Abstract No.69

Role of Osmolytes in The Stabilization of Bovine Pancreatic Ribonuclease A at Different pH values

Somayeh Asgari, Behzad Shareghi, Nayereh Bahamin, Parisa Nooraei, Sadegh Farhadian*

Shahrekord University, IR
(E-mail: somayeh_asgari@ymail.com)

Bovine pancreatic ribonuclease A (RNase A, EC 3.1.27.1) is composed of 124 amino acids (molecular mass 13686 Da), among which basic residues (10 Lys, four Arg and four His) prevail over acidic residues (five Glu and five Asp), making the protein definitely basic, with a pI of 9.3. The protein's secondary structure consists of three α-helices and seven β-strands, and the molecule contains four disulphide bonds(26–84, 40–95, 58–110 and 65–72) that contribute, in particular the two terminal ones (26–84 and 58–110), to the remarkable stability of the protein.It catalyzes the cleavage of P-O5′ bonds in RNA on the 3′side of pyrimidine to form cyclic 2′,5′-phosphates. In their study of the effect of osmolytes (Butandiol, Butanol, Glycerol at pHs 1.5 and 3.3) by spectrophotometric techniques on thermal stability of enzymes in terms of Tm (the midpoint of the transition curve), have reported Butandiol, Butanol destabilizes RNase A at pH=1.5 and 3.3 and byincreasing concentration of Glycerol at pH 1.5 increases enzyme stability.Polyols are co-solvents that are used to protect organisms from denaturation by harsh environmental stresses. Glycerol stabilize ribonuclease A, not by interacting with that directly but by altering the solvent properties of the surrounding water and hence the protein–solvent interaction. As a rule, branching of the hydrocarbon portion of the alcohols tends to reduce their effectiveness as protein denaturants. The glycerols are found to be less effective than the corresponding alcohols, suggesting that increased polarity or hydrogen-bonding capacity is of secondary importancewhen compared with the effects of increasing hydrocarbon content.

Keywords: Bovine Pancreatic Ribonuclease A, Polyols, Osmolytes, Butandiol, Butanol, Glycerol.

Abstract No.70

Theoretical Investigation of Stability of a Di-Copper(I) Macrocyclic Complex as a Model for Active Site of Tyrosinase Enzyme

Amir Nasser Shamkhali, Marjan Abedi, Mahnaz Mardian*

Department of Applied Chemistry, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, IR
(E-mail: shamkhali@uma.ac.ir)

Tyrosinase is a widespread dinuclear copper enzyme present in microorganisms as well as in plants and mammals. It is able to oxidize most phenols to the corresponding catechols and, eventually, o-quinones. Most of the information about the electronic structure and mechanism of tyrosinase action has been obtained from structural and functional models. In this work the di-copper(I) complex of a 20-membered [2+2] macrocyclic Schiff base ligand with three nitrogen donors is chosen to model the real tyrosinase. The activation of tyrosinase enzyme is accomplished by binding an O_2 molecule as a bridged ligand between two Cu(II)-(Hys)3 complex. Also, the similar active state of model complex has been shown before. All DFT calculations were performed by B3LYP hybrid-GGA functional and SBKJC basis set which included effective core potentials for inner shell of heavy atoms. Then the electronic structure, HOMO-LUMO energy gap, Mulliken charges, and relative stability of model complex, tyrosinase active site, and their oxidized state are calculated and discussed.

Keywords: Tyrosinase, Enzyme, Active Site, Stability, Model Complex, DFT.

Abstract No.71

Interaction of Bisdemethoxycurcumin and Diacetylbisdemethoxycurcumin with β-lactoglobulin: Spectroscopic and Molecular Dynamics Simulation Studies

Mehdi Sahihi, Fakhrossadat Mohammadi, Abdol-khalegh Bordbar, Yousef Ghayeb*

Department of Chemistry Isfahan University of Technology, Isfahan, IR
(E-mail: msahihi@ch.iut.ac.ir)

This study demonstrates the interactions of bisdemethoxycurcumin (BDMC) and diacetylbisdemethoxycurcumin (DABC) as the bioactive constituents of turmeric with bovine β-lactoglobulin (BLG) variant B using fluorescence, circular dichroism (CD), molecular docking, and molecular dynamics simulation methods. The binding constant for formation of BLG-BDMC and BLG-DABC complexes were $8.99 \pm 0.1 \times 10^4$ M^{-1} and $1.87 \pm 0.1 \times 10^2$ M^{-1}, respectively. The distance between BLG and BDMC was obtained based on Förster`s theory of non-radiative energy transfer. The secondary structure contents of BLG in the presence of ligands were obtained by analysis of CD spectra. Molecular docking studies revealed

that BDMC and DABC bind to the surface of the protein by four and one hydrogen bond interactions, respectively. Finally, molecular dynamics simulation results show that the binding of BDMC to BLG causes the conformational change of BLG and the structure of binding site remains rigid during the simulation of two complexes.

Keywords: Bisdemethoxycurcumin, Diacetylbisdemethoxycurcumin, β-lactoglobulin.

Abstract No.72

A Comparison of Denaturation of Trypsin in the Presence of Urea and Guanidine Hydrochloride

Ayeh Bolouki, Behzad Shareghi*

Departement of Biology, University of Shahrekord, Shahrekord, IR
(E-mail: atoona29@gmail.com)

Trypsin (EC 3.4.21.4) is a serine-protease with a polypeptide chain of 223 amino acid residues and six disulfide bridges that hydrolyzes peptide bonds at the carboxylic end of the amino acid residues arginine (R) and lysine (K). It is a globular protein with predominance of antiparallel β-sheet secondry structure and it has two domains with similar structures. In this study, denaturation of trypsin in the presence 2 mM, 4 mM, 6 mM and 7.5 mM of urea and GdnHCl has been studies by spectrofluometry and UV-VIS spectrophotometry in different pH (3, 8 and 10) at 308 K. The intensity of the emission spectrum has a direct relationship with the increase of concentration of urea and GdnHCl. Adding of guanidine to 0.25 mg/ml trypsin solution, the intensity of the spectrum was increased more than adding urea and 13 nm red shift occurred with respect to native curve. Adding of 7.5 mM of urea to trypsin solution at pH 3.0, the intensity of the spectrum was reduced with respect to native curve. The value of absorbance was taken at 280 nm. The final results denote unfolding of trypsin occurred in the presence of GdnHCl and urea and the ionic nature of GdnHCl masks electrostatic interactions in trypsin, a phenomenon that was absent in the presence of urea.

Keywords: Trypsin, Urea, Guanidine Hydrochloride, Spectrophotometry, Spectrofluometry.

Abstract No.73

Synthesis, Characterization, Cytotoxicity and Interaction of a Newly Designed Anti-Cancer Palladium(II) Complex with Calf Thymus DNA

Hassan Mansouri-Torshizi [1], Somaye Shahraki [1], Adeleh Divsalar [2], Ali Akbar Saboury [3]*

1. Dept. of Chemistry, University of Sistan and Baluchestan, Zahedan, IR
2. Dept. of Biological Sciences, Tarbiat Moallem University, Tehran, IR
3. Institute of Biochemistry & Biophysics, University of Tehran, IR
(E-mail: hmtorshizi@gmail.com)

Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer platinum- and palladium-based drugs[1]. Several pd(II) and pt(II) complexes of dithiocarbamate derivatives have been prepared[2]. Here we report Synthesis, Characterization, Cytotoxicity and interaction of a palladium(II) complex of formula [Pd(en)(bpy)](NO3)2(where en is ethylendiamine and bpy is 2,2′-bipyridine) with calf thymus DNA(CT-DNA). The cytotoxicity assay of the complexe has been performed on chronic myelogenous leukemia cell line, K562, at micromolar concentration. This complexe showed cytotoxic activity far better than that of cisplatin under the same experimental conditions. The binding parameters of the complexe with CT-DNA was investigated using UV-visible and fluorescence techniques. It shows the ability of cooperatively intercalating in CT-DNA. Gel filtration studies demonstrated that palladium complex could not cleave the DNA. In the interaction studies between this Pd(II) complexe with CT-DNA, several binding and thermodynamic parameters have been determined, which may provide deeper insights into the mechanism of action of these types of complexes with nucleic acids.

Keywords: DNA Binding and Thermodynamic Parameters, Anti-Tumor Activity, Pd(II) Complexe.

Abstract No.74

Studies on the Anti-tumor Activity of an Endostatin Fragment

Reyhane Chamani, Sedigheh Eskandari, Mohsen Asghari, Majid Taghdir*

Depertment Of Biology, Guilan University, Rasht, IR
(E-mail: creyhane@yahoo.com)

Angiogenesis, a complex multistep process including the proliferation, migration and differentiation of endothelial cells, microtubule formation, and sprouting of new capillary branches, is a critical event in growth and metastasis of cancer and prevention of angiogenesis is one of the best strategies for treatment of cancer. Endostatin, the C-terminal fragment of collagen XVIII, is an endogenous inhibitor of angiogenesis that inhibits tumor growth without toxicity and acquired