Abstract No.9

The Effect of pH on Recombinant C-Terminal Domain of Botulinum Neurotoxin Type E (rBoNT/E-HCC)

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Clostrdium botulinum neurotoxins cause botulism syndrome. One of the recombinant vaccines against these neurotoxins is constructed using 93 residues of C-terminal domain of botulinum neurotoxin type E (rBoNT/E-HCC). In this report, we made an effort to investigate the effect of different pH on protein structure to assess if rBoNT/E-HCC could be used as a vaccine for oral administration. Initially, E.coli BL21 (DE3) containing the synthetic gene of rBoNT/E-HCC were cultured and the expressed protein, rBoNT/E-HCC,was purified by affinity chromatography. Structural changes of rBoNT/E-HCC in the presence of different pH (2, 5, 7.4 and 9) were done by various techniques including circular dichroism (CD), fluorescence, aggregation, thermal denaturation and UV-Vis spectroscopy. The aggregation experiments in the presence and absence of DTT indicated that rBoNT/E-HCC at pH 2 is more resistant to thermal aggregation in contrast to higher pH 9. Fluorescence experiments showed the more compact and more stable structure for rBoNT/E-HCC at pH 2, and loosely folded structure at pH 9. Thermal denaturation of protein indicated that the melting temperature was increased with increasing of pH from 2 to 9 up to 2°C. This increment was caused by stabilization effects of increasing amounts of secondary structures which was caused by pH increments. We hypothesize that this finding is not in contrary with aggregation results, because, the nature of forces acting in aggregation and denaturation are not exactly the same. According to our findings we advise further studies of protein from immunological point of view as an oral vaccine.

Keywords: Botulinum Neurotoxin Type E, Ph, Fluorescence, Circular Dichroism, Aggregation, Thermal Studies.

Abstract No.10

Docking of Cladribine and Fingolimod to P53 and RAS exons of DNA

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Some of the newest drugs that are effective on treating Multiple Sclerosis disease (MS) are Fingolimod and Cladribine, but these drugs are carcinogenic. It seems that due to aromatic rings, these drugs are able to bind DNA and can cause cancer by altering DNA structure. Drugs can bind to DNA by covalent and non- covalent bonds, and may cause thermodynamic instability or damage on structure of DNA. These changes can cause abnormal cell division cycle or tumor. Understanding the effect of drugs from the point of structural and mechanical characteristics on the DNA is an important aspect in designing new drugs. Nowadays, docking is essential for designing of new drugs. Fingolimod and Cladribine were docked on four exons of P53 and RAS genes that theire sequence mentioned in table 1 by Autodock 4 software. Routine methods and default parameters were used for docking procedure. Results of docking indicate that Van der Waals interactions are greater than electrostatic interaction in connection of these drugs to DNA, in all four sequences. Then these drugs are attached to DNA through hydrophobic interactions. These two drugs were bond to DNA through minor groove. Data indicate that binding of Fingolimod to DNA is stronger than Cladribine, thus it appears that its carcinogenesis effect is greater. Results show Cladribine and Fingolimod can bind to exon one of RAS gene more powerful than other exons.

Keywords: Docking, Cladribine, Fingolimod, DNA.

Abstract No.11

Structural and Allergenicity Properties of Recombinant Wild-type and Cys121Ser Mutant β-lactoglobulin

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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