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Standard free energies of binding of solute to proteins in aqueous medium. Part 2. Analysis of data obtained from equilibrium dialysis and isopiestic experiments

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Abstract

In an earlier publication by Chattoraj et al. [Biophysical Chemistry 63 (1996) 37], a generalized equation for standard free energy of (ΔG^0) interaction of surfactant, inorganic salts and aqueous solvent with protein, forming a single phase has been deduced on strict thermodynamic grounds. In the present paper, this equation has been utilized to calculate ΔG^0 in kilojoules per kilogram of different proteins for the change of bulk surfactant activity from zero to unity in the mole fraction scale. Values of binding interactions of CTAB, MTAB, DTAB and SDS to BSA, β-lactoglobulin, gelatin, casein, myosin, lysozyme and their binary and ternary mixtures had already been determined in this laboratory at different surfactant concentrations, pH, ionic strength and temperature using an equilibrium dialysis technique. Values of ΔG^0 for saturated protein–surfactant complexes as well as unsaturated complexes are found to be equal. ΔG^0 is also found to vary linearly with maximum moles of surfactants bound to a kilogram of protein or protein mixture and the slope of this linear plot represents standard free energy ΔG_B^0 for the transfer of 1 mol of surfactant from the bulk for binding reaction with protein; $-\Delta G^0$ values for different systems vary widely and the order of their magnitudes represents relative affinities of surfactants to proteins. Magnitude of $-\Delta G_B^0$ on the other hand varies within a narrow range of 32–37 kJ/mol of surfactant. For interaction of SDS with BSA, close to the CMC, values of ΔG^0 are very high due to the formation of micelles of protein-bound surfactants. Values of ΔG^0 for negative binding of inorganic salts to proteins and protein mixtures have been evaluated using our generalized equation in which excess binding values of water and salts have been calculated from the data obtained from our previous isopiestic experiments. ΔG^0 values in these cases are positive due to the excess hydration of proteins. Negative values of ΔG^0 in surfactant interaction and positive values of ΔG^0 for hydration of proteins in the

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presence of neutral salts represent relative affinities of proteins for solute and solvent since in all cases, the reference state for ΔG^0 is the unit mole fraction of solute in the aqueous phase.

Keywords: Protein-surfactant binding; Solute and solvent binding; Proteins; Free energies; Ligand binding to proteins; Free energies, protein hydration

1. Introduction

Many attempts have been made to determine the extent of binding interaction of surfactants, organic solutes, inorganic salts and other types of ligands to proteins dissolved in aqueous media using different types of physicochemical techniques [1–10]. Utilizing these binding data obtained at different pH, ionic strength, temperature and ligand concentrations, several types of thermodynamic procedures have been used for the calculation of the standard free energies of protein–ligand binding interactions. The Scatchard equation [11] derived in 1949 has still served as the most convenient and elegant thermodynamic approach for calculation of free energy changes of such binding interactions. This equation has subsequently been modified [12] for thermodynamic treatment of more complex binding interactions. The concept of preferential binding [13–16] has been introduced subsequently to deal with ligand–protein binding interaction in multicomponent aqueous systems. Bull [17,18] has used the Gibbs adsorption equation valid for two phase systems for the calculation of standard free energy change of ligand–protein binding interaction. Using the concept of micelle formation of surfactants bound to proteins, Tanford [19] has derived expression for the calculation of standard free energy change of massive surfactant–protein interaction.

In part 1 of this paper [20] a general expression for the standard free energy change for positive and negative binding of surfactant and inorganic salt based on the Gibbs excess concept for a single phase aqueous system has been derived on thermodynamic grounds.

In a series of papers [21–24] published from this laboratory in the last two decades, the extents of binding interactions of cationic and anionic surfactants, respectively to several globular and denatured proteins and protein mixtures [25–27] have been evaluated at several pHs, ionic strengths and temperatures. These data have been earlier treated for the calculation of the free energy change for protein–surfactant interaction at unit solute activity at the reference state. The solute activity in this standard state is assumed to be either unit mole fraction [21–26] or unit mole ratio composition [27]. Protein biocolloid and aqueous solvent in these approaches are also assumed to form two phase model systems so that the Gibbs adsorption equation remains valid. Furthermore, extents of positive and negative binding interactions of different inorganic salts to different proteins have also been evaluated by us using isopiestic vapour pressure experiments [28–34].

Using our generalized equation derived in part 1 of this paper [20], standard free energy change for binding of surfactants, salts and water, respectively, to proteins of various types forming 'single phase systems' will be calculated with respect to a standard state of unit bulk solute activity in a rational activity scale so that all the values of free energy can be arranged in terms of maximum affinities of the solutes to proteins.

2. Protein–surfactant binding isotherm

In equilibrium dialysis experiments of binding studies described in part 1 as well as in other papers [20–26], moles of surfactant (Γ_2^1) bound per kilogram of protein present in the dialysis bag may be calculated directly from the equation

$$\Gamma_2^1 = \frac{W_1^t}{1000}(m_2^t - m_2), \tag{1}$$

where W_1^t is the total weight of solvent inside and outside the dialysis bag per kilogram of protein

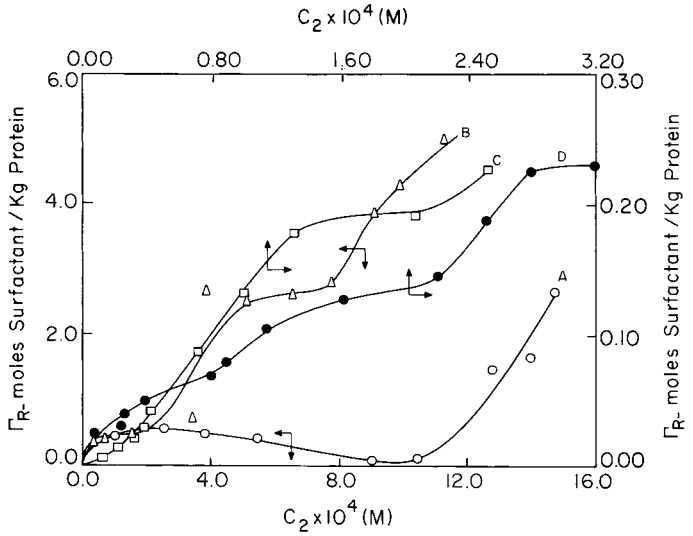

Fig. 1. Plot of Γ_2^1 vs. C_2 for binding of surfactants to BSA (see [21] [22]). (A) SDS, $\mu = 0.0125$, 30°C; (B) SDS, pH = 6.0, $\mu = 0.0625$, 30°C; (C) CTAB, $\mu = 0.125$, 45°C; (D) CTAB, $\mu = 0.125$, 30°C.

present in the bag. Also m_2^t and m_2 are molal concentrations of the surfactant in the total solution before and after binding of surfactant in the system. The solution of surfactant is dilute so that m_2^t and m_2 may be replaced by corresponding molar concentrations and C_2^t and $C_2 \cdot W_1^t$ may be taken to be equal to the total volume V^t of the solution present inside and outside of the dialysis bag so that one can write [20]

$$\Gamma_2^1 = \frac{V^t}{1000}(C_2^t - C_2). \tag{2}$$

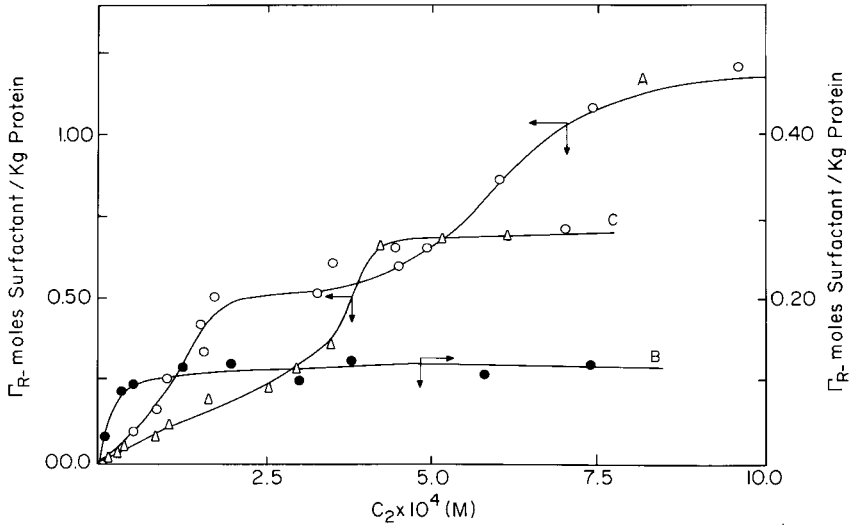

Fig. 2. Plot of Γ_2^1 vs. C_2 for binding of surfactants to proteins at pH 6.0, $\mu = 0.125$. (A) SDS to myosin + BSA at 27°C [23] in a weight ratio of (1:1); (B) CTAB to gelatin at 30°C [26]; (C) CTAB to gelatin + myosin + BSA in a weight ratio of 1:1:1 at 27°C [23].

Since a surfactant has strong affinity for a protein in aqueous medium, C_2^t is greater than C_2 and Γ_2^1 is positive in almost all cases. At a fixed value of pH, ionic strength and temperature, Γ_2^1 changes with C_2 and the curve thus obtained from the $\Gamma_2^1 - C_2$ plot is termed as a binding isotherm. In Fig. 1 the general shape of binding of cetyltrimethyl ammonium bromide (CTAB), and sodium dodecyl sulfate (SDS) to proteins based on our earlier experiments [21–24] have been presented. Γ_2^1 is observed to increase with increase of C_2 until at a higher value of C_2, Γ_2^1 reaches the maximum values Γ_2^m with the apparent formation of a protein–surfactant saturation complex (Fig. 1, curves C and D; Fig. 2). Values of Γ_2^m for binding of ionic surfactants to BSA obtained from our previous measurements [21,22] under different physicochemical conditions are presented in Tables 2 and 3. Sometimes the binding isotherms are found to form two steps [21,22] (see Fig. 1, curve D). In many cases [20,21] the $\Gamma_2^1 - C_2$ plot leads to S-shaped isotherms (see Fig. 1C). At 65°C, BSA binds CTAB by massive cooperative interaction [21] even when C_2 is considerably lower than CMC. Values of Γ_2^m for such cases are indeed quite high (Table 2). Similar types of isotherms are obtained for binding of cationic and anionic surfactants to gelatin, casein [25], myosin [24], binary mixtures of BSA and gelatin [26], BSA and casein [23], BSA and myosin [24], ternary mixtures of casein, BSA and gelatin [23], and myosin, BSA and gelatin [24], respectively (see Fig. 2). In all these cases under different conditions of pH, ionic strength, temperature and composition of binary and ternary mixtures of proteins, values of Γ_2^m are experimentally evaluated using an equilibrium dialysis technique (these were reported earlier [20–24] and are different from each other) (see Tables 4–6).

The shapes of the isotherms (see Fig. 1, curve A) for binding of SDS to BSA are observed to be most striking [22]. With an increase of C_2, Γ_2^1 increases until it reaches an apparent maximum value Γ_2^m. With the further increase of C_2 below the CMC, Γ_2^1 begins to increase sharply without reaching the limiting value again. Recently from EMF measurement it has been shown [35] that binding of CTAB to BSA, lysozyme, β-lactoglobulin, gelatin and hemoglobin exhibit a similar type of massive cooperative interaction near the critical micellar concentration of the surfactant.

3. The Scatchard equation

For the calculation of standard free energy change (ΔG_S^0) due to the binding of Γ_2^1 moles of a ligand to 1 kg (or 1 mol) of a protein at different bulk solute concentrations C_2 of the ligand, the Scatchard equation [Eq. (3)] has frequently been used [11]

$$\Gamma_2^1 = \frac{\Gamma_2^S (e^{-\Delta G_S^0/RT}) C_2}{1 + (e^{-\Delta_S^0/RT}) C_2}. \quad (3)$$

The equation has been derived with the assumption that both ligand and protein are present in a single phase.

If the values of $1/\Gamma_2^1$ for an aqueous system undergoing ligand–protein binding interaction in the equilibrium dialysis and similar types of binding experiments vary linearly with $1/C_2$, then ΔG_S^0 in kilojoules per kilogram (or per mole) of ligand transferred from the bulk to the bound phase as well as maximum moles (Γ_2^S) of binding of ligand per kilogram (or per mole) of the protein may be evaluated.

The Scatchard equation remains valid when the affinity of the ligand for protein is much stronger than that of water. The electrostatic effect as well as conformational change of protein in the binding interaction must be kept to the minimum [12]. All the binding sites of a protein may not possess equivalent affinities for a ligand so that application of Eq. (1) sometimes becomes complicated [12]. The maximum binding ratio of many surfactants to a protein obtained directly from experiments exceeds significantly the corresponding values of Γ_2^S obtained from the Scatchard plot. Eq. (1) derived for a 'single phase' system is strikingly similar to the Langmuir equation for adsorption of a surface-active substance at the interface of 'two phase solid–liquid, liquid–gas and liquid–liquid systems [8]. Modifications of the Scatchard equation for equivalent sets of binding sites, cooperative and non-cooperative interactions and

Table 1
Free energy of binding of anionic ligands to BSA [1]

Ligand	pH	Γ_2^S Moles of ligand/moles of BSA	$-\Delta G_S^0$ kJ/mol ligand	$-\Delta G^0$ kJ/kg BSA
Tetradecyl sulfate	5.6	10.5	34.4	5.3
Dodecyl sulfate	5.6	8.50	34.9	4.4
Decyl sulfate	5.6	5.50	35.2	2.8
Octyl sulfate	5.6	4.50	33.1	2.2
Dodecyl sulfonate	5.6	6.00	31.4	2.8
Decyl sulfonate	5.6	5.00	34.2	2.5
Octyl sulfonate	5.6	3.00	28.7	1.3

homotropic and heterotropic interactions with linked functions have been developed specifically and have [12] become useful for understanding the complex interactions in many systems.

Values of Γ_2^S and ΔG_S^0 obtained for a series of long chain sulfates and sulfonates to serum albumin [1] are presented in Table 1. The observed increase of Γ_2^S with increase of hydrocarbon chain length of the ligand presumably results as a consequence of the hydrophobic effect. However, this regular behaviour is not reflected in the values of ΔG_S^0 which are insensitive to the chain length effect. By multiplication of ΔG_S^0 with 1000 Γ_2^S/M_p, the standard free energy change ΔG^0 per kilogram of protein forming saturated complex may be obtained. Values of ΔG^0 are now observed to increase regularly with an increase of Γ_2^S, the significance of which will be discussed later on. Here M_p stands for the molecular weight of protein.

3.1. The Bull equation

In part 1 of this paper (two phase systems), we have mentioned that in many types of binding experiments, protein molecules regarded as biocolloids are in contact with an aqueous phase containing solvent and solute (or ligand) components 1 and 2, respectively. Component 2 from the aqueous phase is transferred to the boundary region of the protein biocolloid whereby binding of component 2 occurs at the protein–water interface. Following the Gibbs approach of writing the Gibbs–Duhem equations for the free bulk and surface phases, Bull [17, 18] has derived an elegant equation for the change in free energy ΔG due to the transfer of Γ_2^m moles of component 2 from the aqueous solution to the boundary region of the protein. This equation reads

$$\Delta G = -RT\left[\int_0^{a_2^m} \frac{\Gamma_2^1}{a_2}\, \mathrm{d}a_2 + \Gamma_2^m \ln\frac{a_2^{st}}{a_2^m}\right]. \tag{4}$$

Here a_2 is the activity of the solute component 2 in bulk at a particular state of equilibrium and a_2^{st}, is the value of it at its hypothetically selected standard state of reference. Since the solution of a ligand is dilute, a_2 at a given value of Γ_2^1 may be replaced by the molar concentration C_2 (or molal concentrations m_2). Γ_2^m in Eq. (4) represents the maximum amount of a ligand bound per kilogram of protein when its activity in solution attains the value a_2^m. For dilute solution, a_2^m is equal to C_2^m, molar concentration in the bulk for the attainment of the value Γ_2^m. In Eq. (4), a_2^{st} is taken as unit molar concentration of a ligand in a practical scale of bulk activity. In deducing Eq. (4) for ΔG, Bull [17,18] has also assumed that Γ_2^m remains hypothetically constant as a_2 is increased from C_2^m to its standard state of unit molar concentration in the bulk phase. Bull and Breese [36] have calculated the free energy of binding of guanidine chloride to egg albumin using the molal instead of the molar scale of activity. In part 1 of this paper [20] a slightly modified derivation of Eq. (4) based on two phase models has been presented [see Eqs. (17), (35) and (36)].

The integral part of Eq. (4) can be solved using measured values of Γ_2^1 at different values of C_2 (or m_2) by using an appropriate computer package until Γ_2^1 attains the maximum value Γ_2^m when C_2 becomes equal to C_2^m. Values of ΔG in the unit kJ/kg of protein can be estimated from the experimental data in the range of C_2 (or m_2) equal to zero to unity. Like Scatchard, Bull [17,18] has expressed the standard free energy change ΔG_B^0 for the transfer of 1 mol of ligand from the bulk to the protein bound phase using the relation

$$\Delta G_B^0 = \frac{\Delta G}{\Gamma_2^m}. \tag{5}$$

Unlike the Scatchard equation, the Bull equation does not require linear variation of $1/\Gamma_2^1$ with $1/C_2$ or other similar relations so that direct evaluation of ΔG_B^0 from the experimental data may have more general application. Γ_2^1 in the Bull equation represents the Gibbs excess quantity per kilogram of protein so that effect of competitive interaction of both ligand and water for the occupation of protein binding sites is implicity involved in the values of ΔG_B^0. It shall be seen later on in the paper that ΔG_B^0 will also be meaningful even when Γ_2^1 is negative in sign. In the original derivation of the Scatchard equation [11], the role of solvent in the binding process has not been explicitly taken into account. It will be of interest to note that when the integral in Eq. (4) for an ideally cooperative process becomes negligible, $\Delta G/\Gamma_2^m$ in Eq. (3) representing ΔG_B^0 becomes equal to $+RT$ ln $a_2^m \cdot \Delta G_B^0$ under this condition for interaction of the surfactant with protein becomes equal to ΔG_T^0, the standard free energy change for massive cooperative interaction of the surfactant with a protein. This relation for the massive interaction has been independently derived by Tanford [19] from the concept of micelle formation in the surfactant bound phase of protein. ΔG_B^0 in general represents the free energy change when 1 mol of the surfactant is transferred from the bulk to the protein bound phase (forming a saturated complex) due to the change of the activity of the ligand from zero to unity (in molar scale) in the bulk aqueous phase.

Values of ΔG_S^0 and ΔG_B^0 both expressed in the molar scale have been compared by Sen et al. [21] for binding of the cationic surfactants cetyl trimethyl ammonium bromide (CTAB), myristyl trimethyl ammonium bromide (MTAB), dodecyl trimethyl ammonium bromide (DTAB) and the anionic surfactant sodium dodecyl sulphate (SDS), respectively to BSA at different physicochemical conditions. They have also noted that the Scatchard linear plot remains valid only when C_2 values are low and in agreement with the same observations made by other authors [37]. Values of ΔG_B^0 are always observed by Sen et al. [21] to be higher than those of $\Delta G_S^0 \cdot \Delta G_B^0$ derived on the basis of the Gibbs adsorption equation which involves the free energy changes, ΔG_{int}^0 ΔG_{el}^0, ΔG_{conf}^0 and ΔG_{hy}^0 per kilogram of protein due to the intrinsic binding interaction, electrostatic effect, conformational change and protein hydration–dehydration effects etc. whereas ΔG_S^0 contains only the ΔG_{int}^0 term. Values of ΔG_B^0 for the surfactant–protein interaction for BSA and many other proteins have been evaluated from the extensive equilibrium dialysis data [21–26]. This will be discussed in a subsequent section.

3.2. ΔG^0 for a multicomponent system

Binding interaction of a ligand to a protein usually occurs in an aqueous phase whose pH and ionic strength are maintained by the addition of neutral salt and weak acid or alkali. Most of the globular proteins spontaneously dissolve in water so that the free energy of mixing becomes negative. On the other hand powdered solids and colloids on dispersion in the aqueous phase form a large surface area at the boundary between the particles and the aqueous solution whereby the free surface energy of the system will significantly increase and the dispersion process will become non-spontaneous. Application of Eq. (4) based on the Gibbs adsorption equation involving a 'two phase' model may not be acceptable for a protein–surfactant binding interaction in aqueous solution unless this equation is shown to be valid for the binding process occurring in a 'single phase' system.

In part 1 of this paper, we have shown from an elaborate mathematical analysis that Eq. (4) will remain valid for the binding of a ligand to protein forming a single phase provided the solution contains a solvent and a non-electrolyte solute component. We have also shown [20] that for a three component single phase system (protein + ionic surfactant RNA + neutral salt NaCl), the equation for the standard free energy change reads [20]

$$\Delta G^0 = -mRT\left[\int_0^{\times_2^m} \frac{\Gamma_{R-}}{X_{R-}}\, \mathrm{d}X_{R-} - \Gamma_{R-}^m \ln X_{R-}^m\right], \tag{6}$$

where X_{R-} stands for mole fraction of the sur-

factant ions whose value for the dilute solution is equal to $C_2/55.5$. Furthermore, for the derivation of this equation, the concentration (C_3) of the neutral salt should be high and constant so that the ratio C_2/C_3 is negligible and the value of the coefficient m [see Eq. (20) part 1] becomes unity. ΔG^0 in this equation represents the standard free energy change per kilogram of protein for the formation of a surfactant–protein saturated complex when the bulk mole fraction of the surfactant is altered from zero to unity in the rational scale of activity. Values of Γ_{R-} at different values of X_{R-} are known from experiments. From the graphical plot of Γ_{R-} against X_{R-}, maximum values of Γ^m_{R-} can be evaluated at a critical value of X^m_{R-} (equal to $C^m_{R-}/55.5$). The integral in Eq. (6) can thus be evaluated using computer analysis. Values of ΔG^0 in kilojoules per kilogram of bovine serum albumin [21,22] for binding of CTAB, MTAB, DTAB and SDS measured from an equilibrium dialysis technique used by Sen et al. [21,22] are given in Tables 2 and 3 along with the corresponding experimental values of Γ^m_{R-}. From these tables, one notes with interest that magnitudes of ΔG^0 increase regularly with increase of the maximum binding ratio Γ^m_{R-} of the protein directly obtained from experiments carried out at varying temperatures, ionic strength and pH. In Fig. 3, values of ΔG^0 for binding interaction of these surfactants to BSA have been found to vary linearly with Γ^m_{R-}. The slope of this plot represents ΔG^0_B, the standard free energy change [see Eq. (5)] for the transfer of 1 mol of surfactant from the bulk to the protein bound

Table 2
Binding interaction parameters for interaction between BSA and cationic surfactants [21]

μ, ionic strength or M, molarity	pH	Temp (°C)	Γ^m_{R-} Moles of surfactant/kg BSA	$-\Delta G^0$ kJ/kg of BSA	$-\Delta G^0$ kJ/mol of surfactant
CTAB					
0.125	6.0	65	12.3	413	33.6
0.125	6.0	45	0.222	8.03	36.2
0.125	6.0	30	0.218	7.72	35.5
0.0125	6.0	30	0.109	4.10	37.7
0.0625	6.0	30	0.0868	3.31	38.1
6 M urea	6.0	30	0.381	13.3	34.9
6 M urea + 0.1 M NaCl	6.0	30	0.185	6.15	33.2
0.125 NaCl	4.0	30	0.0427	1.62	37.9
0.125 NaCl	8.0	30	0.0838	3.24	38.6
0.1 M $CaCl_2$	6.0	30	0.152	5.51	36.4
0.1 M NaCl	6.0	30	0.0735	2.84	38.6
0.1 M LiCl	6.0	30	0.0985	3.62	36.7
0.1 M Na_2SO_4	6.0	30	0.166	5.87	35.3
0.1 M KCl	6.0	30	0.0132	0.463	35.0
0.1 M KBr	6.0	30	0.138	4.63	33.5
MTAB					
0.125	6.0	65	3.46	109	31.5
0.125	6.0	30	0.591	18.7	31.7
DTAB					
0.125	6.0	65	4.99	179	36.1
0.125	6.0	30	0.162	5.44	33.6

Table 3
Parameters for binding interaction between BSA and SDS [22]

pH	Ionic strength (μ)	Temp. (°)	Γ^m_{R-} Moles of SDS/kg BSA	$-\Delta G^0$ kJ/kg BSA	$-\Delta G^0_{hi}$ kJ/kg BSA	$-\Delta G^0_{ag} = (\Delta G^0_{hi} - \Delta G^0)$ kJ/kg BSA
6.0	0.625	30	3.12	105	551	390
6.0	0.125	30	1.96	67.9		
6.0	0.0625	30	2.57	86.5	439	353
6.0	0.0125	30	0.471	16.4	407	390
4.0	0.125	30	3.50	122		
4.0	0.625	30	1.31	43.8		
4.0	0.0125	30	1.46	51.5	670	619
4.0	0.125	65	2.25	88.9		
6.0	0.125	45	1.13	44.1	617	573
6.0	0.125	65	1.19	42.4	545	503
6.0	0.125	15	0.265	9.15	1630	1620
6.0	0.1 M NaCl	30	2.32	75.4	522	447
6.0	0.1 M NH_4Cl	30	2.46	83.4	842	759
6.0	0.1 M LiCl	30	0.588	18.1	662	644
6.0	0.1 M Na_2SO_4	30	1.44	50.8	420	369

phase due to the change of X_{R-} from zero to unity in the rational scale [20–26] so that one can write,

$$\Delta G^0 = \Gamma^m_{R-} \Delta G^0_B. \quad (7)$$

ΔG^0 representing maximum free change per kilogram (or per mole) of protein is the product of ΔG^0_B and Γ^m_{R-}, the maximum number of moles of binding sites per kilogram (or per mole) of protein. Unlike ΔG^0, ΔG^0_B the Bull free energy change per kilogram of protein in the mole fraction scale appears to be invariant with alteration of the value of Γ^m_{R-} for various systems (see Table 2).

Using Eq. (6), values of ΔG^0 for binding CTAB, MTAB, DTAB, and SDS to gelatin, casein and myosin, respectively have been calculated [24–26]. Some of their values are presented in Table 4 along with the measured values of Γ^m_{R-}. Corresponding values of ΔG^0_B for each system calculated from Eq. (7) have also been presented in these tables. For each protein, the value of ΔG^0_B is observed to remain constant in the limit of experimental error (see Table 4) which means also that $-\Delta G^0$ for each protein varies linearly according to Eq. (7).

From the analysis of the results, presented in our papers [24–26], we further note with interest that the values of ΔG^0_B for binding cationic and anionic surfactants to BSA, gelatin, casein and myosin vary from 32 to 37 kJ/mol of bound surfactants whereby saturated protein–surfactant complexes of various types are formed (see Tables 2–7). The average value of ΔG^0_B in all these cases may be taken as 35 kJ/mol surfactant in the limits of experimental error.

Molecules of serum albumin are rigid and ellipsoidal-shaped whereas those of myosin are known to be rigid and rod-shaped. Gelatin molecules dissolved in the aqueous solution on the other hand behave as random-coiled denatured protein. Milk protein casein is also extensively unfolded in solution at alkaline pH but it remains insoluble in solution in acidic pH. The isoelectric points of these different proteins are different from each other. At a given pH, the net charge of different proteins must be different from each other. At a given pH, far from the isoelectric point, the potential of the double layer surrounding each protein increases with the decrease of the ionic strength the medium. The conformation of unfolded protein alters significantly with alteration of the ionic strength. Binding of cationic and

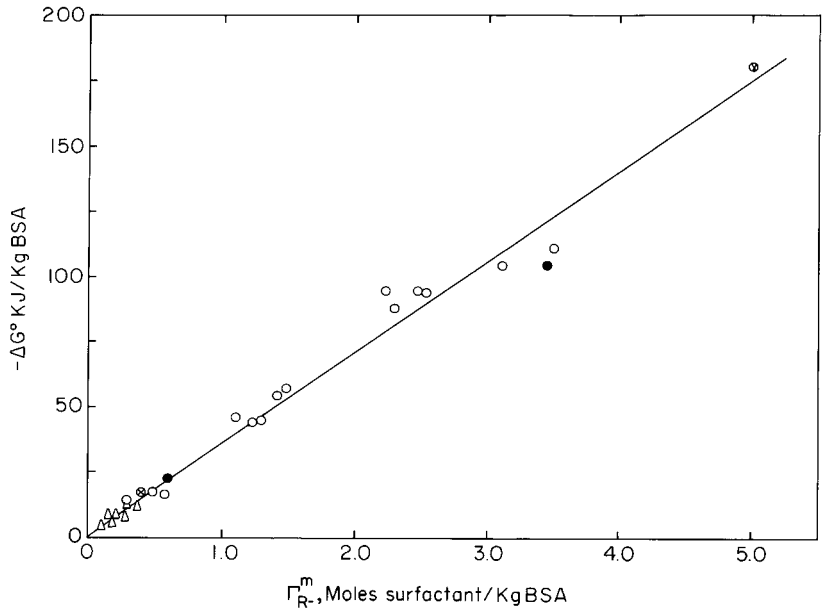

Fig. 3. Plot of ΔG^0 vs. Γ_R^m for binding of surfactants to BSA at different values of pH, ionic strength and temperature (see [21,22]); SDS (○), CTAB (△), DTAB (◇), MTAB (●).

Table 4
Parameters for protein–surfactant binding interaction; $\mu = 0.125$, pH 6.0 [24,26,27]

Protein–surfactant complex (saturated)	Temp (°C)	Γ_{R-}^m Moles of surfactant/ kg protein	$-\Delta G^0$ kJ/kg protein	$-\Delta G_B^0$ kJ/mol surfactant
Gelatin–CTAB	30	0.680	21.8	32.1
Gelatin–MTAB	30	0.210	6.30	30.0
Gelatin–DTAB	30	0.450	13.4	29.9
Gelatin–SDS	30	3.22	102	31.9
Casein–CTAB	30	0.448	16.4	36.6
Casein–CTAB	30	0.989	36.1	36.5
Casein–CTAB	45	0.522	19.1	36.6
Casein–MTAB	30	1.14	44.5	39.0
Casein–DTAB	30	0.487	17.6	36.1
Casein–SDS	30	1.02	36.8	36.1
Myosin–CTAB	27	0.620	2.44	39.4
Myosin–CTAB	45	0.10	3.99	39.9
Myosin–MTAB	27	0.353	11.1	31.4
Myosin–SDS	27	1.57	46.6	29.7

anionic surfactants certainly become responsible for the unfolding of BSA and myosin to different degrees. During binding of CTAB to BSA at 65°C or that in the presence of 6.0 M urea concentration (see Table 2), the conformation of native BSA molecules are expected to alter grossly. Further orientation of different surfactants bound to different proteins having varied conformations must be significantly different from each other.

Because of all these reasons, values of Γ_{R-}^m and also ΔG^0 for different types of protein–surfactant complexes at different values of pH, ionic strength and temperature widely differ from each other (see Tables 2–6). However, the most elegant and interesting observation is that the values of $-\Delta G^0$ increase linearly in all cases with an increase in Γ_{R-}^m and the average value of the unique slope of this line is 35 kJ/mol of surfactant.

Thus the order in the values of maximum affinities (Γ_{R-}^m) of different surfactants for the formation of saturated complexes with different proteins having different conformations and structure linearly varies with the order of $-\Delta G^0$ following a universal general scale of thermodynamics. The values of $-\Delta G_B^0$ in this linear varia-

Table 5
Parameters for binding interaction of surfactants to a binary protein mixture; $\mu = 0.125$, pH 6.0 [23,24]

Protein–surfactant complexes	Temp (°C)	Γ^m_{R-} Moles of surfactant/ kg of protein	$-\Delta G^0$ kJ/kg protein	$-\Delta G^0_B$ kJ/mol surfactant
Gelatin + Casein + CTAB P.wt. ratio 1:1	30	0.221	1.38	33.3
Gelatin + Casein + SDS P.wt. ratio 1:1	30	0.422	16.2	38.4
BSA + Gelatin + SDS P.wt. ratio 1:1	30	0.520	18.9	36.3
BSA + Gelatin + CTAB P.wt. ratio 1:1	30	0.270	8.82	32.7
BSA + Casein + CTAB P.wt. ratio 1:1	30	0.260	9.08	34.9
Myosin + BSA + CTAB P.wt. ratio 1:1	27	0.207	6.78	32.8
Myosin + BSA + SDS P.wt. ratio 1:1	27	1.22	37.6	30.8
BSA–Myosin–CTAB P.wt. ratio 3:1	27	0.144	4.76	33.1
BSA–Myosin–SDS P.wt. ratio 3:1	27	1.11	32.4	29.2
BSA–Myosin–CTAB P.wt. ratio 2:1	27	0.155	5.32	34.3
BSA–Myosin–SDS P.wt. ratio 2:1	27	1.20	37.2	31.0
BSA–Casein–CTAB P.wt. ratio 2:1	30	0.173	5.98	34.6

tion is according to Eq. (7) numerically equal to −35.0.

Using the equilibrium dialysis technique, the binding capacity (Γ^m_{R-}) of different cationic and anionic surfactants to binary mixtures of gelatin and BSA, BSA and casein, gelatin and casein, myosin and BSA, myosin and gelatin mixed in different weight ratios have been estimated at various values of pH, ionic strength and temperature [23,24]. Values of ΔG^0/kg of protein mixture have also been calculated using Eq. (6). Some of the values of and for these mixtures are presented in Tables 5 and 6 along with values of ΔG^0_B calculated using Eq. (7). Average values of ΔG^0_B (see Table 7) are close to 35 kJ/mol of the surfactant so that the universal scale of standard free energy change per kilogram of protein mixture as discussed for individual proteins appears to remain valid. The measurement of Γ^m_{R-} values have also been carried out for ternary mixtures of gelatin, casein, BSA and myosin [23,24]. The values ΔG^0 for different weight ratios of mixtures have been calculated using Eq. (6). Values of ΔG^0 for binary and ternary mixtures of protein in the presence of a surfactant includes a term for the free energy changes ΔG^0_1 for protein–protein interactions whose values can be evaluated [23,24] from experimental data. Average values of ΔG^0_B in such complex cases also calculated from Eq. (7) are close to 35 kJ/mol surfactant. It is now clear to us why ΔG^0 in the practical scale of activity (Table 1) increases regularly with Γ^S_2 and further the average values of ΔG^0_S in the practical scale of activity is close to 33 kJ/mol ligand. This value is slightly lower than that obtained from using Eq. (7) based on a rational scale.

Prior to the states of binding saturation, for any value of Γ_{R-} lower than Γ^m_{R-} (see Fig. 1) the

Table 6
Parameters of binding interaction between ternary protein mixture and different surfactants; pH 6.0, $\mu = 0.125$

Protein–surfactant complex	Γ^m_{R-} moles of surfactant/ kg of protein	$-\Delta G^0$ kJ/kg of protein	$-\Delta G^0_B$ kJ/mol of surfactant
Casein + BSA–Gelatin + CTAB P. Wt. ratio (1:1:1), 30°C	0.180	6.11	33.9
Casein + BSA + Gelatin–SDS P. Wt. ratio 1:1:1, 30°C	0.632	24.8	39.2
Casein + BSA + Gelatin + CTAB P. Wt. ratio 1:2:1, 30°C	0.301	10.9	36.1
Casein + BSA + Gelatin + SDS P. Wt. ratio 1:2:1, 30°C	0.704	27.4	38.9
Myosin + BSA + Gelatin–CTAB P. Wt. ratio 1:1:1, 27°C	0.117	4.26	36.4
Myosin + BSA–Gelatin–CTAB P. Wt. ratio 1:2:1, 27°C	1.36	40.0	29.4
Myosin + BSA + Gelatin + CTAB P. Wt. ratio 1:2:1, 27°C	0.073	2.85	39.0
Myosin + BSA + Gelatin + SDS P. Wt. ratio 1:2:1, 27°C	0.725	22.3	30.8

apparent standard free energy change ΔG^0_{ap} can be calculated using Eq. (6) written in the form: [21–26]

$$\Delta G^0_{ap} = -RT\left[\int_0^{\Gamma_{R-}} \frac{\Gamma_{R-}}{X_{R-}}\,\mathrm{d}X_{R-} - \Gamma_{R-}\,\ln X^m_{R-}\right]. \tag{8}$$

Here Γ_{R-} at a given value of X_R is assumed to remain hypothetically constant when X_R is further increased to unity. For different values of Γ_{R-}, values of ΔG^0_{ap} will be different. ΔG^0_{ap} for such systems have been observed to vary linearly with coverage of the $\Gamma_{R-}/\Gamma^m_{R-}$ fraction of binding sites of a protein or protein mixture (see Fig. 4) so that ΔG^0 for the hypothetically saturated protein–surfactant complex will be given by the equation [20–26]

$$\Delta G^0 = \frac{\Delta G^0_{ap}}{(\Gamma_{R-}/\Gamma^m_{R-})}, \tag{9}$$

or

$$\Delta G^0_{ap} = \Delta G^0 \frac{\Gamma_{R-}}{\Gamma^m_{R-}}. \tag{10}$$

The slope of the linear plot of ΔG^0_{ap} against $\Gamma_{R-}/\Gamma^m_{R-}$ for binding of cationic and anionic surfactants to different proteins and protein mixtures are always found to be equal to ΔG^0, the standard free energy change for the saturated complexes given in Table 3. All these results indicate that ΔG^0 for an actual saturated complex calculated from Eq. (6) is identical with that calculated for a hypothetically formed saturated

Table 7
Values of ΔG^0_B for binding of surfactants (CTAB and SDS) with proteins and their binary and ternary mixtures

Protein systems	$-\Delta G^0_B$ kJ/mol surfactant
BSA [21,22]	34.8 ± 2.3
Gelatin [26]	31.8 ± 3.1
Casein [25]	36.7 ± 2.2
Myosin [24]	33.8 ± 3.6
BSA + Gelatin [26]	37.4 ± 3.8
BSA + Casein [23]	36.0 ± 2.3
Gelatin + Casein [23]	37.1 ± 2.5
Myosin + BSA [24]	32.5 ± 2.3
BSA + Gelatin + Casein [23]	36.0 ± 3.8
BSA + Gelatin + Myosin [24]	33.9 ± 4.0

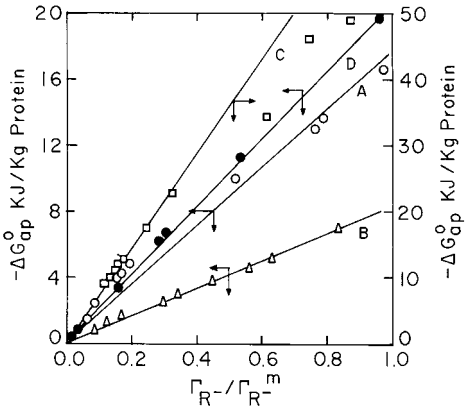

Fig. 4. Plot of ΔG^0_{ap} vs. $\Gamma_{R-}/\Gamma^m_{R-}$ for binding of surfactants to proteins at pH 6.0, ionic strength $\mu = 0.125$ and at 30°C (see [25,21,22,29]). (A) Casein-–CTAB; (B) BSA-–CTAB; (C) BSA–SDS; (D) Gelatin-–CTAB.

complex through Eq. (9) and Eq. (10) when the complex is actually unsaturated [25,26] below X^m_{R-}. From Eq. (10),

$$\frac{\Delta G^0_{ap}}{\Gamma_{R-}} = \frac{\Delta G^0}{\Gamma^m_{R-}} = \Delta G^0_B. \tag{11}$$

This also means that the Bull free energy changes ΔG^0_B expressed in kJ/mol of a surfactant unit for saturated and unsaturated complexes for various systems are close to each other.

In our studies of binding of SDS to BSA, at a given ionic strength, temperature and pH, it has been observed that Γ_{R-} increases from zero to Γ^m_{R-} when X_{R-} increases from zero to Γ^m_{R-} (see Fig. 1). Values of ΔG^0 for such a system calculated with the help of Eq. (6) and Eq. (7) have been presented in Table 3. It has also been noted from Fig. 1 that as X_{R-} is further increased from Γ^m_{R-} so that C_2 is close to the CMC, Γ_{R-} for SDS increases sharply from Γ^m_{R-} without reaching any limiting value [22]. It is assumed by many authors [19] that protein is bound with micellar aggregates of surfactants at higher values of surfactant concentrations. The values of ΔG^0_{ap} in this range of higher concentrations of C_{R-} can be calculated using Eq. (8). In Fig. 5, ΔG^0_{ap} for each such system has been plotted against $1/\sqrt{X_{R-}}$ and its value has been extrapolated to X_{R-} equal to unity whereby the value of the standard free energy change ΔG^0_{hi} for such protein bound surfactant undergoing aggregation have been obtained. These values also included in Table 3 are much larger in magnitude than ΔG^0 obtained for smaller values of X_{R-}. We can then assume $\Delta G^0_{hi} - \Delta G^0$ as the standard free energy of aggregation (ΔG^0_{ag}) of surfactants in the protein bound phase in kJ/kg of a protein unit (see Table 3).

From all these discussions, we can conclude that measured values of Γ^m_{R-} for binding cationic and anionic surfactants, respectively to proteins and protein mixtures include the effects of conformational alterations, protein–protein interactions, surfactant association in the protein bound state, change in ion-atmosphere thickness associated with positively or negatively charged biopolymer, change of temperature etc. Therefore the values of ΔG^0 calculated from Eq. (6) includes all the effects also included in the measured values of Γ^m_{R-}.

From the recent measurement of binding of CTAB and SDS to BSA, lysozyme, hemoglobin and gelatin, respectively [35] at different physicochemical conditions, values of Γ^m_{R-} for protein surfactant complexes are found at a lower range of X_R to vary linearly with ΔG^0 calculated from Eq. (6). Using Eq. (7), Γ^m_R so that using value of ΔG^0_B is observed to be 35 kJ/mol surfactant. Near CMC, aggregation of protein bound surfactant occurs in all cases. Using Eq. (8), values of ΔG^0_{ag} in all cases have been evaluated.

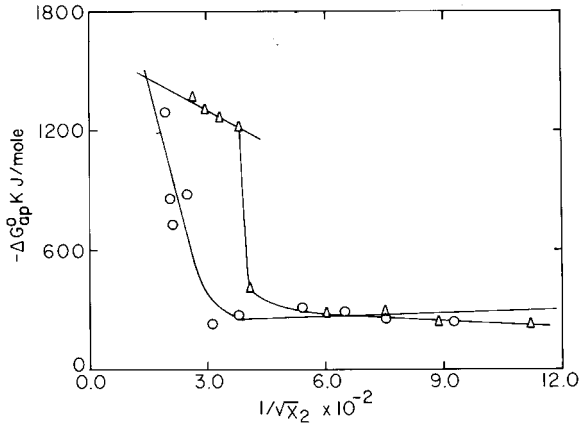

Fig. 5. Plot of ΔG^0_{ap} vs. $1/\sqrt{X_2}$ for binding of SDS to BSA at pH 6.0 and 30°C. $\mu = 0.625$ (○); $\mu = 0.0625$ (Δ).

4. Binding interaction of water and solute to proteins

Following isopiestic vapor pressure technique, the excess binding of inorganic salts to many proteins and protein mixtures [28–34] have been determined at different concentrations of electrolytes dissolved in aqueous media. Concentrations of the salts in contact with proteins are considerably high (ranging from 1.0 to 6.0 molal or more). Both electrolytes and water compete here for the occupation of binding sites of protein. Values of Γ_2^1 are calculated from direct use of Eq. (1), and W_1^t, m_2^t and m_2 can be evaluated from isopiestic experiment [28–34].

From algebraic analysis, it has been shown in part 1 of this paper that Eq. (1) can be transformed into the form

$$\Gamma_2^1 = n_2^t - n_1^t \frac{m_2}{55.5}; \tag{12}$$

where n_2^t and n_1^t are total moles of solute and solvent associated per kilogram of protein and n_2/n_1 is equal to $m_2/55.5$.

Excess moles of water Γ_1^2 bound per kilogram of protein can be similarly calculated using Eq. (13)

$$\Gamma_1^2 = n_1^t - n_2^t \frac{55.5}{m_2}. \tag{13}$$

Combining Eq. (12) and Eq. (13) gives

$$\Gamma_1^2 = -\Gamma_2^1 \frac{55.5}{m_2}; \tag{14}$$

so that from measured values of Γ_2^1 using Eq. (1), corresponding values of Γ_1^2 can be calculated.

It has already been shown in part 1 [see Eq. (5) and Eq. (8)] that if out of n_1^t and n_2^t moles of solvent and solute, respectively, Δn_1 and Δn_2 moles of these components undergo chemical reaction with protein in the aqueous phase then

$$\Gamma_2^1 = \Delta n_2 - \Delta n_1 \frac{m_2}{55.5}; \tag{15}$$

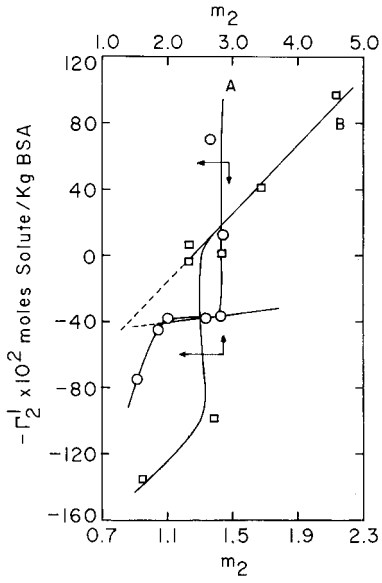

Fig. 6. Plot of Γ_2^1 vs. m_2 for hydration of BSA in the presence of LiCl and KBr at 25°C (see [28]). (A) LiCl; (B) KBr.

$$\Gamma_1^2 = \Delta n_1 - \Delta n_2 \frac{55.5}{m_2}. \tag{16}$$

In Figs. 6 and 7, binding isotherms of different inorganic electrolytes to BSA and a few other proteins based on isopiestic data have been presented. In these isotherms, we note that Γ_2^1 is positive (i.e. $m_2^t > m_2$) when the value of m_2 is very low. With an increase in m_2, the positive value of Γ_2^1 decreases until it becomes zero at an azeotropic state when

$$55.5 \frac{\Delta n_2}{\Delta n_1} = m_2. \tag{17}$$

According to Eq. (17) molality of salt in a protein-bound state at the azeotropic condition becomes equal to molality of the solute remaining free in solution. Values of the molality (m_2^{azeo}) at the azeotropic state for different electrolytes thus evaluated from Eq. (17) are presented in Tables 5 and 6. Values of m_2^{azeo} depend on various factors which need further examination in future.

At $m_2 > m_2^{\text{azeo}}$, values of Γ_2^1 are negative since m_2^t in Eq. (1) in such a situation becomes less than m_2. In terms of Eq. (15), $\Delta n_2 < \Delta n_1 \cdot m_2/55.5$ so that Γ_2^1 becomes negative. The nega-

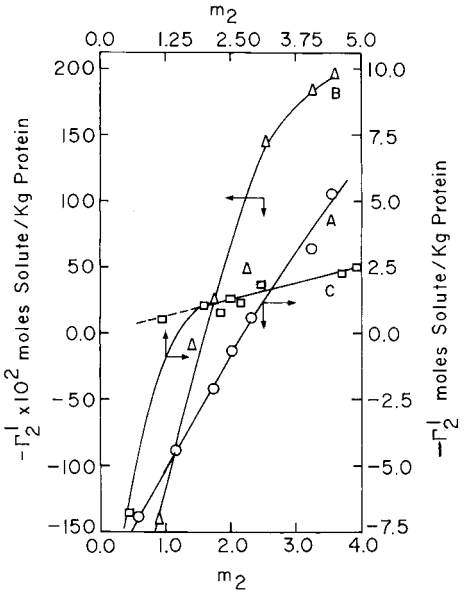

Fig. 7. Plot of Γ_2^1 vs. m_2 for hydration of proteins in presence of KCl. (A) Lysozyme (30°C) [34]; (B) Casein (25°C) [31]; (C) Myosin (27°C) [32].

tive value of Γ_2^1 is found to increase with further increase of m_2 (see Figs. 6 and 7). In most cases, $-\Gamma_2^1$ does not reach a limiting value even after significant increase of m_2.

From Figs. 6 and 7, we also note that in the intermediate range of m_2, Γ_2^1 varies linearly with m_2 for different electrolytes. Values of Δn_1 and Δn_2 can be evaluated [28–34] in such a situation using Eq. (15). These values for BSA, casein, β-lactoglubulin, gelatin, myosin and gelatin–BSA mixtures [28–34] are included in Tables 8 and 9. It appears that in the presence of electrolytes, $\Delta n_1 \gg \Delta n_2$ so that hydration rather than solute binding to protein is the major event in all such systems. Furthermore, in all these cases Γ_1^2 according to Eq. (14) becomes positive due to the excess or preferential hydration of protein in the presence of a neutral salt. The interaction of water and electrolyte and protein forming a multicomponent system is complex in nature so that values of Δn_1 for different systems do not strictly follow the rule for the lyotropic or Hoffmeister series [38]. It may also be pointed out here that due to the strong binding interaction between a surfactant and a protein in a multicomponent aqueous medium, the term $\Delta n_1 m_2/55.5$ is always negligibly smaller than Δn_2 so that Γ_2^1 is very close to Δn_2.

In part 1 of this paper [20], following the isopiertic method the expression for the standard

Table 8
Parameters for excess water binding to proteins in presence of different electrolytes [28,33]

Proteins	Neutral salt	m_2^{azeo}	ΔG^0 kJ/kg protein	Δn_1 Moles H_2O/ kg protein	Δn_2 Moles salt/ kg protein
BSA (at 25°C)	NaCl	0.152	15.9	5.84	0.016
	KCl	0.482	30.9	10.7	0.093
	LiC1	–	25.3	2.87	0.535
	Na_2SO_4	0.431	82.4	32.9	0.256
	$CaCl_2$		55.9	–	–
	KBr	2.61	33.8	22.1	1.04
	KI	6.23	55.9	37.8	4.24
BSA + /Gelatin mixed in wt. ratio (0.5:0.5) at 37°C	$CaCl_2$	1.63	47.0	119	3.54
	LiCl	1.89	28.0	104	3.54
	Na_2SO_4	1.16	210	74.6	1.56
	KBr	2.26	47.0	60.4	2.46
	KCl	2.05	64.0	48.5	1.79
	Kl	2.37	34.0	32.8	1.40
	NaCl	2.65	62.0	11.5	0.55

Table 9
Parameters for excess water binding to proteins in presence of different electrolytes

Proteins	Neutral salt	m_2^{azeo}	ΔG^0 kJ/kg protein	Δn_1 Moles H_2O/ kg protein	Δn_2 Moles salt/ kg protein
Casein (at 25°C) [31]	$CaCl_2$	2.03	38.0	52.0	1.90
	Na_2SO_4	1.23	155	63.0	1.40
	NaCl	2.03	38.0	41.0	1.50
	KSCN	2.11	118	105	4.00
	KCl	1.51	74.0	59.0	1.60
β-lactoglobulin (at 25°C) [29]	NaCl	4.09	16.7	19.8	1.46
	KCl	2.09	88.9	60.6	2.29
	LiCl		55.6	–	–
	Na_2SO_4		96.1	–	–
Myosin (at 27°C) [32]	LiCl	2.28	89.0	34	1.4
	KCl	–	62.0	136	32.5
	NaBr	3.70	17.0	3.00	0.022
	KSCN	0.555	36.0	80.0	0.80
	NaSO	4.08	29.0	34.0	2.50
Lysozyme (at 30°C) [34]	NaCl	2.69	60.4	203	9.86
	KCl	1.51	92.1	340	9.28
	NaBr	2.49	161	96.4	4.32
	NaI	1.77	27.3	58.8	1.88
	LiCl	–	10.4	71.9	4.84
	KSCN	2.92	53.2	123	6.48

free energy change ΔG_{ap}^0 of a protein for different mole fractions ($X_\pm$) of an inorganic salt in bulk from zero to unity have been deduced which reads

$$\Delta G_{ap}^0 = -RT(\nu_+ + \nu_-) \times \left[\int_0^{X_\pm} \frac{\Gamma_2^1}{f_\pm X_\pm} \mathrm{d}f_\pm X_\pm - \Gamma_2^1 \ln(f_\pm X_\pm)\right]. \quad (18)$$

Here $f_\pm$ is the mean activity coefficient in the rational scale whose value for a given value of $X_\pm$ can be obtained from standard tables [39]. Values of the mean mole fraction $X_\pm$ of an electrolyte can also be computed from the experimental value of m_2 [20]. Here ν_+ and ν_- are stoichiometric coefficients of cations and anions, respectively formed as a result of the complete dissociation of a salt molecule in water.

ΔG_{ap}^0 for different values of $X_\pm$ will be different. Its sign will be positive if Γ_2^1 is negative and vice versa. In Fig. 8, ΔG_{ap}^0 has been plotted against $1/\sqrt{X_\pm}$ and from the linear extrapolation of the plots, values of ΔG^0 for $X_\pm$ equal to unity have been evaluated. ΔG^0 represents the standard free energy change in kilojoules per kilogram of protein when mean concentrations of an electrolyte in the mole fraction unit is altered from to zero to unity. Values of ΔG^0 for different systems have been presented in Tables 5 and 6.

From these tables, one finds that values of ΔG^0 in all cases are positive since measured values of Γ_2^1 are negative. Negative values of Γ_2^1 indicate that at the standard state of unit activity, excess solute binding is negative so that excess solvent binding in all cases are positive [see Eq. (14)]. Due to this positive excess binding of water to protein, ΔG^0 for the solute binding appears to be non-spontaneous. The standard free energy change ΔG_{hy}^0 for the excess hydration of protein due to the change of $X_\pm$ from zero to unity may

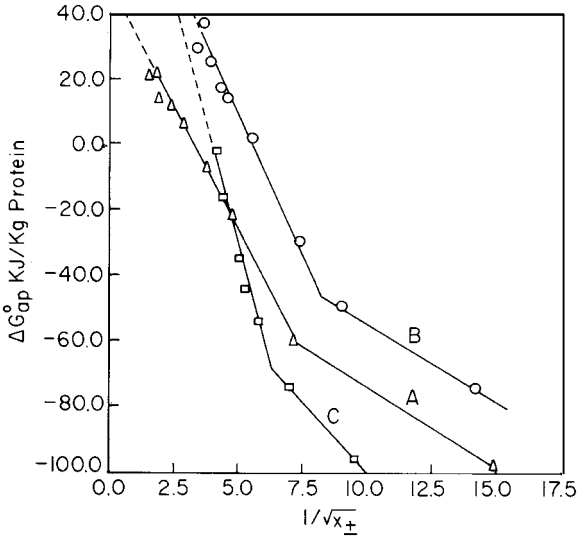

Fig. 8. Plot of ΔG^0_{ap} vs. $1/\sqrt{X_{\pm}}$ for hydration of protein. (A) Lysozyme in the presence of KCl (see [34]); (B) Gelatin in the presence of KCl at 37°C [33]; (C) gelatin + BSA in wt. ratio (1:1) in the presence of NaCl [33].

be related to ΔG^0 since from Eq. (8) and Eq. (11) of part 1 of this paper [20], it can be easily be shown that

$$d\mu_2 = -\frac{n_1^0}{n_2^0} d\mu_1;$$

and

$$-dG_p^i = \Gamma_1^2 d\mu_1 = \Gamma_2^1 d\mu_2. \quad (19)$$

Now

$$\Delta G^0_{hy} = -\int_0^{X_1=1} \Gamma_2^1 d\mu_1 = +\int_1^{X_1=0} \Gamma_1^2 d\mu_1$$
$$= \int_0^{X_2=1} \Gamma_2^1 d\mu_2 = -\Delta G^0; \quad (20)$$

so that by multiplying by ΔG^0 by (-1) in Table 5 and 6, values of ΔG^0_{hy} are found to be all negative. A negative value of ΔG^0_{hy} indicates that in contrast to the excess negative binding interaction of salt with protein, excess hydration is a spontaneous process. Increased order in the magnitude of $-\Delta G^0$ (or ΔG^0_{hy}) presented in Tables 5 and 6 represents increased relative affinities of proteins for water in the presence of different inorganic salts.

We have thus observed in general that when the surfactant or inorganic salt concentration in the solvent is zero, the value of ΔG^0 is zero. As the surfactant concentration in the solvent is increased from zero to the unit mole fraction, values of ΔG^0 which are all negative increase for different systems systematically with increased relative affinities of surfactant to proteins and protein mixtures. On the other hand, with increased addition of inorganic salts to the solvent, values of ΔG^0 become positive because of the decrease in relative affinities of salt to protein or because of the excess hydration of protein. The order of the magnitude of ΔG^0 represents gradual and systematic decrease in affinities of salts to protein. Since ΔG^0 in all situations refers to the same final state of reference (i.e. $X_2 = 1$) values of ΔG^0 are strictly comparable in this well defined unified scale of free energies for interaction of different organic and inorganic solutes to proteins.

We wish to point out that similar reference scales for the values of ΔG^0_S and ΔG^0_B for positive and negative binding of solute and solvent to protein do not exist. These values actually refer to the free energy for the transfer of 1 mol of ligand from the bulk aqueous system to the protein by chemical reaction but they do not include the number of available sites of protein for excess binding interaction with surfactants or neutral salts in the presence of water. However, ΔG^0 includes both binding free energy change per mole of ligand or salt and maximum binding sites available for the protein itself at $X_2 = 1$. Γ_2^m depends implicity on the orientation of the bound ligand and competitive interaction of water, the electrostatic repulsion effect and the tendency of self association of the bound substance for reaction with protein. The units of ΔG^0 and ΔG^0_B are different for all these reasons.

There exists a slight difference in the scale of ΔG^0 for the excess positive binding interaction of surfactant and excess negative interaction of inorganic salts with proteins. In the case of inorganic salts, the solvent is pure water whereas for surfactant solution, the solvent is buffer solution of

constant ionic strength. We assume that ΔG^0 remains unchanged when at the unit mole fraction of surfactant, the standard free energy change for dilution of the solute (forming buffer) reversibly from experimental to zero ionic strength is negligibly small. Under this condition, the free energy scale for positive excess binding of surfactant and negative excess binding of inorganic salt becomes strictly identical.

From this critical analysis, it may be concluded that a general scale for the evaluation of the standard free energy for positive binding of surfactant and negative binding of inorganic salt, respectively to protein or protein mixture can be obtained from the appropriate use of Eq. (6) and Eq. (18) to the experimental data. For positive binding of SDS and CTAB to different types of protein and protein mixtures, the value of ΔG^0 at unit solute activity can represent their maximum affinity of the solute for the protein. For various types of neutral salts at unit solute activity, ΔG^0 actually represents excess affinities of water for various proteins.

Acknowledgements

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