Iranian Journal of Pharmaceutical Research (2005) 4: 239-244
Received: May 2005
Accepted: October 2005

Original Article

The Effects of Acetaminophen on Human Serum Albumin (HSA)

Mostafa Rzaei-Tavirani[a*], Seyed Hassan Moghaddamnia[b], Bijan Ranjbar[c], Said Namaki[b] and Parisa Zolfaghari[d]

[a]*Faculty of Medicine, Medical University of Ilam, Ilam, Iran.* [b]*The Faculty of Paramedical Sciences, Shaheed Beheshti University of Medical Sciences and Health Services, Tehran, Iran.* [c]*Department of Biophysics, Faculty of Science, Tarbiat Moddares University, Tehran, Iran.* [d]*Center of Science and researches, Islamic Azad University, Tehran, Iran.*

Abstract

Thermal conformational changes in human serum albumin (HSA) in present with a 10 mM phosphate buffer, at pH=7 have been investigated via circular dichroism (CD) and UV spectroscopic methods. The results indicate that temperature in a range of 25ºC to 55ºC could induce a reversible conformational change in the structure of HSA. The HSA phase transition corresponds to the physiological and pathological conditions of the body, especially fever. The conformational change observed in HSA is accompanied by a mild conversion of its secondary structures. Acetaminophen is a papular pain killer, and HSA is used as a drug carrier. Hence, acetaminophen could it interact with HSA. The study of HSA – acetaminophen interaction reveals the effects of acetaminophen on HSA structure, preventing it's phase transition. HSA – acetaminophen interaction leads to the stabilization of HAS. This interaction is accompanied with 8 kJ/mol of free energy change. The structural changes within HSA due to it's interaction with acetaminophen could be considered as a drug side effect and it may affect the protein functions.

Keywords: Human serum albumin; Conformation; Temperature**;** Circular dichroism (CD); Acetaminophen.

Introduction

Human serum albumin (HSA) is a major protein component of blood plasma (present at 50 mg/ml (600 μm)), which is also found in the interstitial fluid of body tissues and has a half-life of 19 days in humans (1, 2).

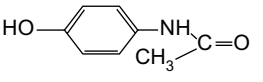
HSA has different roles in the body. The most important ones are: maintenance of normal oncotic pressure, binding to the different substances and transporting drugs and endogenous compounds, metabolic function such as inactivation of some compounds, and acid-base function due to having numerous charged residues and being abundant in plasma. It also has an effective role in the regulation of plasma buffer, anti-oxidant function and an anti-coagulant effect (1, 3, 4). HSA is best known for its ligand binding capacity, because it binds to different substances ranging from metal ions such as calcium (5), zinc (5), copper (6) and nickel (7) to fatty acids (8), amino acids (9), hormones and a wide variety of drugs (1-10).

HSA consists of a single polypeptide chain of 585 amino acids with a molecular weight of 66500 Da. The chain is characterized by having no carbohydrate moiety, a scarcity of triptophan and

* Corresponding author:
E-mail: tavirany@yahoo.com

methionine residues and an abundant of charged residues, such as lysine, arginine, glutamic acid and aspartic acid (1). HSA is monomeric but contains three alpha helical domains (I – III), which are structurally similar. Each domain can be divided into subdomains A and B, which contain six and four alpha helices respectively (11, 12). The flexibility and three-domain structure of HSA makes the molecule have a variety of binding sites. The fatty acid binding sites are distributed throughout the protein and involve all six subdomains, while most drugs have specific binding sites which are known as Sudlow's site I and II (13). The drug sites I and II are located in the subdomains IIA and IIIA respectively (12). Bulky heterocyclic anions bind to site I, whereas site II is preferred by aromatic carboxylates with an extended conformation. Remarkably, ibuprofen, as a nonsteroidal anti-inflammatory agent and warfarin (14) as an anti-coagulant drug, are considered as stereotypical ligands for Sudlow's site II and Sudlow's site I respectively (1, 12, 13, 15-17). warfarin shares its binding site with a range of other drugs including phenylbutazone, tolbutamide, indomethacin, salicylates, digitoxin, furosemide, phenytoin, chlorpropamide and some penicillins (4, 13, 18-20). Other experiments show that low levels of fatty acids or elevated pH may enhance the affinity of HSA for warfarin by up to 3-fold (16, 21-22). Other drugs that bind to the site II include diazepam and other 2, 3 benzodiazepines, naproxen and clofibrate (4). Although examples of drug binding elsewhere on protein have been documented (13, 23-24), most work was focused on the primary drug sites. Dennis et al. showed that study of albumin – drug interaction is a general strategy for improving the pharmacokinetics of proteins (25).

In another study the interaction of 2, 2′-bipyridine glycinate palladium (II) chloride with HSA was investigated and the enthalpy of unfolding was estimated from the comparison of the equilibrium dialysis and the microcalorimetry results. This thermodynamic parameter can be considered as a measure of protein structural change, and hence, as a side effect of the drug (26). Acetaminophen (paracetamol), chemically named as N-Acetyl-P-aminophenol (APAP) (27-28), is a popular pain killer. Its' chemical formula is illustrated below:



In the present study, it is proposed that acetaminophen binds to HAS and as a result alters the structural aspects of the protein. Circular dichroism (CD) and UV absorption spectroscopic methods have been used to determine the structural changes of HSA in the temperature range of 25-60ºC in the presence and absence of acetaminophen.

Experimental

Materials

Serum albumin was obtained from Sigma chemical Co. ,USA, The other substances of reagent grade were obtained from Merck chemical Co., Germany the buffer used throughout the study was a 10 mM phosphate buffer, , pH 7.

Methods

CD spectra were recorded on a Jasco J-715 spectropolarimeter (Japan). Results are expressed as ellipticity, [θ] (degree cm^2dmol^{-1}), based on a mean amino acid residue weight (MRW), assuming an average molecular weight of 113 Da for the serum albumin. The molar ellipticity was determined as $[\theta]_\lambda = (\theta \times 100MRW/cl)$, where c is the protein concentration in mM, l the light path length in cm and θ the measured ellipticity in degree at a given wavelength. The data was smoothened using the Jasco J-715 software, including the fast Fourier-transform noise reduction routine, which allows enhancement of most noisy spectra without distorting their peak shapes. All the experiments were repeated three times. The concentration of the protein solution was 0.5 mg/ml. Acetaminophen concentration for the related experiments was 0.01 mg/ml. Spectrophotometeric experiments were performed by using a recording spectrophotometer (U.Vmodel.3100 Shimadzu, Japan). The sample cell contained 0.8ml of a 1 mg/ml serum albumin at fixed temperatures (25 - 60°C). The reference cell contained the buffer solution and the absorption of sample cell was

recorded at a range of wavelengths, 230nm to 330nm. The extent amounts of absorbance at from each temperature at 280 nm was determined and illustrated as a function of temperature. The experiment was repeated in the presence of 0.01 mg/ml of acetaminophen.

Results and discussion

UV spectroscopy is a suitable method for the study of protein phase transition. This method provides useful information about protein stability and its physical and chemical properties (29). Figure 1 represents the UV absorption spectrum of HSA in the absence (solid line) and presence (dash line) of acetaminophen at 280 nm as a function of temperature. The selected temperature range (25°C -60°C) corresponds to the predenaturation region of temperature (30). In here acetaminophen affects on the HSA and alters its physical properties, as shown from the absorbances obtained through out the range of wavelengths investigated (see Figure 1). It seems that acetaminophen induces contraction of the HSA structure, decreasing the accessibility of its aromatic groups. Figure 2 shows the net effect of acetaminophen on the absorption of HSA, In here the extent of HSA-acetaminophen complex absorption is subtracted from HSA absorption and the results illustrated as a function of temperature. The effect of acetaminophen on HSA structure is significant for lower temperatures (30°C – 40°C), but falls to zero at about 70°C (see extrapolation of Figure 2). It has been reported that HSA unfolding occurs above 70°C (31), hence the effect of acetaminophen on the HSA structure limits to the predenaturation condition. As depicted in Figure 1 (the thick part of solid line) temperature induces phase transition within HSA in the absence of acetaminophen. This transition is a typical sigmoeidal curve starting at 35°C and finishing at 45°C. The sigmoeidal curve in correspondance to the two-state theory (32) could be analyzed for the determination of thermodynamic paremeters. The determination of free energy change (ΔG^o), as a criterion of conformational stability of a globular protein, is based on the two state theory as follows:

$$Native\ (N) \rightleftharpoons Denaturen\ (D) \quad (1)$$

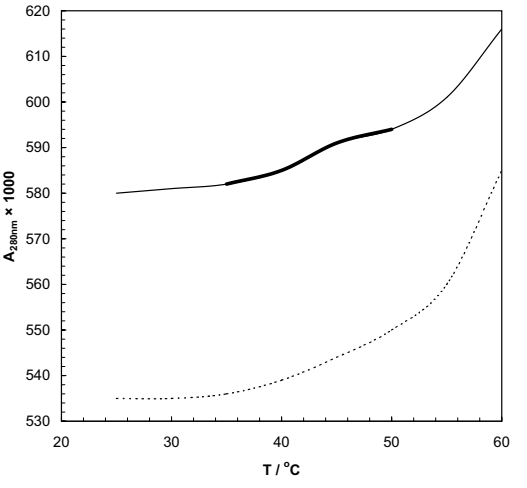


Figure 1. UV absorption of HSA in the absence (solid line) and presence (dashed line) of acetaminophen (0.01 mg/ml) at 280 nm as a function of temperature. The thick part of solid line refers to the temperature induced phase transition in the HSA in the absence of acetaminophen.

Pace (32, 33) described the process as a single denaturant- dependent step according to the two state theory. By assuming the two state mechanism, one could determine the process by monitoring the changes in the absorbance, and hence calculating the denatured fraction of protein (F_d) as well as the determination of the equilibrium constant (K).

$$F_d = (Y_N - Y_{Obs}) / (Y_N - Y_D) \quad (2)$$

$$K = F_d / (1 - F_d) = (Y_N - Y_{Obs}) / (Y_{Obs} - Y_D) \quad (3)$$

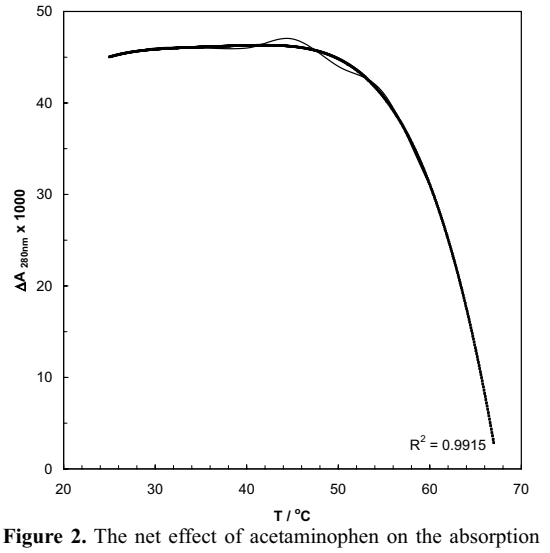


Figure 2. The net effect of acetaminophen on the absorption of HAS. In here the extents of HSA-acetaminophen complex absorption is subtracted from the HSA absorption and the results (solid line) are illustrated as a function of temperature (the concentration of acetaminophen is 0.01 mg/ml). The dashed line is an extrapolation of the solid line.

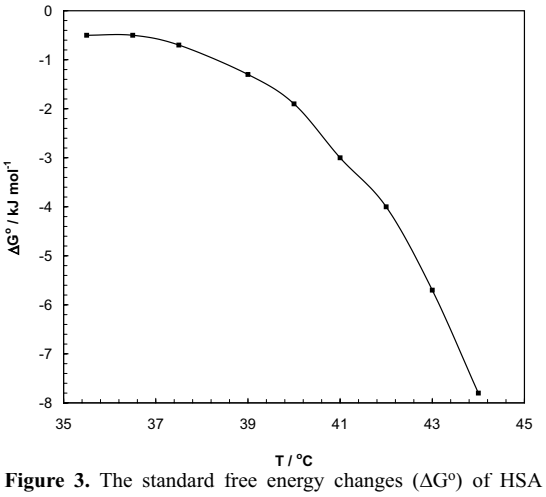


Figure 3. The standard free energy changes (ΔG°) of HSA phase transition as a function of temperature.

In here Y_{obs} is the observed variable parameter (e.g. absorbance) and Y_N and Y_D are the values of the Y characteristic of a fully native and denatured conformation respectively. The free energy change (ΔG°) for equation (1) is given by the following equation:

$$\Delta G^o = - RT\, Ln\, K \qquad (4)$$

where R is the universal gas constant and T is the absolute temperature and:

$$\Delta G^o = G^o_D - G^o_N \qquad (5)$$

where G^o_D and G^o_N are free energies of denatured and native protein respectively. So the larger negative values of ΔG^o refer to the higher affinity of protein for denaturation (phase transition). Figure 3 shows the standard free energy changes (ΔG°) of HSA phase transition as a function of temperature. As depicted in Figure 3 the amount of ΔG° for HSA phase transition lies between 0 to -8 kJ/mol. The amount of ΔG^o for HSA phase transition is equal to zero at 37°C but falls to -8 kJ mol^{-1} at 44°C. UV spectroscopic analysis indicates that the presence of acetaminophen prevents the occurrence of the above-mentioned phase transition.

CD is a suitable technique for detection of structural changes of proteins. Near UV- CD is applied widely for the study of tertiary structural changes of protein while far UV-CD is one of the best ways for studying the of secondary structural changes of proteins during phase transition. (34). Here, far UV-CD technique was employed for the determination of the effect of acetaminophen on the secondary structure of HSA.

Figure 4 represents CD spectra of HSA at the temperature range of 25°C - 60°C in the presence (dashed line) and absence (solid line) of acetaminophen. There is a considerable difference between the obtained spectra, such the effect of acetaminophen on HSA leads to the formation of a new class of spectra which are separated completely from the other one. The obtained CD spectra in the presence of acetaminophen include higher negative values for the CD parameter relative to the spectra in the absence of acetaminophen. This finding refers to the induction of a greater degree of secondary structure within HAS, due to the effect of acetaminophen.

The extent of CD parameter at the wavelength of 222 nm is proportional to the α-Helix structure. Hence, the increase in the negative value of this parameter corresponds to the percentage of α-Helix secondary structure of the protein. (34). Figure 5 represents the CD parameter of HSA in the absence and presence of acetaminophen at 222 nm as a function of temperature. As depicted in Figure 3 there is a considerable difference between the two curves. Therefore, the presence of acetaminophen to a greater extent induces the α –helix structure

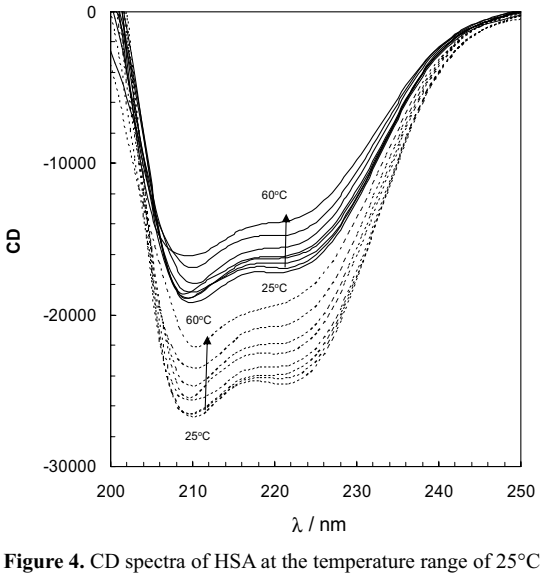


Figure 4. CD spectra of HSA at the temperature range of 25°C - 60°C in the presence (dashed line) and absence (solid line) of acetaminophen (0.01 mg/ml).

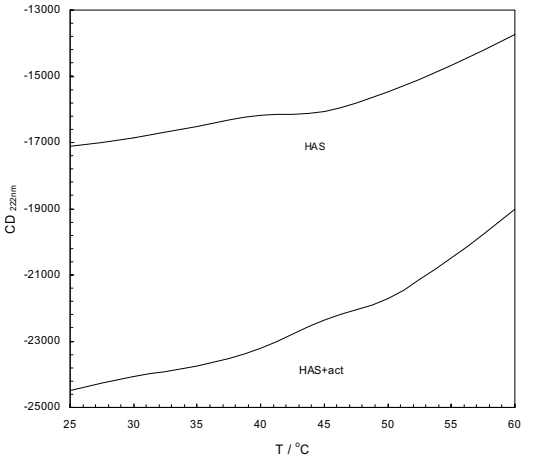


Figure 5. The CD parameter of HSA in the absence and presence of acetaminophen (0.01 mg/ml) at 222 nm as a function of temperature.

present within the HSA structure throughout the range of all applied temperatures used. It seems that the pattern of variation of CD parameter at 222 nm for both curves is similar. For detection of the differences, the dashed line is subtracted from the solid curve. Hence, the obtained curve (Figure 6) refers to the net effect of acetaminophen on the α-Helix content of HSA.

Regarding the UV spectroscopic findings, the CD results (Figure 6) show a critical region in the temperature range of 37ºC – 45ºC, characterized with an increment of α-Helix structure.

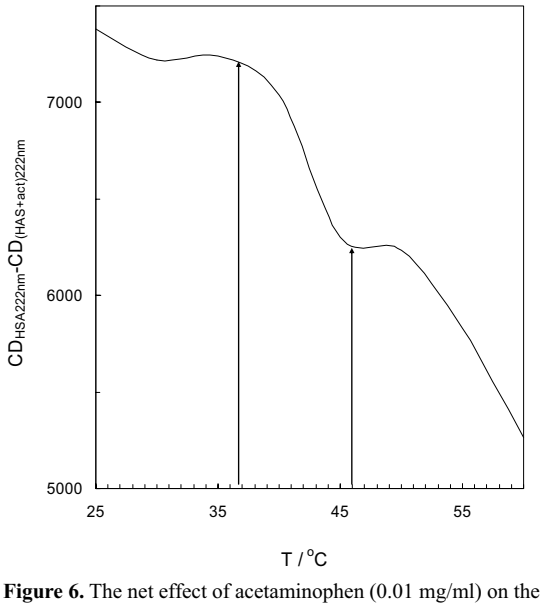


Figure 6. The net effect of acetaminophen (0.01 mg/ml) on the CD parameter of HSA at 222 nm as a function of temperature. In here the extent of HSA-acetaminophen complex CD parameter at 222 nm (Figure 5) is subtracted from the HSA CD parameter at 222 nm (Figure 5).

Both the CD and UV spectroscopic findings, confirm that acetaminophen prevents the HSA phase transition at a critical temperature range, including the physiological and pathological conditions of the human body. The mechanism through which acetaminophen affects the HSA structure is the induction of a greater degree of α-Helix. Acetaminophen is widely used as a pain killer. It affects the HSA structure and could alter the physicochemical properties of HAS. This effect can be considered as an acetaminophen side effect. The capacity of HSA for ligand binding in the presence and absence of acetaminophen at the physiological and pathological condition is currently under investigation in our laboratory.

Acknowledgments

The authors would like to thank Dr. M. Meshkat for her valuable comments. The financial support from the research council of Shaheed Beheshti University of Medical Sciences and Health Services is gratefully acknowledged.

References

(1) Peters T. *All about Albumin: Biochemistry, Genetics and Medical Applications*. Academic Press, San Diego (1995): 1-40

(2) Peter T. Serum Albumin. *Adv. Protein. Chem.* (1985) 37: 161-245

(3) Doweiko JP and Nompleggi DJ. Role of albumin in human physiology and pathophysiology. *J. Parent Enteral Nutr*. (1991) 15: 207–11

(4) Nicholson JP, Wolmarans MR and Park GR. The role of albumin in critical illness. *Brit. J. Anaesthesia* (2000) 85: 599-610

(5) Koch-Wester J and Sellers EM. Binding of drugs to serume albumin. *New Engl. J. Med.* (1976) 294: 311-316

(6) Laussac JP and Sarkar B. Characterization of the copper (II) - and nickel (II)-transport site of human serum albumin. Studies of copper (II) and nickel (II) binding to peptide 1-24 of human serum albumin by 13C and 1H NMR spectroscopy. *Biochemistry* (1984) 5;23: 2832-8

(7) Saboury AA, Hosseini-Kishani F, Rezaei-Tavirani M and Ranjbar B. Thermodynamic studies on the interaction of nickel with human serum albumin. *Prog. Biochem. Biophys.* (2003) 30: 732- 737

(8) Vorum H, Fisker K and Horone B. Palmitate and stearate binding to human serum albumin. Determination of relative binding constants. J Pept Res. (1997) 49: 347-354

(9) Jenkins BG and Lanffer RB. Detection of site-specific binding and co-binding of ligands to human serum albumin using 19F NMR. *Mol. Pharmacol.* (1990) 37:111-118.
(10) Finotti P and Pagetta A. Heparin-induced structural modifications and oxidative cleavage of human serum albumin in the absence and presence of glucose-implications for transcapillary leakage of albumin in hyperglycaemia. *Eur. J. Biochem.* (1997) 247: 1000-1008
(11) Carter DC and He XM. Structure of serum albumin. *Protein Chem.* (1992) 45: 153-203
(12) He XM and Carter DC. Atomic structure and chemistry of human serum albumin. *Nature* (1992) 358: 209-215
(13) Sudlow G, Birkett DJ and Wade DN. The characterization of two specific drug binding sites on human serum albumin. *Mol. Pharmacol.* (1975) 11: 824-832
(14) JamesE and Reynolds F. (Eds.) *Martindale: The Extra Pharmacopoeia,* 31st edn. The Pharmaceutical Press, London (1996)
(15) Dockal M, Chang M, Carter DC and Rüker F. Five recombinant fragments of human serum albumin-tools for the characterization of the warfarin binding site. *Protein Sci.* (2000) 9:1455-1465
(16) Petitpas I, Bhattacharya AA, Twine S, East M and Curry S. Crystal structure analysis of warfarin binding to human serum albumin. *J. Biol. Chem.* (2001) 276: 22804–22809
(17) Baroni S, Mattu M, Vannini A, Cipollone R, Aime S, Ascenzi P and Fasano M. Effect of ibuprofen and warfarin on the allosteric properties of haem-human serum albumin. A spectroscopic study. *Eur. J. Biochem.* (2001) 268: 6214-6220
(18) Fehske KJ, Schläfer U, Wollert U and Müller WE. Characterization of an important drug binding area on human serum albumin including the high-affinity binding sites of warfarin and azapropazone. *Mol. Pharmacol.* (1982) 21: 387-393
(19) Kragh-Hansen U. Evidence for a large and flexible region of human serum albumin possessing high affinity binding sites for salicylate, warfarin, and other ligands. *Mol. Pharmacol.* (1988) 34: 160-171
(20) Kragh-Hensen (ed) Peters TJ. Ligand binding by albumin. In: All *about Albumin, Biochemistry, Genetics and Medical Applications.* Academic Press, San Diego (1996) 76-132
(21) Vorum H and Honoré B. Influence of fatty acids on the binding of warfarin and phenprocoumon to human serum albumin with relation to anticoagulant therapy. *J. Pharm. Pharmacol.* (1996) 48: 870-875
(22) Wanwimolruk S and Birkett D. The effects of N-B transition on human serum albumin on the specific drug-binding sites. *J. Biochim. Biophys. Acta* (1982) 709: 247-255
(23) Sjoholm I, Ekman B, Kober A, Ljungstedt-Pahlman I, Seiving B and Sjodin T. Binding of drugs to human serum albumin: XI. The specificity of three binding sites as studied with albumin immobilized in microparticles. *Mol. Pharmacol.* (1979) 16: 767-77
(24) Bhattacharya AA, Curry S and Franks NP. Binding of the general anesthetics propofol and halothane to human serum albumin. *J. Biol. Chem.* (2000) 275: 38731-38738
(25) Dennis MS, Zhang M, Meng YG, Kadkhodayan M, Kirchhofer D, Combs D and Damico LA. Albumin binding as a general strategy for improving the pharmacokinetics of proteins. *J. Biol. Chem.* (2002) 20;277: 35035-3543
(26) Saboury AA, Shamsaei A, Moosavi-movahedi A and Mansuri-Torshizi H. Thermodynamics of binding 2, 2/-bi pyridineglycinatopalladium (II) chloride on human serum albumin. *J. Chin. Chem. Soc.* (1999) 46: 917-922
(27) Muldrew KL, James LP, Coop L, McCullough SS, Hendrickson HP, Hinson JA and Mayeux PR. Determination of acetaminophen-protein adducts in mouse liver and serum and human serum after hepatotoxic doses of acetaminophen using high-performance liquid chromatography with electrochemical detection. *Drug Metab. Dispos.* (2002) 30: 446-451
(28) Sharma VN. *Essentials of Pharmacology*, 2nd edition, CBS, India (2003): 1-426
(29) Rezaei-Tavirani M, Moosavi-Movahedi AA, Saboury AA, Hakimelahi GH, Ranjbar B and Hosaindokht MR. Thermodynamic domain analysis of fresh and incubated apotransferrin. *Thermochimica Acta* (2002) 383: 108-113
(30) Kragh-Hansen U. Molecular aspects of ligand binding to serum albumin. *Pharmacol. Rev.* (1981) 33: 17-53
(31) Pace CN. Measuring and increasing protein stability. *TIBTech.* (1990) 8: 93-98
(32) Pace CN. Determination and analysis of urea and guanidine hydrochloride denaturation curves. *Methods in Enzymology* (1986) 131: 266-289
(33) Pace CN, Shirly BA and Thomson JA. In: Creighton TE. (Ed.) *Protein Structure, a Practical Approach.* (Chapter 13) Oxford University Press, England (1989): 311-330
(34) Rezaei-Tavirani M, Moosavi-Movahedi AA, Moosavi-Nejad SZ, Chamani J and Ajloo D. Domain analysis of human apotransferrin upon interaction with sodium n-dodecyl sulphate: differential scanning calorimetry and circular dichroism approaches. *Thermochimica Acta* (2003) 408: 9-16

This article is available online at http://www.ijpr-online.com