2. Protein Chemistry Laboratory (PCL), Dept. of Biology, College of Sciences, Shiraz University, IR
3. Dept. of Chemistry, College of Sciences, Shiraz University, Shiraz, IR
4. Dept. of Nuclear Engineering, College of Engineering, Shiraz University, Shiraz, IR
(E-mail: roghayeh.mohammadi@yahoo.com)

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

Zahra sadat Saghaeian, Nahid Saghaiyan, Maryam Nikkhah, Majid Sadeghizadeh*

Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, IR
(E-mail: saghaiyan@yahoo.com)

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

*Mehdi Sadeqi, Bijan Ranjbar**

Department of Biophysics, Faculty of Biological Science, Tarbiat Modares University, Tehran, IR
(E-mail: mehdi.sadeqi65@yahoo.com)

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

technique for kinetic and function study of the DNAzyme compared with conventional methods. The optimum pH and thermal conditions for hybridization of restriction DNAzyme and its catalytic activity was also determined. The results are well in accordance with pervious findings reported by R. R Breaker. The extrinsic fluorescence study of the DNAzyme hybridization and its catalytic function by SYBR GOLD show consistency with Uv-visible experiment results, however this technique offers higher sensitivity compared to that of Uv-visible spectroscopy. Fluorescence intensity of DNAzyme-substrate increased upon hybridization, and decreased when the catalytic activity started. Our findings suggest that spectroscopic techniques, particularly extrinsic fluorescence spectroscopy using SYBR GOLD could be a good alternative to conventional methods for studying kinetic and catalytic activity of DNAzyme, being affordable and time saving.

Keywords: DNAzyme, Hybridization, Catalytic activity, Uv-visible, Fluorescence, Spectroscopy.

Abstract No.21

Interactions of β-lactoglobulin with Hexadecyltrimethylammonium bromide and Cetyltrimethylammonium p-Toluenesulfonate

Zohreh Saadati, Nasrin Sheykhrobati*

Department of Chemistry, Islamic Azad University, Omidieh Branch, IR
(E-mail: zohrehsaadati@gmail.com)

AbstractThe interactions of β-lactoglobulin AB in the presence of cationic surfactants such as Hexadecyltrimethylammonium bromide and Cetyltrimethylammonium p- Toluenesulfonate have been investigated using various experimental techniques such as conductivity, UV-Vis spectrophotometry and fluorimetry. The conductivity of surfactants aqueous solutions with β-lactoglobulin showed that the cmc of cationic surfactants decreased with the increase of counterion size. The results of UV-Vis and fluorescence studies showed a red shift in wavelength and an increase in absorbance and intensity of the emission maximum of protein during the interactions of surfactants with β-lactoglobulin. The results of UV-Vis also showed two distinct conformational changes (that was corresponding to precipitation and solving the precipitation of β-lactoglobulin) at pHs 6.7 and 8.0 and the cooperative character of binding at pH 2.0. The results of fluorescence studies showed that the binding strength of β-lactoglobulin / surfactant complex decreases with the increase of the pH.

Keywords: -lactoglobulin, Hexadecyltrimethyl ammonium bromide, Cetyltrimethyl ammonium p-toluen sulfonate, Conductivity, UV-Vis spectrophotometry, Fluorimetry.

Abstract No.22

Structural Studies on the Interaction of Urea with Lysozyme

Sadegh Farhadian [1], Behzad Shareghi [1], Nayere Bahamin [1], Masoud Salavati-Niasari [2], Somayeh Asgari [1], Parisa Nooraei [1]*

1. Shahrekord University, IR
2. Kashan University, IR
(E-mail: sadeghfarhadian@gmail.com)

The effect of urea on the structure of hen egg white lysozyme has been investigated using the method of UV-Visible detection and fluorescence spectroscopic techniques. The thermal denaturation of hen egg white lysozyme has been investigated in the presence and absence of urea over the temperature range (293-373) K in different buffers and pH values, using temperature scanning spectroscopy. The thermal denaturation of small globular proteins closely approaches a two-state, mechanism. Increasing the concentration of urea decreases the stability of lysozyme to thermal denaturation. The presence of urea caused the destabilization of hen egg white lysozyme resulting in a decrease in the temperature of unfolding with an increase in urea concentration. Fluorescence measurements have been extensively used to obtain information about tryptophan environments in proteins. There are six Trp residues in hen egg white lysozyme, but only two of them, Trp62 and Trp108, appear to dominate the fluorescence spectrum. Emission of lysozyme is dominated by tryptophan residue, which absorbs at the longest wavelength and displays the largest extinction coefficient. Energy absorbed by phenylalanine and tyrosine is often transferred to the tryptophan residues in the same protein. The fluorescence intensity of lysozyme decreases sharply with increasing urea. The result of fluorescence spectroscopy indicated that the structure of the Trp residue environments was altered. The environment of the Trp residues becomes more polar due to neighboring water molecules, and the reorientational relaxation of which after fluorescence excitation and emission leads to a Stokes shift of the fluorescence spectrum.

Keywords: Lysozyme, Fluorescence Spectroscopy, Sodium Dodecyl Sulfate (SDS), Sodium Octyl Sulfate (SOS).
