Abstract No.11

Interactions of β-Lactoglobulin with serotonin and arachidonyl serotonin

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β-Lactoglobulin (β-LG) is the major whey protein of cow's milk. The biological function of β-LG is not clear, but its potential role in carrying fatty acids through the digestive tract has been suggested. β-LG has been found in complexes with lipids and has a high affinity for a wide variety of compounds. Serotonin (5-HT), an important compound found in animals and plants, has various functions, including the regulation of mood, appetite, sleep, and some cognitive functions such as memory and learning. In this study, the interaction of serotonin and one of its derivatives, arachidonyl serotonin (AA-5HT), with β-LG was investigated using circular dichroism (CD) and fluorescence intensity measurements. These two ligands interact with β-LG forming equimolar complexes. The binding constant for the serotonin/β-LG interaction is between 10^5 and 10^6 M^{-1}, while for the AA-5HT/β-LG complex it is between 10^4 and 10^5 M^{-1}. The observed binding affinities were higher in hydroethanolic media. According to far- and near-UV CD results, these ligands have no apparent influence on β-LG secondary structure, however they partially destabilize its tertiary structure. Their binding by β-LG may be one of the peripheral mechanisms of the regulation of the content of serotonin and its derivatives in the bowel of milk-fed animals.

Key words: β-Lactoglobulin, Serotonin, Arachidonyl serotonin, Fluorimetry, Circular Dichroism.

Abstract No.12

Effect of magnesium ion on the structure of apo camel alpha-lactalbumin

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Milk proteins are natural vehicles for the delivery of minerals. Alpha-lactalbumin is one of the major whey proteins in milk. In the present study, the interaction between magnesium ion and metal-freed camel alpha-lactalbumin (apo α-La) has been studied by spectroscopy and isothermal titration calorimetry techniques. It was observed that the magnesium ion has the ability to decrease the fluorescence quantum yield of apo α-La and causes a pronounced spectral shift towards shorter wavelengths. Binding of magnesium ion to apo α-La induced conformational changes in apo α-La like calcium ion. The calorimetry results indicate that magnesium ion cannot bind to α-La in holo state, but there are two sets of binding sites for magnesium ions on the apo α-La structure. The first binding set contains 10 binding sites and the second binding set contains 1 magnesium ion binding site. The association equilibrium constants are 132 and 1.5 mM^{-1} for the first and second binding sets, respectively. The conformational change of apo α-La in the presence of magnesium ion was also accompanied with increases of the thermal stability of the protein.

Key words: Alpha-lactalbumin, Magnesium, Fluorescence spectroscopy.

Abstract No.13

Stabilization of human carbonic anhydrase by glucose as osmolyte

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Human CA II (EC 4.2.1.1) is a zinc metalloenzyme which catalyzes the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions. At least 14 different carbonic anhydrase isoforms were isolated in higher vertebrates. These isozymes have diversified tissue distribution and subcellular positions and they exist in archaea, Eubacteria, animals and plants. Human CA II has a single polypeptide chain of 259 amino acid residues with a molecular mass of about 29 kDa. Organisms and cellular systems which have adapted to stresses such as high temperature, desiccation, and urea-concentrating environments have responded by concentrating particular organic solutes known as osmolytes. These osmolytes are believed to confer protection to

enzyme and other macromolecular systems against such denaturing stresses. In this study, differential scanning calorimetric (DSC) experiments were performed on human carbonic anhydrase (HCA II) in the presence of varying concentrations of the glucose which induced considerable increases in the thermal unfolding transition temperature (Tm) for HCA II. DSC scan of HCA II in the presence of 5.4 M glucose shows the increases of melting point (Tm) up to 10 °C and also enhances the reversibility of thermal unfolded HCA II up to 20 %. The result of circular dichroism CD experiments indicates an increase of 5°C of Tm. Since CD experiment gives informations about secondary structure while DSC profiles show the stability of tertiary structure. The different Tm obtained from CD and DSC indicates the existence of irreversibility for HCA II.

Key words: Carbonic anhydrase, Glucose, Reversibility, T_m.

Abstract No.14

Three-dimensional fluorescence spectroscopy studies of interaction between human serum albumin and Norfloxacin in the presence of counter ions: A novel view of drug therapy

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Human serum albumin (HSA) is the most abundant protein in human blood plasma. HSA has a good binding capacity for water, Ca^{2+}, Na^{+}, K^{+}, fatty acids, hormones, bilirubin and drug. Norfloxacin (Noroxin) is a synthetic broad spectrum antibacterial drug, which is mainly used in the treatment of urinary tract infections. Three-dimensional fluorescence method is sensitive and convenient to use in the study of intermolecular interactions. The aim of this study was to determine the affinities of Norfloxacin in the absence and presence of counter ions to HSA. The binding of Norfloxacin to HSA in presence of various kind of ions (Al^{3+} - Pb^{2+} - Cu^{2+} - Mg^{2+}) under physiological conditions (pH=7.4) has been investigated. Our results show that, with increasing concentration of Nrf, the maximum fluorescence of HSA increased upon binding of Nrf. A slight blue shift in the maximum emission wavelength also suggests a reduction in the polarity of the microenvironment of tryptophan and tyrosin residues. Gradual addition of counter ions to the HSA and drug solution leads to a red shift of the emission maximum associated with an enhancement of fluorescence intensity suggests that a more polar environment of the Trp and tyr residues.These results of three-dimensional fluorescence spectra and Contour map showed that binding of Norfloxacin to HSA in absence and presence of counter ions can induce conformational changes in HSA.

Key words: HSA, Norfloxacin, Ion, Three-dimensional fluorescence.

Abstract No.15

Molecular dynamics studies on the effect of thermal and chemical denaturats on the structure of three poly-peptides with different charges

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One of the main tools of investigation in theoretical level is molecular dynamics which gives single structure properties of macromolecules in biological studies. Denaturation of poly-peptides at different temperatures in the absence and presence of different concentrations of guanidine chloride (GdmCl) was studied by molecular dynamics (MD) implemented in Gromacs 3.3.1. In this paper, thermodynamics of a single molecule using simulation results of molecular dynamics was evaluated. The results were interpreted for an unfolding process of a protein embedded in an isotherm-isobar ensemble. Analyzing the related trajectories showed that the polypeptides was stabilized at low concentration and was unfolded at high concentration of denaturant. Temperature at inflection point (T_m) is 324, 348 and 366K for Poly-lysine (PL), poly-alanine (PA) and poly-glutamate (PG). Respectively. That is mean; PG is more thermodynamic stable than PA and PL. Protein-solvent hydrogen bond (HB) was obtained for polypeptides. It increases for PA, and decreases for PG and PL by temperature, Protein–protein HB decreases by temperature for poly-peptides. Protein solvent and Protein–protein HB for PG are more than PA and PL. Hydrophobic surface increases by temperature for PG and PL while decreases for PA. Also this surface for PL is more than PG and PA. These results show that is PL unfolded faster than PG and PA. Each of hydrogen bonds Poly-peptides increases with GdmCl. This is compatible with literature that decreasing hydration shell coincidence with increasing stability at low concentration but at higher concentration it is decreasing stability.