic association was the major binding force and that the interaction was entropy driven and enthalpically disfavored. The displacement experiment showed that these complexes could bind to the subdomain IIA (site I) of albumin. Furthermore the synchronous fluorescence spectra showed that the microenvironment of the tryptophan residues was not apparently changed. Based on the Förster theory of non-radiation energy transfer, the distance between the donor (Trp residues) and the acceptor metal complexes was obtained.

Key words: Schiff base; Bovine serum albumin; Fluorescence quenching

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Theoretical studies of binding of saccharin to p53 protein and gene via molecular docking method

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ABSTRACT

Saccharin, the first artificial sweetener, has no calories, is 300 times sweeter than sugar, and has been used to sweeten various products, including soft drinks,jams,chewing gum, and medications. Saccharin is carcinogenic for the urinary bladder in rats and mice, and most likely is carcinogenic in human beings. However, excessive consumption of this sweetener may be harmful to human health. The tumor suppressor gene p53 has been identified as the most frequent target of genetic alterations in human cancers. Initial docking studies were performed using AutoDock 4.2.2. In this study, we present a comparative docking study to probe the selective inhibition of p53 protein and gene with SA in order to better understand their binding interactions. Analysis of the molecular docking indicated that SA preferentially bound to the p53 protein and gene promoter and led to the silence P53 gene expression. According to the current study, the possible risk of artificial sweeteners to induce cancer seems to be important.

Key words: Saccharin; P53; Protein; Gene; Molecular docking

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Conformational locks and thermal kinetics of Silica Nanoparticle Ionic Liquid on Horse Liver Alcohol Dehydrogenase

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ABSTRACT

Horse Liver alcohol dehydrogenase (HLADH) is a commercial dimer enzyme, playing role in alcohol to aldehyde/ketones conversion in the body. In this work the stability and the conformational lock of HLADH was studied in presence of silica nanoparticle supported imidazolium ionic liquid. The silica nanoparticle supported imidazolium ionic liquid was utilized as a green additive for studying the thermal reversibility of HLADH by differential scanning calorimetry. The reversibility was enhanced 36% and the Tm was increased 1 °C by adding this nano-ionic liquid to enzyme.Then, the conformational lock of HLADH in the presence of silica nanoparticle was compared to the native form for obtaining the interface conformational changes. For this purpose, the kinetics of thermal inactivation of HLADH was studied in a 50 mM pyrophosphate buffer, pH 8.8, using ethanol as substrate, NAD+ as a cofactor and silica nanoparticle. The temperature range was between 48-55°C and the conformational lock was developed based on Poltrak theory and analysis of the curves was done by the conformational lock method for oligomeric enzymes. The conformational lock numbers was majorly changed that is a clue for the enzyme temperature stability.

Key words: Conformational; Enzyme; Silica nanoparticle