

that this result is in good agreement with obtained results of spectrometric techniques.

**Keywords:** Polyoxometalate, UV-Vis, Fluorescence, Cyclic Voltammetry, Calf Thymus DNA.

---

#### Abstract No.28

##### 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 Cu<sup>2+</sup> 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 Cu<sup>2+</sup> nanobiosensor in aqueous solutions by modification of DNAzyme molecule with fluorescence 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 previous 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.29

##### The Effect of Detergents on ProteinaseK Stability

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

Department of Biology-Shahrekord University-Shahrekord-Iran, IR  
(E-mail: parisanooraei@gmail.com)

Proteinase K EC(3, 4, 21, 14) is a serin proteinase from *Tritirachum album* Limber. This enzyme has two tryptophan residues and has two disulfide bonds, Cys34 - Cys123 and Cys178 - Cys249, which contribute to the stability of the enzyme structure. Virtually all studies of the protein-folding reaction add either heat, acid, or a chemical denaturant to an aqueous protein solution in order to perturb the protein structure. In this study spectroscopic aspect of ProteinaseK as a function of various concentration of SDS and DTAB by spectrophotometer and spectrofluorimeter has been investigated. The reaction takes place at different pHs and various temperature. Results exhibit, upon increasing the concentration of the Sodium n-dodecyl sulphate(SDS), stability of Proteinase K was increased. But in the presence of DTAB stability of Proteinase K was decreased.

**Keywords:** Proteinase K, Stability, Spectroscopic, Detergent.

---

#### Abstract No.30

##### NMR-Monitored Hydrogen Exchange Study on the Conformational Stability of RNase A: The Effect of Cationic Gemini Surfactants

Razieh Amiri\*<sup>1</sup>, Abdol-Khalegh Bordbar<sup>1</sup>, Douglas Laurents<sup>2</sup>

1. Department of Chemistry University of Isfahan, Isfahan, IR
2. Instituto de Química Física Rocasolano, CSIC, Serrano 119, Madrid 28006, ES  
(E-mail: razieh.amiri@gmail.com)

The conformational stability of ribonuclease A (RNase A) has been measured at the per residue level by NMR-monitored hydrogen exchange in the absence and presence of cationic gemini surfactants. The hydrogen/deuterium exchange mechanism of RNase A has been

found EXII in these conditions. We used gemini surfactants alkanediyl- $\alpha,\omega$ -bis(Hydroxyethylmethylhexadecyl ammonium bromide) in this study. 1D NMR experiments showed gemini surfactants bind to DSS. 2D 1H-NMR and Circular Dichroism (CD) spectroscopies show that the conformation of RNase A is unaffected at acidic pH where this protein is positively charged, although hydrogen exchange results shows that the conformational stability of RNase A is slightly lowered at high molar ratios. The denaturation curve of RNase A in the presence of gemini surfactant was analyzed on basis of a two-transition model. These gemini surfactants slightly activate and stabilize RNase A at low molar ratios and acidic pH (Table 1). The gemini surfactant with the shorter spacer interacts more efficiently with RNase A than those with longer spacers. These gemini surfactants neither interact strongly with nor severely destabilize this well folded protein in physiological conditions and we advance that can serve as useful membrane mimetics for studying interactions between membrane components and positively charged proteins.

**Keywords:** Gemini Surfactant, Ribonuclease A, Hydrogen Exchange, Fluorescence Spectroscopy, Circular Dichroism Spectroscopy.

---

#### Abstract No.31

##### Enzymatic Activity of RNase A in the Presence of Sodium N-dodecyl Sulphate

Shadi Salehpour\*<sup>1</sup>, Razieh Amiri<sup>2</sup>, Abdol-khalegh Bordbar<sup>2</sup>,  
Sayyed Hossein Rasa<sup>2</sup>

1. Department of Chemistry, Kashan University, IR
2. Department of Chemistry, University of Isfahan, IR  
(E-mail: salehpourshadi@gmail.com)

Protein-surfactant systems may serve as models for the study of the interactions between membrane proteins and lipids, additionally, the widespread use of the anionic surfactant sodium n-dodecyl sulphate for the solubilization of membrane proteins and in polyacrylamide-gel electrophoresis has stimulated interest in the nature of the interaction between this surfactant and globular proteins. Bovine pancreatic ribonuclease A is an enzyme that catalyses the depolymerization of RNA. In this study the enzymatic activity of RNAase A in the absence and presence of SDS is measured with cytidine 2': 3'-cyclic monophosphate as substrate. The enzyme reaction carried out with UV-VIS spectroscopy and was measured by following the changes in absorption spectra of cytidine 2':3'-phosphate toward time at 284 nm. We obtained initial rate of reaction enzyme from several kinetic spectra and extract Michealis Menten parameters ( $V_{max}$  and  $K_m$ ). We

investigated effect of sodium n-dodecyl sulphate on structure of ribonuclease A with fluorescence spectroscopy and observed changes in quantum yield of ribonuclease A. fluorescence properties of ribonuclease A is due to tyrosin, and this experiment show that SDS has effect on ribonuclease structure specially in tyrosin region.

**Keywords:** RNase A, Cytidine 2': 3'-cyclic Monophosphate, SDS, Enzymatic Activity, Michealis Menten Parameters.

---

#### Abstract No.32

##### Investigation on the Effect of salt Concentration on the Binding Modes of Doxorubicin to DNA with the Use of McGhee-von Hippel Equation

Havva Mehralitabar\*, Olena Krouglova, Saeed Emadi

Department of Biological Sciences, Institute for Advanced Studies in Basic Sciences, Zanjan, IR  
(E-mail: hmehralitabar@gmail.com)

For many years the study on the therapeutic properties of doxorubicin (DOX), like determination of its effective dosage and side effects, were the subject of study in basic and medical sciences. Since these studies are related to the DOX interactions with double stranded DNA, it is interesting to know that intercalation is just one mode of interaction between DOX and DNA at different DOX concentrations. The new method of fitting spectroscopic (spectrophotometric or spectrofluorometric) titration data obtained for DOX-DNA mixtures to different binding models (e.g., McGhee-von Hippel equation describing formation of one or two kinds of complexes, classical chemical equilibrium systems) was used. We investigated the interactions between DOX and salmon testes DNA (%G-C = 41.2) in solutions with various NaCl concentrations at neutral pH. For the fitting we used a set of computer optimization programs worked out by us to estimate the optimal spectra of complexes and thermodynamical binding parameters (binding constants, site sizes and cooperativity factor values). Using these programs we could also take into account the formation of dimeric species in the free DOX and DOX-DNA mixtures with  $\log K_d = 4.3 \pm 0.2$ . We observed that the model in which not only monomeric but also the dimeric DOX species were simultaneously bound to DNA through different mechanisms. We also found that apart from intercalation (with the site size of 3.7-4.0 bp), considerable amounts of aggregated outside bound DOX (with the site size of about 2 bp per one dimeric molecule) were formed with binding constants dependent at different ways on ionic strength. By comparing the Hamilton's Q and Qlim factors, calculated at the end of every iterative process of fitting