Abstract No.102

Spectroscopic Monitoring of Salt Impurity as an IEF Interfering Issue in Tear Sample Preparation

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Human tear film which is composed of three different layers (the innermost glycocalix-mucin, an intermediate aqueous-mucin and an outermost lipid layer) is a valuable specimen for clinical studies. Changes in tear proteome content can be an indicator of many ocular and systemic diseases even in the early stages. Unfortunately high salt content and structural complexity of tear film offers a physicochemical complexity to the sample which makes it difficult to analysis by two-dimensional gel electrophoresis (2DE) as the most powerful technique in biomarker discovery.To seek an appropriate sample preparation, essential for obtaining reliable results in tear proteome analysis, basal tears from healthy volunteers were collected by Schirmer strips and Protein extraction was performed by incubation of each strip in 100 µl of 100 mM ammonium bicarbonate at room temperature for half an hour. All samples were pooled and homogenized to avoid intra individual variability. For tear 2DE interfering removal eight different precipitation methods (acetone, acetone/methanol, TCA/acetone, ammonium sulfate (50, 70 and 90%) tri-n-butyl phosphate (TBP) and chloroform/methanol) were used. A UV-Vis monitoring system (NanoDrop 2000c) was then applied to investigate the probable salt contamination after different sample treatment and 2DE was performed to see if incomplete salt removal can affect the final proteome map or not.Herein we demonstrated the presence of ammonium bicarbonate in samples treated with acetone, acetone/methanol and TCA/acetone methods. Contamination of ammonium sulfate was also observed in those treated with ammonium sulfate precipitations. No salt contamination was observed in TBP and chloroform procedures. Finally comparison of the resulted 2DE pattern revealed the fact that although too much salt in the sample disturbs isoelectric focusing, in the case of salty tear proteome trace amount of salt can improve the protein solubility during the onset IEF separation and affects their transfer to the IPG strips.

Keywords: Tear Film, UV-Vis Spectroscopy, 2D PAGE, Salt Impurity.

Abstract No.103

Spectroscopic studies of the interaction of bovine beta-lactaglobulin with a new designed Pd(II) complex

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Beta-lactoglobulin (BLG) is one of the major soluble cow's milk proteins and also one of the main allergens in milk. This protein seems to be resistant and stable to gastric digestion and denaturation. The purpose of this work is to establish whether the treatment of BLG by Pd(II) complex would affect its allergnicity, as well as its structure. Then, the new Pdcomplex [Pd (bpy) (Hex.Gly)]NO_3 was designed and the interaction of this new synthesized complex with bovine milk carrier protein of BLG was investigated using fluorescence at room and physiologic temperatures. The results of fluorescence studies indicated that Pd(II) complex can quench the intrinsinc fluorescence emission of the protein and also may approved that it can bind to the protein and alter the protein structure. The binding parameters of this interaction have calculated using quenching methods at different temperatures. Above results represented that the new synthesized Pd(II) complex can bind to the milk carrier protein of BLG and also can change the tertiary structure of the protein.

Keywords: β-Lactoglobulin, Pd Complex, Fluorescence, Quenching.

Abstract No.104

A Computational Analysis of Interactions of Curcuminoids with Human Serum Albumin

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A curcuminoid is a curcumin or its derivative with different chemical groups. During the last few decades, curcuminoid have been utilized in biological and pharmaceutical fields, because of its remarkable anti-tumor, anti-oxidant, anti-arthritic, anti-amyloid, anti-ischemic and anti-inflammatory properties. Curcumin is a hydrophobic compound and shows lipid solubility. Being hydrophobic, curcumin needs a carrier system to transport to different parts of the body. Although curcumin has potential for extensive use in disease treatment, there are two major challenges that limit this target. First, curcumin has low solubility in aqueous solution, which poses a severe limitation on the achievable concentration in biological systems. Second, the soluble portion undergoes rapid degradation at physiological pH. Several methods have been proposed to address the issues of low solubility and stability. Recently, the use of serum albumin as a carrier for curcumin has been reported. Results by Wang et al., indicate that degradation of curcumin in buffer solutions is suppressed in the presence of serum, of which the major components are water (92%) and proteins (6-8%).The principal aim of this work is to perform a computational study of the structural and electronic properties of different conformations of curcumin and four other Curcuminoids by DFT Method to find the most stable equilibrium structure and to define the nature of the molecular orbitals, HOMO and LUMO that are important to explain binding characteristic. Then interaction of curcuminoids with albumin protein was studied by using molecular docking and molecular dynamics simulation. By computational methods, we can recognize that group of residues which participate in bonding with curcuminoids and binding energy and kind of interactions. Lys276 and Gln268 amino acids are in suitable position to be involved in making H-bonds with the carbonyl oxygen functions at the center and phenolic ring of curcumin, respectively.

Keywords: Curcumin, Molecular Docking, DFT, Molecular Dynamics, Human Serum Albumin.

Abstract No.105

Investigating the Role of the Salt Bridige Between Asp100 and Arg80 in TMGS Allosteric Behavior

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The Allosterically regulated enzyme methylglyoxal synthase (MGS, EC 4.2.3.3) is a homohexameric enzyme that catalyzes the conversion of dihydroxyacetone phosphate (DHAP) to methylglyoxal and phosphate in the first step of the methylglyoxal bypass of glycolysis pathway. Thermophilic type of MGS was first isolated and expressed from Thermus sp.GH5 (TMGS). Phosphate was found to be a strong inhibitor and negative allosteric effector of the enzyme. According to the crystallographic structure of phosphate bound MGS, conformational changes in the conserved regiones of the protein closes the entry of the channel leading to the active site and affects the interresidual interaction at the monomer-monomer interface. New interaction between residues Arg80 and Asp100 occur upon phosphate binding to the enzyme and may provide a pathway by which allosteric inhibitory signal is transmitted through the intersubunit interface. To test this hypothesis, this two residues were mutated with Quik change site directed mutagenesis and allosteric behavior of mutated variants were investigated.

Keywords: TMGS, Allosteric Inhibitor, Methylglyoxal.

Abstract No.106

Targeting Undifferentiated hBM-MSCs with Polymeric Nanocurcumin

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The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The main reason for diabetes type I is that pancreatic beta cells do not produce insulin sufficiently. Organ/islet transplantation and daily insulin injection are conventional methods have been utilized until now. The search for alternatives have started several years ago and methods based on stem cells differentiation is an ongoing task have suggested, significant goals having been achieved in most experimental settings (e.g. insulin production and euglycaemia restoration). Mesenchymal stem cells (MSCs) are uniquely capable of crossing germinative layers borders and are considered as promising cells for regenerative medicine approaches in several diseases. Type I diabetes therapy should potentially benefit from such differentiated cells. MSCs are obtainable in high numbers via ex vivo culture and can be differentiated towards insulin producing cells (IPCs). An important obstacle on the way of this kind of cell-based therapy, is the risk of tumorigenecity in the patients.