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Biophysical Chemistry 63 (1996) 37–45

Biophysical Chemistry

Standard free energies of binding of solute to proteins in aqueous medium [1]. Part 1: Thermodynamic analysis for multicomponent system

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Received 4 December 1995; revised 5 April 1996; accepted 22 July 1996

Abstract

Using an equilibrium dialysis technique, moles (Γ_2^1) of cationic and anionic surfactants bound per kilogram of proteins of various types in aqueous media have been measured previously in this laboratory under different physicochemical conditions. From a thermodynamic analysis in the present paper, Γ_2^1 has been shown to be equal to the Gibbs relative excess of surfactant per kilogram of protein at a measured value of solute activity, a_2. The values of relative solvent excesses, Γ_1^2 (which are negative for surfactants) can be estimated from values of Γ_2^1 and a_2. Using the Gibbs–Duhem relationship for protein solution inside the dialysis bag and dialysate solutions respectively at equilibrium, an integrated expression for the standard free energy change, ΔG° (in kilojoules per kilogram of protein for binding with ligand as a result of the change of a_2 from zero to unity) can be calculated from experimental data. The isopiestic vapour pressure technique was used extensively for evaluation of negative binding ($-\Gamma_2^1$) of inorganic salts to proteins of different types for various values of a_2 of salts present in the bulk media. With some modifications of our derived equations for free energy of binding in such a system, ΔG° has been evaluated for the change of mean activity of electrolyte from zero to unity in the rational scale. ΔG° is positive since Γ_2^1 is negative and Γ_1^2 is positive for such ionic systems. ΔG° in all cases, however, are expressed in terms of the standard state of reference of unit activity so that their magnitudes and sign can be related to the relative affinities of a solute for binding with proteins in aqueous media.

Keywords: Binding interactions; Standard free energies; Thermodynamic analysis

1. Introduction

In living systems, binding interactions between biopolymers (such as proteins, nucleic acids and polysachharides) 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 [10,11]. The different tech-

[1] Part of this paper was presented at the Tenth International Symposium on Surfactants in Solution, Caracas, Venezuela, June 1994.

PII S0301-4622(96)02207-7

niques used for such study include equilibrium dialysis, measurement of surface tension, electrical conductivity and viscosity, electrophoresis and ultracentrifugation, gel filtration, ion-specific electrodes, solubilisation, 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.

Hayakawa and co-workers [12,13] have made extensive studies on the binding of cationic surfactants to a series of anionic polyelectrolytes under a variety of conditions, such as the variation of ionic strength, salt type and temperature. Thalberg and Lindman [14] have studied the phase behaviour and also the binding interactions of polyelectrolyte–surfactant systems using surface tension measurements, viscometry and conductometry.

The extents of binding of cationic and anionic surfactants to egg albumin, bovine serum albumin, gelatin, casein and myosin from solution have been measured extensively by previous workers [15–18] in this laboratory as a function of pH, ionic strength and temperature. The binding isotherms for most of these systems indicate an increase of surfactant binding to different proteins at low bulk concentrations of ligands, whereas the extent of binding approaches a limiting saturation value when the bulk concentration is high.

Measurements related to binding of water to a biopolymer by various physicochemical techqniques have been carried out earlier in the presence of neutral salts and denaturants. The concept of water binding itself becomes complex in these three-component systems. A useful thermodynamic concept of preferential hydration of a biopolymer has been developed by Schachman and Lauffer [19]. The concept has been further elaborated recently by several workers [20–27]. The treatment has been used to calculate the preferential interaction of water and solutes with proteins and nucleic acids using sedimentation, light scattering, densitometry and other techniques.

In the presence of many inorganic salts, urea and sucrose, the extents of hydration of proteins are observed to be significantly high. Negative excess binding of electrolytes as a result of excess hydration of BSA, haemoglobin, β-lactoglobulin, gelatin, casein, and myosin in aqueous media has been extensively studied in this laboratory using isopiestic vapour pressure techniques [28–30].

The thermodynamic theory of excess binding of solute or solvent at an interface was developed by Willard Gibbs [31] in 1876, and the physical meaning of this excess concept of binding adsorption has subsequently been elaborated by many workers [32–34]. Bull [35,36] used this concept for calculation of the free energy of adsorption of proteins at solid–liquid interfaces, and he also applied it for estimation of the standard free energy change for the binding interaction of guanidine hydrochloride with protein in aqueous media, with the assumption of the existence of a physical boundary between protein and water in the system [37]. An attempt will be made in the present paper to derive a general expression for the standard free energy change for competitive interaction of water and solute with a biopolymer on a thermodynamic basis and also to develop a universal thermodynamic scale for the comparison of the standard free energy changes for simultaneous interaction of water and solute with a biopolymer, forming either single-phase or two-phase systems. In the second and third papers of this series, the application of this theory to treat the experimental data on protein–surfactant binding and water–protein binding interactions will be discussed.

2. Equilibrium dialysis experiments

Using equilibrium dialysis experiments [15–18], the extents of binding of surfactants to different proteins are determined at various values of protein concentration, pH, ionic strength and temperature. In dialysis experiments, a definite volume (V_i ml) of solution containing W_p kg of protein, or sometimes a binary or ternary mixture of several proteins, is taken and the dialysis bag, carefully tied with thread, is dipped into a definite volume V_0 of the dialysis solution containing n_2^1 moles of surfactants per kg of protein. The ionic strength of the internal solution and that of the external surfactant solution maintained by a suitable buffer are the same. The whole system is stirred gently for 24 h or more until a state

of dialysis equilibrium is reached, and the equilibrium molar concentration c_2 of the external dialysate solution is estimated using the dye-partition technique. The total initial molar concentration of the surfactant in the whole system of total volume V^t (equal to $V_i + V_0$) may be taken as c_2^t prior to binding. The number of moles Γ_2^1 of surfactant bound per kg of protein is calculated using Eq. (1)

$$\Gamma_2^1 = \frac{1}{W_p}\frac{V^t}{1000}\left(c_2^t - c_2\right) \tag{1}$$

One important assumption involved in using Eq. (1) is that the binding of the solvent component 1 to the protein component p is effectively zero, so that Γ_2^1 represents the relative excess of the solute component 2 bound per kg of the biopolymer ($W_p = 1$) present in the system.

As the solution in the system is usually dilute, c_2^t and c_2 may be replaced by molal concentrations m_2^t and m_2 respectively of the solute component 2 in the presence and in the absence of protein in the system, so that

$$\Gamma_2^1 = \frac{W_1^t}{1000}\left(m_2^t - m_2\right) \tag{2}$$

Here V^t is replaced by the total weight W_1^t of the solvent component because the solution is dilute.

We know that m_2^t and m_2 in Eq. (2) can be replaced by $1000n_2^t/n_1^t$ and $1000n_2/n_1$ respectively, where n_1^t and n_2^t are the total moles of the solvent and solute components per kg of protein at dialysis equilibrium and are directly known. Also, n_1 and n_2 are moles of solvent and solute respectively in the system of volume V^t at binding equilibrium. One can replace W_1^t by $n_1^t M_1$, where M_1 is the molecular weight of the solvent component. Substituting the values of m_1^t, m_1 and W_1 in Eq. (2) one obtains

$$\Gamma_2^1 = n_2^t - n_1^t\frac{n_2}{n_1} \tag{3}$$

The Γ_2^1 in this expression actually represents the Gibbs relative excess of the surfactant bound per kg of protein (in the two-phase model), where the excess of solvent component 1 becomes zero by suitable placement of the Gibbs dividing plane. The superscript 1 in Γ_2^1 represents this condition. Eq. (3) can be written in the form

$$n_1^t - n_2^t\frac{n_1}{n_2} = -\Gamma_2^1\frac{n_1}{n_2} = \Gamma_1^2 \tag{4}$$

Here Γ_1^2 is the Gibbs excess of the solvent bound per kg of protein when the excess of component 2 becomes zero by suitable placement of the Gibbs dividing plane [32–34]. Γ_2^1 and Γ_1^2 are not independent but are related to each other by Eq. (4). If the value of Γ_2^1 determined from a binding experiment is positive, the value of Γ_1^2 calculated from Eq. (4) automatically becomes negative and fixed, and vice versa.

For a multicomponent solution one assumes that, out of n_1^t, n_2^t, n_3^t..... moles of components present per kg of the solution forming a single phase, Δn_1, Δn_2, Δn_3..... moles are undergoing reaction with 1 kg of the biopolymer component p, so that n_1, n_2, n_3..... moles of the components remain in the free state in the aqueous system. One can then write $n_1^t = n_1 + \Delta n_1$, $n_2^t = n_2 + \Delta n_2$, $n_3^t = n_3 + \Delta n_3$ etc. so that, inserting these expressions in Eq. (3), we find

$$\Gamma_2^1 = \Delta n_2 - \Delta n_1\frac{n_2}{n_1} \tag{5}$$

Similarly

$$\Gamma_3^t = n_3^t - n_1^t\frac{n_3}{n_1} = \Delta n_3 - \Delta n_1\frac{n_3}{n_1} \tag{6}$$

For the solution present inside the dialysis bag at equilibrium, we can write the Gibbs–Duhem equation in the form

$$n_1^i\mathrm{d}\mu_1^i + n_2^i\mathrm{d}\mu_2^i + n_3^i\mathrm{d}\mu_3^i + \ldots\ldots + n_p^i\mathrm{d}\mu_p^i = 0 \tag{7}$$

Here n_1^i, n_2^i, n_3^i..... are the total moles of water, surfactant and neutral salt components respectively present inside the bag in the single-phase mixture with n_p^i (or W_p/M_p) moles of the protein component and μ_1^i, μ_2^i, μ_3^i..... and μ_p^i are the corresponding chemical potentials. M_p stands for the molecular weight of the protein. The solutions inside and outside the bag form the aqueous phase but, because of the inaccessibility of protein from the inside through the dialysis membrane, an osmotic pressure Π will

be developed. The Gibbs–Duhem equation for the dialysate solution at equilibrium may be written as

$$n_1^\circ \mathrm{d}\mu_1 + n_2^\circ \mathrm{d}\mu_2 + n_3^\circ \mathrm{d}\mu_3 + \ldots\ldots = 0 \qquad (8)$$

where n_1°, n_2° and n_3° moles of water, surfactant and neutral salt components respectively are present in the dialysate solution per kg of protein at equilibrium and μ_1, μ_2 and μ_3 are their respective chemical potentials. We also know that [36]

$$\begin{aligned} \mu_1 &= \mu_1^i + \bar{v}_1 \Pi \\ \mu_2 &= \mu_2^i + \bar{v}_2 \Pi \\ \mu_2 &= \mu_3 + \bar{v}_3 \Pi \end{aligned} \qquad (9)$$

so that on differentiation

$$\begin{aligned} \mathrm{d}\mu_1 &= \mathrm{d}\mu_1^i + \bar{v}_1 \mathrm{d}\Pi \\ \mathrm{d}\mu_2 &= \mathrm{d}\mu_2^i + \bar{v}_2 \mathrm{d}\Pi \\ \mathrm{d}\mu_3 &= \mathrm{d}\mu_3^i + \bar{v}_3 \mathrm{d}\Pi \end{aligned} \qquad (10)$$

Here $\bar{v}_1$, $\bar{v}_2$ and $\bar{v}_3$ are the partial molar volumes of components 1, 2 and 3 respectively, and these may be taken as constant.

Inserting Eq. (10) in Eq. (7), we get

$$\begin{aligned} -n_p^i \mathrm{d}\mu_p^i &= n_1^i \mathrm{d}\mu_1 + n_2^i \mathrm{d}\mu_2 + n_3^i \mathrm{d}\mu_3 \\ &\quad - \left(n_1^i \bar{v}_1 + n_2^i \bar{v}_2 + n_3^i \bar{v}_3\right) \mathrm{d}\Pi \\ &= n_1^i \mathrm{d}\mu_1 + n_2^i \mathrm{d}\mu_2 + n_3^i \mathrm{d}\mu_3 - V_i \mathrm{d}\Pi \end{aligned} \qquad (11)$$

Here the volume V_i of the internal solution is equal to $n_1^i \bar{v}_1 + n_2^i \bar{v}_2 + n_3^i \bar{v}_3$.

We can replace $n_p^i \mathrm{d}\mu_p^i$ by $W_p \mathrm{d}G_p^i$, where G_p^i is the partial specific free energy of the protein. Also, from Eq. (8)

$$\mathrm{d}\mu_1 = \frac{n_2^\circ}{n_1^\circ}\mathrm{d}\mu_2 - \frac{n_3^\circ}{n_1^\circ}\mathrm{d}\mu_3 \qquad (12)$$

So that, combining Eq. (11) and Eq. (12)

$$-\left(\mathrm{d}G_p^i\right) = \frac{1}{W_p}\left[\left(n_2^i - n_1^i \frac{n_2^\circ}{n_1^\circ}\right)\mathrm{d}\mu_2 + \left(n_3^i - n_1^i \frac{n_3^\circ}{n_1^\circ}\right)\mathrm{d}\mu_3 - V_i \mathrm{d}\Pi\right] \qquad (13)$$

As $n_1^t = n_1^i + n_1^\circ$, $n_2^t = n_2^i + n_2^\circ$ and $n_3^t = n_3^i + n_3^\circ$ and $W_p = 1$, then inserting the values of n_1^i, n_2^i and n_3^i in Eq. (13) one finds

$$\begin{aligned} \left(\mathrm{d}G_p^i\right) &= \left[\left(n_2^t - n_2^\circ\right) - \left(n_1^t - n_1^\circ\right)\frac{n_2^\circ}{n_1^\circ}\right]\mathrm{d}\mu_2 \\ &\quad + \left[\left(n_3^t - n_3^\circ\right) - \left(n_1^t - n_1^\circ\right)\frac{n_3^\circ}{n_1^\circ}\right]\mathrm{d}\mu_3 \\ &\quad - V_1 \mathrm{d}\Pi = \left(n_2^t - n_1^t \frac{n_2^\circ}{n_1^\circ}\right)\mathrm{d}\mu_2 \\ &\quad + \left(n_3^t - n_1^t \frac{n_3^\circ}{n_1^\circ}\right)\mathrm{d}\mu_3 - V_i \mathrm{d}\Pi \end{aligned} \qquad (14)$$

As $(n_2^\circ)/(n_1^\circ)$ and $(n_3^\circ)/(n_1^\circ)$ are respectively equal to $(n_2)/(n_1)$ *and* $(n_3)/(n_1)$, Eq. (14) in combination with Eq. (3) and Eq. (6) leads to the relationship of Eq. (15)

$$-\left(\mathrm{d}G_p^i\right) = \Gamma_2^1 \mathrm{d}\mu_2 + \Gamma_3^1 \mathrm{d}\mu_3 - V_i \mathrm{d}\Pi \qquad (15)$$

Eq. (15) derived for the interaction of ligand with protein in the single aqueous phase has some close similarity to the Gibbs equation [33,34] for the adsorption of surfactant or other ligands at the surface formed at the contact point of two phases, which we shall discuss soon. Neglecting the term of osmotic pressure

$$-\left(\mathrm{d}G_p^i\right) = \Gamma_2^1 \mathrm{d}\mu_2 + \Gamma_3^1 \mathrm{d}\mu_3 + \ldots\ldots \qquad (16)$$

In the special case of a binary solution containing solute and solvent components besides protein, one can write

$$-\left(\mathrm{d}G_p^i\right) = \Gamma_2^1 \mathrm{d}\mu_2 \qquad (17)$$

In the experimental arrangement for equilibrium dialysis, c_2 for an anionic surfactant of type RNa is gradually increased, keeping c_3 of NaCl (or buffer) constant. By definition, μ_2 and μ_3 are equal to $(\mu_{R^-} + \mu_{Na^+})$ and $(\mu_{Na^+} + \mu_{Cl^-})$ respectively, so that Eq. (15) now assumes the form

$$\begin{aligned} -\left(\mathrm{d}G_p^i\right) &= \Gamma_{R^-} \mathrm{d}\mu_{R^-} + \left(\Gamma_{R^-} + \Gamma_{Cl^-}\right)\mathrm{d}\mu_{Na^+} \\ &\quad + \Gamma_{Cl^-} \mathrm{d}\mu_{Cl^-} - V_i \mathrm{d}\Pi \end{aligned} \qquad (18)$$

At constant concentration of NaCl, $\mathrm{d}\mu_{Cl^-}$ may be taken as zero for a dilute solution. Using Eq. (3) and Eq. (6) respectively, Γ_2^1 and Γ_3^1 may be shown to

be equal to Γ_{R^-} and Γ_{Cl^-} respectively. Eq. (18) may be written as

$$-\left(dG_p^i\right) = mRT\Gamma_{R^-} \cdot d\ln c_{R^-} - V_i d\Pi \qquad (19)$$

when m, the coefficient of RT, is given by Eq. (20)

$$m = 1 + \left(1 + \frac{\Gamma_{Cl^-}}{\Gamma_{R^-}}\right)\frac{c_{R^-}}{c_{Na^+}} \cdot \frac{dc_{Na^+}}{dc_{R^-}} \qquad (20)$$

Applying the electroneutrality condition for the dialysate solution

$$c_{Cl^-} + c_{R^-} = c_{Na^+} \qquad (21)$$

so that dc_{R^-} is equal to dc_{Na^+} at constant c_{Cl^-} and

$$\frac{c_{R^-}}{c_{Na^+}} = \frac{c_{R^-}}{c_{R^-} + c_{Cl^-}} = \frac{1}{1 + \dfrac{c_{Cl^-}}{c_{R^-}}} = \frac{1}{1 + \dfrac{c_3}{c_2}} \approx 0 \qquad (22)$$

if the ratio between the concentrations of NaCl and RNa is infinitely large. This is usually the case in all measurements carried out in this laboratory and elsewhere for binding experiments, so that m in Eq. (20) becomes unity and Eq. (19) on integration between c_2 being equal to zero and to 1 takes the form

$$\Delta G_p^\circ = \left(G_p^i\right)_{c_2=1} - \left(G_p^i\right)_{c_2=0}$$

$$= -RT\left[\int_{c_2=0}^{c_2=1} \frac{\Gamma_{R^-}}{c_{R^-}} dc_{R^-} - \frac{V_i}{RT}\int_{c_2=0}^{c_2=1} d\Pi\right] \qquad (23)$$

In most cases, the isotherms for binding of various surfactants to proteins indicate that Γ_{R^-} increases with increase of c_{R^-} until a maximum value $\Gamma_{R^-}^m$ is reached at a critical concentration $c_{R^-}^m$. In several cases, this critical condition is not reached under experimental conditions. A special procedure has been followed to obtain $\Gamma_{R^-}^m$ for such cases discussed elsewhere. When c_{R^-} is further increased from $c_{R^-}^m$, $\Gamma_{R^-}^m$ remains constant and $d\Gamma_{R^-}/dc_{R^-}$ becomes zero. This condition operationally represents the state of the saturation of binding reaction. Eq. (23) may then be written as

$$\Delta G_p^\circ = -RT\left[\int_0^{c_{R^-}^m} \frac{\Gamma_{R^-}}{c_{R^-}} dc_{R^-} - \Gamma_{R^-}^m \ln c_{R^-}^m - \frac{V_i}{RT}(\Pi_1 - \Pi_0)\right] \qquad (24)$$

As the concentration of protein is small and constant, Π_1 and Π_2 at c_{R^-} equal to 1 or to zero are small and their difference is negligible at high NaCl concentration, so that

$$\Delta G_p^\circ = -RT\left[\int_0^{c_{R^-}^m} \frac{\Gamma_{R^-}}{c_{R^-}} dc_{R^-} - RT\Gamma_{R^-}^m \ln c_{R^-}^m\right] \qquad (25)$$

ΔG_p° in Eq. (23), Eq. (24) and Eq. (25) represents the standard free energy due to the excess binding reaction of surfactant with 1 kg of protein when the concentration of the surfactant is altered from zero to one molar in the practical scale, whereby a protein–surfactant saturated complex is formed at an intermediate stage during concentration variation. Using measured values of Γ_{R^-} at different concentrations c_{R^-} of the ligand, ΔG_p° can be estimated using Eq. (25) in the units of kJ per kg of protein. As the solutions are relatively dilute with respect to components 2 and 3 respectively, the mole fraction X_2 of component 2 may be taken as $c_{R^-}/55.5$. Inserting this in Eq. (25)

$$\Delta G_p^\circ = -RT\int_0^{X_{R^-}^m} \frac{\Gamma_{R^-}}{X_{R^-}} dX_{R^-} + RT\Gamma_{R^-}^m \ln 55.5 X_{R^-}^m$$

$$= -RT\int_0^{X_{R^-}^m} \frac{\Gamma_{R^-}}{X_{R^-}} dX_{R^-} + RT\Gamma_{R^-}^m \ln X_{R^-}^m + RT\Gamma_{R^-}^m \ln 55.5 \qquad (26)$$

The standard free energy change ΔG° in the rational mole fraction scale is therefore expressed by the equation

$$\Delta G^\circ = -RT\int_0^{X_{R^-}^m} \frac{\Gamma_{R^-}}{X_{R^-}} dX_{R^-} + RT\Gamma_{R^-}^m \ln X_{R^-}^m$$

$$= \Delta G_p^\circ - RT\Gamma_{R^-}^m \ln 55.5 \qquad (27)$$

Thus, from the knowledge of ΔG_p°, ΔG° in the rational scale can be further estimated using Eq. (27). This will be elaborated further in Part 2 of this paper, where the standard free energy of formation of surfactant–protein complexes will be estimated from experimental data.

3. Isopiestic experiments

We now consider the application of Eq. (18) for understanding the excess negative interaction of inorganic salts, sugar and urea respectively with different proteins because of their excess hydration as a result of water–protein interaction. Isopiestic experiments were used by several workers [28–30] to study such phenomena. In the arrangement for isopiestic experiments, a definite weight of protein, along with salt and water, is taken in a weighing bottle (sample system) without a lid, which was allowed to float in the reference solution contained in a desiccator. The desiccator was then evacuated so that exchange of water vapour between the reference solution and the sample could take place until vapour pressure equilibrium was reached. From the known water and solute contents of the sample containing protein, the total molality m_2^t of the solution in contact with protein becomes known. The molality m_2 of the solute in the reference solution can also be estimated from experiments, so that the value of Γ_2^1 for inorganic salts at isopiestic equilibrium can also be calculated using Eq. (2) directly with the assumption that the protein in the sample does not contribute significantly to the equilibrium vapour pressure of the system [38–40].

For the calculation of ΔG° from the previous treatment based on the "one-phase model", we can write Eq. (17) in the form

$$-\left(\mathrm{d}G_p^i\right) = \Gamma_2^1 \mathrm{d}(\nu_+ \mathrm{d}\mu_+ + \nu_- \mathrm{d}\mu_-) \tag{28}$$

Here, μ_+ and μ_- are the chemical potentials of cations and anions respectively and ν_+ and ν_- are the stoichiometric coefficients of cations and anions when one molecule of electrolyte is dissociated completely. Here μ_2 in Eq. (17) for the adsorption of electrolyte component 2 has been replaced by $\nu_+\mu_+ + \nu_-\mu_-$ for obtaining Eq. (28), so that

$$\begin{aligned} -\left(\mathrm{d}G_p^i\right) &= RT\Gamma_2^1(\nu_+ \mathrm{d}\ln f_+ X_+ + \nu_- \mathrm{d}\ln f_- X_-) \\ &= RT\Gamma_2^1 \mathrm{d}\ln(f_+^{\nu+} \cdot f_-^{\nu-})(X_+^{\nu+} \cdot X_-^{\nu-}) \\ &= RT(\nu_+ + \nu_-)\Gamma_2^1 \mathrm{d}\ln(f_\pm \cdot X_\pm) \end{aligned} \tag{29}$$

Mole fractions X_+ and X_- of cation and anion respectively can be calculated from the relationships

$$X_+ = \frac{\nu_+ m_2}{(\nu_+ + \nu -)m_2 + 55.5} \tag{30}$$

$$X_- = \frac{\nu_- m_2}{(\nu_+ + \nu_-)m_2 + 55.5} \tag{31}$$

The mean mole fraction $X_\pm$ of the electrolyte may be calculated from the relationship

$$X_\pm = (X_+^{\nu_+} \cdot X_-^{\nu_-})^{\frac{1}{\nu_+ + \nu_-}} \tag{32}$$

and $f_\pm$, the mean activity coefficient, is given by

$$f_\pm = (f_+^{\nu_+} \cdot f_-^{\nu_-})^{\frac{1}{(\nu_+ + \nu_-)}} \tag{33}$$

In isopiestic experiments, m_2 can be obtained directly from experiment, so that X_+ and X_- can be obtained using Eq. (30) and Eq. (31). $X_\pm$ is estimated using Eq. (32). The mean activity coefficients of different electrolytes in the practical scale can be obtained from standard tables [41] so that, from these values, $f_\pm$ in the rational scale can be computed by multiplication with appropriate conversion factors.

One can now fix the equation for the standard free energy changes $\Delta G^\circ$for interaction with electrolyte and water by integration of Eq. (29) between the limits $X_\pm = 0$ to $X_\pm = 1$, so that

$$\Delta G^\circ = -RT(\nu_+ + \nu_-) \times \left[\int_0^{X_\pm^m} \frac{\Gamma_2^1}{f_\pm X_\pm} \mathrm{d}f_\pm X_\pm - \Gamma_2^m \ln(f_\pm X_\pm^m)\right] \tag{34}$$

Here $X_\pm^m$ stands for the critical concentration of the electrolyte when the value of Γ_2^1 reaches the maximum value Γ_2^m.

Using this equation, the standard free energy change due to the excess positive hydration and the negative electrolyte binding interaction with protein as a result of the change in bulk mole fraction of the solute from zero to unity have been evaluated. This will be discussed in Part 3 of this paper for lysozyme and other proteins.

4. Two-phase systems

So far we have discussed the thermodynamic treatment for the results obtained from two widely used experimental techniques, i.e. equilibrium dialysis and the isopiestic method. In these two techniques, protein inside the dialysis bag or in the sample bottle (respectively) may remain dissolved in water, thus forming a single phase. Within this aqueous phase, 1 kg of protein may undergo chemical reaction with Δn_1 and Δn_2 moles of solvent and solute components. The composition of these components remaining free can be estimated from an analysis of the concentration of the solute in either the dialysate or the reference solution, so that the relative excesses Γ_2^1, Γ_1^2 etc. can be estimated from experimental data using Eq. (3) and Eq. (4). In the absence of the boundary between protein and water forming a single aqueous phase, Γ_2^1 and Γ_1^2 should not be strictly identified with the term "binding". These may be termed as "extents of excess interaction" with protein in the appropriate units.

However, in many types of binding experiment, protein is in direct contact with solvent and solute. As, for example, using a CTAB reversible electrode, we have recently determined Γ_2^1 from the concentration of CTAB in contact with a protein biocolloid solubilised in aqueous media [42]. In the absence of a dialysis bag or reference solution in such experiments, application of Eq. (15) and Eq. (16) as originally derived for single-phase systems may become questionable.

To deal with such systems, the biopolymer in water may be assumed to remain as a separate phase (pseudo-phase) and the term γ in ergs cm^{-2} stands for the surface free energy of the protein–water interface. Bull and Breese [43] were able to estimate γ for such complex systems from contact angle measurement. Following Defay and Prigogine [32] and Chattoraj [33] one can write the Gibbs–Duhem equation for the system containing n_1^t and n_2^t moles of solvent and solute respectively, as before, per kg of protein of interfacial area S in the form

$$n_1^t d\mu_1 + n_2^t d\mu_2 + n_3^t d\mu_3 + S d\gamma = 0 \qquad (35)$$

For the free bulk aqueous phase of the two-phase (pseudo) system, one finds the validity of Eq. (8), so that, combining this with the relationship of Eq. (35), we find

$$-S d\gamma = \Gamma_2^1 d\mu_2 + \Gamma_3^1 d\mu_3 \qquad (36)$$

Comparing Eq. (36) with the relationship of Eq. (1), we find that $S d\gamma$ for a two-phase system is in fact equal to $(dG)_p^l$ for a one-phase system, so that Eqs. (15)–(36) derived for a one-phase system remain valid for two-phase systems also. A general value of m similar to that for Eq. (19) has already been derived by Chattoraj [44,45] for the adsorption of electrolytes at a liquid surface.

Thus we find that Eq. (26) and Eq. (27) may be used in general for the calculation of standard free energy change for binding interaction of ligands with protein when the ligand activity in the bulk phase is altered from zero to unity. Also, Eq. (34), derived from Eq. (17), can be used to calculate the value of ΔG° for negative binding of electrolyte and other solutes due to the excess positive hydration of protein. The experimental data will be treated in detail in the light of these equations in Parts 2 and 3 of this series of papers.

It is thus clear from all these discussions that the standard free energy change ΔG° for the composite interaction of solute and solvent with 1 kg kilogram of protein resulting from the change of the bulk solute concentration (or activity) from zero to a hypothetical standard state of unit solute activity in the mole fraction scale can be evaluated from the experimental data for different proteins at several values of pH, ionic strength and temperature. All these values of ΔG° are strictly comparable in this standard state. ΔG° may represent the relative affinities of the solute and solvent components for interaction with 1 kg of protein.

Previously, an attempt has been made by Chattoraj et al. [46] to calculate the standard free energy change for interaction of solute and solvent with protein with respect to a new arbitrary standard state of unit mole ratio composition of both solute and solvent. However, further analysis has shown this approach to be inconvenient and semiquantitative in nature.

The standard free energy for the transfer of one mole of ligand from the bulk solution to protein is frequently calculated using the Scatchard equation [36,46]. This actually represents the chemical part of

the free energy due to the binding interaction, and its unit of kJ per mole of ligand transfer is different from that of ΔG° expressed in kJ mol^{-1} of protein. The expression for the standard free energy change ΔG_B° has also been derived by Bull [35] for the adsorption of protein from the solution to the surface of the solid, and it is based on the Gibbs adsorption equation. Its unit is also kJ mol^{-1} of adsorbate transferred from the solution to the surface of the solid. ΔG_B° includes all types of interaction, but values of ΔG_B° appear not to be comparable with each other because no account is taken of the different orientations of the adsorbates on the surface of the adsorbent [46]. Further, application of the equations of Bull and Scatchard for interpretation of negative binding of solute has not been considered by them. This subject has been discussed in detail by Chattoraj et al. [46].

For binding experiments in three-component systems, Bull and Breese earlier derived an equation which according to the present notation reads [36,38,39]

$$m_2 n_1^t - 55.5 n_2^t = \Delta n_1 m_2 - 55.5 \Delta n_2 \qquad (37)$$

When m_2 is replaced by $55.5(n_2)/(n_1)$, the equation can be written in the form

$$n_1^t - n_2^t \frac{n_1}{n_2} = \Delta n_1 - \Delta n_2 \frac{n_1}{n_2} \qquad (38)$$

According to Eq. (4) and Eq. (5) (written in the proper form), each side of Eq. (38) is equal to Γ_1^2, so that Bull's experimental data on hydration can be used for the calculation of ΔG° using Gibbs excess quantities.

Kuntz and Kauzman [47] have made an attempt to correlate Bull's equation for isopiestic experiment with that derived from the defined term for preferential hydration [19–21] rearranged for fitting data on isopiestic experiments. This equation in the notation followed in this paper now reads

$$\Gamma_\omega = W_1^t - 1000 \frac{n_2^t}{m_2} \qquad (39)$$

where Γ_ω is the grams of water excess per gram of protein and W_1^t is the total water uptake (at a particular water activity) in grams per gram of protein. Here n_2^t is the moles of salt per gram of macromolecule. Dividing both sides by 18, the molecular weight of water, and replacing the molality m_2 of free water in contact with 1 g of protein by $55.5(n_2)/(n_1)$, we find

$$\Gamma_1^2 = n_1^t - n_2^t \frac{n_1}{n_2} \qquad (40)$$

This simple treatment indicates that the preferential hydration term in appropriate units may be identified with the Gibbs excess of water. Alternatively, it can be related with Γ_2^1, the salt excess, which will be negative in this case (see Eq. (4)). We thus find that the preferential binding of a component can be identified with the Gibbs excess without any difficulty from thermodynamic consideration and that our derived expression for ΔG° will also remain valid for such a concept.

In Part 2 of this series we shall show the quantitative relationship between ΔG_B° and ΔG° and their relationships for the relative measurement of affinities of ligands to different systems of proteins dissolved in agueous media.

Acknowledgements

One of the authors (D.K.C.) is indebted to the Indian National Science Academy for financial assistance. We are grateful to the referee for his very useful comments.

References

[1] L. Stryer, Biochemistry, 4th edn., W.H. Freeman, New York, 1994.
[2] J. Steinhardt and J.A. Reynolds, Multiple equilibria in proteins, Academic Press, New York, 1969.
[3] J. Steinhardt, N. Stocker and K.S. Birdi, Biochemistry, 13 (1974) 4461.
[4] M.D. Reboiras, H. Pfisher and H. Pauly, Biophys. Chem., 9 (1978) 37.
[5] G.S. Manning, Biophys. Chem., 9 (1978) 65.
[6] T. Gilanyi and E. Wolfram, Colloids Surf., 3 (1981) 181.
[7] J.D. McGhee and P.H. von Hippel, J. Mol. Biol., 86 (1974) 469.
[8] S.J. Gill, H.T. Gaud, J. Wyman and B.G. Barias, Biophys. Chem., 8 (1978) 53.
[9] A.A. Spector and E.C. Santos, Ann. N.Y. Acad. Sci., 226 (1973) 247.

[10] E.D. Goddard, Colloids Surf., 19 (1986) 301.
[11] E.D. Goddard, in K.L. Mittal (Ed.), Surfactants in solution, Plenum Press, New York, 1992.
[12] K. Hayakawa, J.P. Santerre and J.C.T. Kwak, Biophys. Chem., 17 (1983) 175.
[13] J.P. Santerre, K. Hayakawa and J.C.T. Kwak, Colloids Surf., 13 (1985) 35.
[14] K. Thalberg and B. Lindman, in K.L. Mittal and D.O. Shah (Eds.), Surfactants in Solution, Vol. 11, Plenum Press, New York, 1991, p. 243.
[15] M. Sen, S.P. Mitra and D.K. Chattoraj, Colloids Surf., 2 (1981) 259.
[16] M. Sen, S.P. Mitra and D.K. Chattoraj, Indian J. Biochem. Biophys., 17 (1980) 370.
[17] B.K. Sadhukhan and D.K. Chattoraj, in K.L. Mittal and B. Lindman (Eds.), Surfactants in Solution, Plenum Press, New York, 1986, p. 1249.
[18] M. Das and D.K. Chattoraj, Colloids Surf., 61 (1) (1991) 15.
[19] H.K. Schachman and M.A. Lauffer, J. Am. Chem. Soc., 71 (1949) 536.
[20] J.E. Hearst and J. Vinograd, Proc. Natl. Acad. Sci. U.S.A., 47 (1964) 999.
[21] S.N. Timasheff, Acc. Chem. Res., 3 (1970) 62.
[22] M.T. Record, C.F. Anderson and T.M. Lohman, Q. Rev. Biophys., 11 (1978) 103.
[23] J.C. Lee, K. Gekko and S.N. Timasheff, Methods Enzymol., 61 (1979) 26.
[24] E.F. Cassassa and H. Eisenberg, Adv. Protein Chem., 19 (1964) 287.
[25] E.P. Pittz and S.N. Timasheff, Biochemistry, 17 (1978) 615.
[26] H. Inoue and S.N. Timasheff, Biopolymers, 11 (1972) 737.
[27] S.N. Timasheff, J.C. Lee, E.P. Pittz and N. Tweedy, J. Colloid Interface Sci., 55 (1976) 658.
[28] S.P. Mitra, D.K. Chattoraj and M.N. Das, Indian J. Biochem. Biophys., 14 (1977) 101.
[29] B. Sadhukhan and D.K. Chattoraj, Indian J. Biochem. Biophys., 20 (1983) 66.
[30] M. Das and D.K. Chattoraj, J. Biosci., 6 (1984) 589.
[31] J.W. Gibbs, The Collected Works of J.W. Gibbs, Vol. 1, Longmans Green, New York, 1931.
[32] R. Defay, I. Prigogine and A. Bellemans, Surface Tension and Adsorption, translated by D.H. Everett, Longmans Green, London, 1966.
[33] D.K. Chattoraj, Indian J. Chem., 20A (1981) 941.
[34] D.K. Chattoraj and K.S. Birdi, Adsorption and the Gibbs Surface Excess, Plenum Press, New York, 1984.
[35] H.B. Bull, Biochim. Biophys. Acta, 19 (1956) 464.
[36] H.B. Bull, in An Introduction to Physical Biochemistry, F.A. Davis Co., Philadelphia, 1971.
[37] H.B. Bull and K. Breese, Biopolymers, 15 (1976) 1573.
[38] H.B. Bull and K. Breese, Arch. Biochem. Biophys., 137 (1970) 299.
[39] H.B. Bull and K. Breese, Arch. Biochem. Biophys., 139 (1970) 93.
[40] D.K. Chattoraj and H.B. Bull, Arch. Biochem. Biophys., 142 (1971) 363.
[41] R.A. Robinson and R.H. Stokes, Electrolyte Solutions, Butterworth, London, 1959.
[42] S. Maulik, P.K. Jana, S.P. Moulik and D.K. Chattoraj, Biopolymers, 35 (1995) 533.
[43] H.B. Bull and K. Breese, Arch. Biochem. Biophys., 202 (1977) 116.
[44] D.K. Chattoraj, J. Phys. Chem., 70 (1966) 2687.
[45] D.K. Chattoraj, J. Colloid Interface Sci., 29 (1969) 399.
[46] D.K. Chattoraj, S.P. Mitra and B. Sadhukhan, Indian J. Biochem. Biophys., 22 (1985) 127.
[47] I.D. Kuntz and W. Kauzman, Adv. Protein Chem., 28 (1974) 239.