Abstract No.16

The Effect of Temperature and pH on Porcine Pancreatic Alpha-Amylase

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Alpha-amylase (EC 3.2.1.1) is the most important enzyme in starch digestion. This enzyme is distributed all over various organisms. In this study the effect of temperature and pH were tested on activity toward porcine pancreatic alpha-amylase (PPA).Bernfeld method was used for the enzyme assay. To determine the optimum temperature, amylase activity was measured at different temperatures. For thermal stability, the enzyme solution was kept at different temperatures for 60 min and then immediately cooled and the residual activity was measured. To determine the optimum pH, amylase activity was measured at different pH values. For pH stability, the enzyme solution at a desired pH, was kept at 4ºC for 24 h, and then activity was measured. The optimum temperature of the enzyme was 40.7ºC. The rates of thermal inactivation, the enzyme retained 100, 80 and 30% activity for 120 min when heated to 35,45 and 55ºC respectively. At 65 and 75ºC the enzyme lost activity after heating for 120 and 60min respectively. The optimum pH of the enzyme was 7.6. The enzyme was stable over a wide pH range. The enzyme was not stable below pH 4.5 or above pH 10.0. The present study show that more than 50% of activity is between 15 and 55ºC. The enzyme also exhibited high activity in basic or near neutral conditions.

Keywords: Alpha-amylase, Temperature, Stability, Activity.

Abstract No.17

Structure-Function Relationship of B-Lactoglobulin in the Presence of Dodecyltrimethyl Ammonium Bromide

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Bovine β-Lactoglobulin (β-LG) present in milks has been found "in vivo" in complexes with lipids such as butyric and oleic acids. To elucidate the still unknown structure-function relationship in this protein, the structural changes of β-lactoglobulin in the presence of cationic surfactant such as dodecyltrimethyl ammonium bromide (DTAB) have been investigated using various experimental techniques such as UV-Vis spectrophotometry, fluorimetry, isothermal titration calorimetry (ITC) and circular dichroism (CD). Subsequently, the retinol binding by β-LG has been investigated in the presence of various amounts of this surfactant as its extrinsic functional binding fluorophore. Comparison of the results allowed to determine the binding of retinol by β-LG in the presence of DTAB. The results of UV-Vis and fluorescence studies showed a red shift in wavelength and an increase in absorbance and enhancement in the intensity of the quantum yield of protein during its interaction with DTAB. The results of UV-Vis indicated the cooperative character of binding at pH 2.0. The results of fluorescence studies showed that the binding strength of β-LG/DTAB complex increases with the increase of the pH. CD results showed the shifts in positions of the major minima and change in magnitude of ellipticity and subsequently signified two significant changes in structure of β-LG between 10 to 30 and 50 to 100 molar ratio of [DTAB]/[β-LG]. ITC measurements indicated the endothermic nature of β-LG/DTAB interactions at pH 6.7 and the exothermic nature of β-LG/DTAB interactions at pH 8.0. The analysis of the binding data demonstrates the absence of significant changes in retinol binding properties of β-LG in the presence of various amounts of this surfactant. This implies that surfactant binding does not change the conformation of β-LG in the regions defining retinol-binding site nor interferes with retinol binding by a competition for the same binding site(s).

Keywords: β-Lactoglobulin, Isothermal Titration Calorimetry, Circular Dichroism, Interaction, DTAB.

Abstract No.18

The Anticancer Activity and DNA/HSA Binding Properties of Benzo [H] Quinoline (Bhq) Pt (II) Complexes

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In this study, three structurally related platinum (II) complexes, containing benzo [h] quinoline (bhq) moieties as the non-leaving carrier groups were synthesis and their biological activities evaluated. The anti-proliferation activity against K562 and MCF-7 cell lines was measured using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Moreover, fluorescence study was performed to obtain the binding parameters of Pt-(II) complexes to both human serum albumin (HSA) and DNA, as well as to explore role of hydrophobic character in their binding to HSA. The results revealed that MCF-7 cell line resists more than K562 cancer cells against the cytotoxic effect of the Pt-(II) complexes. Also depends to type of the substitution made, different anti-proliferation activities and binding properties were observed. Moreover, the environments of the Pt center, charge and hydrophobicity of the complexes were suggested to play significant roles in their biological properties. Overall, the substitutions on the synthetic Pt- (II) complexes which associated with the marked improvement of anticancer activity can be considered as the significant point in construction of a novel generation of antineoplastic agents.

Keywords: Platinum- (II) Complexes, Benzo [H] Quinoline (Bhq), Human Serum Albumin, Anticancer Activity, Fluorescence Study.

Abstract No.19

Study of Effect of The Mutation Amino Acidis18and 125 on Specific Biological Activity of Human Interlukin-2

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Interleukin-2 (IL-2) is a 133 amino acid alpha-helical protein secreted by activated T-cells. This protein possesses three cysteine residues. Cysteines 58 and 105 are two residues involved in forming an intramolecular disulfide bridge, whereas cysteine 125 owns a free sulphydryl group. Formation of this disulfide bond is critical for biological activity of the protein. In this study, we constructed two mutant proteins of IL-2 through site-directed mutagenesis. In the mutant C125 A, the free sulphydryl group was eliminated. This change will probably prevent the formation of mispaired disulfide bonds during the refolding process. In the mutant L18C, a new cysteine residue was introduced for formation of disulfide bond with cysteine 125. This mutation was designed to study the effect of an extra disulfide bond on the structure and function of IL-2. These mutant analogs were then expressed isolated as solution phase in E.coli-derived pLysS strain. Subsequently, the expressed protein was purified using NI-NTA columns. In order to investigate, the effects of introduced modifications on the structure of the protein, Circular Dichroism (CD) and Fluorescence Spectroscopy were exploited. The data obtained exhibited no alteration in the structure of IL-2 variants. As well, the analysis of biological activity of the mutants is under examination on CTLL-2 cells.

Keywords: Human Interleukin-2, Site-directed Mutagenesis, Disulfide Bond, Biological Activity.

Abstract No.20

Structural and Activity Study of The Restriction DNAzyme by Spectroscopic Methods

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Beyond the preservation of genetic information in the double helix structure in cells, DNA could also have catalytic role in the single stranded form taking different 3-D structures. This artificial catalytic biomolecule is known as DNAzyme or deoxyribozyme. The Cu2+ dependent DNA cleavage DNAzyme is the unique example of known DNAzymes. It is introduced as a restriction DNAzyme due to the site specific cleavage of single strand DNA molecule. It is also used as Cu2+ nanobiosensor in aqueous solutions by modification of DNAzyme molecule with florescence dye. Herein, we studied the structure and catalytic function of DNAzyme using Uv-visible and extrinsic fluorescence spectroscopy. Hyperchromic and hypochromic effects of DNA have been traced by Uv-visible spectroscopy to investigate structure, hybridization phenomenon and catalytic function. Absorbance intensity at 260 nm decreases upon hybridization of DNAzyme with substrate (hypochromic effect), which increased upon addition of cofactor and starting catalytic activity (hyperchromic effect). This result confirmed the efficiency of this spectroscopic