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Oral Talk – L1 Open Access

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

Shiraz University 10 & 11 December 2014

A collection of bioinformatics tools to protein engineering

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ABSTRACT

Protein engineering is an important tool for overcoming the limitations of natural enzymes as biocatalysts. In this regard computational tools are becoming increasingly important in order to create improved or novel enzymes. We developed a useful collection of bioinformatics tools in protein engineering. These applications are freely available at http://bioinf.modares.ac.ir. A short description will come in the following: **PUTracer** is a useful and easy to use web based application to assign number of domains and domain boundaries in a protein. Recognition of domains in proteins is a critical step in chimeric protein engineering. **LinDa** (Linker Design Assistant) is a database of protein fragments in 4-20 amino acid length. Linda is an effective tool to chimeric protein engineering and peptide design. **MPDB** (Mutation Proposer on Disulfide Bond): Disulfide bonds formation is one of the solutions to increase the thermal stability of enzymes. MPDB gives a list of amino acids pair with potential to form a disulfide bond. **CFinder** (Contact Finder) Accurate identification of amino acids involved in the binding of the two proteins has wide applications, including antibody optimization, analysis of docking studies, increasing protein stability and peptide design. CFinder identifies amino acids involved in binding in a protein complex and also an individual amino acid neighbors too. **Mini Mutate** is a user friendly application to have the structure of a mutated protein. The input of application is native protein structure in PDB format and desired mutation. It will give the mutated protein structure after replacing amino acid and optimizing the structure.

Key words: Protein engineering; Chimeric protein engineering; Peptide design; Protein stability; Docking analysis

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Oral Talk – L2 Open Access

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

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Interaction of metal Schiff base complexes with bovine and human serum albumin

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ABSTRACT

Since it is commonly accepted that DNA and proteins are considered as the main molecular targets in the action of drugs, and many compounds exert their drug effects through binding to DNA or proteins, which is the basis of designing and discovering new and more efficient drugs, the studies on syntheses and interactions of metal complexes with DNA and proteins have been an active field of research. Albumin is the most abundant plasma/serum protein; it is responsible for maintenance of the oncotic pressure in plasma. In addition, albumin provides a large variety of binding and transport functions therefore have a major influence on the pharmacokinetics of numerous endogenous ligands (like thyroid hormones, fatty acids and bilirubin). Since albumin serves as a transport carrier for drugs, it is important to study the interactions of these drugs with this protein. The effectiveness of these compounds as pharmaceutical agents depends on their binding ability.The medicinal properties of metal Schiff base complexes depend upon the nature of the metal ions and the ligands. Binding of the Schiff base metal complexes with serum albumins have 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. The quenching of the intrinsic fluorescence of protein was used to determine the values of Stern–Volmer constants, quenching rate constants, binding constants, number of binding sites and average aggregation number of protein. Also, the thermodynamic parameters, binding modes, high-affinity binding site, intermolecular distances, and conformational changes can be determined.

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

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Oral Talk – L3 Open Access

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Engineering of anti-angiogenic peptides for cancer therapy: structure-function and therapeutic evaluations

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ABSTRACT

Inhibition of tumor angiogenesis is amongst the most effective approaches for treatment of cancer. Endostatin, an endogenous inhibitor of angiogenesis, has shown to inhibit the proliferation and angiogenesis of endothelial cells and growth of tumors. However, application of human endostatin for clinical trials is limited by some obstacles such as formation of inclusion bodies in the production of recombinant protein and low stability in circulation. Previous studies have shown that endostatin fragments such as the 27 amino-acid N-terminal fragment mimic the biological activity of full length endostatin. Here, we report an applied strategy to obtain anti-angiogenic peptide(s) for treatment of breast cancer. For this purpose, a series of peptides derived from endostatin was designed. The structure of peptide variants were investigated by spectroscopic and theoretical approaches including far-UV CD, FTIR, steady state fluorescence, molecular dynamic simulations and molecular docking. To assess the functional consequences of the sequence modifications, in vitro studies including the inhibition of HUVEC proliferation and tube formation in the presence of peptides were performed. In addition, induction of intrinsic and extrinsic apoptosis in HUVECs was extensively investigated. Further functional studies were done by comparing the antitumor properties of peptides. Based on the in vivo results, breast tumor growth and in vivo angiogenesis were strongly inhibited by some of the peptide variants. Accordingly, toxicity and further efficacy experiments were done in animal models and pharmacokinetic studies is in progress.

Key words: Peptide engineering; Endostatin; Angiogenesis; Cancer treatment

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Oral Talk – L4 Open Access

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

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Firefly luciferase complementation assay for IP3 detection

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ABSTRACT

Luciferase is the key enzyme that catalyzes the oxygenation of luciferin, generating energy-rich peroxidic intermediates, emitting a photon of visible light. Inositol 1, 4, 5-trisphosphate (IP3) plays a vital role in the control of a large number of cellular processes. Alteration in its content has been observed in metabolic disorders. Hence, development of high-throughput screening systems to monitor temporal changes of IP3 is essential for screening of new potential therapeutic compounds. Toward a sensitive, rapid and simple method to measuring IP3, we describe the development and application of a luciferase-fragment complementation strategy, which converts the ligand-induced conformational changes to light. In order to create a good performance sensor for IP3 monitoring, IP3 binding domain (IP3BD) of IP3R that consist of all the specific binding site of IP3 was selected as a ligand binding domain. The NLuc of firefly luciferase is fused to the N-terminus of IP3BD whiles the CLuc of luciferase is attached to the C-terminus of IP3BD (NLuc-IP3BD-CLuc). A flexible peptide linker $(G_4S)_2$ was inserted between the fragmented luciferase and IP3BD to assist in proper protein folding and luciferase complementation. According to the results, the screening time was very fast and maximum response was obtained up to 11-fold higher than background. Moreover, the designed biosensor was able to monitor release of IP3 upon induction by different inducer like Bradykinin and ATP. The current biosensor not only provides a specific IP3 detector *in vitro*, but also facilitates to monitoring of the response of IP3 in living organisms.

Key words: Luciferase; IP3; Biosensor

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Oral Talk – L5 Open Access

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

Shiraz University 10 & 11 December 2014

Synthesis of novel peptides and investigation of their biological activities

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ABSTRACT

The importance of peptides as pharmaceutical has increased significantly and peptide drugs have an essential role in pharmaceutical market. However, the pharmacokinetic profile and selectivity of peptide drugs is limited by their low metabolic stability due to the amide bond hydrolysis by peptidases and low bioavailability. In this way, finding of a suitable way for drug delivery and modification of peptide structure is the subject of the recent researches. Now, some glycopeptides and nucleopeptides are introduced as novel drugs.We intend to use multicomponent reaction approach to construct products with further functional groups which have lipophilic moieties and in some cases are prone to carrying out further reactions such as additional ring closure reactions. This strategy allows us to prepare in a very economic and ecologic way complex systems. This lecture will highlight some of our contributions to this area which contained:

a) Ugi-4CR as an approach for the synthesis of some novel GnRH analogues and investigation of their anti-cancer activity.

b) Design and synthesis of novel fentanyl analogues based on Ugi-4CR, and also functionalization of natural analgesic peptides.

c) Synthesis of novel cyclopeptides through Ugi ligation/click reaction to construct the cyclopeptides which have a triazole moiety and also lipophilic moieties.

Key words: Biologically active peptide synthesis; GnRH analogues; Anti-cancer peptides; Sequential Ugi/Dipolar Huisgen reaction; Cyclopeptide contained

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Oral Talk – L6 Open Access

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

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Preferential packaging of negative sense RNA by viral nucleoproteins

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ABSTRACT

Crimean-Congo Hemorrhagic Fever is an infectious disease of high virulence and a high mortality rate caused by Crimean-Congo Hemorrhagic Fever Virus (CCHFV). CCHFV is a negative sense RNA virus with genomic RNA enwrapped by a viral nucleoprotein. Positively charged residues on CCHFV nucleoprotein make multiple binding sites to facilitate genomic RNA encapsidation. In the present work, we investigated the mechanism underlaying preferential packaging of negative sense genomic RNA by CCHFV nucleoprotein in the presence of host cell RNAs upon viral assembly. The work includes genome sequences analyses for different families of negative and positive sense RNA viruses, using serial docking experiments and MD simulations. Our results indicate that the main determinant parameter of nucleoprotein binding affinity for negative sense RNA is the ratio of purine/pyrimidine in the RNA molecule. Negative sense RNA with a ratio of purine/pyrimidine (>1) higher than that of positive sense RNA (<1) exhibits higher affinity for nucleoprotein. Our calculations indicate that negative sense RNA express about 0.5KJ/Mol higher binding energy per nucleotides compared to positive sense RNA. This energy difference produces a binding energy high enough to make negative sense RNA the preferred substrate for packaging by CCHFV nucleoprotein in the presence of cellular or complementary positive sense RNA.

Key words: Crimean-Congo hemorrhagic fever virus; Nucleoprotein; Negative sense RNA, Positive sense RNA; Molecular dynamic simulation

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Oral Talk – L7 Open Access

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

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Immunogenicity of recombinant ISS protein as potential vaccine against colibacillosis in poultry

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ABSTRACT

Colibacillosis is a disease caused by avian pathogenic *Escherichia coli* (APEC) and depending on the virulence status of the strain, host status and presence and type of predisposing factors, is followed by either sudden death or localized inflammation in multiple organs. Virulence factors described so far for APEC include adhesions (type 1, curli and P fimbriae), flagella, Aerobactin system, capsule, outer membrane protein, temperature sensitive haemagglutination, serum resistance and colicin production but the exact mechanism of pathogenesis is not yet fully elucidated. One of the major virulence factors, which cause resistance of APEC to serum complement, is *ISS* gene located on ColV plasmid. The objectives of this study were to clone, sequence, expression and immunogenicity of ISS protein. The *ISS* gene cloned and sequenced into pTZ57R/T and pGEX-3X vectors, after that protein expression were analyzed by SDS-PAGE and western blot. Finally ISS protein sequence was confirmed by Ms-Ms. Sequence analysis showed that the *ISS* gene from *E. coli* (O78:K80) isolated from Iranian poultry does not differ from previous reported genes submitted in GenBank database. GST: ISS fusion protein was purified by Glutathione sepharose column. To investigate immunity, purified ISS injected to animal model and checked by Dot-blot assay. The results showed that ISS protein could be used for detection and control of colibacillosis in poultry.

Key words: Colibacillosis; APEC; ISS; Immunogenicity

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Oral Talk – L8 Open Access

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

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A novel intermediary amylase capable of completely hydrolyzing starch, pullulan and cyclodextrin

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ABSTRACT

The intermediary amylolytic enzymes (mostly classified in the subfamily GH13_36) with conserve sequence of MPDLN in their fifth conserved region, are known by their mixed enzyme specificity of α-amylase and some others from the oligo-1,6-glucosidase and neopullulanase subfamilies (e.g., cyclodextrin-hydrolyzing or transglycosylating ones). Since these enzymes can act on wide range of substrates they have many applications in starch processing industries. BMW-amylase had been isolated from *Bacillus megaterium* WHO based on its ability to hydrolysis both starch and pullulan. Using the sequence of the fifth conserved sequence region, the enzyme has been classified as intermediary group. The enzyme activity analysis revealed hydrolyzes of starch, amylose, amylopectin, pullulan and cyclodextrine. Catalytic efficiency of the enzyme for starch, amylopectin and amylose was about 5.5 fold higher than pullulan. In addition, the enzyme can produce glucose from maltose via condensation of two maltose molecules by transglycosylation. HPLC and TLC analysis showed that BMW-amylase is a novel intermediary amylase that produces glucose as a main degradation product of all mentioned substrates.

Key words: Intermediary amylase; Substrate specificity; HPLC analysis; Recombinant α-amylase; *Bacillus megaterium*

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Oral Talk – L9 Open Access

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

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Investigation of Artemisia genus effects on iNOS and COX-2 expression using RAW 264.7 macrophage cells

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ABSTRACT

Cancer prevention and treatment using traditional medicines have attracted increasing interest. Artemisinin is a sesquiterpene lactone with a peroxide bridge, which is the effective moiety against both sensitive and multi-drug resistant *Plasmodium falciparum*, the malarial agent. Although artemisinin absorbs light in the UV region between 190 and 210 nm, its extinction coefficient is poor. As a result, the methodology of standard UV detection is ineffective in the quantitative analysis of artemisinin. Therefore, it is of prime importance to introduce a simply available, rapid, and cost efficient, yet dependable quantitative method for its analysis. In this research, we have developed a TLC/UV_{258nm} method for the simultaneous determination of artemisinin. Artemisinin concentration was analysed and compared Artemisia species 16. The highest artemisinin concentration was detected in Artemisia annua. *Artemisia annua* and *khorassanica* selected for cell culture treatment. We examined the effects of essential oil on the production of nitric oxide (NO), cyclo-oxygenase 2 (COX-2) and inducible NO synthases (iNOS) in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Western blotting and Real time PCR tests indicated that *Artemisia annua* has potent inhibitory effects on COX-2 and iNOS and *A. khorassanica* did not affect the iNOS protein level. These findings suggest that *A. annua* may exert an anti-inflammatory effect by inhibiting the expression of pro-inflammatory cytokines.

Key words: Artemisia; COX-2; iNOS; Inflammatory

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Oral Talk – L10 Open Access

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Differential immune reactivity pattern of SW48 and SW1116 colorectal cancer cell lines with colorectal cancer patients sera

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ABSTRACT

Colorectal cancer (CRC) is one of the most common types of cancer and the second cause of cancer deaths in Europe and other Western countries, because it is not possible to detect the cancer in the early stages by present methods. Therefore, finding a fast, convenient and affordable way to detect colorectal cancer allocated a vast amount of investigations. Among these methods using of biomarkers are important for early detection and prognosis. In this study we used different stages of CRC patient's sera as a source of auto-antibody and two colorectal cancer cell lines (SW48 and SW1116) with different invasive properties as source of antigens. The pattern of immune-reactivity between SW48 and SW1116 cell lines with CRC patient's sera were evaluated by software. The band intensity of pattern of immune-reactivity between SW48 and SW1116 cell lines with CRC patients sera was different and most immune-reactivity was seen in SW48 cell lysate with stage III of CRC patients sera.In result of humoral immune response sera of CRC stage III contain auto antibodies that shown higher immune-reactivity also high aggressive behavior cell line (SW48) reacted with CRC patients sera with greater intensity in compared to less aggressive behavior cell line (SW1116). Therefore require the using techniques such as two-dimensional electrophoresis and mass spectrometry to identify the details of differences in the immunoblot patterns of colorectal cancer patient's sera with these two cell lines.

Key words: Colorectal cancer; Immune reactivity pattern; SW48 cell line; SW1116 cell line

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Oral Talk – L11 Open Access

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

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In silico and *in vitro* analysis of structure based designed peptides on TrkB receptor of Ov-car-3 and Sk-ov-3 cell lines

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ABSTRACT

Tropomyosin receptor kinase B (Trk B) is one of the significant oncogen proteins. Over-expression of Trk B was occurs in thyroid, ovarian and prostate cancers and multiple myeloma. The interaction of BDNF/TrkB leads to activation of angiogenesis and proliferation pathways in tumor cell lines. The aim of this study is design of novel peptides as TrkB inhibitor. At first, the peptide library was generated by backrub protocol, and then the binding of the designed peptides with TrkB was investigated by molecular Docking. In the experimental evaluation cytotoxic and apoptotic effects of designed peptides on Sk-ov-3 and Ov-car-3 cell lines were assessed by MTT and Annexin -PI staining flowcytometry techniques, respectively. The expression of phosphorylated and un-phosphorylated AKT, EIF4E and MAPK proteins was compared in TrkB signaling pathway by using western-blotting. The designed peptides induced cytotoxicity on Sk-ov-3 and Ov-car-3 cell lines and showed inhibitory effects on their TrkB signaling pathway in cell lines. From the sort of designed and tested peptides the peptide II is more effective than the others and they are efficient in development of anticancer drugs.

Key words: Backrub protocol; Docking; Peptides; Sk-ov-3; TrkB receptor; Ov-car-3

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Oral Talk – L12 Open Access

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

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Performance of three nanoparticle based immuno-sensors for detection of hepatitis B surface antigen

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ABSTRACT

Three simple immuno-biosensors were developed using voltammetry, capacitance and chemilumunescence methods and the results were compared: (I) Biotinilated antibody (anti-hepatitis B, Ab_1) was connected to the streptoavidin coated magnetic nanoparticle (MNPs). Then, horseradish peroxidase (HRP) was used as a label for the secondary antibody (Ab_2). In the presence of hepatitis B surface antigen (HBsAg) the immunoreaction was occurred and a sandwich was formed in the order of: Ab_1-MNPs/HBs/Ab_2-HRP). The redox current generated by Ab_2-HRP was measured electrochemically. The immuno-sensor responded towards antigen in the linear range of 1–150 pg/ml. (II) Two planar gold electrodes were used as capacitor plates. Surface of the gold electrodes was covered by insulating molecule layer. Then Ab_1 was immobilized on isolating layer. By addition of HBsAg, the distance and also the average surface area of plates were changed. Alteration of these factors leads to change in capacitance which could be used as an indicator of antibody-antigen interaction. The linear range for the immunosensor was from 10 to 60 ng/ml. (III) HBsAg was added to the wells (plates), covered with Ab_1, then to remove the unbound antigens the wells were washed. Thereafter, the gold nanoparticles (GNPs) labeled with secondary antibody and luminol (Ab_2/GNP/L) were added to the well to complete the immune sandwich. The emission was recorded at 425 nm. The linear range for the immunosensor was from 0.12 to 30 ng/ml. Among the nanoparticle based immuno-biosensors developed for detection of HBsAg, the voltametric method presented a higher sensitivity.

Keywords: Chemiluminescence, Capacitive immunosensor, Magnetic nanoparticle, Gold nanoparticles, Hepatitis B

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Oral Talk – L13 Open Access

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

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How do proteins involve in promotion of type 2 to 3 diabetes?

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ABSTRACT

In type 2 diabetes, the pancreas produces insulin, but the cells become resistant to insulin, leading to hyperglycemia that consequence neurodegeneration and Alzheimer's disease (AD) as type 3 diabetes. Hyperglycemia brings about protein glycation and turns the proteins from friend to foe and provides a toxic condition. Here we present possible fortune of proteins, including serum albumin (SA), hemoglobin (Hb) and insulin under diabetic condition. Serum albumin (BSA) glycation causes lipid peroxidation and its product kills microglia upon a RAGE involved receptor binding mechanism. Hemoglobin (Hb) glycation destroys heme to provoke hypoxia and the product of Hb-glycation inhibits platelet aggregation. Insulin glycation disables it to cause insulin resistance and even destroys adjacent membranes through a lipid peroxidation mechanism to trigger an observed glial apoptosis. Also possible preventive strategies for type 3 diabetes are discussed.

Key words: Glycation; Glial cells; Apoptosis; Lipid peroxidation

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Oral Talk – L14 Open Access

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Molecular docking studies of quercetin as a xanthine oxidase inhibitor

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ABSTRACT

Xanthine oxidase (XO) catalyses the conversion of hypoxanthine to xanthine and subsequently xanthine to uric acid. The increase of uric acid can lead to gout and other diseases. Allopurinol, an analogue of hypoxanthine, is a specific potent inhibitor and substrate for XO. The most common adverse effects of allopurinol include hypersensitivity reactions, skin rashes, hepatotoxicity and gastrointestinal distress. Thus, there is a need to discover compounds with XO inhibitory activities which devoid of the undesirable effects of allopurinol. It has been shown that flavonoids act as the inhibitors of xanthine oxidase, So quercetin as a flavonoid was chosen and the modes of interaction of quercetin was investigated at the atomic level using *in silico* means. The aim of this study is to investigate the Inhibitory effect and toxicity of quercetin compared with allopurinol by using molecular modeling techniques. The virtual screening analysis using AutoDock 4.2 and *in vitro* xanthine oxidase inhibitory activities of allopurinol and quercetin as the substrate were used in this study. In addition, the kinetics of the enzymatic reactions were analyzed using Lineweaver-Burk plot and related to the structural features of the studied inhibitors. The results showed that the inhibitory effect of quercetin is more potent than allopurinol (a standard inhibitor), while showing less toxicity effect.

Key words: Molecular docking; Xanthine oxidase; Quercetin; Allopurinol

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Oral Talk – L15 Open Access

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

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Design and construction of a cell based biosensor for rapid identification of *Vibrio cholerae* using CANARY technology

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ABSTRACT

Cell-based biosensors have emerged as powerful functional tools for the rapid detection of hazards. The CANARY (Cellular Analysis and Notification of Antigen Risks and Yields) sensor, an engineered B-cell biosensor, enabled the specific detection of different pathogens. This report describes the construction of a sensor for *vibrio cholerae* detection that achieves an optimal combination of speed and sensitivity through the use of hybridoma cells. Binding of bacteria to the antibodies initiates a signal transduction cascade, which causes the calcium sensitive photoproteins emit light. The efficiency of hybridoma cells for replacement with engineered B-cells were successfully proved using Fura2-AM. *V. cholerae* detection using this system performed for the first time. First, hybridoma cells were transfected with pBudCE4.1 vector which engineered to have two calcium sensitive photoproteins (aequorin and i-Photina). Then, the stable cell lines were selected after exposure of the cells into zeocine selection marker after 4 months. PCR, SDS-PAGE and western blotting techniques were performed for analysis of these photoproteins in the cells. In addition, the biosensor was screened for generation of blue light and dose response and limit of detection curve were plotted. This biosensor detected as few as 50 CFU in a total assay time of less than 20s which means that it responded to *V. cholera* with high speed and sensitivity. Also, this biosensor would detect *V. cholerae* from different environment such as sea water and stool and did not respond to other bacteria that used as negative controls.

Key words: CANARY; Cell based biosensor; Hybridoma *Vibrio cholera*; Photoprotein

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Oral Talk – L16 Open Access

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

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Molecular dynamics simulations of Mn (II) coordination in the absence of Ca (II) in human calprotectin binding sites

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ABSTRACT

Recent investigations indicate that Mn (II) acquisition by invading microbial pathogens contributes to their virulence and successful colonization of their host. The host antimicrobial protein, Calprotectin (CP), is shown to be involved in the competition between host and pathogen for the transition-metal nutrients Mn (II). CP is a tetramer made up of two heterodimer of S100A8 and S100A9. Crystallographic characterization of the CP heterotetramer revealed the presence of four transition metal-binding sites at the S100A8/S100A9 interface. Using molecular dynamics simulations, we investigated the Mn (II)-binding properties of CP and the role of Mn (II) on the dynamics of CP. The simulations were performed on Mn(II)-free and Mn(II)-bound CP models, using default GROMACS parameters modeled only the electrostatic binding to the Mn (II) at the Mn-CP and trajectories at constant temperature (310 ^{o}K) and pressure (1 atm) in a rectangular box (6.163* 6.721* 6.268 cm^3) for 10ns period. Our simulation analysis demonstrated that Mn (II) can stabilize the conformation of their binding site via conferring more negative potential energy compared to free CP, so that enabling interaction as an electrolyte with negatively charged groups. Due to CP structure alteration, the RMSD for Mn (II)-CP bond was more than its free state, throughout the simulation. Also, the Mn (II)-binding to CP leads to increased activity of the protein. The RMSF graph showed that Mn (II)-CP complex has higher flexibility and mobility. This study showed that despite Mn (II) being a member of hofmeister series of destabilizing nature for proteins, its natural presence provides the protein structure flexibility and stability.

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

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Oral Talk – L17 Open Access

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

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Effect of conformational free energy differences on binding and drug resistance of the HIV-1 protease

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ABSTRACT

Many proteins, such as HIV-1 protease, undergo conformation change through inhibitor binding. Mutation can effect on the energy landscape of the conformational states of the protein. So, a central question is how mutation-induced changes in the energy landscape of unbound and inhibitor-bind states of protein confer with protein-inhibitor interaction and what is its relation with drug resistance. Here, we investigated the effects of three resistant point mutations of protease including L90M, G48V and V82A. By comparing the binding free energies of 5 clinically approved inhibitors to wild-type and mutated structures, $\Delta\Delta G_b$ (mutation-induced changes in binding free energy) was estimated, using molecular docking. Then, by comparing $\Delta\Delta G_b$ with the experimental binding constant data, $\Delta\Delta G_c$ (mutation-induced changes in conformational free energy) of protease was estimated, indirectly. Also, $\Delta\Delta G_c$ values were estimated using Concoord-PBSA method. The highest effect on protein stability was observed for G48V mutation whereas V82A mutation induced very small changes in protein conformation. The observed resistance by L90M mutation could be attributed to decreasing in both close contact interactions and stability of protein in the inhibitor-bind state. However, for G48V and V82A mutation, the main source of drug resistance could be attributed to decreasing of protein stability and decreasing of close contact interactions, respectively.

Key words: HIV-1 protease; Conformation change; Inhibitor; Mutation; Free energy

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Oral Talk – L18 Open Access

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

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How well can DFT-D methods predict protein geometries and energetics?

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ABSTRACT

The recent advances in software as well as hardware developments are making quantum calculations possible on systems of ever-increasing size: thus there is a growing need of procedures providing reliable treatments of solute-solvent interaction. Density Functional Theory (DFT) has been proven as a most considerable method as time and reliability of results in large biomolecular computations especially in solvent models. Although DFT yield reliable results for interactions that are largely of an electrostatic nature, it generally fail to produce accurate results for attractive interactions derived from dispersion forces. DFT dispersion (DFT-D) and van der Waals Density Functional (vdW-DF) methods provide the accurate treatment of non-covalent interactions in aqueous solution and in a protein-like environment without any significant additional computational cost. Alanine dipeptide molecule (N-acetyl-alanine-N'-methylamide) was proposed as a model for computational studies of protein structure and dynamics. We selected α'_R, α_L, C_{7eq}, P_{II} and β_A conformers of alanine dipeptide, and examined the ability of ten density functionals including B3LYP, M06-L, M06, M06-2X, TPSS, CAM-B3LYP ,wB97XD, M06+vdw, M06-2X+vdw, and M06-L+vdw to evaluate optimized structures. The solvation effects were added using three continuum models: the Onsager model, the polarizable continuum model (PCM), and the conductor polarizable continuum model (CPCM); so 200 calculations (5 conformer's × 10 functionals × 4 solvent models) were performed. Also, our results showed that the P_{II} conformer is preferred in these two models: input β_A, $C7_{eq}$ and P_{II} conformers converted to this conformation during optimization using different methods that is in good agreement with experimental data.

Key words: Alanine dipeptide; DFT-D; Continuum solvent model; Ramachandran plot

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Oral Talk – L19 Open Access

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

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Flexibility switch of firefly luciferase

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ABSTRACT

In this study, different approaches including addition of disulfide bridges, saturation of surface charges, and decrease of segmental flexibility have been used to increase thermostability of firefly luciferase. A disulfide bridge is introduced into *Photinus pyralis* firefly luciferase to make two separate mutant enzymes. One of the designed mutant (A103C/S121C) showed remarkable thermal stability, its specific activity decreased, whereas the A296C/A326C mutant showed tremendous thermal stability and 7.3-fold increase of specific activity. Analysis of enzyme according to residues B-factors shows that its C-terminal is much more flexible than its N-terminal. Two mutations in the most flexible region of luciferase were designed. Analysis shows that D476N mutation doesn't have any significant effect but D474K mutation destabilized protein. On the other hand, flexibility analysis using dynamic quenching and limited proteolysis demonstrates that D474K mutation became more flexible than wild type. Protein engineering studies have shown that thermostable proteins have a higher frequency of Arg, especially in exposed states. To further elucidate the arginine saturation effects on stability of firefly luciferase, some of hydrophobic solvent-exposed residues in luciferase are changed to arginine. All of these residues are located at the external loops of *L. turkestanicus* luciferase. Introduction of double mutation (-Q35R/I232R) and triple mutation (-Q35R/I232R/I182R) were reserved specific activity of enzyme while its stability was enlarged and its flexibility was declined. Structural and functional properties of the mutants were investigated using different spectroscopic methods. It seems a clear relationship between stability and segmental flexibility in firefly luciferase controls its activity under different conditions.

Key words: Mutation; Firefly luciferase; Thermostability; Flexibility

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Oral Talk – L20 Open Access

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

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Protein tailoring as a tool for extracellular expression of an archeal lipase in *E. Coli* and its secretion optimization by response surface methodology

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ABSTRACT

Extracellular expression of recombinant proteins using fusion protein approach has several advantages including significant increase in the yield of functional protein and easier downstream processing. Towards having recombinant *Thermoanaerobacter thermohydrosulfuricus* lipase (TtL) in culture medium of *E. coli*, the *Salinivibrio* metalloprotease (SVP2) signal peptide was used as fusion partner. Two sets of primers were designed for amplification of related genes by PCR. Firstly, PCR product of *TtL* gene with restriction sites of SacI and HindIII was cloned into pQE80L plasmid, named as pQE80L-TtL. Afterward, the SVP2 signal peptide nucleotide fragment along with EcoRI and SacI restriction sites was amplified and cloned into pQE80L-TtL and the final construct pQE80L-STL was obtained. Study on the extracellular expression of recombinant TtL revealed that the most of enzyme active form placed in periplasmic space. So, effect of seven different factors including glycine, Triton X-100, yeast extract and IPTG concentration, incubation time, induction time, and temperature on the extracellular expression of TtL was evaluated using statistical fractional factorial and response surface methods. Based on the results, concentration of glycine and IPTG and time of induction were the most important factors. In overall, the optimum condition for secretion of recombinant TtL in *E.coli* BL21 cells is 0.2 mM of IPTG, 1.27 % glycine and 24 hours of induction time which reveals 29.6-fold improvement (44.5 U/L:1.5 U/L) over the basal medium.

Key words: Extracellular expression; *Thermoanaerobacter thermohydrosulfuricus*; Fusion protein; *Salinivibrio* metalloprotease (SVP2); RSM

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Oral Talk – L21 Open Access

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

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Peroxidase activity of "heme-ferritin" complex: a possible mechanism of pathogenesis in neurodegenerative diseases

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ABSTRACT

Oxidative stress is at the forefront of neurodegeneration research. While its implications in the characteristic neurodegeneration of Alzheimer disease (AD) are vast, the most important aspect is that it seems increasingly apparent that oxidative stress is in fact a primary progenitor of the disease, and not merely an epiphenomenon. It has been previously suggested that oxidation of neurotransmitters by uncontrollable peroxidase systems in the presence of H_2O_2 is a possible molecular link between amyloid structures and the abnormal neurotransmitters and oxidative damage seen in AD/PD brain. Heme and sometimes heme-protein complexes possess peroxidase activities which cause irreversible damages *in vivo*. It has been previously shown that ferritin molecule can bind to 15-17 heme moieties. In the present study, we reported the peroxidase activity of "heme-ferritin" complex using several oxidizable substrates. First, the ferritin was purified from liver homogenate of sheep by ammonium sulphate precipitation and DEAE ion-exchange chromatography. The peroxidase activity of ''heme-ferritin" complex was then characterized by using H_2O_2 or *t*-BHP as oxidant substrates and TMB, L-Dopa or DOPA as a reductant substrates. Results showed that the non-specific peroxidase activity of the ''heme-ferritin" system can oxidize the neurotransmitters with high catalytic efficiency. Since, the concentrations of ferritin, free heme, and peroxide species increase in neurodegenerative diseases such as AD. There is this possibility that the peroxidase activity of ''heme-ferritin" complex play a significant role in development of oxidative damage within involved cells. We will discuss the importance of our observations.

Key words: AD; Peroxidase activity; Ferritin

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Oral Talk – L22 Open Access

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

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Formation of heme degradation products during the interaction of human hemoglobin with methyl tertiary-butyl ether (MTBE)

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ABSTRACT

Methyl *tertiary*-butyl ether (MTBE) is widely used in gasoline as oxygenate and octane enhancer and reduce emissions of carbon monoxide. The number of people exposed to gasoline is large and includes mainly employees in gasoline companies and gasoline station attendants, but also the general public. Acute effects, such as headache, nausea, and nasal and ocular irritation, have been associated with the exposure to gasoline containing MTBE. The aim of this study was to assess the effect of MTBE on Hemoglobin (Hb) structure and heme degradation. Hemoglobin is a tetramer protein that has a 3D structure consisting of two alpha and two beta subunits, which are non-covalently associated within erythrocytes and arranged around a central cavity. Measurement of fluorescence during the reaction of MTBE with hemoglobin displayed fluorescence emission peaks around 465 nm (excitation wavelength at 321 nm). Following the addition of MTBE, the fluorescence in this region increased with different concentration of MTBE. The detection of one fluorescent product during the Hb reaction with MTBE suggested that the heme degradation may involve the reaction of reactive oxygen species (ROS) with oxyHb. Based on our experimental results, it was confirmed that the interaction between Hb and MTBE leads to heme degradation.

Key words: MTBE; Human hemoglobin; Heme degradation

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Oral Talk – L23 Open Access

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

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Tau, amyloid-β and neurotrophin dysregulation in alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is one of the most costly diseases and a great burden on caregivers. At present, there is no cure for AD. Early diagnosis of the disease is important for an effective treatment. Knowing the mechanisms involved in pathology of AD is necessary in order to have an early diagnosis and proper treatment. Amyloid-β (Aβ) aggregation is known to be the major culprit in Alzheimer's disease (AD) and tau pathology is considered as a downstream event. Both amyloid-beta and tau dysfunction can cause dementia. Neurotrophins such as nerve growth factor (NGF), brain derived neurotrophic factor and their precursors (proNGF and proBDNF) are also dysregulated in AD leading to cognitive impairment. Amyloid-β reduces BDNF levels and can interrupt axonal transport which could lead to abnormal level of proNGF. Tau may also affect normal levels of neurotrophins in the brain. This review will focus on the relation between these alterations in AD and their impact on cognitive ability.

Key words: Alzheimer’s disease; Tau; Amyloid-Beta; Neurotrophins

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Oral Talk – L24 Open Access

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

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Recent discovery of α-glucosidase inhibitors with pyrimidine scaffold

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ABSTRACT

In our ongoing program which is aimed at the design, synthesis and biological evaluation of novel and selective α-glucosidase (α-Gls) inhibitors, eight new pyrimidine fused heterocycles (PFHs) were synthesized and found to be potent and selective inhibitors of α-Gls. The action of these agents would reduce the liberation of glucose in the blood stream which in turn decreases the postprandial hyperglycemia in diabetic patients. Enzyme kinetic assays on yeast and mouse α-Gls proved that these compounds have an IC50 value in micro molar range and since they have no inhibitory activity on porcine pancreatic α-amylase, they may be considered as specific and selective inhibitors of α-Gls which in medical usage mean that it may result in fewer side effects for diabetic patients. The addition of different substructures to the pyrimidine fused core significantly altered the action of the compounds, ranging from zero to considerable inhibitory action. The inhibitory action of these PFH derivatives on yeast α-Gls was up to 30 folds improved than commonly used anti-diabetic drug, acarbose. Hence, in search of new, selective and easily accessible anti-diabetic α-Gls inhibitors, pyrimidine fused derivatives were proved to be a new scaffold for potent and specific α-Gls inhibitors which can efficiently decrease the blood glucose levels and result in fewer side effects.

Key words: α-Glucosidase (α-Gls); Inhibitors; Antidiabetic compounds; Pyrimidine-fused heterocycles

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Oral Talk – L25 Open Access

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

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Lemon balm (*Melissa Officinalis*, L.) extract prevents formation of haemoglobin A1c

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ABSTRACT

Among the strategies to combat protein glycation are medicinal herbs with a broad spectrum of functional molecules. This work represents how balm (Melissa officinalis) may help in preventing the negative effects of AGE-induced toxicity in human hemoglobin. The progress of Millard reaction during the initial stage of protein glycation was attenuated by applying the herb extract. However, mitigation of the incidence of heme loss at the later stage of glycation yielded hints for the herb preventive effect. After treatment with balm extract, an obvious quenching in the tryptophan fluorescence intensity of the glycated Hb was observed. Increment in ThT-related fluorescence intensity with increasing the time of incubation reveals the progressive formation of the amyloid-conformation, remained constant when the samples treated with the balm extract. Fructose-induced conformational alterations at the secondary structure of Hb were studied by CD spectropolarimetry. Noticeable differences in intensity of the signals between samples glycated with and without balm extract indicate the herb inhibitory effects on transition of α to β conformer during fructation. The efficacy of Melissa extract in protecting the structural integrity of hemoglobin during glycation is in accordance with its known antioxidant properties, suggest an outstanding natural resource capable to delay or prevent the onset of diabetic complications. Targeting each step of the molecular mechanism(s) underlying the detrimental effects of the sugar by the balm extract suggest a promising tool for preventing the complications of diabetes and related anemia, deleterious dietary behaviors and sugar-induced cellular damage.

Key words: *Melissa officinalis*; Protein Glycation; Hemoglobin; Advanced glycation end products

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Oral Talk – L26 Open Access

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

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Oxidative stress stimulates protein glycation

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ABSTRACT

Urbanization especially in great cities and consequent events like all of chemical and non-chemical pollutions, terrific and intellectual engagement, are the main factors for stress and any concerns in recent word. Oxidative stress arises from transferring of any external stress to the body's internal system which leads to consequent body imbalances and various diseases. In the other word, oxidative stress can be considered as the minor part of all disease procedures and also as a serious imbalance situation between oxidants and anti-oxidants systems. This imbalance increases the formation of unconventional toxic products entitled “free radicals” and especially “ROS”. These compounds are removed from body control system and their interactions with biological macromolecules, especially proteins cause the incontrollable cascade interactions as the new source of toxic free radicals. These results make further damage of biomacromolecules structure, greater toxic compounds, and disrupt the structure and function of tissues and organs eventually. Different types of proteins oxidative modifications can be made directly by interaction with ROS or indirectly via oxidative stress secondary products. Both of these pathways produce the intermediates which are the important toxic products of protein glycation with glucose. So the presence of free radicals and protein oxidation can be the main linkage between protein oxidation and oxidative stress even in the absence glucose. Our research has also demonstrated the protein glycation effect; plenty advanced glycation end products (AGEs) generation and harsh fibrillation of human serum albumin (HSA) upon incubation with industrial preservatives (e.g. potassium sorbate, sodium benzoate, etc) in the absence of glucose. This effect is much more effective relative to incubation of HSA with glucose. Therefore we conclude the industrial preservatives (oxidative stress) can play important and extensive role in diabetes type2 and its complication.

Key words: Protein Glycation; Oxidative Stress; HSA; ROS; Protein fibrillation; Potassium sorbate; Diabetes type2

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Oral Talk – L27 Open Access

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

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Recombinant expression of light chain of *Botulinum neurotoxin* type-A in *E. coli* and evaluation of its enzymatic activity

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ABSTRACT

Botulinum neurotoxins, specifically type-A, are one of the most dangerous biological agents in nature that cause paralysis. These toxins have endopeptidase activity which can inhibit secretion of acetylcholine neurotransmitter through neuron synapsis. This study focuses on recombinant expression of catalytic domain (light chain) of botulinum neurotoxin type-A with high yields in order to evaluate its catalytic activity. Sequence of light chain of botulinum neurotoxin type-A was obtained from NCBI databank. Optimizing the codon preferences for *E. coli* and final sequence were ordered for subcloning in pET28a (+). Vector including mentioned gene was transferred into *E. Coli BL21-DE3* for expression. Protein expression conditions were optimized for solubilization of protein. Expressed protein were purified with Ni-NTA, proved with specific antibody and analyzed for its activity with HPLC technique. Best soluble expression conditions of protein are achieved in induction OD of 0.5, 0.5 mM of IPTG for 18 hours in 18° C. Soluble protein were purified with Ni-Agarode affinity chromatography and proved with western blotting and ELIZA techniques. Results represent soluble expression of light chain of botulinum neurotoxin type-A with high yields over 98%. Analysis of HPLC data demonstrates 3 µmol min^{-1} mg^{-1} specific activity of light chain of botulinum neurotoxin type-A.

Key words: Botulinum neurotoxin type-A; Catalytic domain; Endopeptidase activity; Recombinant expression

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Oral Talk – L28 Open Access

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

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Worldwide research on camel proteins

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ABSTRACT

During the last two decades, camel has become very important species worldwide. It is considered as a source of functional food and a factory to produce pharmaceutical products for human being. Camel population increased during the last 14 years by 10 million (26,944,669 heads, FAO, 2012). Unfortunately, camel population in Iran remained constant during the last 2 decades (150,000 heads)! UAE is the only country in the world that makes strong move toward industrialization of camel milk and received the import permit for the milk to Europe. Although Africa has the greatest camel population (80%), its role in producing science in this species is very limited. In contrast, America and Europe with nearly no camel population, have great impact on producing science in camel proteins! During the last 2 decades, 283 articles on camel proteins were indexed by ISI (web of science). The contribution of Asia, Europe, America and Africa in producing such articles was 44, 37, 14 and 5%, respectively. Among 202 articles published in camel milk, 48 articles were on camel milk proteins, 33 articles were on the effect of camel milk on treating the diseases such as diabetes and allergies and 121 articles were on milk component analysis. Iran produced 18 articles in this topic. All articles published regarding proteins in camel blood (81 articles) were on immunoglobulins and nano-bodies. Iran produced 8 articles in this subject. With long term programs to study about camel proteins, we are able to produce more scientific articles and wealth for our country.

Key words: Camel; Milk; Blood; Protein

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Oral Talk – L29 Open Access

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

Shiraz University 10 & 11 December 2014

Hairy roots efficient tool for the production of recombinant proteins

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ABSTRACT

A specific protein is rarely easily enriched from natural host cells. Therefore, recombinant protein production is the only practical procedure. Different recombinant proteins have been produced in prokaryotic hosts but many of them accumulate in the form of inactive inclusion bodies. The inability of prokaryotes to produce native versions of eukaryotic proteins is because of unsuitable posttranslational protein processing, including inappropriate protein cleavage and folding, and to the lake of proper mechanisms that add chemical groups to specific amino acids. Consequently, eukaryotic expression systems were devised for fungal, insect, mammalian cells, and plant hairy roots. Although functional recombinant proteins have been achieved using fungal and insect cells, some mammalian proteins are not correctly glycosylated. In the other side, the mammalian host cells have the high costs. All of these cell hosts can become contaminated with the animal pathogens and sometimes are so costly and complex to be optimized. Hence, hairy roots for recombinant protein production are as one of the interesting technologies. They result from the genetic transformation of plant cells after infection by *Agrobacterium rhizogenes*. They have interesting advantages including fast growth rate on hormone-free medium, highly branched, and phenotypically and genetically stability. Hairy roots can do all the posttranslational protein processing. The foreign proteins expressed are usually secreted in the culture medium and can be harvested from it with the low cost. This model of recombinant protein production is relatively safe because they are non-host for animal pathogens.

Key words: Protein expression hosts; Hairy roots; Recombinant proteins

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Oral Talk – L30 Open Access

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

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Disruption of mitochondrial membrane integrity induced by amyloid aggregates arising from variants of SOD1

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a progressive lethal, degenerative disorder of motor neurons. Although most cases are classed as sporadic ALS (SALS), 10% of cases are inherited (known as familial ALS; FALS). The cause of most cases of ALS is as yet undefined. About 20–25% of all FALS cases arise because of mutations in Cu, Zn superoxide dismutase (SOD1) which is the primary cytoplasmic scavenger of super oxide radical. It has been shown that mutations in SOD1 can induce ALS through a gain of cytotoxic function, the molecular basis of which remains unknown. Increasing evidences indicate that the interaction of amyloid aggregates with membranes is critical in the onset and progression of amyloid diseases. In the present report, we describe interaction of the fibrillation products of the wild type (WT) and four mutants (E100K, D125H, D102N and L127S) of SOD1 obtained under destabilizing condition with mitochondrial membranes, as an *in vitro* biological model, with the aim of gaining insight into possible mechanisms of cytotoxicity at the membrane level. Release of mitochondrial enzymes upon exposure to SOD1 aggregates demonstrates that these aggregates could affect membrane integrity.

Key words: Superoxide dismutase; Familial amyotrophic lateral sclerosis; Aggregation; Mitochondria; Membrane permeability

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Oral Talk – L31 Open Access

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Genipin; a natural cross-linking agent for tissue engineering

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ABSTRACT

Healing and regeneration of the cutaneous wounds and those defects related to the tendons, bones and other connective tissues are regarded as a considerable challenge for the cosmetic and orthopedic surgeons. Tissue engineering, as a novel technology, by fabrication of the artificial skin or synthetic grafts or implants is attempting to help the medicinal society. For this purpose, tissue engineering employs different biomaterials effective in accelerating the healing process of these defects. In addition to the properties such as biodegradability, biocompatibility and bioactivity, these agents should be cross-linked together to exert their cumulative effects. Therefore, there is an essential need for a non-toxic or low toxic and non-expensive cross-linker to establish a link among these biomaterials. Genipin, a natural cross-linking agent obtained from gardenia (*Gardenia jasminoids*) fruit extract, has received an increasing interest in biomedical applications. Genipin could be used as an excellent cross-linker for proteins such as collagen, gelatin, chitosan and other materials that are commonly used in tissue engineering with various purposes. It can efficiently cross-link cellular tissues and biomaterials containing amino acids and because of its nature, genipin exhibits the least cytotoxicity than other cross-linking agents such as glutar aldehyde. Moreover, due to the cross-linking property of genipin, it can be applied as a regulating agent for constant and continuous drug delivery. In conclusion, genipin could be considered as a promising cross-linking reagent in tissue engineering of different soft and hard tissues such as skin, tendon, ligament, cartilage, and bone.

Key words: Tissue engineering; Cross-linking; Genipin; Proteins; Collagen

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Oral Talk – L32 Open Access

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Pyrimidine-fused heterocycle derivatives as potent and selective antidiabetic α-glucosidase inhibitors

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ABSTRACT

α-Glucosidase (a-Gls) has a crucial role in digestion of carbohydrates and biosynthesis of glycoproteins. Therefore, the inhibition of this enzyme plays an important role in treatment of degenerative diseases such as type-II diabetes mellitus. The inhibitors can reduce the complications of diabetes as they interfere with the enzymatic action of intestinal a-Gls. Consequently, the liberation of glucose into bloodstream will be retarded, resulting in delaying glucose absorption and decreasing postprandial hyperglycemia. In this study, a new scaffold containing 3 substructures was introduced to be efficient antidiabetic a-Gls inhibitors. In the structure of this class of compounds there is a pyrimidine-fused heterocycle (PFH) ring as main substructure which the activity of synthesized compounds completely depended to the existence of it in the structure. Dives derivatives of PFH were synthesized using a multicomponent reaction by change of 3 substructures and their activity evaluated against a-Gls and a-Amy enzymes. For synthesis of these compounds, a convenient and efficient method was described which open up a new direction in the synthesis of divers compounds based on this scaffold. It should be mentioned that compared to Acarbose, these compounds weakly inhibit the activity of pancreatic a-Amy. Overall, these compounds can be considered as a base for the synthesis of novel a-Gls inhibitors which may efficiently and specifically inhibit a-Gls and more importantly they may cause less side effects compared to the conventional a-Gls inhibitors such as Acarbose.

Key words: Pyrimidine-fused heterocycle; Antidiabet; α-glucosidase inhibitor; Multicomponent reaction

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Oral Talk – L33 Open Access

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

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Chemical analogs design of curcumin as effective inhibitors related to lysozyme amyloid fibrillation

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ABSTRACT

The self-association of misfolded proteins into fibrils is an essential event of many human diseases including Alzheimer's and Parkinson diseases. Several clinical studies proved that curcumin has a chemotherapeutic activity in AD. It has been suggested that chemical scaffold of curcumin, containing two aromatic end groups separated by a rigid, planar linker is necessary for its inhibition role. Clinical application of curcumin for AD treatment is severely limited because of its poor bioavailability, high rate of metabolism, and instability under neutral condition. In the current study, we found three compounds in which diketone moiety of curcumin was replaced by cyclohexanone and the linker length of the molecules are optimal; further, substitution dioxolane for hydroxyl groups on compound 3 should prevent metabolic inactivation. We used hen egg white lysozyme (HEWL) to induce fibrillation and investigated the inhibitory effect of the compounds against HEWL fibrillation. The molecules were screened via ThT, AFM and MTT assay. We found that all three compounds able to inhibit HEWL aggregation in a dose-dependent manner. These compounds also inhibit the cytotoxic activity of aggregated HEWL toward cell culture. Docking results also demonstrated that the compounds bind into lysozyme and occupy the whole active site groove. In conclusion, we found chemical analogs of curcumin with various modifications in the spacer and the phenolic rings in order to identify improved inhibitors of amyloid aggregation.

Key words: Drug discovery; Curcumin analog; Amyloid disaggregation; Cytotoxicity; Docking

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Oral Talk – L34 Open Access

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Introducing biomarker panel in esophageal, gastric, and colon cancers; a proteomic approach

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ABSTRACT

Cancer research is an attractive field in molecular biology and medicine. By applying large-scale tools such as advanced genomics and proteomics, cancer diagnosis and treatment have been improved greatly since. Cancers of esophagus, gastric, and colon accounted for major health problem globally. Biomarker panel could bring out the accuracy for cancer evaluation tests as it can suggest a group of candidate molecules specified to particular malignancy in a way that distinguishing malignant tumors from benign, differentiating from other diseases, and identifying each stages with high specificity and sensitivity. Here, a systematic search of unique protein markers reported by several proteomic literatures in addition to original findings related to cancers of esophagus, gastric, and colon are classified in their specific cancer type group as novel panels for feasible accurate malignancy diagnosis and treatment. About thousands of candidate proteins were studied; however, a little numbers of them were belonged to a specific kind of malignancy. In conclusion, despite of the fact that combinatorial biomarkers appear to be hopeful, more evaluation of them is crucial to achieve the suitable biomarker panel for clinical application. This effort needs more investigations and researches for finding a specific and sensitive panel.

Key words: Biomarker panel; Esophagus cancer; Gastric cancer; Colon cancer, Proteomics

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Oral Talk – L35 Open Access

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

Shiraz University 10 & 11 December 2014

The effects of γ-aminobutyric acid (GABA*)* and its derivatives on microtubule polymerization in rat brain

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ABSTRACT

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the brain. This molecule is essential for the overall balance between neuronal excitation and inhibition which is vital for normal brain function. At least 40% of inhibitory synaptic processing in the mammalian brain employs GABA. In vertebrates, GABA acts at inhibitory synapses in the brain by binding to specific transmembrane receptors in the plasma membrane of both pre- and postsynaptic neuronal processes. GABA binding to the receptors causes the opening of ion channels to allow the flow of either negatively charged chloride ions into the cell or positively charged potassium ions out of the cell. The action results in a negative change in the transmembrane potential, usually causing hyperpolarization. Two general classes of GABA receptor are known: GABAA in which the receptor is part of a ligand-gated ion channel complex, and GABAB metabotropic receptors, which are G protein-coupled receptors that open or close ion channels via intermediaries. Neurons that produce GABA as their output are called GABAergic neurons, and have mainly inhibitory action at receptors in the adult vertebrate. GABA (A) receptors and microtubules interact together via a GABARAP (GABA (A) receptor-associated protein). Inhibitory system in the brain has been theorized for time perception and GABA is an important part of this system. In this study, we investigated GABA and its derivatives effects on microtubule Polymerization resulting in time induction.

Key words: GABA's derivatives; GABA (A) receptors; Microtubule; Time perception

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Oral Talk – L36 Open Access

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

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In vitro cell response to a new protein nanocomposite

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ABSTRACT

Current artificial materials used in the fabrication of orthopedic implants involve a range of various materials based on ceramics, metals, conventional polymers, and their combinations. In contrast to the protein-based hydrogels, none of these materials can be adapted neither for fabrication of hydrated structure nor for call-laden constructs. Moreover, most of these materials typically do not bear any functionality that encourages communication with their cellular environment. Therefore, in this study, composite of collagen-based protein and calcium phosphate (CaP) nano-ceramic was fabricated and preosteoblastic MC3T3-E1 cells were subsequently encapsulated at a density of 3×10^6 cell/mL in the resulting nanocomposite. In vitro cell response to the fabricated protein nanocomposite was subsequently determined by Alamar blue assay, DNA quantification, and fluorescent labeling of F-actin fibers. According to the results, CaP cannot significantly induce cell apoptosis. Moreover, cells remained mainly round and isolated from each other after one day of encapsulation. However, encapsulated cells readily elongated, proliferated, and formed a well-interconnected network with neighboring cells after 4 days of culture, showing the ability of the protein/CaP nanocomposites to provide natural cell binding motifs for cellular growth. In addition, after a longer culture period, cells became first confluent in the protein matrix, and then migrated and formed a confluent cell layer over the hydrogel. Accordingly, the protein-based composites developed in this study were quite compatible with bone cells; and hence they may be considered for preparing cell-laden scaffolds with increased osteoconductivity to induce bone formation in vivo.

Keywords: Protein hydrogels; Nanocomposite; Calcium phosphate; Preosteoblastic cells; Cell-laden scaffolds

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Oral Talk – L37 Open Access

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Antigen nature effect on immunological memory

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ABSTRACT

Heat labile toxin B subunit (LTB) as binding domain of toxin induce 6 months memory longevity in humans; while botulinum toxin binding domain (BoNT A-Hc) and tetanus toxin binding domain (THc) can induce 2 and 10 years memory longevity, respectively. It seems nature of antigen can effect on immunological response and memory (huge of memory cell population). So, this study, in identical circumstance, investigates and determined memory B cell population that induced by each three antigens. Recombinant proteins of THc, BONT/A-Hc and LTB were expressed in *E. coli* Bl21 (DE3). Then, these three proteins were injected into mice separately and their immunogenicity and immunological memory were evaluated by ELISA and flow cytometry. After immunization, the ELISA results showed that antibody titer was higher in serum of THc mice than BoNT/A-Hc, and LTB immunized mice had lower antibody titer. Flow cytometry results indicated memory B cell population of immunized mice was increased in LTB, BONT/A-Hc and THc respectively. Huge of memory B cell population was affected by antigen nature.

Key words: Recombinant protein; LTB; BONT/A-Hc; THc; Immunological memory

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Oral Talk – L38 Open Access

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

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Evidences for a dynamic role of the histone 2A variant H2A.Z in regulation of cell proliferation/differentiation switch

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ABSTRACT

Differentiation of eukaryotic cells from an unspecified to a committed state involves global changes in genome expression patterns, critically dictated by specific incorporation of different types of chromatin proteins, totally named as epigenetic marks. Histone variation, in which canonical histones are replaced with their variant counterparts, is an entire branch of epigenetics that has received several functional significances on gene regulation. Recent studies suggest the incorporation of histone variants into the chromatin regulate cellular proliferation, differentiation, and de-differentiation. Here we show that histone H2A.Z, a variant of histone H2A, is actively exchanged in response to Retinoic Acid (RA)-induced differentiation of human embryonal carcinoma cells, as well as through the proliferative phase of menstrual cycle in human endometrial cells. Also, we demonstrate that the chromatin incorporation level of this histone variant is significantly higher in ectopic endometrial tissues of patients with endometriosis. Collectively, these data support a strong correlation of H2A.Z expression with undifferentiated or unspecified cells, rather than with committed cells, and provide evidences for the dynamic role of H2A.Z in cellular proliferation/differentiation switch.

Key words: Histone variant; H2A.Z; Proliferation; Differentiation

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Oral Talk – L39 Open Access

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

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Heterologous expression and metal-binding characterization of four different rice metallothionein isofroms

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ABSTRACT

Metallothioneins (MTs) are a superfamily of low-molecular-weight, cysteine (Cys)-rich proteins that are believed to play important roles in protection against metal toxicity and oxidative stress. Plants have several MT isoforms, which are classified into four types based on the arrangement of Cys residues. In this study, four rice (Oryza sativa) MT isoforms OsMTI-1b, OsMTI-2a, OsMTI-3a and OsMTII-1a that belong to type 1, type 2, type 3 and type 4, respectively, were heterologously expressed in *Escherichia coli* as carboxy-terminal extensions of glutathione-S-transferase (GST). The tolerance of *E. coli* cells expressing GST–OsMTI-1b, GST-OsMTI-2b and GST-OsMTI-3a considerably increased to $Ni^{2+}$and Cd^{2} through the accumulation of more metal ions compared with cells expressing GST alone. However, heterologous expression of GST–OsMTII-1a had no significant effects on metal tolerance or ion accumulation. The recombinant MTs were extracted from *E. coli* cells and purified using affinity chromatography. The apo forms of these MTs were provided by acidification and then were exposed to Cd^{2+} and Ni^{2+}. The UV absorption spectra and competitive reactions of in vitro Cd^{2+}/Ni^{2+}-incubated proteins with 5-5_-dithiobis (2-nitrobenzoic) acid revealed that GST–OsMTI-1b, GST-OsMTI-2b and GST-OsMTI-3a are able to form Cd/Ni-thiolate clusters. However these different rice MT isoforms differ in binding to different metals.

Key words: Metallothionein; Rice; Metal-binding characterization

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Oral Talk – L40 Open Access

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

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Design of oligonucleotide ligands with affinity for coagulation factor VIII

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ABSTRACT

Aptamers are oligonucleotide ligands of synthetic single-stranded DNA/RNAs (ssDNA/RNA) with specific conformations which were created by *in-vitro* selection from a large random sequence pool, based on the affinity between oligonucleotides and the specific target molecules. This screening method is called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Aptamers' unique characteristics in protein binding have made them highly valuable tools for different diagnostic applications, downstream purification processes, analysis, new drug development and drug delivery. One of the important and valuable therapeutic plasma proteins is human coagulation factor VIII (FVIII). In this investigation, FVIII was prepared from plasma source or recombinant production media and considered as a model protein for designing and development of relevant oligonucleotide aptamers. As the first step, random oligonucleotide library and its specific primers were designed and then chemically synthesized. After induction of 3D conformation by a specific temperature treatment, the random library was incubated with plasma derived FVIII. Gel filtration method was then employed as a partitioning step. During SELEX process, for the elimination of nonspecific cross interactions by other plasma proteins, two steps of negative selections were run. Finally, SELEX process was stopped when no more increase in the affinity constant (K_d) was observed and therefore the enriched pool was cloned. As the final step, protein affinity of enriched pools was determined using fluorescence method. It was found that the calculated K_d for enriched pools were 0.5 to 60nM and that they are comparable to the other specific ligands of the target protein.

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

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Oral Talk – L41 Open Access

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

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E-cadherin protein an appropriate marker for epithelial cancer cells and a good candidate for cancer treatment

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ABSTRACT

E-cadherin is a marker for epithelial cells which expressed at the cell surface and adherens junctions. β-catenin is a partner for E-cadherin and binds to its cytoplasmic domain. Lack of E-cadherin expression in malignant epithelial tumors and invasive cancer cell lines such as PC-3 (prostate cancer cell), has been reported. Also it has been reported that PC-3 cells are negative for E-Cadherin. We showed that the E-cadherin protein does not appear to be localized at the cell membranes and it has a diffuse staining pattern inside the PC-3 cells. We demonstrated that treatment of PC-3 cells leads to restoration of this protein and also translocation of β-catenin protein from the nucleus to the cell membrane, formation of E-cadherin/catenin complex and adherens junction strengthening. In this study, we showed that increasing the cellular levels of E-cadherin and β-catenin proteins in PC-3 cells induces a mesenchymal to epithelial transition (MET). This led to inhibition of cell invasion capability of PC-3 cells. We also indicated that formation of E-cadherin/catenin complex leads to changing the expression of its target genes. Taken together, this study shows that E-cadherin is an appropriate diagnostic marker for epithelial cancers and inducing the translocation of E-cadherin induces the formation of E-cadherin/ β-catenin complex. We found that inducing the expression of E-cadherin could be a strategy to inhibit invasive cancer cells.

Key words: E-cadherin; E-cadherin-β-catenin complex; Protein translocation; Epithelial cancers-invasion

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Oral Talk – L42 Open Access

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

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Enhancement of stability and catalytic activity of immobilized xylanase on modified magnetite nanoparticles

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ABSTRACT

The covalent binding of xylanase to silica-coated modified magnetite nanoparticles via cyanuric chloride activation was investigated. The structure, size, and magnetic properties of the support and immobilized xylanase were characterized by TEM, FTIR spectroscopy, TGA, and VSM analysis. The TEM images showed that the synthesized functionalized nanoparticles (CC-Fe_3O_4@SiO_2) possessed three dimensional core–shell structures with an average diameter of ~9 nm. Results from FTIR, Bradford protein assay, and TGA indicated that xylanase was covalently attached to the surface of magnetic nanoparticles with a immobilization yield of 280 mg enzyme/g MNPs. The VSM analysis revealed that Fe_3O_4, Fe_3O_4@SiO_2 and xylanase-MNPs had high saturation magnetization of 69.4, 63.84 and 46.56 emu/g, respectively. The properties of the immobilized xylanase were investigated in comparison with the free enzyme counterpart. Enzymatic activity, reusability, thermo-stability, pH-stability, and storage stability of the immobilized xylanase were found significantly superior to those of the free one. The xylanase-MNPs exhibited maximal catalytic activity at pH 6.5 and 60 °C and the immobilized enzymes were found to keep as high as 80% of the activity of free ones. Notably, xylanase-MNPs showed quite impressive stability, even after 9 reaction cycles, it could still retain about 65% of the initial activity. The measurement of Michaelis–Menten parameters (K_m and v_{max}) also revealed the considerable improvement of immobilized enzyme. The results suggested that xylanase-MNPs could be used in an interesting range of application allowing both using in broader temperature and pH ranges, facilitating long-term storage, while permitting magnetic recovery of the enzyme for reuse or purification of the product.

Key words: Magnetite nanoparticles; Immobilization; Xylanase; Enzyme activity; Enzyme stability

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Oral Talk – L43 Open Access

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

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The impact of homocyteinylation and peroxynitrytation on structure, function and aggregation of lens crystallins; implications for their possible contribution in the pathogenesis of cataract disorders

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ABSTRACT

Due to limited turn-over of lens crystallins, various post-translational modifications (PTMs) accumulate on these proteins throughout the whole life span. Accordingly, PTMs can affect the structure and functionality of lens crystallins which result in coloration, aggregation and insolubilization. Eventually, the formation of large water-insoluble crystallin aggregates leads to lens clouding and light scattering. In the current study, the impact of homocyteinylation and peroxynitrytation was examined on structure, function and aggregation/amyloidgenic properties of lens crystallins, using various spectroscopic techniques and gel mobility shift assay. As indicated in this study, homocysteinylation results in significant structural alteration, formation of detectable protein aggregates/amyloid-like entities and significant loss in chaperone activity of α-crystallin. The results of this study may suggest lens proteins homocysteinylation as a possible mechanism to explain the relationship between hyperhomocysteinimia and various impairments of the visual system. Also, the peroxynitryte-modified lens crystallins demonstrate considerable structural destabilization and significant aggregation propensities, particularly in the presence of calcium ion which has been known to play an imperative role in the pathophysiology of eye lens. Therefore, the simultaneous raise of calcium and peroxynitrite in eye ball seems an important pathological event, underlying the pathomechanism of cataract development.

Key words: Lens crystallins; Peroxynitrite; Homocysteine thiolacton; Calcium; Cataract

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Oral Talk – L44 Open Access

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

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Toward a strategy to describe and predict activities of peptide drugs contain L/D and unnatural residues: QSAM of antimicrobial hexapeptides

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ABSTRACT

The fact of increasing use of antibiotics in immunosuppressant patients has resulted in the prevalence and drug resistance of bacterial/fungal superbugs. Cationic antimicrobial peptides (CAPs) as a wide category of compounds with their primitive defense mechanism could be an effective immune wall against the superbugs-associated infections. Design and introduction of new CAPs with better therapeutic activity is always demanding and is in progress. Between the different strategies for peptide design, applications of quantitative structure-activity relationship (QSAR) studies which provide information on activities of CAPs based on descriptors for each individual amino acid are inevitable. In this study, the quantitative sequence-activity modeling (QSAM) of 60 CAPs derived from O-W-F-I-F-H(1-Bzl)-NH_2 sequence which showed excellent activities against a broad range of hazardous microorganisms; e.g., MR*SA*, MR*SE*, *E. coli* and *C. albicans* are discussed. The peptides contained natural and non-natural amino acids (AAs) of the both isomers D and L. In this study, a segmented principal component (SPCR) strategy was performed on the structural descriptors of AAs to extract AA's indices. Our results showed that constructed models covered more than 82%, 94%, 80% and 78% of the cross-validated variance of *C. albicans*, *MRSA*, *MRSE* and *E. coli* data sets respectively. The results were also used to determine the important AAs which are important in CAPs activities. According to the best of our knowledge it is the first successful attempt in the QSAM studies of peptides contain both natural and non-natural AAs of the both L and D isomers.

Key words: Cationic antimicrobial peptides; Superbugs; Chemoinformatic; Quantitative sequence-activity modeling

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Oral Talk – L45 Open Access

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Molecular dynamics simulations studies of substrate and inhibitor interactions with monoamine oxidase-B active site

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ABSTRACT

Monoamine oxidase-B (MAO-B) is a mitochondrial outer membrane flavoenzyme that is a well-known target for anti-depressant and neuroprotective drugs. MAO-B is one of two flavin-dependent isozymes (the other being MAO-A) that function in the oxidative determination of neurotransmitters and exogenous arylalkylamines. Mammalian MAOs are bound to the outer mitochondrial membrane through a C-terminal transmembrane poly peptide. These oxidases have long been a pharmacological focus because both reversible and irreversible inhibitors of MAO-A and B have been used clinically in the treatment of neurological disorders. The active site of MAO-B consists of a 420 $Å^3$-hydrophobic substrate cavity interconnected to an entrance cavity of 290$Å^3$. Advances in computer hardware, in force fields, and in molecular dynamics simulation methods and software have enabled increasingly more sophisticated studies of protein and drug structures and dynamics. On the basis of the experimental information about the substrate and inhibitor binding site of human MAO-B atomistic molecular dynamics simulation was performed to determine what factors govern inhibitor specificity and the inhibitor binding process, the active site loop, and several key residues within the binding pocket. The simulations demonstrate that the average energy of interaction between inhibitor and MAO-B residues provide researchers with valuable tools for designing effective MAO-B inhibitors as well as outline a method that can be translated to the study of other enzyme-inhibitor complexes.

Key words: Molecular dynamics simulation; Monoamine oxidase-B (MAO-B)

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Oral Talk – L46 Open Access

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The mechanism of antioxidant defense of glutathione peroxidase and superoxide dismutase induced by nano selenium

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ABSTRACT

Glutathione peroxidase and superoxide dismutase (SOD) are the most important antioxidant enzymes of the cell. The aim of this study is to find the antioxidant mechanism and ameliorative role of selenium nanoparticles on oxidative stress in Wistar rats treated with isoniazid. This experimental study was done on 40 adult Wistar rats. Animals were divided into 5 groups. Control group: This group received only enough food and water. Reference group received physiology serum. The first Experimental group received isoniazid with 50 mg/kg dose of the animal weight for 14 days. The second Experimental group received Selenium nanoparticle with 0.1 mg/Kg/ bw dose and the third Experimental group received Selenium nanoparticle with 0.2 mg/Kg/ bw dose for 13 days and after 24 hours both experimental group 2 and experimental groups 3 received, in addition to Selenium nanoparticle, 50 mg/kg isoniazid. Blood samples were used to measure the concentration of nitric oxide, glutathione peroxidase and superoxide dismutase activity and total antioxidant capacity by Spectroscopic methods and ELISA kit. The results of this study shows that isoniazid with antioxidant enzymes restrain, decrease of the total antioxidant capacity and NO oxidant production induced lipid peroxidation causes oxidative damage. Selenium nanoparticles, by increasing GPx activity and decreasing SOD activity lead in reduction of damages produced by NO. In conclusion, decreasing of superoxide dismutase activity leads to decrease in hydrogen peroxide concentration and control antioxidant defense by the negative feedback.

Key words: Antioxidant; Glutathione peroxidase; Superoxide dismutase; Nitric oxide; Nano selenium