Thermodynamics of the Interaction of Globular Proteins with Powdered Stearic Acid in Acid pH

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Adsorption isotherms of different globular proteins and gelatin on strearic acid particles have been studied as a function of biopolymer concentration, ionic strength of the medium, and temperature. The effect of neutral salts including $CaCl_2$, Na_3PO_4, and urea on the adsorption isotherms has been also investigated. It is observed that the extent of adsorption (Γ_2^1) increases in two steps with the increase of biopolymer concentration (C_2) in the bulk. Γ_2^1 increases with an increase of C_2 until a steady maximum value Γ_2^m is reached at a critical concentration C_2^m. After initial saturation, Γ_2^1 again increases from Γ_2^m without reaching any limiting value due to the surface aggregation of the protein. The values of the standard free energy change for adsorption have been calculated on the basis of the Gibbs equation. The standard entropy and enthalpy changes are also calculated.

Introduction

Long-chain fatty acids bind with serum albumin of blood, and following a complex mechanism, they are transferred to cell membranes of different regions of the body.[1−5] In the cell membrane, different proteins are in complex interaction with lipids containing various types of fatty acids.[6−9] Digestion of insoluble fats present in food materials is also controlled by adsorbed protein.[10] For a thorough understanding of such fundamental biological processes, the interaction of fatty acid with protein needs to be investigated in vitro. Binding interaction of long and short chain fatty acid anions with protein has been widely studied since 1946 and onward.[12,13] It was reported in the literature that fatty acid anions stabilize serum albumin against denaturation by urea, guanidine hydrochloride, and heat.[12] Different techniques including partition analysis using radio tracer technique, electron spin resonance (ESR), and the spin label method were used to determine binding isotherms of long chain fatty acid anions and their derivatives with bovine and human serum albumin.[12−16]

Moreover protein−fatty acid interactions have also been studied from technological viewpoints. Recently Yoo and coworkers[17] reported that lysozyme−oleate complexes exhibit much higher conformational stability at elevated temperatures compared with that for free lysozyme in the same organic solvent. They have also prepared biodegradable nanoparticles by a spontaneous and solvent diffusion method from the lysozyme−oleate complex and free lysozyme and showed that lysozyme loading in the nanoparticle was much higher in the case of the lysozyme−oleate complex compared to free lysozyme. These protein-enriched nanoparticles could be useful for oral protein delivery. Protein-fatty acid Langmuir Blodgett (LB) films on different substrates can be used as a biosensor.[18] The long chain fatty acids including stearic and palmitic acid in acidic pH are practically insoluble in the aqueous phase, but the particles may be suspended in water to prepare colloidal suspensions of low stability. The work presented here is aimed at investigating the adsorption of proteins on the surface of insoluble stearic acid particles at various physicochemical conditions.

Although the interaction of protein with fatty acid anions in solution has been extensively studied, to the best of our knowledge, so far there has been no report on the adsorption study of protein on insoluble long-chain fatty acid surfaces at the solid−water interface. Thermodynamic parameters due to such adsorption interactions are also estimated to understand energetic aspects of such interactions in a more quantitative manner.

Materials and Method

In our present investigations, highly pure stearic acid from Loba Chemical was used. Bovine serum albumin (BSA; lot no. 116F-9390, $M_w = 68\,000$), β- lactoglobulin (lot no.75H 7155, $M_w = 36\,000$), gelatin (lot no. 47F-0001, $M_n = 45\,000$, measured by osmometry), and lysozyme (lot no. 111H-7010, $M_w = 13\,900$) were obtained from Sigma Chemicals Company. Inorganic salts such as NaCl, $CaCl_2$, and Na_3PO_4 were of analytical grade and hence used directly without any further purification. Urea was twice recrystallized from warm ethanol. Double-distilled water was used throughout the experiment.

All experiments were performed at pH 4.0, which was obtained by suitable addition of HCl. Ionic strength (μ) of the solution was maintained by the addition of a suitable amount of NaCl.

Adsorption Experiment. To a definite volume, V (equal to 20 mL), of the solution of protein, taken in 100 mL standard joint conical flask was added a fixed amount, w (equal to 5×10^{-4} kg), of dry stearic acid. The flasks were shaken gently on a horizontal shaker for 24 h at constant temperature. After 24 h shaking, the flasks were kept undisturbed for another 24 h at the same temperature. The temperature of the system was maintained using an air thermostat (±0.1 °C accuracy). After 24 h settlement, the supernatant solution from each flask was taken and centrifuged at 5000 rpm for removal of any suspended particles. The concentration of the protein in the supernatant was estimated using the Lowry method.[19] Before measurement, proper dilution of the solution was always done. For the Lowry method, 1

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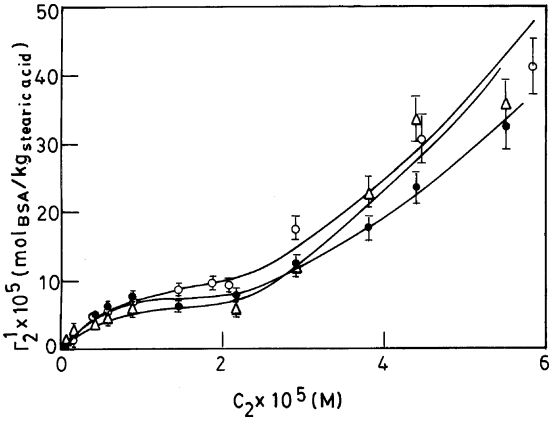

Figure 1. Plot of Γ_2^1 vs C_2 for adsorption of Bovine serum albumin at the stearic acid−water interface, pH 4.0, $\mu = 0.01$, (●) 300, (○) 305, and (△) 316 K.

mL of protein solution was taken in a test tube and 4 mL of a solution containing 2% Na_2CO_3 in 0.1 N NaOH (50 parts) and 0.1% $CuSO_4$ in 2% Na−K tartrate (1 part) was added to it. The solution was mixed gently and kept for 10 min. Then after the addition of 0.5 mL of folin reagent with constant shaking, it was kept in the dark for 45 min. The absorbance of blue color developed in the protein solution was measured with a U-2000 Hitachi spectrophotometer at 750 nm against the blank. Six sets of protein solutions of known concentrations were prepared as standards. All of these solutions were treated with folin reagent in a manner similar to that described earlier. To obtain the standard curve, the absorbance of these solutions measured at 750 nm against that of a blank solution were plotted against known protein concentration. The molar concentration of protein (C_2) was calculated using this standard curve obtained for known concentrations of the solution.

The moles (Γ_2^1) of protein adsorbed per kilogram of dry stearic acid can be calculated using eq 1

$$\Gamma_2^1 = \frac{(C_2^t - C_2)V^t}{1000} \quad (1)$$

Here C_2^t is the molar concentration of the protein in a solution of volume V, in mL, before addition of the solid adsorbent and C_2 is its molar concentration in the bulk solution at adsorption equilibrium after the addition of W grams of solid adsorbent. V^t equal to V/W, represents volume of solution per kg of solid powder.

Average value of Γ_2^1 at a given concentration is based on four sets of adsorption experiments; standard deviation in the values of C_2 remained in the range 2−3%, so that standard error in the average value of Γ_2^1 did not exceed 7−10% percent. The range of the error is also shown in Figure 1.

Results and Discussion

It is well-known that monobasic fatty acids in the homologous series contain a hydrophilic COOH group. All of these fatty acids also contain a hydrophobic group, $CH_3-(CH_2)_n^-$, of increasing chain length. The fatty acids with low values of n (less than 9) are reasonably soluble in water medium, and the surface activity of these short chain soluble fatty acids at air−water and oil−water interfaces have been extensively studied with many interesting conclusions.[20−22] When values of n are 10 or higher, the small amount of fatty acids and their salts soluble in water begin to be associated forming micelles. Values of the critical micelle concentration (cmc) of the acid and krafft points sharply decrease with an increase of n until the solubility in water medium is negligibly small for stearic and higher fatty acids.[23,24] Powdered stearic acid in acid pH is practically

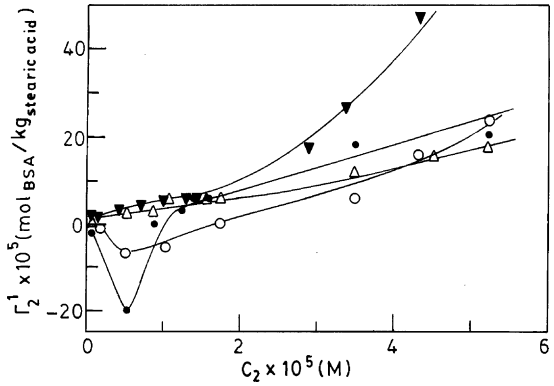

Figure 2. Plot of Γ_2^1 vs C_2 for adsorption of Bovine serum albumin at the stearic acid −water interface, pH 4.0, $T = 305$ K, (●) 6 M urea, (○) 2 M urea, (△) $CaCl_2$, $\mu = 0.05$, and (▼) Na_3PO_4, $\mu = 0.05$.

insoluble in aqueous phase, and the solid powder exposes a large extent of the solid−liquid interface for the adsorption of proteins from an aqueous solution, so that C_2^t is significantly different from C_2 and Γ_2^1 can be measured directly using eq 1.

In Figure 1, moles of bovine serum albumin, Γ_2^1, adsorbed per kg of stearic acid powder have been plotted against C_2 at different temperatures keeping the pH and ionic strength (μ) 4.0 and 0.01, respectively. Such plots have also been compared at different ionic strengths of the medium. In Figure 2, plots of Γ_2^1 vs C_2 have been compared in the presence of additives $CaCl_2$, Na_3PO_4, and urea. Except in the presence of urea, the nature and shapes of curves in all cases are strikingly similar with that observed for adsorption of cationic surfactant to powdered stearic acid.[25] $\Gamma_2{}^1$ increases with an increase in C_2 until a steady maximum value Γ_2^m is reached at a critical concentration C_2^m. For $C_2 \gg C_2^m$, Γ_2^1 again increases from Γ_2^m without reaching any limiting value due to the surface aggregation of protein. At pH 4.0, BSA like cetyl trimethylammonium bromide (CTAB) behaves as a cation. The adsorption isotherms for BSA and other proteins for silica and alumina surfaces[26] are similar to the adsorption of BSA on the stearic acid surface. Even previously reported adsorption isotherms of BSA at the polar oil−water interface are also similar in nature.[27] However, unlike the stearic acid surface, Γ_2^1 for different globular proteins on carbon, silica, alumina, and resin surfaces and at polar oil−water interface does not increase further with C_2 beyond C_2^m, so that surface aggregation of the protein on all of these interfaces is absent.[26,27]

Recently, the adsorption of CTAB on a powdered stearic acid surface suspended in aqueous media has been measured at different values of C_2.[25] The maximum Γ_2^m value of CTAB adsorbed per kg of stearic acid at pH 4.0 and at 42 °C was found to be 2.23 mol. Taking the cross sectional area of the CTAB molecule as 0.30 nm^2 and further assuming the vertical orientation of the CTAB ion on the stearic acid surface at the state of maximum saturation, approximate average surface area per kg of stearic acid powder has been calculated to be 41×10^{20} nm^2. The compact monolayer of ellipsoid shaped BSA molecules with vertical and horizontal orientations would correspond to 12.3×10^{-8} and 3.67×10^{-8} mol of BSA, respectively, packed per square meter of surface plane.[28] However, the more accurate determination of quantitative adsorption of protein on different solid surfaces can be obtained by sophisticated optical tecnique.[29,30] The moles of BSA adsorbed per square meter of stearic acid surface varies from 1.5×10^{-8} to 2.8×10^{-8} for different conditions. This result indicates that BSA has undergone lateral expansion on stearic

Table 1. Parameters for Adsorption of Protein at the Stearic Acid−Water Interface at pH 4.0

protein	additives	temp (K)	ionic strength (μ)	$C_2^m \times 10^5$ mol/lit	$\Gamma_2^m \times 10^5$ mol protein/ kg of stearic acid	$-\Delta G^0 \times 10^3$ kJ/ kg of stearic acid	$-\Delta G_B^0$ kJ/mol protein	$(\Gamma_2^m)_{hi} \times 10^5$ mol protein/ kg of stearic acid	$-\Delta G_{hi}^0 \times 10^3$ kJ/ kg of stearic acid	$-(\Delta G_0^B)_{hi}$ kJ/mol protein	$-(\Delta G_{hi}^0 - \Delta G^0) \times 10^3$ kJ/ kg of stearic acid
BSA	NaCl	300	0.01	1.37	7.00	2.9 ± 0.1	41	106	37	35	37
BSA	NaCl	305	0.01	1.87	9.33	3.9 ± 0.2	41	107	36	33	36
BSA	NaCl	316	0.01	1.66	6.00	2.8 ± 0.1	46	109	35	32	35
BSA	NaCl	305	0.05	1.87	12.0	4.9 ± 0.1	40	205	74	36	73
BSA	NaCl	305	0.2	1.33	5.83	2.4 ± 0.1	40	142	54	38	54
BSA	$CaCl_2$	305	0.05	1.33	6.33	2.6 ± 0.1	40	86.0	32	37	32
BSA	Na_3PO_4	305	0.05	1.37	6.00	2.6 ± 0.1	43	174	62	35	62
BSA	6 M urea + NaCl	305	0.05	1.38	6.16	2.4 ± 0.2	39	65.4	24	37	24
β-lactoglobulin	NaCl	305	0.05	0.71	5.00	2.2 ± 0.1	45	151	49	32	49
gelatin	NaCl	305	0.05	0.92	4.00	1.7 ± 0.2	43	399	140	35	140
lysozyme	NaCl	305	0.05	3.47	17.5	6.8 ± 0.3	39	933	261	28	260

acid surface. Das and Chattoraj[27] obtained a value of Γ_2^m close to 1.03×10^{-8} mol/m^2 for BSA at polar oil−water interface at the pH 4.0. They also explained that the significant alteration of the BSA structure by surface denaturation at this pH at oil−water interface may be responsible for this low value of Γ_2^m. The specifically deformed stearic acid surface becomes suitable for surface aggregation and co-aggregation of proteins. Stearic acid particles serve as a nucleus for protein aggregation and critical aggregation concentration of different proteins have thus been estimated.

From Table 1, it is observed that the values of Γ_2^m increases with increase of temperature from 27 to 32 °C but with further increase of temperature, Γ_2^m decreases due to the desorption effect. Value of Γ_2^m also changes when NaCl is replaced by $CaCl_2$, but with Na_3PO_4, its value does not further alter (vide Table 1 and Figure 2). Different extents of hydration effect of BSA by different inorganic salts may cause of such variation of Γ_2^m value. Hydration effect is possibly controlled by lyotropic or Hoffmiester series effect by ions of different valency. Such study was carried out earlier in our laboratory for protein adsorption on various surfaces in the presence of variety of electrolytes of different valency.[26,27] Interesting results regarding effect of Hoffmiester series for protein adsorption have also been found by Ramsden et al.[31] In the presence of urea, Γ_2^m is initially negative due to the excess water adsorption but at higher values of C_2, positive values of Γ_2^1 shoots up without reaching any limit due to extensive surface aggregation of denatured BSA (Figure 2).

The adsorption isotherms for β-lactoglobulin, gelatin (vide Figure 3), and lysozyme have been compared under similar physicochemical conditions, and corresponding Γ_2^m values are presented in Table 1. Γ_2^m for proteins stand in the increasing order lysozyme > BSA > β-lg > gelatin.

The standard free energy change ΔG^0 for adsorption saturation of protein per kg of stearic acid powder can be calculated[32,33] using the integrated form of the Gibbs adsorption eq 2

$$\Delta G^0 = -RT \int_0^1 \Gamma_2^1 \, \mathrm{d} \ln a_2 \qquad (2)$$

where a_2 stands for the activity of the solute component in solution. For a dilute protein solution, its rational activity may be taken to be equal to the mole fraction, X_2, whose value may be taken to be equal to $C_2/55.5$. Here C_2 stands for the molar concentration of the bulk protein solution. Equation 2 can be converted to the form[32,33]

$$\Delta G^0 = -RT \int_0^{X_2^m} (\Gamma_2^1/X_2) \, \mathrm{d}X_2 + RT\Gamma_2^m \ln X_2^m \qquad (3)$$

The first term on the right side of eq 3 represents the change of free energy when the mole fraction of the protein (X_2) in the bulk phase is altered from 0 to X_2^m, so that the surface becomes hypothetically saturated and Γ_2^1 becomes equal to Γ_2^m. The second term represents the free energy change due to the change of mole fraction of the protein from X_2^m to unity at the standard state. This involves the assumption that Γ_2^m, remains constant when the X_2 is altered from X_2^m to unity. Values of ΔG^0 for different systems in kJ per kg calculated with the help of this equation are included in Table 1.

The effect of temperature, ionic strength, neutral salts, and urea on the values of ΔG^0 is also noted from this table. From the ratio $\Delta G^0/\Gamma_2^m$, ΔG_B^0 the standard free energy change for the transfer of one mole of protein from bulk to the surface of stearic acid powder can be calculated with the assumption that the adsorption process is reversible.[32,33] Here average values of ΔG_B^0 for protein adsorption are 41.5 ± 4.5 kJ/mol. Similar values of ΔG_B^0 per mole of protein at different surfaces of solid have been obtained earlier by Chattoraj and co-workers.[34]

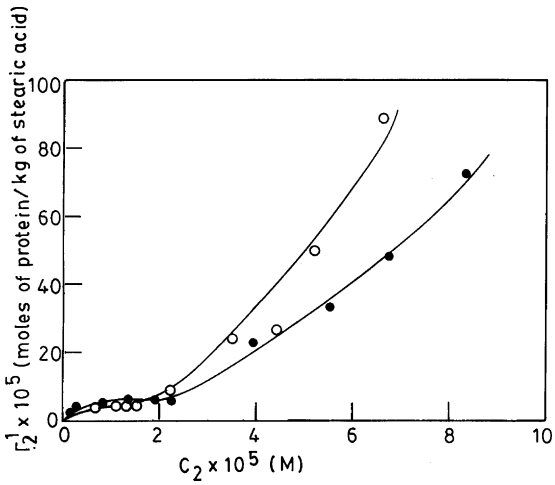

Figure 3. Plot of Γ_2^1 vs C_2 for the adsorption of different proteins at the stearic acid−water interface at pH 4.0, $T = 305$ K, $\mu = 0.05$. (●) β-Lactoglobulin and (○) gelatin.

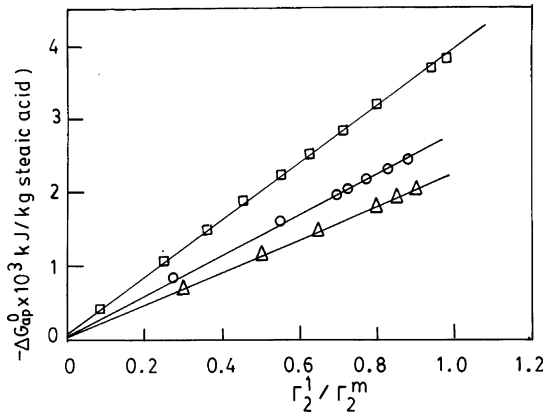

Figure 4. Plot of ΔG^0_{ap} vs Γ_2^1/Γ_2^m for the adsorption of proteins at the stearic acid−water interface at pH 4.0, (●) BSA at $\mu = 0.01$, $T = 305$ K; (○) BSA at $\mu = 0.01$, $T = 316$ K; (△) β-lactoglobulin at $\mu = 0.05$, $T = 305$ K.

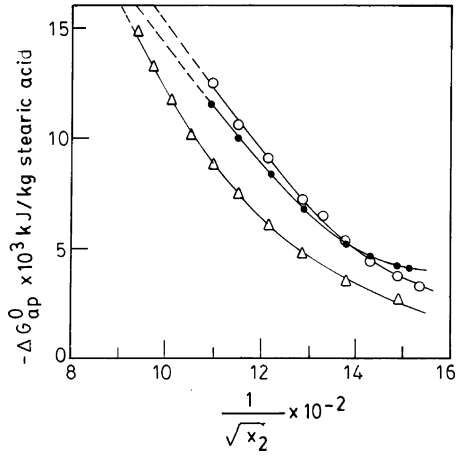

Figure 5. Plot of ΔG^0_{ap} vs $1/\sqrt{X_2}$ for the adsorption of proteins at the stearic acid−water interface at pH 4.0, (●) BSA at $\mu = 0.01$, $T = 305$ K; (○) BSA at $\mu = 0.01$, $T = 316$ K; (△) β-lactoglobulin at $\mu = 0.05$, $T = 305$ K.

When Γ_2^1/Γ_2^m (or fraction of surface coverage θ) is less than unity, the monolayer is unsaturated and apparent standard free energy change ΔG^0_{ap} for adsorption at any given state can be calculated using eq 4

$$\Delta G^0_{ap} = -RT \int_0^{\Gamma_2^1} (\Gamma_2^1/X_2)\, dX_2 + RT\Gamma_2^1 \ln X_2 \qquad (4)$$

Values of ΔG^0_{ap} estimated from this equation is less than ΔG^0 at a given value of Γ_2^1. ΔG^0_{ap} is observed to vary linearly with Γ_2^1/Γ_2^m (Figure 4), and the slope of this line represents standard free energy change for the unsaturated monolayer. Those are always observed to be equal to ΔG^0, obtained by using eq 3 for the saturated monolayer. We have already pointed out that the error involved for calculation of Γ_2^1 (and also Γ_2^m) may occur in the range of 7−10% depending upon the system concerned (see Figure 1). Using these experimental values, ΔG^0 for saturated and ΔG^0_{ap} for unsaturated states of adsorption have been calculated. For unsaturated states of adsorption $\Delta G^0 = \Delta G^0_{ap}/(\Gamma_2^1/\Gamma_2^m)$ so that ΔG^0 for saturated and unsaturated states of adsorption have been calculated for each protein under a fixed physicochemical condition. In both cases, ΔG^0 remains same, and the average value of ΔG^0 for each system up to the state of saturation have been presented in Table 1 along with their errors.

When $\Gamma_2^1 > \Gamma_2^m$, the multilayer adsorption of proteins on the stearic acid surface takes place. Even at this state, ΔG^0_{ap} can be calculated using eq 4.[35] Values of ΔG^0_{ap} in this range of multilayer adsorption states have been empirically plotted against $1/\sqrt{X_2}$, and the linear region of the plot (the initial five or six points) has been extrapolated (see Figure 5) to X_2 equal to unity for evaluation of standard free energy change, ΔG^0_{hi} at higher values of X_2. The error, calculated by regression analysis for ΔG^0_{hi} is within 0.3×10^{-3}. Difference of $(\Delta G^0_{hi} - \Delta G^0)$ is the standard free energy change of adsorbed protein at the interface for aggregation presented also in Table 1.

From Table 1, we note with interest that maximum value of adsorption Γ_2^m for different proteins (possessing different molecular weights and different molecular structure) per kg of stearic acid are different from each other. Further, the ΔG^0 values in Table 1 are observed to increase linearly with increase of different values of Γ_2^m for different proteins, so that the ratio $\Delta G^0/\Gamma_2^m$ equal to ΔG^0_B varies only in the range of 39−45 kJ/mol of protein. We thus conclude that the wide variations of ΔG^0 (equal to $\Gamma_2^m\, \Delta G^B_0$) for various proteins are mainly due to variation of Γ_2^m since ΔG^0_B for various proteins are close to each other.

The close values of ΔG^0_B in Table 1 may plausibly indicate that the free energy change for the specific interaction of one binding site of the biomacromolecule with an active spot of the stearic acid surface is more or less constant. This may lead to the primary attachment of the molecule at the interface, and the free energy change per mole of protein for such binding process will be ΔG^0_B. The attached molecules on the surface of the solid will then orient, expand, and unfold on the surface so that the packing of those molecules per square meter indicated by the value of Γ_2^m will be widely different for different systems at the state of saturation. For this reason, ΔG^0 in kJ per square meter of the surface equal to $\Gamma_2^m \Delta G^0_B$ at the state of surface saturation will differ from each other for different systems.

The standard enthalpy change for proteins at the stearic acid−water interface can be calculated using eq 5

$$\frac{\Delta G_1^0}{T_1} - \frac{\Delta G_2^0}{T_2} = \Delta H^0_{av}(1/T_1 - 1/T_2) \qquad (5)$$

ΔG_1^0 and ΔG_2^0 are standard free energy changes at adjacent temperatures T_1 and T_2 so that the average enthalpy change ΔH^0_{av} at an average temperature T_{av} equal to $^1/_2(T_1 + T_2)$ can be estimated. The average free energy change ΔG^0_{av} is equal to $^1/_2(\Delta G^1_0 + \Delta G^2_0)$. Values of ΔH^0_{av} and $T_{av}\Delta S^0_{av}$ have been presented in Table 2 for adsorption of proteins at stearic acid−water interfaces. ΔS^0_{av} standing for entropy change for adsorption is equal to $(\Delta H^0_{av} - \Delta G^0_{av})/T_{av}$.

The calculation of the values of ΔG^0_{av}, ΔH^0_{av}, and ΔS^0_{av} all based on the estimated values of Γ_2^1 (and Γ_2^m) involves error (vide Figure 1 and Table 1) so that their magnitudes may be taken only up to two significant figures. However, the interesting thing is that, in all cases of proteins, the magnitude of ΔG^0_{av} is considerably lower than those of ΔH^0_{av} and $T_{av}\Delta S^0_{av}$. These results indicate that the adsorption is largely controlled both by entropy and enthalpy and their effects are compensated to each other, so that values of ΔG^0 become quite low. These compensation effects are shown to be same for other systems undergoing adsorption of biopolymers.[26,32] When $\Gamma_2^1 > \Gamma_2^m$, this compensation effect is relatively lower.

Table 2. Thermodynamic Parameters for Adsorption of Bovine Serum Albumin at the Stearic Acid−Water Interface at pH 4.0, $\mu = 0.01$

T_{av} (K)	$\Delta G^0_{av} \times 10^3$ (kJ/kg)	$\Delta H^0_{av} \times 10^3$ (kJ/kg)	$T_{av}\Delta S^0_{av} \times 10^3$ (kJ/kg)	$\Delta S^0_{av} \times 10^3$ (kJ kg^{-1} K^{-1})	$(\Delta G^0_{av})_{hi} \times 10^3$ (kJ/kg)	$(\Delta H^0_{av})_{hi} \times 10^3$ (kJ/kg)	$(T_{av}\Delta S^0_{av})_{hi} \times 10^3$ (kJ/kg)	$\Delta S^0_{av} \times 10^3$ (kJ kg^{-1} K^{-1})
302.5	−3.4	57	60	0.2	−36	−100	−64	−0.2
310.5	−3.5	−34	−30	−0.1	−35	−60	−25	−0.1

We have already noted that ΔG^0 for $\Gamma_2^1 \leq \Gamma_2^m$ remains unchanged when X_2 has been altered from zero to X_2^m(or $C_2^m/55.5$). The adsorbed protein molecules are in direct contact with the stearic acid surface in this region of the protein concentration in the bulk. At an average temperature of 302.5 K, values of ΔH^0_{av} and $T_{av}\Delta S^0_{av}$ in Table 2 are observed to be 57×10^{-3} and 60×10^{-3}kJ kg^{-1}. This means that, at any state in this concentration range, the adsorption is an exothermic process with a large increase of entropy so that the net value of $-\Delta G^0_{av}$ becomes relatively small due to the entropy−enthalpy compensation effect. At an average temperature of 310.5 K, however, values of ΔH^0_{av} and $T_{av}\Delta S^0_{av}$ are -34×10^{-3} and -30×10^{-3}, respectively, so that the adsorption interaction becomes an endothermic and ordered process with considerable decrease in entropy. However, due to the compensation effect, value of $-\Delta G^0_{av}$ still remains unaltered in the higher average temperature.

For $\Gamma_2^1 > \Gamma_2^m$, we note from Table 2 that at both average temperatures of 302.5 and 310.5 K the process is highly endothermic and ordered in structure since both ΔH^0_{av} and $T\Delta S^0_{av}$ are negative and high in magnitude. However, due to the compensation effect of these two parameters, the value of $-\Delta G^0_{av}$ is relatively low.

It may be pointed out that, at $\Gamma_2^1 \leq \Gamma_2^m$, adsorbed protein molecules are always attached to the active spots of the stearic acid surface so that ΔG^0 represents the protein−stearic acid interaction for hypothetical or real saturation of the solid surface by the biomacromolecules. On the other hand, at $\Gamma_2^1 > \Gamma_2^m$, protein molecules from the bulk solution are adsorbed on the protein-covered stearic acid surface so that $-\Delta G^0_{ap}$ will shift, in increasing direction due to this process, to a hypothetically maximum value of ΔG^0_{hi} at $X_2 \rightarrow 1$. The large difference between $-(\Delta G^0_{hi} - \Delta G^0)$ thus represent standard free energy decrease for protein aggregation at the interface per kg of the stearic acid powder. Plotting the experimental values of Γ_2^1 against $1/(\sqrt{X_2})$ for adsorption systems, one can calculate an extrapolated hypothetical value of Γ_2^1 equal to $((\Gamma_2^m)_{hi}$ at $X_2 \rightarrow 1$. These are also included in Table 1. The ratio $\Delta G^0_{hi}/(\Gamma_2^m)_{hi}$ equal to $(\Delta G^0_B)_{hi}$ in kJ per mole of adsorbed protein varies between 28 and 37 kJ per mole of the protein which is surprisingly close to the value of ΔG^0_B found for $\Gamma_2^1 \leq \Gamma_2^m$. We can thus conclude that the free energy change for the transfer of one mole of protein to the surface of bare or protein-coated surface are very close to each other although ΔG^0 and ΔG^0_{hi} in those two cases are widely different due to the different values of Γ_2^m and $(\Gamma_2^m)_{hi}$ for various systems as a result of the various types of interactions between the biomacromolecules in the adsorbed states including molecular aggregation. By dividing ΔH^0_{av} and $T_{av}\Delta S^0_{av}$ by Γ_2^m, average values of ΔH^0_B and $T_{av}\Delta S^0_B$ for $\Gamma_2^1 \leq \Gamma_2^m$ are found to be 700 and 735 kJ/mol at T_{av} equal to 302.5 K, whereas at $\Gamma_2^1 > \Gamma_2^m$, these quantities are −93 and −60 kJ/mol, respectively. At 310.5 K, the magnitudes of ΔH^0_B and $T_{av}\Delta S^0_B$ are −440 and −394 kJ/mol, but at $\Gamma_2^1 > \Gamma_2^m$, these thermodynamic quantities are −56 and −20 kJ/mol, respectively. Thus, unlike the unique values of ΔG^0_B and $(\Delta G^0_B)_{hi}$ values of ΔH^0_B, $(\Delta H^0_B)_{hi}$ and $T_{av}\Delta S^0_B$, $(T_{av}\Delta S^0_B)_{hi}$ vary widely for $\Gamma_2^1 \leq \Gamma_2^m$ and $\Gamma_2^1 > \Gamma_2^m$, respectively, for these two different temperatures although the entropy−enthalpy compensation effect seems to be effective in all of these cases.

Conclusion

The results show that different globular proteins can be accumulated as positive excesses on the surface of stearic acid particles at acidic pH. The extent of such adsorption depends on the temperature, the ionic strength, and the nature of the additives and neutral salts and urea. The standard free energy of adsorption, ΔG^0, of proteins adsorbed on stearic acid is found to vary linearly with the value of Γ_2^m. At higher values of X_2, adsorbed protein undergoes aggregation at interfaces. Hypothetical values of the standard free energy change ΔG^0_{hi} of adsorption and maximum value $(\Gamma_2^m)_{hi}$ for different proteins can be evaluated from the linear extrapolation of the experimental data. Values of ΔG^0_B and $(\Delta G^0_B)_{hi}$ in kJ/mol of protein for low as well as high values of Γ_2^1 are found to be close to each other. These observations could be interesting for biological and biomedical applications.

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