Abstract No.227

Disulfide Bonds Modulate Dynamics of Catalytic site in Native State of Pseudomonas Aeruginosa Elastase

*Hossein Rahmani**, *Ammar Mohseni*, *Majid Taghdir*, *S. Mohsen Ashrafi*

Department of Biology, Faculty of Science, University of Guilan, Rasht, IR
(E-mail: rahmani65@msc.guilan.ac.ir)

Pseudomonas aeruginosa Elastase (PAE) is a Thermolysin like protease (TLP) containing two disulfide bonds. A disulfide bond is located between Cys30-Cys57 which connects two b-strands within the N-terminal domain, while another disulfide bond, Cys270-Cys297, is located close to the end of C-terminal domain and connects two a-helices. Previous experimental studies have demonstrated the role of disulfide bonds in enzyme stability and activity. In this study a series of short time molecular dynamics simulations were performed in the native and disulfide bond broken states at 298 and 333 °K. Results revealed that removal of the disulfide bonds in the native state cause a significant change in dynamics of stability determinant loop. Moreover, flexibility andcorrelation of motions significantly altered within the active site regionin low as well as high temperatures.Theseresultssuggestthatdisulfidebonds are crucial for structural stability and activity tuning of PAEin thenative state by adjustingforce distribution in structure.

Keywords: Disulfide Bonds, Molecular Dynamics Simulation, Stability Determinant Loop, Correlation of Motions.

Abstract No.228

Purification and Characterizations of 50-kDa Extracellular Metalloprotease from Serratia Marcescens ZF03

Navabeh Salarizadeh[1], *Sadegh Hasannia**[2,3], *Kambiz Akbari Noghabi*[3], *Reza Hassan Sajedi*[2]

1. Dept. of Biology, Faculty of Science, University of Guilan, Rasht, IR
2. Dept. of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, IR
3. National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, IR
(E-mail: salarinavabeh@yahoo.com)

Proteolytic enzymes have been widely used in management of enzyme deficiencies and therapeutic applications. These enzymes are co-administered with non-steroidal anti inflammatory agents. one of these enzymes is Strain-dependent extracellular metalloproteases which secreted by varied S.marcescens strains and has been purified and characterized by some scholars. This metalloprotease (serrapeptidase) is an important pharmaceutical agent. Serrapeptidase is useful for a wide range of inflammatory conditions. It is an effective drug for the treatment of breast engorgement. serrapeptidase relieve swelling and pain after surgery buccal, swelling, after maxillary sinus antrostomy. Enzyme may be effective to break down atherosclerotic plaques and fibrin on the inside of the arteries. In general, this major metalloprotease (SMP) has been detected in various strains of S. marcescens which is now manufactured for commercial exploitation because of its economic importance. In this research, the first, protease gene encoding a zinc-metalloprotease from the red-pigmented Serratia marcescens ZF03, which is isolated from hot-spring waters has been sequenced and reported to the GenBank. This fragment encodes an extracellular zinc-metalloendopeptidase with a molecular weight of approximately 50 kDa. This metalloprotease purified by ammonium sulphate precipitation, dialysis and DEAE-Sepharose chromatography and characterized. Proteolytic activity was determined by skim milk agar medium and zymography. Protease activity was optimum at temperature 50-55 °C and a range of pH 8-10. The effects of various compounds on protease activity and Kinetic parameters were determined. Considering high expression of metalloprotease and proteolytic activity, this protease can be used in the pharmaceutical Industries.

Keywords: Metallopeptidase, Serratia marcescens, Extracellular protease, Characterization, Pharmaceutical.

Abstract No.229

Structural Changes of Ribonucleoprotein ,LMG160, in the Presence of Sodium Chloride

*Sayeh Abdossamadi**, *Azra Rabbani-Chadegani*

Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, IR
(E-mail: abdossamadi@ibb.ut.ac.ir)

Low mobility group (LMG) proteins have been studied less intensively as they are very heterogeneous with low solubility. A fraction of these proteins named LMG160 (160 kDa) has been isolated from rat liver in pure form and characterized as a ribonucleoprotein (RNP) which detected in RNP-containing nuclear matrix of hepatocyes.In this study, we have investigated the effect of sodium chloride on the structure of

intact and RNase-treated LMG160 using circular dichroism in the range of far UV (190-260 nm) and fluorescence spectroscopy.The results showed that α-helical content was abundant in both intact and RNase-treated LMG160 in the absence of NaCl although the helicity of RNase-treated LMG160 was more than intact LMG160. Intrinsic emission fluorescence spectra imply that although both emission maxima were almost the same, intact LMG160 showed 8 nm red-shift compared to the RNase-treated form. It means that accessibility of tryptophan residues to the bulk solvent is increased.By increasing sodium chloride concentration from 0-1 M, the α-helical content of the intact LMG160 was decreased almost 8% by increasing %2.1 β-sheets, %1.3 β-turn and %4.7 random coil. The addition of increasing amount of sodium chloride to the intact and RNase-treated LMG160 exhibited 50% and 25% emission maxima reduction, respectively. Using modified Stern-Volmer equation, intact and RNase-treated LMG160 Ksv was 6.19M-1 and 1.67M-1, respectively. Also the amount of fa parameter indicated that the fraction of the initial fluorescence that is accessible to NaCl is 66% further for the intact LMG160 compared to RNase-treated.The result suggests that RNA moiety is important in this ribonucleoprotein structure implying that the intact LMG160 exhibits more relaxed structure compared to RNase treated protein.

Keywords: Ribonucleoprotein, Low Mobility group (LMG) proteins, Circular dichroism, Fluorescence spectroscopy, Ionic strength.

Abstract No.230

The Effect of some Synthetic Benzochromene Compounds on ROS and NO generation in Breast Cancer Cell Lines (MCF-7, MDA-MB231 and T47D)

*Asma Kheirollahi**, *Sussan K.Ardestani*

Depatment of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, IR
(E-mail: kheirollahi.asma@ibb.ut.ac.ir)

The cytotoxic activity of many chemotherapeutic drugs is resulted of their potential in apoptosis induction. Apoptosis is defined as programmed cell death that can occur in response to a variety of insults, such as cytotoxic compounds. Reactive oxygen species (ROS) and nitric oxide (NO) generation are some of the signs observed in cells subjected to anticancer drugs treatment. In contrast to their role on promoting cell growth under non-stress conditions, ROS are powerful inducers of apoptosis when cells are under stress. Nitric oxide, which is one of the smallest biological products of mammalian cells, plays various roles such as an intracellular or transcellular messenger and apoptosis inducer in cells.Intracellular ROS generated by some synthetic benzochromene compounds were measured by a non-fluorescence dye, DCFH-DA, which is permeable in cells and interacts with intracellular ROS to generate fluorescent 2′, 7′-dichlorofluorescein (DCF). DCF can then be measured by spectrophotometer (λmax = 525 nm). We also measured the nitrite concentration in the culture medium as an indicator of NO production using the Griess reaction method.Previously we showed that these compounds induce apoptosis in these cell lines. In this study we demonstrated that the amount of ROS generations in MCF-7 and MDA-MB cells treated for 4 and 24 h were significance and time-dependent. Nevertheless, T47D cells that were treated for 4 h didn't produce ROS but after 24 h ROS production was considerable. None of the cell lines produced NO after 4 h. NO generation by MCF-7 cell line treated for 24 h, was significant. However, these compounds did not induce NO generation in MDA-MB231 and T47D cell lines.This study indicated that one of the ways that these compounds can induce apoptosis is by increasing ROS generation. However this needs to be worked on in detail.

Keywords: Breast Cancer, Benzochromene Derivatives, ROS, NO.

Abstract No.231

Study on the Interaction of L-Proline-Derived Aminophosphinic Acid Ligand with Bovine Serum Albumin

*Fakhrossadat Mohammadi**, *Babak Kaboudin*, *Khavar Moradi*, *Mohammad Reza*

Department of Chemistry, Institute for Advanced Studies in Basic Sciences Zanjan, IR
(E-mail: fmohammadi@iasbs.ac.ir)

Organo-phosphinic acids are known as organic derivatives of hypophosphorus acid (H3PO2) and have found potential applications in the areas of industrial, agricultural and medicinal chemistry. The structure of the phosphinic functional group mimics the transition state of peptide hydrolysis. Among the a-functional phosphinic acids, a-aminophosphinic acids are an interesting class of compounds possessing broad biological activities. Herein, we report synthesis and the study on the interaction of a carrier protein with a new L-proline based aminophosphinic acid ligand. Serum albumins are one type of proteins possessing various physiological functions. Serum albumins act as carriers for transporting of many of compounds such as fatty acids, amino acids and drugs. Bovine serum albumin (BSA) is used in biophysical and biochemical studies as a model protein because of low