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LETTER TO THE EDITOR

Structural Stability of β -lactoglobulin in the Presence of Cetylpyridinium Bromide: Spectroscopic and Molecular Docking Studies

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Introduction

Protein-surfactant interaction is very important in wide range of industrial aspects, including drug delivery, cosmetics, food industry, preparation of pharmaceutical substances, as well as in biotechnology and biosciences. Proteins are often used as a component of healthcare products due to their appropriate affinity to bind various molecules (Chamani, Moosavi-Movahedi, & Hakimelahi, 2005; Sarzei & Chamani, 2010). Hydrophobic part of surfactant molecules tends to bind hydrophobic residues of proteins and forms surfactant-protein complex (Chamani & Heshmati, 2008; Chamani & Moosavi-Movahedi, 2006). Studying the surfactant-protein interaction can help in understanding the effect of surfactants on protein denaturation, solubilisation and aggregation processes. Although, the molecular mechanism of protein denaturation in the presence of surfactants is still not entirely interpreted.

Bovine β -lactoglobulin (BLG) is the major whey protein, forms up to 50% of whey and 12% of whole cow milk proteins. BLG consists of 162 amino acids with about 18.4 kDa of molecular weight, and is a member of the lipocalin superfamily. This protein has a three-dimensional structure consisting of one α -helix and nine anti-parallel β -strands with eight β -sheets folded into a cone-shaped barrel forming a hydrophobic pocket (Chanphai, Froehlich, Mandeville, & Tajmir-Riahi, 2017; Chanphai & Tajmir-Riahi, 2017; Jameson, Adams, & Creamer, 2002; Loch et al.,

2015). Its easy purification and significance in food industry explain in part the huge amount of literature relating to it.

Interactions between BLG and surfactants are highly diverse and have received much attention due to their significance in food systems. At high concentrations, ionic surfactants such as the cationic *n*-decyltrimethyl ammonium bromides (C_n TABs) and anionic sodium dodecylbenzenesulfonate (SDBS) unfold the protein (Sahihi, Bordbar, & Ghayeb, 2011; Sahihi, Bordbar, Ghayeb, & Fani, 2012). The results represented the increase in the denaturizing power of C_n TABs with an increase in hydrocarbon chain length (*n*). Moreover, the relevant results represented the enhancement of retinol binding affinity in the presence of these cationic surfactants. Also, the denaturation effect of SDBS in acidic pH was greater than that in other pHs because of the positive net charge of BLG at this pH and represented the predominate role of electrostatic interaction. Although, there are many works that investigate the role of surfactant structure in protein-surfactant interactions, little works dealt with the effect of surfactant counter ion. The effect of the counter ions of dodecyl sulfate on PEO-dodecyl sulfate interaction is investigated. They showed that increasing the size of surfactant counterion causes the weakening of interaction. Also, researches showed that counterions strikingly affected the properties of perfluorooctanoate surfactants and when the size of counterions increased from NH_4^+ to $N(C_3H_7)_4^+$ the BLG-perfluorooctanoate surfactants interaction was weakened. In our previous work, the stability parameters of BLG in the presence of cetylpyridinium chloride (CPC) were determined (Sahihi, Bordbar, & Ghayeb, 2010). Results show that the minimum value of ΔG_{tr} occurs at $T=328$ K.

Based on the above mentioned references, here, we report a study of interaction between BLG and cetylpyridinium bromide (CPB) as a denaturant cationic surfactant using UV-Vis

spectrophotometry technique at various temperatures. Also, fluorescence titration, near-UV CD spectroscopy and molecular docking were used as efficient methods to indicate the details of this interaction. These data provide useful information about effects of counterion in cetylpyridinium surfactants, conformational stability of BLG and forces which contribute to its stability.

Experimental

Materials

The BLG genetic variant A and CPB were purchased from Sigma Chemical Co., and used without further purification. All salts used for buffer preparation were analytical grade and dissolved in distilled water. All of the solutions were used freshly after preparation.

Methods

The concentration of BLG was determined experimentally using the value of $17600 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for the molar absorption coefficient (ϵ) at 279 nm. For all measurements, solutions were prepared in 50 mM phosphate buffer at pH, 8.0. The CPB denaturation curves were determined by measuring the maximum absorption of BLG solutions by Varian Cary 500UV-Vis Spectrophotometer and 1-cm cuvettes thermostated to maintain the temperature at $\pm 0.1 \text{ K}$. In typical experiments, 2.0 cm^3 of BLG solution ($5 \mu\text{M}$) were placed in the cuvette, absorbance spectra were recorded between 200 nm and 400 nm after each addition of CPB stock solutions (1 mM), and the observed absorbance was corrected for dilution. The CPB stock solution was added to both reference and sample cells. Fluorescence spectroscopy was also used to study the conformational changes of BLG during the CPB denaturation.

The intrinsic fluorescence of indol chromophores in tryptophan (Trp) residues is particularly sensitive to their microenvironments. During fluorescence measurements, 2.0 cm³ of BLG solution (2 μM) were placed into the cuvette and emission spectra were recorded after each addition of CPB stock solutions (0.5 mM). BLG was excited at 295 nm and the emitted fluorescence was recorded between 300 nm and 400 nm. The band slits for excitation and emission were set at 5 nm. To avoid of inner filtering effect, absorbances of the BLG solutions in 279 nm should not exceed 0.1. The final CPB concentration was about 122.8 μM and there is no inner filter effect due to the presence of CPB.

Furthermore, the near-ultraviolet circular dichroism (near-UV CD) spectrum was recorded using Aviv spectropolarimeter model 215 (ProterionCorp., USA) at 25 °C. The scan speed was 20 nm min⁻¹ at room temperature in the range of 250–330 nm, using 0.1 cm quartz cell. The CD spectrum of BLG solution was recorded before and after addition of the CPB with molar ratio 0, 1, 2, 4 and 20.

Molecular docking

In this work, docking study was carried out to indicate the binding site for the surfactant. The 3D structures of the CPB was plotted using the Gauss view 5.0.8 software. The geometry of the CPB was optimized using Gaussian 09 at the level of B3LYP/ 6-31G**. The crystal structure of BLG (PDB ID:1CJ5) was taken from the Brookhaven Protein Data Bank (<http://www.rcsb.org/pdb>). Water molecules of the protein pdb file were removed, polar hydrogen atoms and Gasteiger charges were added. Flexible-surfactant docking was performed by AutoDock 4.2.6 molecular docking program using the implemented empirical free energy function and the Lamarckian Genetic Algorithm. The Autogrid was used to calculate Grids. A blind docking with 126 lattice points along X, Y, and Z axes was performed to find the active site of surfactant on BLG. After

determination of the active site, the dimensions of the grid map were selected 60 points with a grid point spacing of 0.375 Å, to allow the ligand to rotate freely. 250 docking runs with 25,000,000 energy evaluations for each run were performed (Hosainzadeh, Gharanfoli, Saberi, & Chamani, 2012).

Results and discussion

Fluorescence Spectroscopy

The fluorescence properties of tryptophan residues are sensitive to changes of protein structure (Omidvar, Asoodeh, & Chamani, 2013; Pasban Ziyarat, Asoodeh, Sharif Barfeh, Pirouzi, & Chamani, 2014). Interaction of CPB with BLG changes the environment of tryptophan residue and causes to fluorescence enhancement and slight blue shift of BLG emission. Actually, Trp residues of protein has transferred to nonpolar environment due to the BLG interaction with CPB. The relative emission intensity (F/F_0), where F and F_0 are the emission intensities in the presence and absence of CPB, respectively, at 331 nm in fluorescence spectra of BLG solution versus mole ratio of $[CPB]/[BLG]$, was plotted (figure is not shown). Its sigmoid look is related to the characteristic of a two-state mechanism and shows the denaturing power of CPB that induces a cooperative unfolding in tertiary structure of BLG. Moreover, the binding constant (K_b) has been determined using the following equation:

$$\ln(F-F_0/F)=\ln(K_b)+n \ln[E] \quad (1)$$

where, F and F_0 are the fluorescence intensity of BLG in absence and presence of CPB, respectively. $[E]$ is the concentration of enhancer that enhancer is CPB here. “ K_b ” is obtained from the plot of $\ln((F-F_0)/F)$ versus $\ln[E]$ as a y-intercept. Furthermore, “ n ” which is the number

of binding site per protein is slope of the plot. The value of n is nearly 1, indicating that the CPB binds to BLG with molar ratio of 1:1.

UV-Vis Spectroscopy

The CPB denaturation curves for BLG are shown in figure 1. In all cases, we measured the difference absorption ($\Delta\epsilon$) at λ_{\max} . The Gibbs free energy of denaturation, ΔG_D , was calculated as a function of CPB concentration by assuming a two-state mechanism and using the following equations:

$$F_D = (\Delta\epsilon_N - \Delta\epsilon_{\text{obs}}) / (\Delta\epsilon_N - \Delta\epsilon_D) \quad (2)$$

$$\Delta G_D = -RT \ln K = -RT \ln(F_D / (1 - F_D)) \quad (3)$$

Where K is the equilibrium constant, R is the gas constant, $\Delta\epsilon_{\text{obs}}$ is the observed difference absorption, $\Delta\epsilon_N$ and $\Delta\epsilon_D$ are the difference absorption of the native and denatured protein molecules, respectively. The latter two have been extrapolated from the pre- and post-transition baselines to the considered concentration of CPB. These pre and post-transition baselines were determined by a linear fit of the corresponding data before and after the transition, according to the following equations:

$$\Delta\epsilon_N = a_N + b_N[\text{CPB}] \quad (4)$$

$$\Delta\epsilon_D = a_D + b_D[\text{CPB}] \quad (5)$$

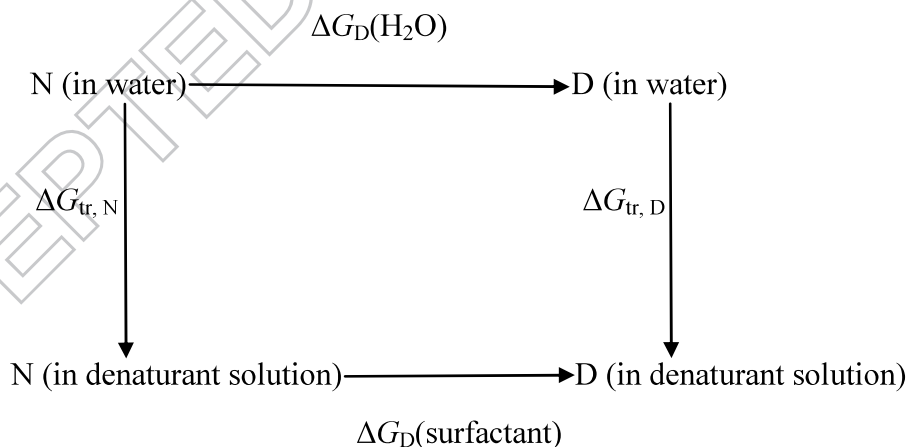
Figure 2a shows the Gibbs free energy of denaturation, ΔG_D , based on the data of figure 1, which varies linearly with CPB concentration. The ΔG_D was plotted against $[\text{CPB}]$ and a linear least-squares analysis was used to fit the ΔG_D and $[\text{CPB}]$ data to the following relation:

$$\Delta G_D = \Delta G_D(\text{H}_2\text{O}) - m [\text{CPB}] \quad (6)$$

Where $\Delta G_D(\text{H}_2\text{O})$ is the value of ΔG_D at 0 M CPB and m is the slope of the curves which gives the linear dependence of ΔG_D on CPB concentration. Furthermore, m reflects the cooperativity and also hydrophobicity of the transition state. The m value correlates very strongly with the amount of protein surface exposed to the solvent upon unfolding (Chamani et al., 2006). Thus, for proteins that undergo a simple two-state unfolding mechanism, the amount of surface exposed to solvent upon unfolding is a main structure determinant for m values and hydrophobicity. In addition, at the transition concentration, $[\text{CPB}]_{1/2}$, we have: $[\text{N}]=[\text{D}]$, $F_D=0.5$, $K=1$ and $\Delta G_D=0$. Thus, at the midpoint of the transition we get:

$$\Delta G_D(\text{H}_2\text{O}) = m[\text{CPB}]_{1/2} \quad (7)$$

Unfolding of BLG in an isothermal chemical denaturation process and can be divided into thermodynamically defined stages according to the below scheme.



It follows that ΔG_D , for the transition of BLG from water to surfactant is given by the below relationship:

$$\Delta G_{tr, N} + \Delta G_D(\text{surfactant}) = \Delta G_D(\text{H}_2\text{O}) + \Delta G_{tr, D}$$

or

$$\Delta G_{tr} = \Delta G_D(\text{surfactant}) - \Delta G_D(\text{H}_2\text{O}) = \Delta G_{tr, D} - \Delta G_{tr, N} \quad (8)$$

Where $\Delta G_{tr, N}$ and $\Delta G_{tr, D}$ represent the transfer Gibbs free energies of native and denatured states of BLG from water to CPB solution at a given concentration of CPB. The $\Delta G_D(\text{surfactant})$ is the Gibbs free energy change for transformation of native BLG (in surfactant solution) to denatured BLG in surfactant solution.

Figure 2b shows the linearity of the plot of ΔG_D versus $\ln[\text{CPB}]$. The Gibbs free energy in the surfactant solution, $\Delta G_D(\text{surfactant})$, was obtained at $\ln[\text{CPB}] = 0$, from the least squares analysis of these plots. A concentration of CPB of 1 M is in good agreement for the transition of the native state of protein (from water) to the denatured state of protein to CPB solution.

We can use the Gibbs–Helmholtz equation to calculate the ΔH_D and ΔS_D :

$$d(\Delta G_D/T) / d(1/T) = \Delta H_D \quad (9)$$

$$d\Delta G_D/dT = -\Delta S_D \quad (10)$$

Figures 2c and 2d show the variation of $\Delta G_D/T$ versus $1/T$ and the ΔG_D versus T , respectively. The BLG denaturation in the presence of CPB is an endothermic process and ΔH_D increases with increasing concentration of surfactant (Table 1). Increasing the surfactant concentration causes more intermolecular interactions and higher values of ΔH_D . The positive values of ΔH_D and ΔS_D represent the main role of hydrophobic interactions in stability of BLG-CPB complex. This result is in consistent with molecular docking results (see section 3.2). The $\Delta H_D(\text{H}_2\text{O})$ and $\Delta S_D(\text{H}_2\text{O})$ are found from extrapolating the ΔH_D and ΔS_D to $[\text{CPB}] = 0$. The values of $86.02 \text{ kJ.mol}^{-1}$ and

212.24 J.mol⁻¹.K⁻¹, were obtained for $\Delta H_D(\text{H}_2\text{O})$ and $\Delta S_D(\text{H}_2\text{O})$, respectively. These values represent the entropy term dominates the enthalpy term.

Table 2 represents the estimated values of m , $\Delta G_D(\text{surfactant})$, $\Delta G_D(\text{H}_2\text{O})$, ΔG_{tr} and $[\text{CPB}]_{1/2}$. The $\Delta G_D(\text{H}_2\text{O})$ has positive values but $\Delta G_D(\text{surfactant})$ and ΔG_{tr} have negative values. The $\Delta G_D(\text{H}_2\text{O})$ shows unusual changes that have been reported for the interaction of CPC with BLG but have not seen before for any other proteins (Sahihi et al., 2010). The minimum in $\Delta G_D(\text{H}_2\text{O})$ occurs at $T=328$ K that shows the minimum stability of BLG at $T=328$ K. Upon heating, BLG undergoes intramolecular and intermolecular changes. Raising the temperature shifts the BLG (monomer + dimer) equilibrium at BLG concentrations below 10 g.dm⁻³ toward monomers. Upon heating above $T=333$ K, the molecule undergoes conformational changes and partially unfolds (i.e. it denatures); the nonpolar groups and the thiol group become exposed. Denaturation can be followed by an irreversible aggregation reaction so that the whole heat-induced process becomes irreversible. So, above $T=328$ K in the presence of CPB, aggregation of the protein molecules causes increasing of stability. Thus BLG has minimum stability at $T=328$ K in the absence of CPB. Also, the values of $\Delta G_D(\text{surfactant})$ show that the minimum stability at the presence of surfactant occurs at $T=328$ K (the protein is completely surrounded by denaturant). The ΔG_{tr} is also less negative at this temperature which is the best state for a denatured protein. Comparison of the ΔG_{tr} value at 328 K with our previous study on the interaction of BLG and CPC (-246.00 and -222.67 kJ.mol⁻¹ for CPB and CPC, respectively) shows that counterions strikingly affect the properties of cetylpyridinium surfactants and when the size of counterions increased from Cl⁻ to Br⁻ the BLG-cetylpyridinium surfactants interaction was stabilized.

Near-UV CD measurements

The near-UV CD spectra of a protein characterize its tertiary structure mainly because of constrained asymmetries in the environment of the aromatic amino acids. In the native state of BLG, the indole group of Trp-19 is located inside the calyx, which is the main hydrophobic cavity where ligands such as retinol can bind. On the other hand, the indole group of Trp61 protrudes into the protein exterior surface, adjacent strand β_I , and so this residue believed to play a significant role in dimer bonding. The water accessibility to Trp-19 is, accordingly, much lower than that to Trp-61. It is generally assumed that Trp-19 is the residue mainly responsible for these spectra with this simplified assumption, the spectral data observed in the presence CPB would point to a less constrained Trp-19 than in the native protein; this could mean that the calyx structure (or β -barrel) of BLG has partially loosened. The peaks at about 293 nm in the spectrum of native BLG in water (figure 3) are mainly due to tryptophan (Trp-19 and) absorbance.

Molecular docking

Molecular docking results suggest that, CPB binds on the surface of BLG with the binding energy of $-23.07 \text{ kJ.mol}^{-1}$. This result is in consistent with obtained binding constant from fluorescence measurements ($K_b=1.5 \times 10^4 \text{ M}^{-1}$). Analysis of the molecular docking results represents that TYR102 interacts with CPB by hydrogen bond interaction with bond length 2.206 Å. Moreover, figure 4 represents that this compound is nearby to some hydrophobic residues of BLG (LEU143, LEU147, LEU104, LEU95, MET145, ALA142, ALA139, VAL3, TYR102, TYR2). This result is in good agreement with findings of UV-Vis spectroscopy experiments (see section 3.1).

Conclusions

The studied surfactant (CPB) denatured BLG in a cooperative manner at various temperatures. The denaturation effect of this surfactant at $T=328$ K was greater than the other temperatures. Above this temperature in the presence of CPB, aggregation of the protein molecules causes increasing of stability. The value of about $16.74 \text{ kJ.mol}^{-1}$ was obtained at this temperature for $\Delta G_D(\text{H}_2\text{O})$, as thermodynamic stability of BLG in the absence of surfactant. ΔG_{tr} represented the less stability of BLG in the presence of CPB in comparison with CPC. Actually, when the size of counterions increased from Cl^- to Br^- the BLG-cetylpyridinium surfactants interaction was increased. Also, the unfolding transition of BLG in the presence of CPB was investigated by near-ultraviolet circular dichroism spectroscopy. CPB forced conformational changes in the protein tertiary structure that expose the tryptophans to a less constrained environment. Furthermore, molecular docking results suggest that, CPB binds on the surface of BLG with the binding energy of $-23.07 \text{ kJ.mol}^{-1}$. The atomic detail of this interaction represent that H-bond and hydrophobic interactions have dominant role in the interaction of BLG and CPB.

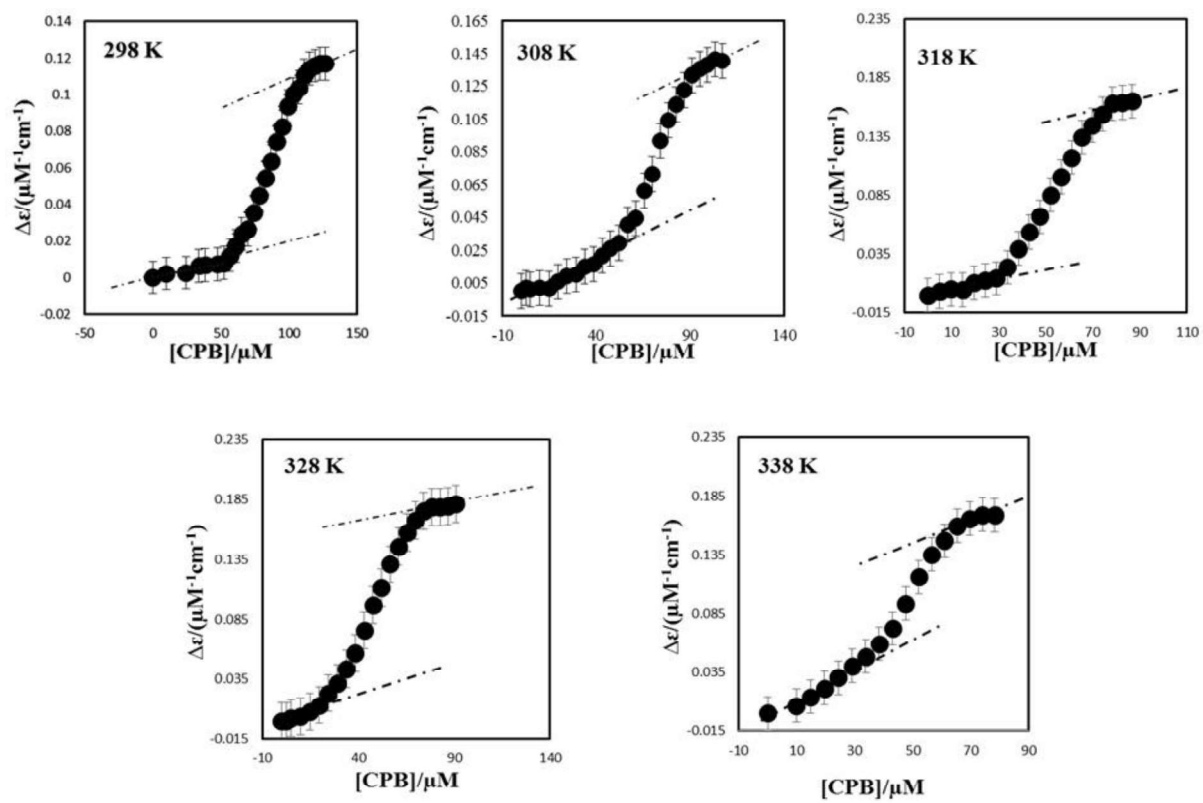
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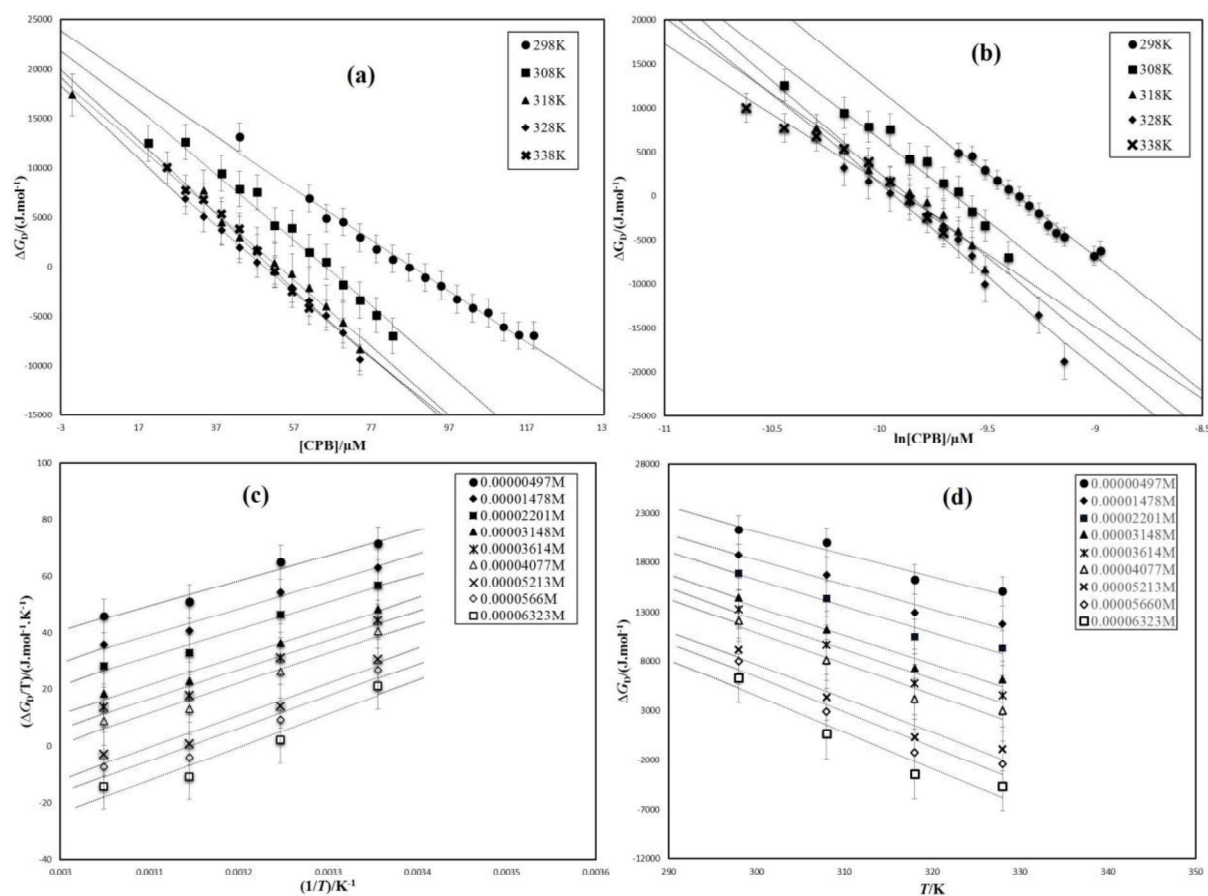
The financial support of Research Council of University of Isfahan is gratefully acknowledged.

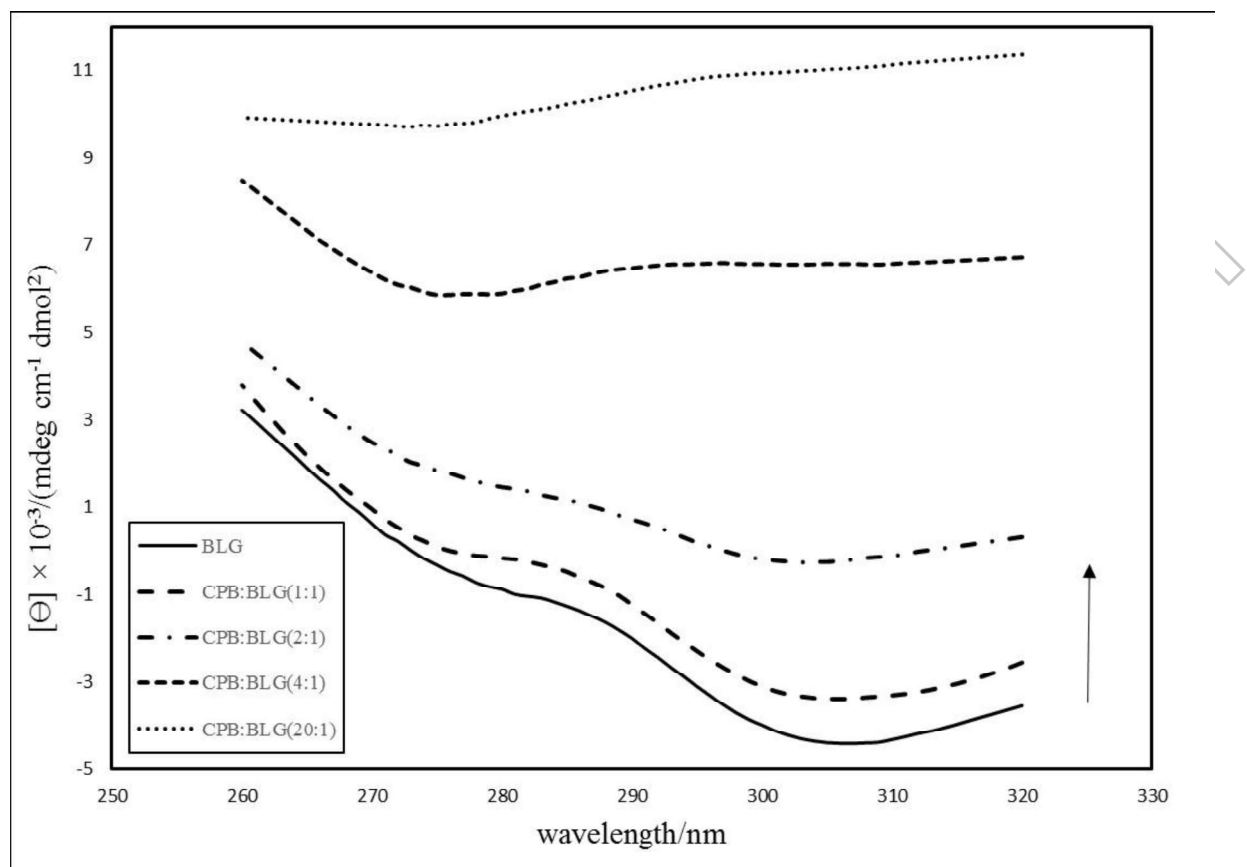
References

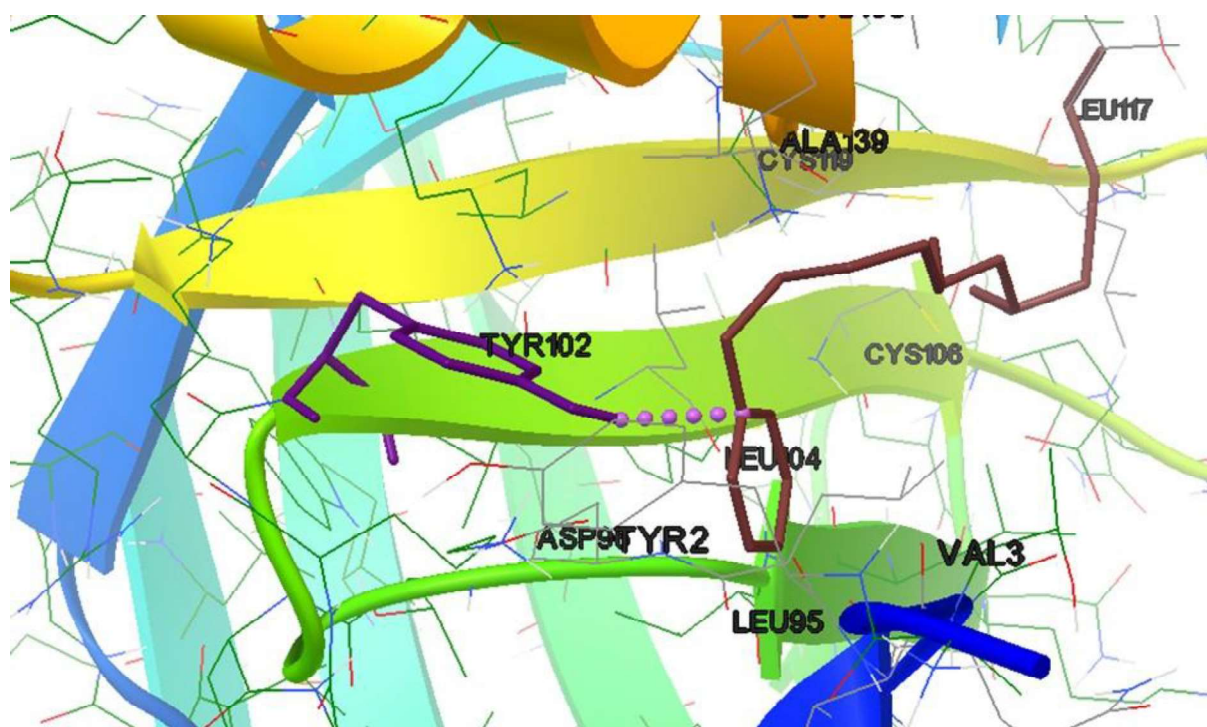
Chamani, J., & Heshmati, M. (2008). Mechanism for stabilization of the molten globule state of papain by sodium n-alkyl sulfates: spectroscopic and calorimetric approaches. *Journal of colloid and interface science*, 322(1), 119-127. doi: 10.1016/j.jcis.2008.03.001

- Chamani, J., & Moosavi-Movahedi, A. (2006). Effect of n-alkyl trimethylammonium bromides on folding and stability of alkaline and acid-denatured cytochrome c: A spectroscopic approach. *Journal of colloid and interface science*, 297(2), 561-569. doi: 10.1016/j.jcis.2005.11.035
- Chamani, J., Moosavi-Movahedi, A., & Hakimelahi, G. (2005). Structural changes in β -lactoglobulin by conjugation with three different kinds of carboxymethyl cyclodextrins. *Thermochimica acta*, 432(1), 106-111. doi: 10.1016/j.tca.2005.04.014
- Chamani, J., Moosavi-Movahedi, A., Rajabi, O., Gharanfoli, M., Momen-Heravi, M., Hakimelahi, G., ... Varasteh, A. (2006). Cooperative α -helix formation of β -lactoglobulin induced by sodium n-alkyl sulfates. *Journal of colloid and interface science*, 293(1), 52-60. doi: 10.1016/j.jcis.2005.06.015
- Chanphai, P., Froehlich, E., Mandeville, J., & Tajmir-Riahi, H. (2017). Protein conjugation with PAMAM nanoparticles: Microscopic and thermodynamic analysis. *Colloids and Surfaces B: Biointerfaces*, 150, 168-174. doi: 10.1016/j.colsurfb.2016.11.037
- Chanphai, P., & Tajmir-Riahi, H. (2017). Trypsin and trypsin inhibitor bind milk beta-lactoglobulin: Protein-protein interactions and morphology. *International journal of biological macromolecules*, 96, 754-758. doi: 10.1016/j.ijbiomac.2016.12.075
- Hosainzadeh, A., Gharanfoli, M., Saberi, M. R., & Chamani, J. (2012). Probing the interaction of human serum albumin with bilirubin in the presence of aspirin by multi-spectroscopic, molecular modeling and zeta potential techniques: Insight on binary and ternary systems. *Journal of Biomolecular Structure and Dynamics*, 29(5), 1013-1050. doi: 10.1080/073911012010525029
- Jameson, G. B., Adams, J. J., & Creamer, L. K. (2002). Flexibility, functionality and hydrophobicity of bovine β -lactoglobulin. *International Dairy Journal*, 12(4), 319-329. doi: 10.1016/S0958-6946(02)00028-6
- Loch, J. I., Bonarek, P., Polit, A., Świątek, S., Czub, M., Ludwikowska, M., & Lewiński, K. (2015). Conformational variability of goat β -lactoglobulin: Crystallographic and thermodynamic studies. *International journal of biological macromolecules*, 72, 1283-1291. doi: 10.1016/j.ijbiomac.2014.10.031
- Omidvar, Z., Asoodeh, A., & Chamani, J. (2013). Studies on the antagonistic behavior between cyclophosphamide hydrochloride and aspirin with human serum albumin: time-resolved fluorescence spectroscopy and isothermal titration calorimetry. *Journal of Solution Chemistry*, 42(5), 1005-1017. doi: 10.1007/s10953-013-0009-7
- Pasban Ziyarat, F., Asoodeh, A., Sharif Barfeh, Z., Pirouzi, M., & Chamani, J. (2014). Probing the interaction of lysozyme with ciprofloxacin in the presence of different-sized Ag nano-particles by multispectroscopic techniques and isothermal titration calorimetry. *Journal of Biomolecular Structure and Dynamics*, 32(4), 613-629. doi: 10.1080/07391102.2013.785919
- Sahihi, M., Bordbar, A., & Ghayeb, Y. (2010). Thermodynamic denaturation of β -lactoglobulin in the presence of cetylpyridinium chloride. *The Journal of Chemical Thermodynamics*, 42(12), 1423-1428. doi: 10.1016/j.jct.2010.06.010
- Sahihi, M., Bordbar, A., & Ghayeb, Y. (2011). Thermodynamic stability and retinol binding property of β -lactoglobulin in the presence of cationic surfactants. *The Journal of Chemical Thermodynamics*, 43(8), 1185-1191. doi: 10.1016/j.jct.2011.03.004
- Sahihi, M., Bordbar, A., Ghayeb, Y., & Fani, N. (2012). Structure-function relationship of β -lactoglobulin in the presence of sodium dodecylbenzenesulfonate. *The Journal of Chemical Thermodynamics*, 52, 16-23. doi: 10.1016/j.jct.2011.12.017
- Sarzehi, S., & Chamani, J. (2010). Investigation on the interaction between tamoxifen and human holo-transferrin: determination of the binding mechanism by fluorescence quenching, resonance light scattering and circular dichroism methods. *International journal of biological macromolecules*, 47(4), 558-569. doi: 10.1016/j.ijbiomac.2010.08.002









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Table 1. The values of ΔH_D and ΔS_D in various concentrations of CPB.

[CPB]/ μM	$\Delta H_D/(\text{kJ.mol}^{-1})$	$\Delta S_D/(\text{J.mol}^{-1}.\text{K}^{-1})$
4.97	88.46	224.56
14.78	93.24	248.83
22.01	96.78	266.73
31.48	101.40	290.18
36.14	103.68	301.74
40.77	105.94	313.18
52.13	111.49	341.33
56.60	113.67	352.40
63.23	116.91	368.81

Table 2. Parameters characterizing the CPB denaturation for BLG at various temperatures.

T /K	m /(kJ.mol ⁻¹ .M ⁻¹)	$\Delta G_D(\text{H}_2\text{O})$ /(kJ.mol ⁻¹)	$\Delta G_D(\text{Surfactant at 1M CPB})$ /(kJ.mol ⁻¹)	ΔG_{tr} /(kJ.mol ⁻¹)	$[\text{CPB}]_{1/2}/\text{M}$
298	257.72	22.58	-176.46	-199.04	0.087611
308	331.77	21.63	-179.41	-201.04	0.065196
318	337.91	17.92	-186.33	-204.25	0.053041
328	338.21	16.74	-229.26	-246.00	0.049496
338	383.82	19.61	-150.58	-170.19	0.051092