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β-Lactoglobulin (β-Lg) is a lipocalin, which is the major whey protein of cow milk and the milk of other mammals. However, it is absent from milk of primates. This globular protein of about 18 kDa is folded forming a b-barrel (or calyx) structure. Each monomer contains two disulphide bonds and one cysteine at position 121 (Cys121). This free thiol plays an important role in the heat-induced aggregation of β-Lg, and, possibly, in the maintenance of its conformational stability. β-Lg is one of major allergens in bovine milk. In this study, the expression in the yeast Pichia pastoris of a mutant bovine β-Lg, in which Cys121 was changed into Ser (Cys121Ser) was accomplished. Analysis of recombinant proteins by mass spectrometry has confirmed their purity, matching the calculated molecular mass with their mass theoretical, and the lack of post-translational modifications. Circular dichroism (CD) and high performance liquid chromatography (HPLC) experiments showed that the recombinant wild-type (WT) and Cys121Ser mutant retain native-like fold. The far- and near-UV CD spectra of WT and Cys121Ser were very similar to that of the standard, indicating a similar secondary and tertiary structure. The mutation on the position 121 in amino acid chain completely blocks the irreversible aggregation induced by heat treatment according to electrophoresis results. Compared to the recombinant wild-type protein, the mutant is less stable to temperature and disulphide reducing agents, and is much more sensitive to peptic digestion. Binding of IgE from patients with cow milk allergy to native β-Lg, wild-type β-Lg and Cys121Ser mutant β-Lg was aslo measured by ELISA. The calculated IC50 values for native and recombinant proteins were almost the same and the difference was not significant, indicating that the recognition of β-Lg by IgE from CMA patients is not impaired by recombinant WT and Cys121Ser mutant β-Lgs.

Keywords: β-Lactoglobulin, Allergy, Recombinant Protein, Circular Dichroism, ELISA test.

Abstract No.12

Kinetics and Spectrophotometric Studies on The Interaction of Sodium Dodecyl Sulfate With Chicken Egg White Lysozyme in Aqueous Solution

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The binding of surfactant to protein is a widespread phenomenon and plays a particularly important role in the activity of an enzyme. In this study the turbidimetric assay (activity) of lysozyme followed by the decreased optical density (OD) of a turbid cell suspension (about .3 mg/ml Micrococcus lysodeikticus) photometrically at 450 nm. Effect of sodium dodecyl sulfate (SDS) as an anionic surfactant on lysozyme enzyme was investigated by UV-Vis spectrophotometry at pH 7.25 at 35°c using sodium phosphate buffer. Measurements were carried out using 6×10^{-8} M concentration of lysozyme and a range of SDS solution concentration between 0.4 and 0.8 mM. It was found that by increasing of SDS concentration, the rate of Micrococcus lysodeikticus lyses will be decreased. Vmaxvalue will be reduced by increasing of SDS concentration. Lysozyme consists of two domains: a α-domain with helical structure and a β-domain with predominantly β-sheet, separated by the active cleft. The cleft between the two domains includes the binding site for the substrate. Probably the contents of α-helix decreased sharply with the increase of concentration of SDS.

Keywords: Lysozyme, Sodium Dodecyl Sulfate, Spectrophotometry, Activity, Protein.

Abstract No.13

Effects of Sodium Selenate on the Structure and Activity of Acetylcholinesterase

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Acetylcholinestrase (AChE) (EC 3.1.1.7), is one of the most important enzymes in nervous system, and plays a role in the signal transduction in the somatic nervous system by termination of signal transduction in the synapse. It has been reported that the function of this enzyme plays a role in Alzimer's disease. Selenium is one of the most important micronutrient. Many investigations have been performed about the physiological, biochemical and behavioral effects of this element, such as postponing the Alzheimer's symptoms in the elderly and delaying the initiation signs of skin aging. In this study the effects of different concentrations of Sodium selenate (0, 390, 870, 1300 μM) on AChE

activity investigated using UV-visible spectroscopy, fluorescence spectroscopy and circular dichroism spectroscopy. The results demonstrated that the Sodium selenate decreased enzyme activity and the main inactivation take place in the 1300μm concentration of Sodium selenate ($p<0.05$). It can be concluded that increasing the concentrations of Sodium selenate caused to increase helicity of AChE, therefore, in the presence of different concentrations of Sodium selenate the α-helix structure can induce in the secondary structure of AChE. Also the intrinsic fluorescence decreased by increasing the concentrations of Sodium selenate. In conclusion, according to changes observed in the secondary and tertiary structure of enzyme it is proposed that Sodium selenate are able to affect the activity of the AChE . Therefore, we suggest that the Sodium selenate could be useful in treatment of Alzimer's disease.

Keywords: Acetylcholinestrase, Alzimer's Disease, Circular Dichroism Spectroscopy, Fluorescence Spectroscopy, Sodium Selenate, UV-Visible Spectroscopy.

Abstract No.14

Extremely Low Frequency of Electromagnetic Fields (ELF-EMF) Decreased Acetylcholinesterase Activity

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Daily exposure to electromagnetic field is unavoidable as a consequence of living in a society that depends heavily on the use of the electricity. The exposure to extremely low frequency electromagnetic field (ELF-EMF, frequencies less than 200-300 Hz) can alter enzyme activities. Acetylcholinesterase (AChE) (EC 3.1.1.7), is responsible for the hydrolysis of acetylcholine to form choline and acetate. AChE is mainly found at cholinergic synapses in the central nervous system (CNS). The aim of this study was to examine the effects of ELF-EMF (frequency of 75, 120, 217 Hz, induction 0.3 mT) on AChE activity. AChE activity was measured by using acetylthiocholine chloride as substrate and monitoring the thiocholine production according to the Elman method with UV-visible spectroscopy. We also use circular dichroism spectroscopy and fluorescence spectroscopy for investigating of secondary and tertiary structure of enzyme. The results demonstrated that the ELF-EMF decreased enzyme activity and the main inactivation took place in 217 Hz ($p<0.05$). According to changes observed in the secondary and tertiary structure of enzyme it is proposed that these fields are able to affect structure and dynamics of the active site gorge of AChE and result in changing enzyme activity.In conclusion, AChE plays an important role in nervous system and EMF changes can affect on some biochemical processes in decreasing the severity of diseases such as Alzheimer's disease.

Keywords: Acetylcholinestrase, Circular Dichroism Spectroscopy, Elman Method, Extremely Low Frequency Electromagnetic Field, Fluorescence Spectroscopy, UV-Visible Spectroscopy.

Abstract No.15

Effect of Shear Stress on Stem cells

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The stem cells especially Mesenchymal stem cells (MSCs) or stromal cells, can be used as a therapeutically in tissue engineering and regenerative medicine to substitute and relief the damage tissues and also differentiate to verify cell lineages to idealization of tissue engineering. All cells in body are exposed to tangential mechanical forces, called shear stress because of the blood flowing across the cells surface. These force effect on cell culture conditions, differentiation, proliferation and cells expression way. Considering the essential role of stem cells, understanding and investigation of these cells responses to mechanical forces are very important. The present article reviews the mechanisms of gene expression changes the MSCs in response to different amounts of laminar shear stress during periods and its role in proliferation, culture and differentiation of cells under different shear stress.

Keywords: Stem cell, Mesenchymal, Stress.
