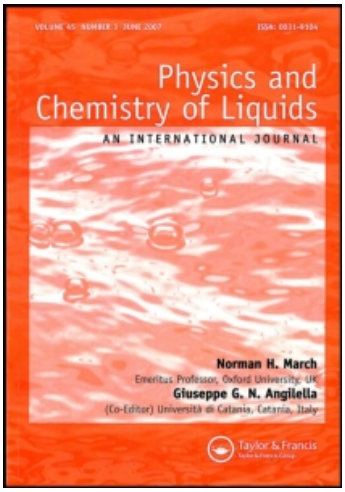

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Thermodynamic analysis for cationic surfactants binding to bovine serum albumin

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In the present study, the binding isotherms for interaction of a homologous series of *n*-alkyltrimethyl ammonium bromides with bovine serum albumin (BSA) have been analyzed on the basis of intrinsic thermodynamic quantities. In this regard, the intrinsic Gibbs free energy of binding, $\Delta G^{(i)}_{b,v}$, has been estimated at various surfactant concentrations and its trend of variation for both binding sets have been interpreted on the basis of cooperativity and hydrophobicity of the process. Subsequently, the contribution of electrostatic and hydrophobic interactions in $\Delta G^{(i)}_{b,v}$, have been estimated using a published method which has been previously introduced by us for analysis of jack bean urease–cationic surfactant system. The results represent the favouring predominate role of hydrophobic interactions and minor rule of electrostatic interaction in binding affinity of both sets. The predominate role of hydrophobic interactions in the second binding set can be related to entropy statistical effect, which arises from numerous number of binding sites in this set but it may be referred to a large amount of positive charge density and accessible hydrophobic surface area of BSA in first binding set.

Keywords: Bovine serum albumin; Cationic surfactants; Gibbs free energy of binding; Electrostatic interactions; Hydrophobic interactions

1. Introduction

In living systems, binding interactions between biopolymers (such as proteins, nucleic acids and poly sachharides) and organic solutes (such as hormones, sugars and fatty acid salts) frequently occur in aqueous media [1]. Such interactions are responsible for the occurrence of many types of bioactive phenomena. Positive binding of many ligands to proteins has been extensively investigated from both the experimental and the theoretical standpoint [2–9]. Excellent reviews on the physicochemical aspects of polymer–surfactant interactions have recently been presented by Goddard [l0,11]. The different techniques used for such study include equilibrium dialysis, measurement of surface tension, electrical conductivity, viscosity, electrophoresis and

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ultracentrifugation, gel filtration, ion-specific electrodes, solubilization, fluorescent probes, electro-optic effects, NMR, small angle neutron scattering, calorimetry, ESR and X-ray diffraction. The effects of surfactant chain length and structure, interaction models and causes for polymer–surfactant complex formation have been discussed in these reviews. It has been suggested that the mechanism of interaction is due to binding charge head groups of the surfactant to the sites with opposite charge at the protein surface, with simultaneous interaction of hydrophobic tail of the surfactant to hydrophobic patches at the protein surface [12]. The above statement of these initial interactions are followed by unfolding and exposure of the hydrophobic interior and hence generation of numerous hydrophobic binding sites [13,14].

In many studies of protein denaturation and its folding, bovine serum albumin (BSA), which is composed of 583 amino acids and 17 disulfide bonds, has been used with different physicochemical methods [15–18] perhaps, most importantly, due to its well-established primary structure [17,19]. BSA is largely helical and thermally more stable at pH 7 [18]. Recently, we have investigated the interaction of a series of *n*-alkyl trimethyl ammonium bromides with BSA using ion selective membrane electrodes as a simple, fast and accurate method [20]. The obtained accurate binding curves have been analyzed on the basis of two sets binding sites and the role of both electrostatic and hydrophobic forces have been shown in binding affinity of sites. In the present study, at first, the intrinsic Gibbs free energy of binding has been calculated for both binding sets and its trend of variation has been interpreted on the basis of binding mechanism. Subsequently, the contribution of electrostatic and hydrophobic interactions in intrinsic Gibbs free energy has been estimated using an approach which was successfully applied for interaction of cationic surfactants with jack bean urease (JBU), previously [21].

2. Data analysis and results

It has previously been shown that the intrinsic Gibbs free energy of binding per mole of surfactant ions for first, $\Delta G^{(1)}_{b,\nu}$, and second, $\Delta G^{(2)}_{b,\nu}$, binding sets can be calculated from the following formula [22]:

$$\Delta G^{(1)}_{b,\nu} = -RTn_{H1}\ln K_{H1} + RT(1-n_{H1})\ln[S]_f \quad \text{if } 0 < \nu \le g_1 \tag{1a}$$

$$\Delta G^{(2)}_{b,\nu} = -RTn_{H2}\ln K_{H_2} + RT(1-n_{H2})\ln[S]_f \quad \text{if } 0 < \nu \le g_1 + g_2 \tag{1b}$$

where R, T, $[S]_f$ and ν are gas universal constant, absolute temperature, free surfactant concentration and average number of bound surfactant ions per each macromolecule in these formulas, respectively. Where g_1, $n_{H1}$and K_{H1} are the number of binding sites, Hill coefficient and the Hill binding constant, for first binding set and g_2, n_{H2} and K_{H2} are the corresponding parameters for second binding set, respectively. With respect to the nature of interaction, $\Delta G^{(i)}_{b,\nu}$ can be considered as a summation of two parts, as follows:

$$\Delta G^{(i)}_{b,\nu} = \Delta G^{(i)}_{b,\nu}(\text{ele}) + \Delta G^{(i)}_{b,\nu}(\text{hyd}) \tag{2}$$

where $\Delta G^{(i)}_{b,\nu}(\text{ele})$ and $\Delta G^{(i)}_{b,\nu}(\text{hyd})$ are the electrostatic and hydrophobic contribution to intrinsic Gibbs free energy of binding for ith set, respectively. For binding of a homologous series of *n*-alkyl trimethyl ammonium bromide, $\Delta G^{(i)}_{b,\nu}(\text{hyd})$ depends on

hydrocarbon tail length of surfactant, while $\Delta G^{(i)}_{b,\nu}$(ele) does not. This dependency can be represented by the following relation:

$$\Delta G^{(i)}_{b,\nu}(\text{hyd}) = f(C_n) \tag{3}$$

where C_n and $f(C_n)$ are the number of carbon atoms in the hydrocarbon tail of surfactant and any arbitrary function of C_n, respectively. It is obvious that:

$$\lim_{C_n \to 0} \Delta G^{(i)}_{b,\nu}(\text{hyd}) = 0 \tag{4}$$

or

$$\lim_{C_n \to 0} \Delta G^{(i)}_{b,\nu} = \Delta G^{(i)}_{b,\nu}(\text{ele}) . \tag{5}$$

This simple idea can be used for estimation of $\Delta G^{(i)}_{b,\nu}$(ele) and $\Delta G^{(i)}_{b,\nu}$(hyd). Figures 1 and 2 show the variation of $\Delta G^{(1)}_{b,\nu}$ and $\Delta G^{(2)}_{b,\nu}$ *versus* $\log[S]_f$ for interaction of dodecyl trimethyl ammonium bromide (DTAB), tetradecyl trimethyl ammonium bromide (TTAB) and hexadecyl trimethyl ammonium bromide (HTAB) with BSA, respectively. The required data for calculation of $\Delta G^{(i)}_{b,\nu}$ have been directly taken from previous study [20]. The values of $\Delta G^{(i)}_{b,\nu}$ at any specified value of $[S]_f$ have been extracted from these figures and plotted *versus* C_n (figures 3 and 4). The points relate to the specified value of $[S]_f$ were fitted in a linear equation using least-square fitting program. With respect to equation (5), $\Delta G^{(i)}_{b,\nu}$(ele) should be equal to *Y*-intercept of these lines, and subsequently, the values of $\Delta G^{(i)}_{b,\nu}$(hyd) can be estimated by subtracting of $\Delta G^{(i)}_{b,\nu}$(ele) from $\Delta G^{(i)}_{b,\nu}$. Figures 5 and 6 show the variation of $\Delta G^{(i)}_{b,\nu}$(ele) and $\Delta G^{(i)}_{b,\nu}$(hyd) *versus* $\log[S]_f$ for first and second binding sets, respectively.

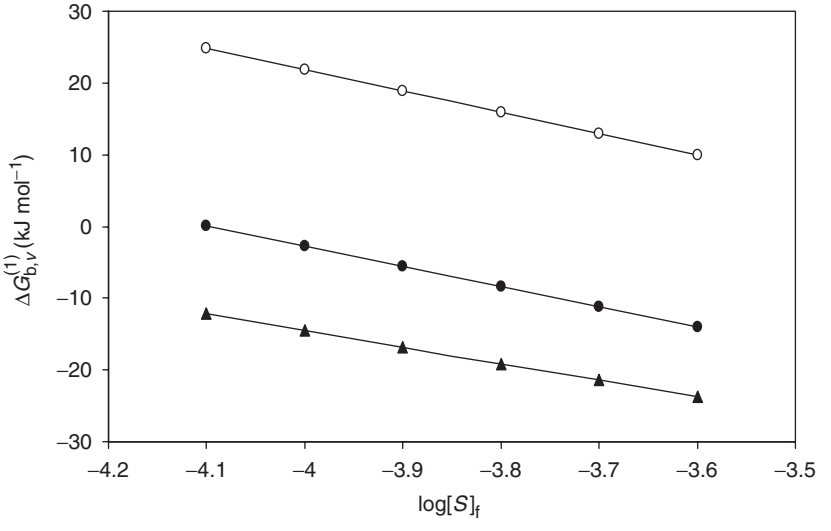

Figure 1. The variation of $\Delta G^{(1)}_{b,\nu}$ kJ mol^{-1} *vs.* $\log[S]_f$ for interaction of BSA with DTAB (○), TTAB (●) and HTAB (▲).

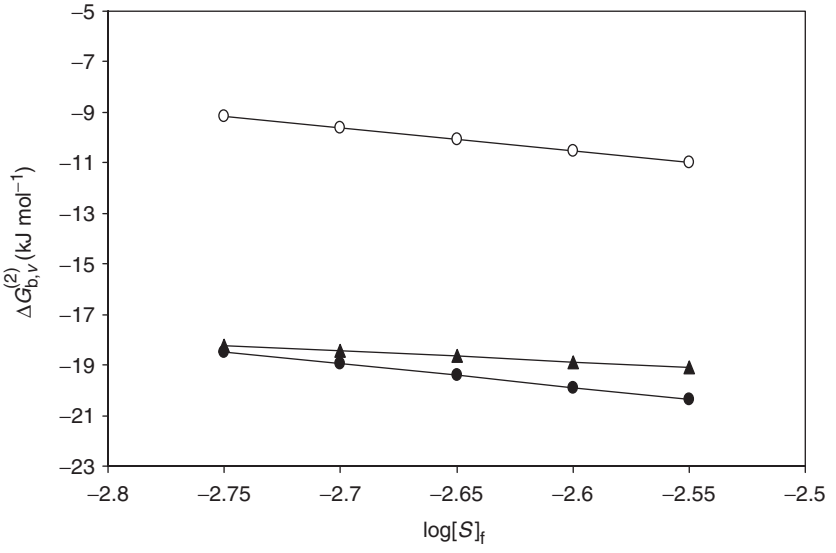

Figure 2. The variation of $\Delta G^{(2)}_{b,v}$ kJ mol^{-1} *vs.* log[S]$_f$ for interaction of BSA with DTAB (○), TTAB (●) and HTAB (▲).

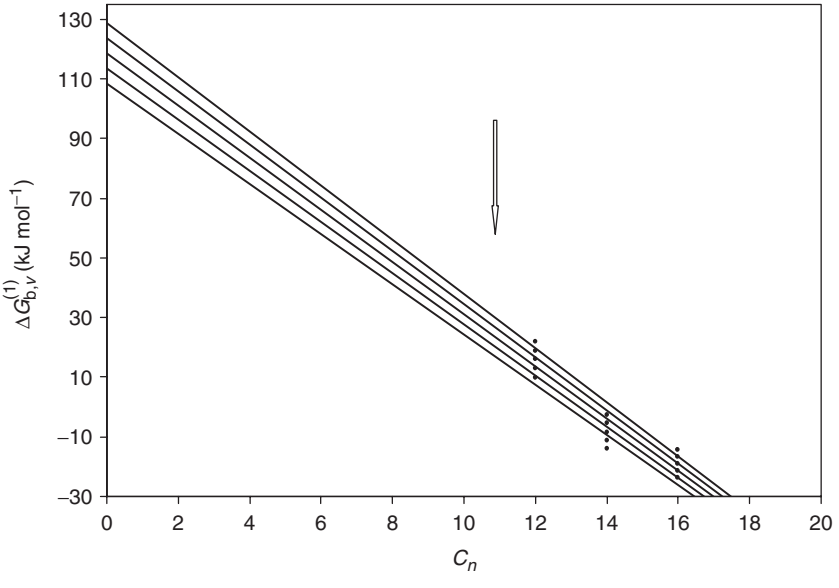

Figure 3. The variation of $\Delta G^{(1)}_{b,v}$ kJ mol^{-1} *vs.* C_n for interaction of BSA with cationic surfactants at various [S]$_f$.

3. Discussion and conclusion

The negative slope of the lines in figures 1 and 2 represent the positive cooperativity in the binding process. The positive cooperativity in both sets can be related to special role

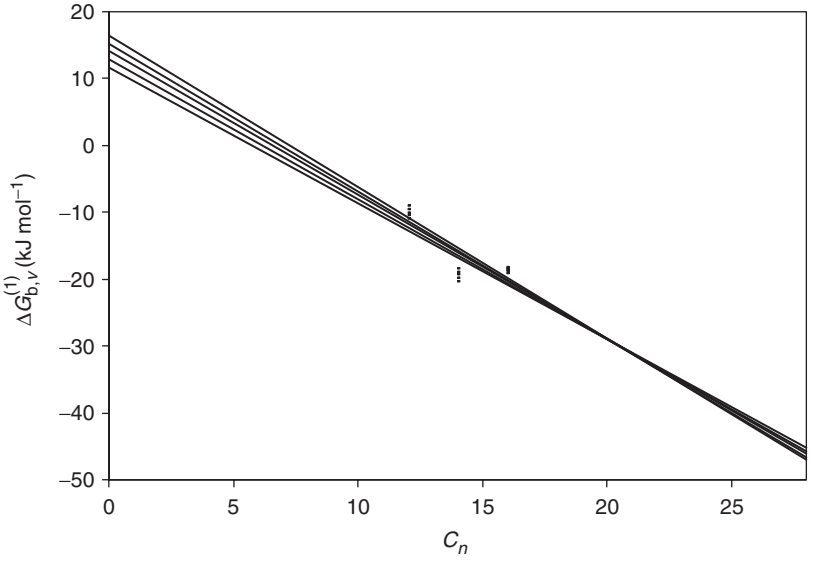

Figure 4. The variation of $\Delta G^{(2)}_{b,\nu}$ kJ mol^{-1} *vs.* C_n for interaction of BSA with cationic surfactants at various $[S]_f$.

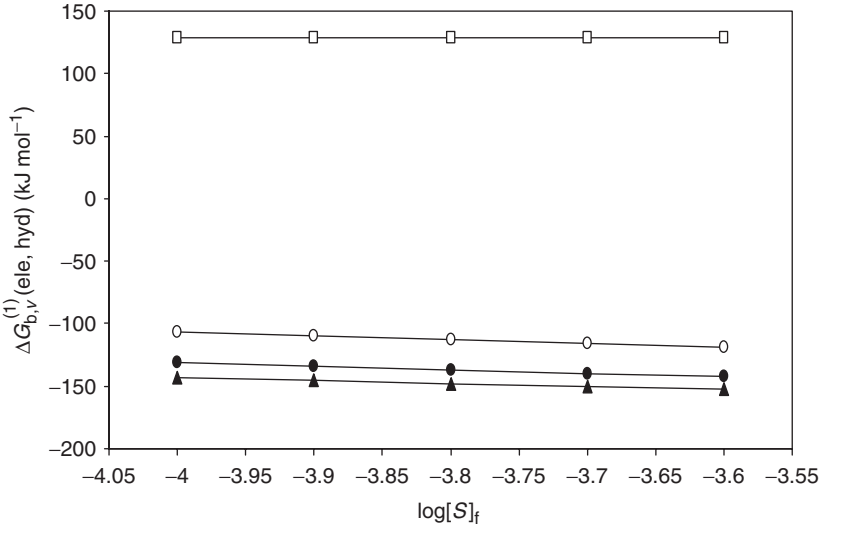

Figure 5. The variation of $\Delta G^{(1)}_{b,\nu}$(ele) kJ mol^{-1} (□) and $\Delta G^{(1)}_{b,\nu}$(hyd) kJ mol^{-1} for interaction of BSA with DTAB (○), TTAB (●) and HTAB (▲) with log$[S]_f$.

of hydrophobic forces in the formation of BSA-surfactant complexes. However, the greater degree of steepness of the lines in figure 1 represents the more predominate rule of hydrophobic interactions in the first binding set. This observation is in contradiction with JBU-cationic surfactants system. With respect to these observations, the following assumptions of the model regarding the changes in the state of the protein

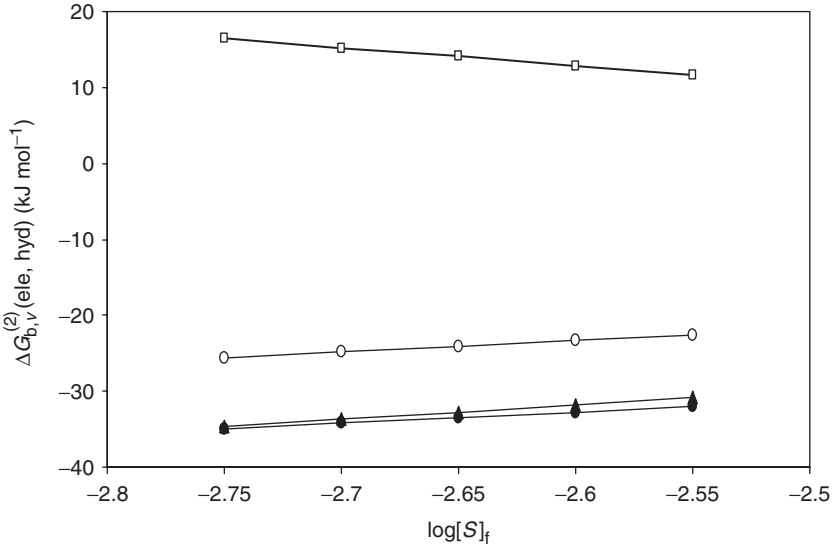

Figure 6. The variation of $\Delta G^{(2)}_{b,\nu}$(ele) kJ mol^{-1} (□) and $\Delta G^{(2)}_{b,\nu}$(hyd) kJ mol^{-1} for interaction of BSA with DTAB (○), TTAB (●) and HTAB (▲) with log[S]$_f$.

at different concentrations of surfactant can be defined as follows: the first type of binding sites is present in the native protein. The saturation of these binding sites is cooperative, then, surfactant binding induce a considerable change in conformational state of BSA. This large conformational change can be occurred with unfolding and exposure of numerous non-specific binding sites.

The more negative values of $\Delta G^{(1)}_{b,\nu}$ with respect to $\Delta G^{(2)}_{b,\nu}$ for any surfactant, represents the stronger initial interactions, which are usually due to attractive electrostatic forces between cationic head group of surfactant ions with negative charge centres at the protein surface. The most interesting part of this article is the use of experimental data (obtained with a series of ionic surfactants differing in the length of the hydrocarbon tail) to obtain the hydrophobic and electrostatic components of binding energies. In this regard the novel feature of figure 4 is the existence of iso-affinity point. This point is at C_n equal to 19.57. It seems that this point is inflection point for kind of cooperativity. The meaning of iso-affinity point in figure 4 can be interpreted as follows: it is well known that denaturation power of ionic surfactant increased by increasing of the hydrocarbon tail. However, the stability of protein has a limited value so that it is expected that after a specified value of C_n all of the surfactants with various tail lengths behave identically. Hence, it can be suggested that this limiting value for C_n of surfactant relate to the denaturating power of homologous surfactants and the extent of structural stability of protein. However, such inflection point has not been observed in figure 3 that corresponds to the first binding set. This is in contradiction with JBU-surfactant system. Figure 5 represents that the contribution of electrostatic interactions is less than hydrophobic in the first binding set for all of the surfactants. Moreover, an inhibition effect is observed for electrostatic interactions in the second binding set. So, the predominant driving force in first and second binding sets is hydrophobic interactions. However, the variation trend of $\Delta G^{(1)}_{b,\nu}$(ele) to less positive values is not in agreement with increasing of positive charge density in the BSA

due to binding of cationic surfactants. This may be related to conformational changes of BSA that reduces the positive charge density on BSA. The values of $\Delta G^{(1)}_{b,v}$(hyd) are going up to more negative values due to increasing C_n or hydrocarbon tail length, which is expected.

With respect to figure 6 the positive values of $\Delta G^{(i)}_{b,v}$(ele) represents the net positive charge in protein in all binding stages of second binding set. In other words, the repulsive electrostatic forces between cationic head group of surfactant and positive charges in the BSA-surfactant complexes inhibited the binding of next surfactant ions.

However, these values are less positive corresponding to the first binding set. This may be related to the unfolding of protein and reduction of its positive charge density. The values of $\Delta G^{(i)}_{b,v}$(hyd) are negative and represents the favouring effects of hydrophobic interaction in binding process for both the binding sets and its values are sufficient that can compensate the repulsive electrostatic forces, effectively. It can be concluded that hydrophobic interactions have an essential role in binding process of cationic surfactant to BSA. Part of this role can also be related to the numerous numbers of binding sites in the second binding set, which increased the statistical entropy part of macroscopic Gibbs free energy. However, in comparison with JBU-surfactant, it seems that the role of hydrophobic interactions is much more, especially in first binding set.

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