

## **BIOPHYSICAL CHEMISTRY LAB**

**ANNUAL REPORT 2006** 

Institute of Biochemistry and Biophysics Tehran, Iran

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# FOREWORD

The Laboratory of Biophysical Chemistry (LBC) was established in 1986 in the Institute of Biochemistry and Biophysics (IBB) and now functions as the main base and the mother of Biophysical Chemistry in Iran. This laboratory is well-known in the national and international level. It is also famous worldwide in the research area of Thermodynamics of Protein Denaturation and Proteinligand Interaction.

This laboratory enjoys from advanced facilities and is equipped with advanced apparatuses for the research on Biothermodynamics and Biomacromolecular Interactions. LBC is equipped with Nano and Micro Differential Scanning Calorimeters (DSC); Nano and Micro Isothermal Titration Calorimeters (ITC); modern Circular Dichroism (CD) Spectropolarometry, Sensitive Densitometer and Tensiometer; Fluorescence and Uv-vis Spectrophotometers, Microviscometers and biochemical and biophysical methods as well as and computational facilities.

LBC is an appropriate place for the promotion of the research and science in the field of Biochemistry, Nanobiophysics, Biotechnology and Biophysical Chemistry. LBC is a suitable laboratory for training PhD students and postdoctorate researchers, associate researchers and sabbatical leaves for faculty members at national and international levels.

Up to the present time, 30 Ph.D and 50 Master students have developed their theses in this laboratory and graduated from the university. Faculty members, postdoctorates and students and foreign research associates using the facilities of this laboratory which have published hundreds of full research articles in international prestigious journals. LBC is an appropriate laboratory for supporting and promoting the research of scientists and researchers at national and international levels.



# Biophysical Chemistry Lab

## A research group 2006







Ali A. Moosavi-Movahedi Professor of Biophysics *Protein Thermodynamics* 



Ali A. Saboury Professor of Biophysics *Protein- ligand binding* 



### Lab Colleagues

\* Professor M. Shamsipur

Razi University Department of Chemistry

\* Dr. M. Habibi-Rezaei

University of Tehran College of Science, Department of Biology

✤Dr. H. Ghourchian University of Tehran Institute of Biochemistry and Biophysics (I.B.B)

★Dr. S. Safarian University of Tehran College of Science, Department of Biology \* Professor A. Shafiei University of Tehran

Faculty of Pharmacy, Medical Sciences

#### \* Professor M. R. Ganjali

University of Tehran College of Science, Department of Chemistry

\* Dr. K. Nazari Research Institute of Petroleum Industry, Tehran, Iran

✤ Dr. P. Norouzi University of Tehran College of Science, Department of Chemistry

\* Dr. G. Ataei Medical Science Shahid Beheshti University Department of Premedical Science



### INTERNATIONAL COLLABORATION

#### **\***Professor G. Floris:

Department of Applied Science in Biosystem, University of Cagliari, Cagliari, Italy

#### ★Professor T. Haertle

National Research Institute of Agronomique, 44316 Nantes Cedex 03, France

#### ★ Professor F. Ahmad

Director, Centre for Interdiscipinary Research in Basic Sciences, Jamia Millia Islamia (A Central University), Jamia Nagar, NEW DELHI - 110 025, India

#### ★Professor G. Hakimelahi

Taigen Biotechnology X, 7F, 138 Hsin Ming Rd. Neihu Dist, Taipei, Taiwan

#### ★Dr. N. Sheibani

Department of Ophthalmology and Visual Science, University of Wisconsin, Madison, WI S370S, USA





### STUDENTS (Year 2006)

## Doctor of Philosophy (Ph.D)

※J. Moosavi
※M. Amani
※M. Atri
※J. Badraghi
※A. Barzegar
※A. Divsalar
※N. Gheibi

## Master of Science (MSc)

☆M. Alijanianzadeh
☆M. Mojtahedi
☆H. Sepasi Tehrani
☆A. Hekmat





### Projects (2006)

**1- Molten globule states of proteins induced by surfactants** *Institute of Biochemistry and Biophysics, University of Tehran* 

**2- Interaction of adenosine deaminase with inhibitors and substrate analogs** *International joint project, Institute of Biochemistry and Biophysics, University of Tehran; Institute of Biochemistry, National Academy of Science, Yeravan, Armenia; Department of Chemical Sciences, University of Camerino, Camerino, Italy* 

## 3- The stablization and activation of enzyme via metal ions: industrial, medical and bioenvironmental application

Interchange joint project between Institute of Biochemistery and Biophysics, University of Tehran and Department of Chemistry, Razi University.

#### 4- Stabilization of industrial and therapeutical enzymes by osmolytes

International project entitled "Stabilization of industrial and therapeutical enzymes by osmolytes" was approved by Iran National Science Foundation (INSF). This project is between Dept of Bioscience, Jamia Millia Islamia, New Delhi, India and Institute of Biochemistry and Biophysics, University of Tehran, Iran.



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1. J. Chamani, A.A. Moosavi-Movahedi, O. Rajabi, M. Gharanfoli, M. Momen-Heravi, G.H. Hakimelahi, A. Neamati-Baghsiah and A.R. Varasteh, "Cooperative alpha helix formation of beta- lactoglobulin induced by sodium dodecyl sulfate" J. Colloid and Interface Science 293, 52-50 (2006)

2. H. Tavakoli, H. Ghourchian, A.A. Moosavi-Movahedi and A.A. Saboury, "Histidine and serine roles in catalytic activity of choline oxidase from Alcaligenes species studied by chemical modifications" Process Biochemistry 41, 477-482 (2006)

3. A.A. Saboury, M.S. Atri, M.H. Sanati, A.A. Moosavi-Movahedi, G.H. Hakimelahi and M. Sadeghi, "A thermodynamic study on the interaction between mangnesium ion and human growth hormone" Biopolymers, 81, 120-126 (2006)

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8. A.A. Moosavi-Movahedi, M. Gharanfoli, S. Jalili, F. Ahmad, J. Chamani, G.H. Hakimelahi, M. Sadeghi, M. Amani, A.A. Saboury, "The correlation of Rnase A enzymatic activity with the changes in the distance between  $N_{\delta 2}$ -His<sub>12</sub> and  $N_{\delta 1}$ -His<sub>119</sub> upon addition of stabilizing and destabilizing salts" The Protein Journal 25(2), 117-125 (2006)



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11. J. Chamani, A.A. Moosavi-Movahedi "Effect of n-alkylammonium bromide on the folding and stability of alkaline and acid –denatured cytochrome c. A spectroscopic approach" J. of Colloid and Interface Science 297, 561-569 (2006)

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14. S. Hashemnia , A.A. Moosavi-Movahedi, H. Ghourchian, F. Ahmad, G.H. Hakimelahi and A.A. Saboury "Diminishing of Aggregation for Bovine Liver Catalase through Acidic Residues Modification" International Journal of Biological Macromolecules 40, 47-53 (2006)

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16. Jun Hong, H.Ghourchian and A.A.Moosavi-Movahedi "Direct electron transfer of redox proteins on a Nafion-cysteine modified gold electrode" Electrochemistry Communication 8,1572-1576(2006)

18. N. Gheibi, A.A. Saboury, K. Haghbeen and A.A.Moosavi-Movahedi "The effect of some osmolytes on the activity and stability of mushroom tyrosinase "J. Bioscience 31(3),355-362 (2006)

19. A. A. Saboury and M. S. Atri, "Metal ions binding study on human growth hormone by isothermal titration calorimetry", Journal of Physical and Theoretical Chemistry **2** (2006), 169-182.

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2. O. Karima, G. H. Riazi, A.A. Moosavi-Movahedi, F. Mokhtari and B. Fakurian "The effect of beta-boswellic acid on microtubule polymrization" The FEBS Journal, vol 273, supplement 1, page 61 (2006) .This paper was presented orally at 31th FEBS Congress, Istanbul ,Turkey, 24-29 June 2006

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7. S. Rezaei-Zarchi, A.A. Saboury, H. Ghourchian, P. Norouzi, A.A. Moosavi-Movahedi, M.R. Ganjali and A. Javed "Electrochemical investigation on the ligand binding by hemoglobin "The FEBS Journal, vol 273, supplement 1, page 353 (2006) This paper was presented at 31th FEBS Congress, Istanbul ,Turkey, 24-29 June 2006



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9. A. A. Saboury, "Isothermal titration calorimetry study on metal ions binding by human growth hormone", 20th IUBMB International Congress and 11th FAOBMB Congress: Life: Molecular Integration and Biological Diversity, Kyoto, Japan (18-23 June 2006).

10. Mahdi Alijanianzadeh, A.A. Saboury, H. Mansoori-Torshizi and K. Haghbeen, "Inhibition study on the cresolase activity of mushroom tyrosinase by new synthesized alkyl xanthates", 31th FEBS Congress: Molecular in Health and Disease, Istanbul, Turkey (24-29 June 2006).

11. S. Zolghadri and A.A. Saboury, "The Inhibitory effect of thiophenol on the activity of tyrosinase", 31th FEBS Congress: Molecular in Health and Disease, Istanbul, Turkey (24-29 June 2006).

12. P. S. Pourhosseini, M. N. Sarbolouki and A.A. Saboury, "Polymersomes as drug delivery vehicles", 33rd Annual Meeting of Exposition of the Controlled Release Societ<u>y</u>, Vienna, Austria (22-26 July 2006).

13. K. Mahnam, A.A.Moosavi-Movahedi "Surface esterification of adenosine deaminase by Woodward reagent K" 50th Annual Meeting Biophysical Society, Salt lake City, Utah, USA, Feb 18-22, 2006.

14. P.Pirzadeh, A.A.Moosavi-Movahedi, B. Hammatinejad, M. Shamsipur, A. Cooper "A chemometric study of the interaction of lysozyme with sodium dodecyl sulfate and beta-cyclodextrin" 50th Annual Meeting Biophysical Society, Salt lake City,Utah,USA, Feb 18-22, 2006.

15. A. Barzegar and A.A.Moosavi-Movahedi "Multiple unfolded states of yeast and horse liver alcohol dehydrogenase by heat treatment" 20th IUBMB International Congress of Biochemistry and Molecular Biology, June 18-23, 2006 Kyoto, Japan



1. A. Barzegar, A. A. Moosavi-Movahedi, M. R. Ganjali, P. Norouzi, A. A. Saboury and S. Sobhanian, "Monitoring horse liver alcohol dehydrogenase aggregation during thermal unfolding by dynode voltage in circular dichroism spectroscopy", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

2. F. S. Nematpour, K. Haghbeen, N. Gheibi and A. A. Saboury, "Application of enzymatic inhibitors for catechol derivaties production", *Seventh*\_Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

3. A. A. Saboury, "A general theory for the enzyme inhibition", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

4. H. Mansouri-Torshizi, A. A. Saboury and M. Eslami-Moghaddam, "Thermodynamics of Binding in the interaction of 2,2'bipyridineoctyldithiocarbamato palladium (II) choloride with calf thymus DNA", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

5. S. Nafisi, A. A. Saboury, N. Keramat, J.-F. Neault and H. A. Tajmir-Riahi, "Stability and structural features of DNA interaction with ethidium bromide, acridine orange and methylene blue", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

6. H. Mansouri-Torshizi, A. A. Saboury and M. Eslami-Moghaddam, "Binding studies of 2,2'-bipyridinehexyldithiocarbamato palladium (II) choloride with calf thymus DNA", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

7. H. Mansouri-Torshizi, A. A. Saboury and M. Eslami-Moghaddam, "Binding parameters in the interaction of 2,2'-bipyridinebuthyldithiocarbamato palladium (II) choloride with DNA", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

8. R. Khoshneviszadeh, K. Haghbeen, M. Sadeghi and A. A. Saboury, "Quaternary structure of mushromm tyrosinase", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006

9. D. Ajloo, H. Dayani, A. A. Saboury, A. A. Moosavi-Movahedi, A. Aghapour, A. Shokravi and M. Ahmadi, "Structure activity relationship study on some of adenosine deaminase inhibitors", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).



10 A. Divsalar, A. A. Saboury, H. Mansouri-Torshizi, and A. A. Moosavi-Movahedi, "Refolding studies of chemically denatured -lactoglobulin types A and B", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

11. S. Zolghadri and A. A. Saboury, "Activation and inhibition of mushroom tyrosinase by different concentration of thiophenol", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

12. H. Mansouri-Torshizi, A. A. Saboury, M. Eslami-Moghaddam and F. Zeidali, "Interaction studies of 2,2'-bipyridinebenzyldithiocarbamato palladium (II) nitrate with DNA", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

13. N. Sattarahmadi, A. A. Moosavi-Movahedi, M. Habibi-Rezaei and A. A. Saboury, "Specialization of Maillard reaction for HAS with glucose at different days of incubation", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006

14. H. Mansouri-Torshizi, A. A. Saboury, M. Eslami-Moghaddam and M. Saeidifar, "Spectroscopic studies on the interaction of DNA with 2,2'bipyridinephenyldithiocarbamato palladium (II) nitrate", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

15. M. S. Atri, A. A. Saboury and M. Kordbacheh, "Thermodynamics of metal ions binding to human growth hormone", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

16. M. Alijanianzadeh, A. A. Saboury, H. Mansouri-Torshizi and K. Haghbeen, "Inhibition study on the catecholase activity of mushroom tyrosinase by new synthesized alkyl xanthates", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

17.) N. Mortazavi, A. A. Saboury, A. Nasehzadeh, H. Naghibi Beidokhti and A. Abdollahpour, "Study of coordination of tranferrin components with metal ions by using different methods", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

18. K. Mahnam, A. A. Moosavi-Movahedi, H. Bahrami, G. Ataei, S. Jalili, A. A. Saboury, "Site determination of modified acidic residues of adenosine deaminase: hydration free energy calculation", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

19. Z. Moosavi-Movahedi, M. Zahedi, H. Bahrami, K. Mahnam, A. A. Saboury and A. A. Moosavi-Movahedi, "Lysine site determination of human serum albumin apon interaction with bilirubin in aqueous solution: Theoretical calculations", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).



20. D. Ajloo, L. Najafi, A. A. Moosavi-Movahedi, A. A. Saboury and S. J. Moosavi, "The effect of mobile electromagnetic field on the activity and structure of adenosine deaminase", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

21. R. Yousefi, M. Amani, A. A. Moosavi-Movahedi, A. Mura, G. Floris and A. A. Saboury, "Conformational changes and transitional switch between regular secondary structures of LSAO induced by TFE: Relation to the protein aggregation", Seventh Iranian Biophysical Chemistry Conference, Tabriz University, Tabriz (June 18-19, 2006).

22. S. Rezaei-Zarchi, A. A. Saboury, S. Ahmadian, H. Ghourchian, J. Hong, P. Norouzi, A. A. Moosavi-Movahedi, M. R. Ganjali and A. Javed, "Silver nanoparticle as electron transfer in electrochemical ligand binding investigation of hemoglobin", 14<sup>th</sup> National & 2<sup>nd</sup> International Conference of Biology, Tarbiat Modares University, Tehran (August 29-31 2006).

23. M. Bakhti, M. Habibi-Rezaei, S. Rezaei-Zarchi, H. Ghourchian, A. A. Saboury, A. A. Moosavi-Movahedi and J. Zargar, "Electrochemical analysis of the glycation induced protein unfolding", 14<sup>th</sup> National & 2<sup>nd</sup> International Conference of Biology, Tarbiat Modares University, Tehran (August 29-31 2006).



### Selected Papers (year 2006)

#### A thermodynamic study on the interaction between magnesium ion and human growth hormone

Author(s): <u>Saboury AA</u>, <u>Atri MS</u>, <u>Sanati MH</u>, <u>Moosavi-Movahedi AA</u>, <u>Hakimelahi GH</u>, <u>Sadeghi M</u>

Source: BIOPOLYMERS 81 (2): 120-126 FEB 5 2006

Abstract: A thermodynamic study on the interaction between magnesium ion and human growth hormone (hGH) was studied at 27 degrees C in NaCl solution (50 mM) using different techniques. Two techniques of ionmetry using a Mg2+ selective membrane electrode and isothermal titration calorimetry were applied to obtain the binding isotherm for hGHMg(2+); results obtained by both techniques were found to be in good agreement. There is a set of three identical and noninteracting binding sites for magnesium ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 46 mu M and - 17.7 kJ/mol, respectively. Temperature scanning UV-visible spectroscopy was applied to elucidate the effect of Mg2+ binding on the protein stability, and circular dichroism (CD) spectroscopy was used to show the structural change of hGH due to the metal ion interaction. Magnesium ion binding increased the protein thermal stability by increasing the alpha-helix content as well as decreasing both beta and random coil structures. However, the secondary structural change of the protein returns to its native form, including a small change in the tertiary structure, in high concentrations of magnesium ion. (C) 2005 Wiley Periodicals, Inc.

**Author Keywords:** human growth hormone; magnesium; protein stability; titration calorimetry; circular dichroism



### Stability of proteins in the presence of polyols estimated from their guanidinium chloride-induced transition curves at different pH values and 25 degrees C

Author(s): Haque I, Islam A, Singh R, Moosavi-Movahedi AA, Ahmad F

Source: BIOPHYSICAL CHEMISTRY 119 (3): 224-233 FEB 1 2006

Abstract: We have recently concluded from the heat-induced denaturation studies that polyols do not affect Delta G(D)degrees (the Gibbs free energy change (Delta G(D)) at 25 degrees C) of ribonuclease-A and lysozyme at physiological pH and temperature, and their stabilizing effect increases with decrease in pH. Since the estimation of Delta G(D)degrees of proteins from heat-induced denaturation curves requires a large extrapolation, the reliability of this procedure for the estimation of Delta G(D)(o) is always questionable, and so are conclusions drawn from such studies. This led us to measure Delta G(D)degrees of ribonuclease-A and lysozyme using a more accurate method, i.e., from their isothermal (25 degrees C) guanidinium chloride (GdmCl)-induced denaturations. We show that our earlier conclusions drawn from heat-induced denaturation studies are correct. Since the extent of unfolding of heat- and GdmCl-induced denatured states of these proteins is not identical, the extent of stabilization of the proteins by polyols against heat and GdmCl denaturations may also differ. We report that in spite of the differences in the structural nature of the heat- and GdmCl-denatured states of each protein, the extent of stabilization by a polyol is same. We also report that the functional dependence of Delta G(D) of proteins in the presence of polyols on denaturant concentration is linear through the full denaturant concentration range. Furthermore, polyols do not affect the secondary and tertiary structures of the native and GdmCl-denatured states. (C) 2005 Elsevier B.V. All rights reserved.

Author Keywords: protein stability; guanidinium; chloride denaturation; protein structure; polyol osmolyte; ribonuclease-A; lysozyme



#### A unique molten globule state occurs during unfolding of cytochrome c by LiClO4 near physiological pH and temperature: Structural and thermodynamic characterization

Author(s): Moza B, Qureshi SH, Islam A, Singh R, Anjum F, Moosavi-Movahedi AA, Ahmad F

Source: BIOCHEMISTRY 45 (14): 4695-4702 APR 11 2006

Abstract: We have carried out denaturation studies of bovine cytochrome c (cyt c) by LiClO4 at pH 6.0 and 25 degrees C by observing changes in difference molar absorbance at 400 nm (Delta epsilon(400)), mean residue ellipticities at 222 nm ([theta](222)) and difference mean residue ellipticity at 409 nm (Delta[theta](409)). The denaturation is a three-step process when measured by Delta epsilon(400) and Delta[theta](409), and it is a two-step process when monitored by [theta](222). The stable folding intermediate state has been characterized by near- and far-UV circular dichroism, tryptophan fluorescence, 8-anilino-1-naphthalene sulfonic acid (ANS) binding, and intrinsic viscosity measurements. A comparison of the conformational and thermodynamic properties of the LiClO4-induced molten globule (MG) state with those induced by other solvent conditions (e.g., low pH, LiCl, and CaCl2) suggests that LiClO4 induces a unique MG state, i.e., (i) the core in the LiClO4-induced state retains less secondary and tertiary structure than that in the MG states obtained in other solvent conditions, and (ii) the thermodynamic stability associated with the LiClO4-induced process, native state-MG state, is the same as that observed for each transition between native and MG states induced by other solvent conditions.

**KeyWords Plus:** GUANIDINE-HYDROCHLORIDE; HYDROGEN-EXCHANGE; FERRICYTOCHROME C; PROTEIN STABILITY; ALPHA-LACTALBUMIN; A-STATE; DENATURATION; APOMYOGLOBIN; BINDING; HORSE



#### Effect of n-alkyl trimethylammonium bromides on folding and stability of alkaline and acid-denatured cytochrome c: A spectroscopic approach

Author(s): Chamani J, Moosavi-Movahedi AA

Source: JOURNAL OF COLLOID AND INTERFACE SCIENCE 297 (2): 561-569 MAY 15 2006

Abstract: The molten globule (MG) state can be an intermediate in the protein folding pathway; thus, its detailed description can help understanding protein folding. Alkyl trimethylammonium bromides including dodecyl trimethylammonium bromide, trimethylammonium bromide, TTAB; DTAB; tetradecyl and hexadecyl trimethylammonium bromide, HTAB; cationic surfactants that are commonly used to mimic hydrophobic binding environments such as cell membranes, are known to denature some native state proteins, including horse cytochrome c (zyt c). In this article, refolding of alkaline and acid-denatured cyt c are studied under the influence of n-alkyl trimethylammonium bromides to form MG-like states at both low concentration (pH 11) and above the critical micelle concentration (pH 2) using ultraviolet and visible absorption, fluorescence and circular dichroism (CD). The addition of n-alkyl trimethylammonium bromides to the unfolded state of cyt c in alkaline and acidic condition appears to support the stabilized form of the MG state. The m-values of the refolded state of cyt c by DTAB, TTAB and HTAB showed substantial variation. The enhancement of m-values as the stability criterion of the MG state corresponded with increasing chain length of the cited n-alkyl trimethylammonium bromides. Based on the results obtained, the merits of two models of the protein-surfactant structure are discussed for various n-alkyl trimethylammonium bromides concentration in inducing the MG state at two different pH conditions. Therefore, hydrophobic interactions play a dominant role in stabilizing the MG state. (c) 2005 Elsevier Inc. All rights reserved.

**Author Keywords:** cytochrome c; cationic surfactants; molten globule-like state; protein folding; hydrophobic interaction; stabilization



## Molten globule-like state of bovine carbonic anhydrase in the presence of acetonitrile

Author(s): Safarian S, Saffarzadeh M, Zargar SJ, Moosavi-Movahedi AA

Source: JOURNAL OF BIOCHEMISTRY 139 (6): 1025-1033 JUN 2006

Abstract: We have evaluated the effects of acetonitrile on the structure and function of bovine carbonic anhydrase II. The potential structural and functional changes in carbonic anhydrase in the presence of different acetonitrile/buffer ratios (0%, 17.5% and 47.5% v/v) were determined using a variety of methods. These included simple spectrophotometric methods to record enzyme velocity, fluorescence measurements and calculation of accessible surface area (ASA) to identify possible alterations in tertiary structure of the protein, CD measurements to search for secondary structure conversions, and thermal scanning to determine structural stability of the protein in different media. The Far-UV CD studies indicated that carbonic anhydrase, for the most part, retains its secondary structure in the presence of acetonitrile. Fluorescence measurements using iodide ion and ANS along with ASA calculations revealed that in the presence of acetonitrile some degree of conformational change occurs in the carbonic anhydrase structure. In addition to the hydrophobic pockets, two additional tryptophanyl residues become exposed to the solvent, thereby increasing the surface hydrophobicity of the protein. These alterations dramatically reduce the catalytic activity, thermal stability, and aggregation velocity of the enzyme. Thus, our results support a molten globule-like structure of carbonic anhydrase in the presence of acetonitrile.

**Author Keywords:** acetonitrile; carbonic anhydrase; molten globule; organic solvent; thermal stability



## Calorimetric and binding dissections of HSA upon interaction with bilirubin

Author(s): <u>Moosavi-Movahedi Z</u>, <u>Safarian S</u>, <u>Zahedi M</u>, <u>Sadeghi M</u>, <u>Saboury AA</u>, <u>Chamani J</u>, <u>Bahrami H</u>, <u>Ashraf-Modarres A</u>, <u>Moosavi-Movahedi AA</u>

Source: PROTEIN JOURNAL 25 (3): 193-201 APR 2006

Abstract: The interactions between bilirubin and human serum albumin (HSA) were studied by isothermal titration calorimetry (ITC) and UV-vis spectrophotometry at 27 degrees C in 100 mM phosphate buffer pH 7.4 containing 1 mM EDTA. The biphasic shape of the HSA-bilirubin binding curve depicted the existence of two bilirubin binding sets on the HSA structure which had distinct binding interactions. Each binding set contained one or more bilirubin binding site. The first binding set at subdomain IIA included one binding site and had a more hydrophobic microenvironment than the other two binding sites in the second bilirubin binding set (subdomain IIIA). With our method of analysis, the calculated dissociation constant of the first binding site is 1.28x10(-6) M and 4.80x10(-4) M for the second and third binding sites. Here, the typical Boltzmann's equation was used with a new approach to calculate the dissociation constants as well as the standard free energy changes for the HSA-bilirubin interactions. Interestingly, our calculations obtained using the Wyman binding potential theory confirmed that our analysis method had been correct (especially for the second binding phase). The molar extinction coefficient determined for the first bound bilirubin molecule depicted that the bilirubin molecules (in low concentrations) should interact with the nonpolar microenvironment of the first high affinity binding site. Binding of the bilirubin molecules to the first binding site was endothermic (Delta H-circle > 0) and occurred through the large increase in the binding entropy established when the hydrophobic bilirubin molecules escaped from their surrounding polar water molecules and into the hydrophobic medium of the first binding site. On the other hand, the calculated molar extinction coefficient illustrated that the microenvironment of the second binding set (especially for the third binding site) was less hydrophobic than the first one but still more hydrophobic than the buffer medium. The binding of the third bilirubin molecule to the HSA molecule was established more through exothermic (electrostatic) interactions.

**Author Keywords:** bilirubin; binding sites; entropically driven; HSA; microcalorimetry



# The inhibitory effect of benzenethiol on the cresolase and catecholase activities of mushroom tyrosinase

Author(s): Saboury AA, Zolghadri S, Haghbeen K, Moosavi-Movahedi AA

**Source:** JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY 21 (6): 711-717 DEC 2006

Abstract: The inhibitory effect of benzenethiol on the cresolase and catecholase activities of mushroom tyrosinase (MT) have been investigated at two temperatures of 20 and 30 degrees C in 10 mM phosphate buffer solution, pHs 5.3 and 6.8. The results show that benzenethiol can inhibit both activities of mushroom tyrosinase competitively. The inhibitory effect of benzenethiol on the cresolase activity is more than the catecholase activity of MT. The inhibition constant (K-i) value at pH 5.3 is smaller than that at pH 6.8 for both enzyme activities. However, the Ki value increases in cresolase activity and decreases in catecholase activity due to the increase of temperature from 20 to 30 degrees C at both pHs. Moreover, the effect of temperature on Ki value is more at pH 6.8 for both cresolase and catecholase activities. The type of binding process is different in the two types of MT activities. The binding process for catecholase inhibition is only entropy driven, which means that the predominant interaction in the active site of the enzyme is hydrophobic, meanwhile the electrostatic interaction can be important for cresolase inhibition due to the enthalpy driven binding process. Fluorescence and circular studies also show a minor change in the tertiary structure, without any change in the secondary structure, of the enzyme due to the electrostatic interaction in cresolase inhibition by benzenethiol at acidic pH.

**Author Keywords:** mushroom tyrosinase; benzenethiol; competitive inhibition; inhibition constant; fluorescence; circular dichroism



## Diminishing of aggregation for bovine liver catalase through acidic residues modification

Author(s): <u>Hashemnia S</u>, <u>Moosavi-Movahedi AA</u>, <u>Ghourchian H</u>, <u>Ahmad F</u>, <u>Hakimelahi</u> <u>GH</u>, <u>Saboury AA</u>

**Source:** INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES 40 (1): 47-53 DEC 15 2006

**Abstract:** The tendency of proteins to aggregate is an important problem in biotechnology and the pharmaceutical industry. Because proteins in the aggregated state generally do not have the same biological activity as proteins in the native state. In order to prevent aggregation, it is essential to know the effective parameters in anti-aggregation mechanism. Using a chemical protein modification approach, UV-vis and fluorescence spectroscopies and circular dichroism spectropolarimetry, this study investigates the parameters involved in anti-aggregation mechanism of bovine liver catalase. Our findings clearly indicate that the modified bovine liver catalase provides better protection than the native enzyme against thermal aggregation. It seems that a decrease in hydrophobicity resulting in chemical modification plays an important role in preventing aggregation. (c) 2006 Elsevier B.V. All rights reserved.

**Author Keywords:** bovine liver catalase; chemical modification; hydrophobicity; modification number; thermal aggregation; Woodward's reagent K



## Chemometric studies of lysozyme upon interaction with sodium dodecyl sulfate and beta-cyclodextrin

Author(s): <u>Pirzadeh P</u>, <u>Moosavi-Movahedi AA</u>, <u>Hemmateenejad B</u>, <u>Ahmad F</u>, <u>Shamsipur M</u>, <u>Saboury AA</u>

Source: COLLOIDS AND SURFACES B-BIOINTERFACES 52 (1): 31-38 SEP 1 2006

**Abstract:** The interaction of hen egg-white lysozyme with sodium n-dodecyl sulfate (SDS) as an anionic surfactant was investigated by UV-vis spectrophotometry at different pHs at 25 degrees C using HCl/glycine and NaOH/glycine for acidic and basic pH ranges, respectively. Analysis of the spectral data using chemometric method gave the evidence for the existence of intermediate components during the cited interaction. Results also indicated a connection between turbidity of the protein solution upon interaction with SDS and distribution of our newly found intermediates. As intermediates are important in aggregation of proteins, beta-cyclodextrin was employed as an anti-aggregation agent and the results obtained for the lysozyme-SDS-beta-cyclodextrin ternary system were compared with those obtained in the absence of beta-cyclodextrin on distribution and mole fraction of intermediates with. It is also shown that as the distribution of intermediates broadens in a range of SDS concentrations, the turbidity and aggregation state of solution are reduced. (c) 2006 Elsevier B.V. All rights reserved.

Author Keywords: lysozyme; intermediates; turbidity; SDS; beta-cyclodextrin; chemometry



## Direct electron transfer of redox proteins on a Nafion-cysteine modified gold electrode

Author(s): Hong J, Ghourchian H, Moosavi-Movahedi AA

Source: ELECTROCHEMISTRY COMMUNICATIONS 8 (10): 1572-1576 OCT 2006

**Abstract:** A novel Nafion-cysteine functional membrane was constructed. Rapid and direct electron transfer of horseradish peroxidase, Euphorbia latex amine oxidase, superoxide dismutase and cytochrome c was carried out on the functional membrane modified gold electrode with good stability and repeatability. The immobilized protein modified electrodes showed quasi-reversible electrochemical redox behaviors with formal potentials of 60, 35, 66 and 38 mV (vs. Ag/AgCl) in 20 mM potassium phosphate buffer solution, pH 7.0 at 25 degrees C. The cathodic transfer coefficients were 0.42, 0.42, 0.45, 0.44 and electron transfer rate constants were evaluated to be 1.2, 1.6, 1.0 and 0.6 s(-1). (c) 2006 Elsevier B.V. All rights reserved.

**Author Keywords:** direct electron transfer; horseradish peroxidase; Euphorbia latex amine oxidase; superoxide dismutase; cytochrome c; Nafion