Tris-HCl buffer solution (pH=7.0) containing 20 mM sodium chloride at 300 and 310K. Studies of antitumor activity of this complex against human cell tumor lines(k562) have been carried out. It shows IC50 value lower than that of cisplatin.There is a set of 6 binding sits(g) for the complex on the DNA with positive cooperativity in binding. n,the Hill coefficient (as a criterion of cooperativity) find out to be 5.6 at 300 K and 7.3 at 310 K, respectively. K app the apparent equilibrium constant are 21.4 mM^{-1} and 39.3 mM^{-1} at 300 and 310K respectively.The above compound can denature the DNA and the concentration of this ligand in the midpoint of transition ($[L]_{1/2}$), is decreased by improving temperature, from 0.25 mmol/L at 300K to 0.28 mmol/L at 310K. the conformational stability of DNA in the interaction with ligand (ΔG° H_2O) determined to be 32.5 kJ/mol and 35.7 kJ/mol at 300 and 310 K, respectively.Presence of ligand led to less stability of the DNA . values for m, (a measure of ligand strength for DNA denaturation) are 121 and 153 (kJ/mol).$(mmol/L)^{-1}$ at 300 and 310 K, respectively.Enthalpy of DNA denaturation by the complex (ΔH° coformation or ΔH° denaturation) in the range of 300 and 310 K is find out to be 42.2 kJ/mol. In addition, the calculated entropy (ΔS° H_2O) of DNA denaturation by complex is -0.23 kJ/mol at 300 K. the negative value of entropy change is related to the more disorder of denatured DNA with respect to the native DNA.Fluorescence titration spectra and fluorescence Scatchard plots suggest that the Pd(II) complex intercalate in DNA. The gel chromatograms obtained from Sephadex G-25 column experiments showed that the binding of metal complex with DNA is so strong that it does not readily break.

Keywords: Thermodynamic Parameters, Spectroscopic Techniques, Anti-Tumor, DNA-Binding, Palladium (II) Complex.

Abstract No.87

Spectroscopic Studies on the Thermodynamic and Thermal Denaturation of the CT-DNA Binding with [Pd(en)(dppz)](NO3)2

*Somaye Shahraki**, *Hassan Mansouri Torshizi*

Department of Chemistry, University of Sistan and, Baluchestan, Zahedan, IR
(E-mail: somaye_shahraki@yahoo.com)

The development of new metal complexes which can selectively interact with nucleic acid is of much current interest. During recent years, the interest for metal complexes containing planar extended polyaromatic ligands has increased tremendously, mainly for their usage as probes capable to utilize the nucleic acid structures and as DNA-molecular light switches. Many efforts have been directed towards the design of complexes containing modified bpy or phen ligands that bind DNA primarily via-base pair intercalation.In this study, we report the results of investigation of interaction between calf thymus DNA(CT-DNA) and $[Pd(en)(dppz)](NO_3)_2$, complex (where en is ethylendiamine and dppz is dipyrido[3,2-a:2',3'-c]phenazine). Electronic absorption, fluorescence titration and gel filtration experiments were employed to determine the thermodynamic parameters, binding parameters and the mode of binding between this complex and DNA in Tris-HCl buffer solution containing 20mM sodium chloride (pH=7.0) at 300 and 310 K. Studies of antitumor activity of this complex against human cell tumor lines(k562) have been carried out. It shows IC50 value lower than that of cisplatin.The above compound can denature the DNA and the concentration of this ligand in the midpoint of transition ($[L]_{1/2}$), is 0.046 mmol/L at 300 K and 0.045 mmol/L at 310 K. the conformational stability of DNA in the interaction with ligand (ΔG° H_2O) determined to be 485.5 kJ/mol and 385.2 kJ/mol at 300K and 310K respectively.There is one set of 5 binding sits (g) for the complex on the DNA(per 1000 nucleotides) with positive cooperativity in binding. n,the Hill coefficient (as a criterion of cooperativity) find out to be 4 at 300 K and 6 at 310 K respectively. K app the apparent equilibrium constant are 14.37 mM^{-1} and 18.9 mM^{-1} at 300 and 310 K, respectively.Presence of complex led to less stability of the DNA. Values for m, (a measure of ligand strength for DNA denaturation) are 162 and 155 (kJ/mol).$(mmol/L)^{-1}$ at 300 and 310 K, respectively.Enthalpy of DNA denaturation by the complex (ΔH° coformation or ΔH° denaturation) in the range of 300 and 310 K is find out to be 42.1 kJ/mol. In addition, the calculated entropy (ΔS° H_2O) of DNA denaturation by complex is 0.12 kJ/mol at 300K.Fluorescence titration spectra and fluorescence Scatchard plots suggest that the Pd(II) complex intercalate in DNA. The gel chromatograms obtained from Sephadex G-25 column experiments showed that the binding of metal complex with DNA is so strong that it does not readily break.

Keywords: Thermodynamic Parameters, Spectroscopic Studies,Anti-Cancer, Palladium (II) Complex, DNA-Binding.

Abstract No.88

Comparative Structural Analysis of two forms (m1/m2) of p55 Domain of Helicobacter Pylori Vacuolating Toxin

Saeid Latifi-Navid [1], Shiva Mohammadi [1], Hamid Latifi-Navid [1], Saber Zahri [1], Amir Nasser Shamkhali [2]*

1. Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, IR

2. Department of Applied Chemistry, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, IR
(E-mail: shivamohamadi66@yahoo.com)

Helicobacter pylori VacA, a pore-forming toxin causing several alterations in human cells, plays an important role in the development of peptic and duodenal ulcerations as well as gastric adenocarcinoma. The N- and C-terminal cleavage of VacA during the secretion process yields a mature 88-kDa toxin which its partial proteolysis produces p33 and p55 domains. The latter is encoded by the middle (m) region of the vacA gene which includes m1/m2 polymorphisms. The m1 and m2 VacA proteins bind different cell-surface receptors. This is relevant clinically because the strains harboring m1 vacA genotype are associated with gastric cancer and VacA is a candidate antigen for vaccine development. The crystallographic structure of the m1 VacA p55 domain has been released and docked into the 19-Å cryo-EM map of the wild-type dodecamer in order to discern the p55 structure in the context of VacA oligomers. The purpose of this study was to produce the theoretical three-dimensional structure of m2 VacA p55 domain in order to perform a comparative structural analysis of two forms of the domain. The models were constructed using comparative modeling method and the stereochemical quality of protein structures and molecular dynamics simulation of the refined model was performed. The structural analysis showed the key features of m2 relative to m1 domain that may illustrate their different potentiality for interaction with host receptors and formation of oligomeric structures.

Keywords: Helicobacter pylori, VacA, Gastric Cancer, Comparative Modeling.

Abstract No.89

The Proteoletic Analysis of Firefly Luciferase with Disulfide Bridge and Hydrophilic Residue Substitution

Somayeh Karimzadeh, *Saman Hosseinkhani**, *Farangis Ataei*

Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, IR
(E-mail: somayeh.karimzadeh@gmail.com)

Luciferase from the North American firefly Photinus pyralis is a well-characterized enzyme that catalyzes the emission of yellow–green light. It is a useful protein that has been extensively used for sensitive in vitro and in vivo applications. Limited proteolysis has become a powerful tool for probing the higher order structure of proteins which determine the location of particular peptide bonds within the overall fold of the protein. In other to determine the effect of disulfide bridge and substitution of hydrophilic residue with surface hydrophobic residue on stability against protease, limited proteolysis of native, A296C/A326C (to create disulfide bridge), I232R (hydrophilic residue substitution) and A296C/A326C/I232R mutant luciferases was carried out using trypsin at 23◦C in different length of times. In this regards, first of all mutant luciferases were created using site direct mutagenesis. Afterward, they were expressed and purified. At last, proteolysis was done. Based on the analysis of SDS-PAGE and the percent of full-length remaining enzyme (using UN-SCAN-IT software (Silk Scientific,Inc)), all mutants shown different sensitivity against trypsin hydrolysis over a period of time, and apparently they were more stable than native one (especially the enzymes containing I232R mutation). In the case of Luciferases with disulfide bridge, some fragments were found to be more resistant to further degradation, whereas in native and I232R mutant, fragments released from the intact protein were subdigested by trypsin, giving rise to smaller peptides. Therefore disulfide bridge caueses increased interactions and consequently decreased accessibility of digest sites of trypsin. This results along with fluorescence and CD data suggest that these mutations exert an important role in decreasing structural felexibility and consequently proteolysis.

Keywords: Firefly luciferase, Photinus Pyralis, Proteolysis.

Abstract No.90

A Comparative Study of Non-hydrolytic Activities of Acetyl and Butyrylcholinesterase Enzymes and their Impact on the Formation of Beta-amyloid Aggregation

Soheila Davari, *Saeed Emadi**

Institute for Advanced Studies in Basic Sciences (IASBS), IR
(E-mail: emadi@iasbs.ac.ir)

Alzheimer disease (AD) is one of the most common neurodegenerative dementias, caused by silent plaques that are created by fibril formation of beta amyloid (Aβ) peptide. Aβ, is a portion of transmembrane receptor-like amyloid precursor protein (APP) in neuron cells. Recent investigations have shown that acetylcholinesterase (AChE) plays a crucial role in the promotion of Aβ aggregation beside its role in the rapid hydrolysis of the neurotransmitter acetylcholine (ACh). Butyrylcholinesterase (BChE), because of similar structure and function to AChE may also have role in thia phenomena. Both AChE and BChE have a peripheral anionic site, beside their active site, and it is proposed that it is involved in the promotion of amyloid fibrillation