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Modeling of the salt effects on hydrophobic adsorption equilibrium of protein

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Abstract

A two-state protein model is proposed to describe the salt effects on protein adsorption equilibrium on hydrophobic media. This model assumes that protein molecules exist in two equilibrium states in a salt solution, that is, hydrated and dehydrated states, and only the dehydrated-state protein can bind to hydrophobic ligands. In terms of the two-state protein hypothesis and the steric mass-action theory, protein adsorption equilibrium on hydrophobic media is formulated by a five-parameter equation. The model is demonstrated with the adsorption of bovine serum albumin to Phenyl Sepharose gels as a model system. The effects of salt type (sodium chloride, sodium sulfate and ammonium sulfate) on the model parameters are discussed. Then, the model formulism is simplified in terms of the small magnitude of the protein dehydration equilibrium constant in the model. This simplification has returned the model derived on the basis of the two-state protein hypothesis to its original mechanism of salt effects on the hydrophobic adsorption of protein. This simplified model also creates satisfactory prediction of protein adsorption isotherms.

Keywords: Salt effects; Hydrophobic adsorption; Adsorption equilibrium; Albumin; Proteins

1. Introduction

Hydrophobic interaction chromatography (HIC) was pioneered by Hjerten [1] and Porath et al. [2]. This technology is based on the hydrophobic interactions between hydrophobic ligands and non-polar regions on the surface of biomolecules [3,4]. Because weaker interactions than affinity, ion-exchange and reversed-phase chromatography modes are involved in HIC, the structural damage to biomolecules can be minimized and the biological activities of the biomolecules be highly maintained in the separation process [5,6]. Therefore, it has been recognized as an important and powerful technique in the separation and purification of biomolecules, especially proteins.

The mechanism of hydrophobic interactions between solutes has long been studied because of its importance in protein precipitation by salting-out. In 1960s, Sinanoglu and Abdulnur [7] presented a solvophobic theory (or cavity theory), which describes the formation of a cavity in the solvent to make it possible for the solute molecule to enter the cavity and to interact with its environment by

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electrostatic and van der Waals forces. They assumed that the hydrophobic interactions originate from the repulsion between solvent and the hydrophobic ligand as well as the non-polar moiety of the solute. Therefore, the properties of the solvent, especially the role of salt in the solvent, become the most intriguing field in the studies of hydrophobic interactions. Then, a thermodynamic model based on the solvophobic theory was developed to describe the relationship between salt concentration and protein adsorption equilibrium on hydrophobic media [8,9]. In addition, Arakawa and coworkers [10,11] proposed a hydration theory to explain the preferential interaction of proteins with certain salts in HIC as well as in protein salting-out. In the theory, free energy augmentation of proteins related to the hydrophobic surface area of protein molecules was taken into account.

It is well known that the type of salt and salt concentration greatly influence the hydrophobic interactions between proteins and hydrophobic media, and HIC processes are often carried out by gradient elution with decreasing salt concentration [12,13]. Hence, for the analysis and optimization of HIC processes, theoretical models dealing with the salt effects on protein adsorption equilibrium are demanding. Though a quantitative relation between protein adsorption and salt concentration in mobile phase has been given [9], this statistical thermodynamic model cannot be readily used in a practical HIC process. As a macroscopic model, the Langmuir equation is the most widely adopted model to simulate protein adsorption equilibrium. However, this equation provides an unsatisfactory description of the protein adsorption to hydrophobic adsorbents due to the following two reasons. First, the binding of most proteins to hydrophobic adsorbents is based on multivalent interactions [14,15]. That is, the mechanism of protein adsorption to hydrophobic media does not really obey the Langmuir premises. Second, protein adsorption to hydrophobic media is highly affected by salt concentration [2,9,16], but the Langmuir equation cannot express this behavior and the model parameters are implicit functions of salt concentration. This makes the model unsatisfactory in a practical application to the analysis of hydrophobic interaction chromatography by gradient elution. To overcome this problem, an exponentially modified Langmuir isotherm has been proposed to bring salt contribution in protein adsorption isotherms [17]:

$$Q = \frac{\lambda b \exp(-kC_S)C_p}{1 + b \exp(-kC_S)C_p} \quad (1)$$

where λ, b and k are equation parameters. The empirical equation has been employed in the analysis of hydrophobic displacement chromatography [18].

The present work is aimed at the development of a simplified thermodynamic model to account for the salt effects on protein adsorption to hydrophobic media. To this end, a two-state protein assumption is proposed. The model assumes that there are only two states of protein in a salt solution, that is, the hydrated one and dehydrated one. Only the dehydrated-state protein can bind to the hydrophobic ligands on a stationary phase, and its fraction increases with increasing salt concentration. Obviously, in this model the increase of protein surface hydrophobicity with increasing salt concentration is regarded as the increase of the dehydrated-state protein fraction. Based on the two-state protein hypothesis and the steric mass action model proposed by Brooks and Cramer [19], we have formulated the salt effects on the hydrophobic adsorption equilibrium of protein. Bovine serum albumin was used as a protein, sodium chloride, sodium sulfate and ammonium sulfate were used as salts, to test the model validity. The model parameters are determined using adsorption equilibrium data obtained from static adsorption experiments with Phenyl Sepharose gels, and the effects of the type of salts on the model parameters are discussed. Moreover, the model is simplified with respect to the small magnitude of a model parameter. It is found that this model, directly taking salt concentration as a variable, can well describe the hydrophobic adsorption equilibrium of protein.

2. Theoretical development

2.1. Model assumptions

Considering a hydrophobic adsorption system where a hydrophobic adsorbent and a protein solu-

tion containing a protein and a specific concentration of salt are present, we first make assumptions for this hydrophobic adsorption system as follows.

1. The liquid and solid-phases are thermodynamically ideal, allowing the use of concentrations instead of activities.
2. The ion-exchange effect on protein adsorption to a pure hydrophobic matrix such as Phenyl Sepharose used in this work (see below) is negligible.
3. The effect of salt on the hydrophobicity of the hydrophobic ligand groups is assumed negligible. In contrast, it is considered that protein hydrophobicity increases with increasing salt concentration.

It is well known that protein molecules are associated with a hydration shell in solution [20]. The bound water prevents protein molecules from binding to the hydrophobic ligands on an adsorbent surface. However, in the presence of a salt, the protein will be dehydrated due to the hydration effect of the salt molecules surrounding the protein [3]. Thus, the hydrophobic zones of the protein will be naked gradually with increasing salt concentration. That is, the naked hydrophobic surface increases with increasing salt concentration, making the hydrophobic interactions between the protein and the adsorbent surface become stronger. To simplify the salt effect on the dehydration of protein surface, we assume that there are only *two states* of the protein in solution: the hydrated protein, which hydrophobic zones are completed covered by the hydration shell, and the dehydrated protein, which hydrophobic zones have been completely exposed by the hydration of the salt molecules surrounding the protein. Thus, instead of the gradual increase of protein hydrophobicity with increasing salt concentration, the assumption suggests that the dehydrated protein fraction increases with increasing salt concentration. As shown in Fig. 1, the hydrated-state protein exists as a complex with water, while the dehydrated-state protein as a complex with the hydrated salt molecules. The two states of the protein are in equilibrium in a salt solution, and only the dehydrated protein can interact with hydrophobic ligands. Based on this assumption, the interactions between protein and salt molecules in solution can be written as:

$$P(H_2O)_\beta + \alpha S \Leftrightarrow P \cdot \alpha S(H_2O)_{\beta/\alpha} \quad (2)$$

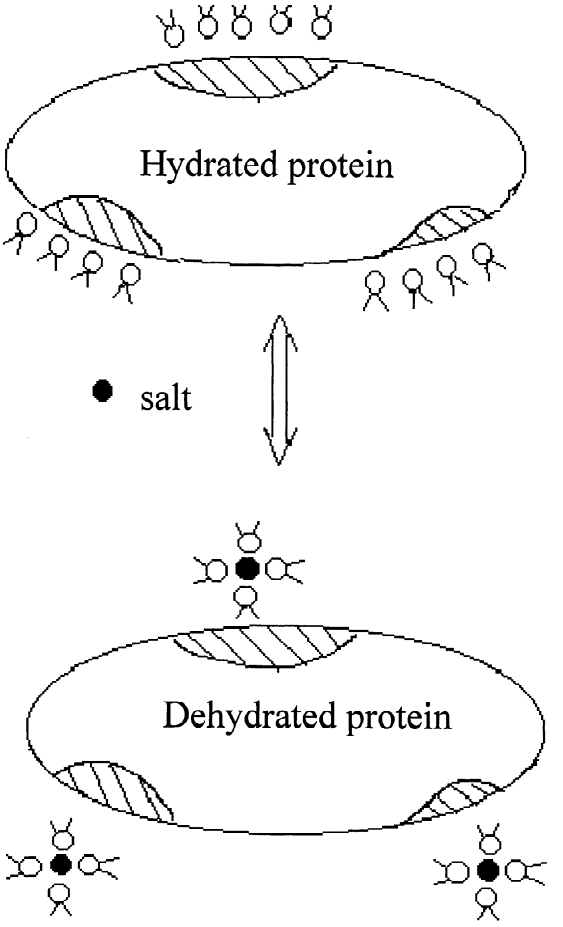

Fig. 1. Schematic presentation of the two-state protein model. The hydrated protein molecule is associated with a hydration shell, so its hydrophobic zones are completely covered by water, preventing it from binding to any hydrophobic ligand. The hydrophobic zones of dehydrated-state protein are exposed due to the hydration effect of salt in solution, so it can bind to hydrophobic ligands through the exposed hydrophobic zones. Note that the hydration shell on the hydrophilic and charged surfaces (white area) is not indicated.

or

$$C_{P-H_2O} + \alpha C_S \Leftrightarrow C_{P-D} \quad (3)$$

where $P(H_2O)_\beta$, S, $P \cdot \alpha S(H_2O)_{\beta/\alpha}$ represent hydrated protein, salt molecule and dehydrated protein, respectively; C_{P-H_2O}, C_S and C_{P-D} are their corresponding concentrations; β is the protein hydration factor, which characterizes the number of water

molecules needed for the complete coverage of the hydrophobic surfaces of a protein molecule; α is the salt coefficient, which characterizes the number of salt molecules participating in the dehydration of a protein to expose the hydrophobic surfaces of a protein.

In terms of the steric mass-action model [19], we make additional assumptions for the hydrophobic adsorption system:

4. Protein binding can be represented by mass-action equilibrium. Adsorption equilibrium holds between the hydrophobic adsorbent surface and the protein, and the adsorption is reversible.
5. The multipoint nature of protein binding can be represented by a characteristic number of binding sites ''*n*'' for each dehydrated-state protein molecule.
6. The binding of protein to the hydrophobic adsorbent surface may cause steric hindrance of the hydrophobic ligands and the number of blocked sites is proportional to the adsorbed protein concentration [19]. As indicated in Fig. 2, these blocked sites are subsequently unavailable for protein adsorption.

Based on Assumptions (1)–(5), the equilibrium expression representing the stoichiometric interaction of protein with the available hydrophobic ligands can be written as:

$$\mathrm{P}\cdot\alpha\mathrm{S}(\mathrm{H_2O})_{\beta/\alpha} + nL \Leftrightarrow \mathrm{P}\cdot\alpha\mathrm{S}(\mathrm{H_2O})_{\beta/\alpha}\cdot L_n \qquad (4)$$

or

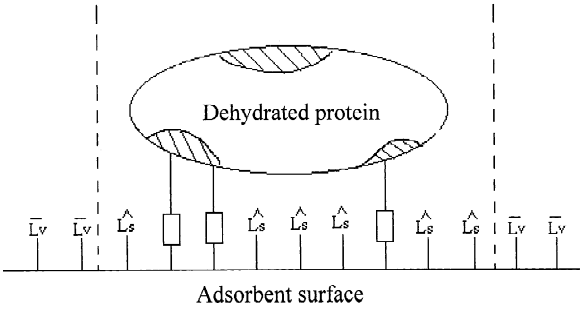

Fig. 2. Schematic presentation of protein adsorption on hydrophobic adsorbent surface. Hydrophobic ligands available for protein binding are denoted as $\overline{L_v}$, while sterically hindered ligands are denoted as $\hat{L}_s$. Ligands that contribute to protein binding are shown as rectangles. (Schematic diagram redrawn after Brooks and Cramer [19]).

$$C_{\mathrm{P-D}} + n\overline{L_v} \Leftrightarrow Q \qquad (5)$$

where L and $\mathrm{P}\cdot\alpha\mathrm{S}(\mathrm{H_2O})_{\beta/\alpha}\cdot L_n$ represent vacant hydrophobic ligands on adsorbent surface and the protein–ligand complex, respectively; $\overline{L_v}$ and Q are their corresponding concentrations.

2.2. Model formulism

Based on Assumption (3), the protein dehydration equilibrium constant K_{S} for Eq. (3) can be written as:

$$K_{\mathrm{S}} = \frac{C_{\mathrm{P-D}}}{C_{\mathrm{P-H_2O}}C_{\mathrm{S}}^{\alpha}} \qquad (6)$$

The total protein concentration in solution C_{p} is given by

$$C_{\mathrm{P}} = C_{\mathrm{P-H_2O}} + C_{\mathrm{P-D}} \qquad (7)$$

So substituting Eq. (7) into Eq. (6) yields the following expression of K_{S}:

$$K_{\mathrm{S}} = \frac{C_{\mathrm{P-D}}}{(C_{\mathrm{P}} - C_{\mathrm{P-D}})\overline{C_{\mathrm{S}}^{\alpha}}} \qquad (8)$$

Besides, the adsorption equilibrium constant K_{P} for Eq. (5) can be written as

$$K_{\mathrm{P}} = \frac{Q}{C_{\mathrm{P-D}}\overline{L_v}^{n}} \qquad (9)$$

In terms of Assumptions (5) and (6), once binding, one protein molecule interacts with n ligand sites accompanied by blockage of σ sites. The concentration of the sterically hindered hydrophobic ligand by solute is thus given by:

$$\hat{L}_{\mathrm{s}} = \sigma Q \qquad (10)$$

where σ is the steric factor. Thus, the total concentration of the ligand on the adsorbent phase, Λ, is given by the following expression according to mass balance:

$$\Lambda \equiv \overline{L_v} + (\sigma + n)Q \qquad (11)$$

Substituting Eqs. (9) and (11) into Eq. (8) and rearranging yields the following implicit adsorption isotherm:

$$\frac{Q}{C_P} = \frac{K_S K_P C_S^{\alpha}[\Lambda - (n+\sigma)Q]^n}{1 + K_S C_S^{\alpha}} \quad (12)$$

Eq. (12) is the model formulism describing protein adsorption equilibrium on a hydrophobic medium. Clearly, salt concentration effect is included in this model.

Furthermore, the above model can be extended to a multicomponent adsorption system. That is, for a system of N proteins and one salt, Eq. (11) can be written as [19]:

$$\Lambda \equiv \overline{L_v} + \sum_{i=1}^{N} (\sigma_i + n_i) Q_i \quad (13)$$

Correspondingly, the isotherm for each protein can be described by:

$$\frac{Q_i}{C_{P,i}} = \frac{K_{S,i} K_{P,i} C_S^{\alpha_i}[\Lambda - (n_i + \sigma_i)Q_i]^{n_i}}{1 + K_{S,i} C_S^{\alpha_i}}, \quad i = 1, 2, \ldots, N \quad (14)$$

3. Materials and methods

3.1. Materials

Phenyl Sepharose Fast Flow gels of low and high substitutions were provided by Amersham Pharmacia Biotech (Uppsala, Sweden). The phenyl group densities (that is, Λ values in the model) on the gels are 0.02 mol dm^{-3} for the low-substitution gel (low sub) and 0.04 mol dm^{-3} for the high-substitution gel (high sub) (according to the manufacturer catalog Amersham Pharmacia Biotech 1999, p. 564). Bovine serum albumin (BSA) and anhydrous sodium sulfate were provided by Sigma (St Louis, MO). All other reagents were of analytical grade from local sources.

3.2. Adsorption experiments

A series of adsorption equilibrium experiments of BSA to the two hydrophobic adsorbents (that is, Phenyl Sepharose FF low sub and high sub) were performed in 0.02 mol dm^{-3} Tris–HCl buffer pH 7.5 with different salts, NaCl, $(NH_4)_2SO_4$ and Na_2SO_4. For each gel and salt, adsorption experiments were carried out to obtain the adsorption isotherms at a series of salt concentrations. The adsorption equilibrium experiments were performed by the stirred batch adsorption method described by Zhang and Sun [21]. Generally, about 0.1 to 0.3 ml drained gel, previously equilibrated for 24 h in the Tris–HCl buffer with a specific salt concentration, was introduced to 10 ml protein solution of known concentration. The suspension was allowed to equilibrate at 25±0.2 °C on a shaking incubator at 120 rev./min. After 22-h incubation, protein concentration in the supernatant was determined with a UV–Vis spectrophotometer at 280 nm, and the adsorbed density of protein was calculated by mass balance.

4. Results

4.1. Determination of model parameters

In order to determine the model parameters, we simplify the model formulism (Eq. (12)) under the following two limiting cases:

(1) In a dilute single-component protein solution, $Q \to 0$ when $C \to 0$. As a result, Eq. (12) reduces to

$$\lim_{C_P \to 0} \frac{Q}{C_P} = \frac{K_S K_P \Lambda^n C_S^{\alpha}}{1 + K_S C_S^{\alpha}} \quad (15)$$

(2) Under an overloaded condition, $\overline{L_v} \to 0$ and $Q \approx Q_m$ when $C \to \infty$, so the isotherm becomes

$$\lim_{C_P \to \infty} Q = \frac{\Lambda}{\sigma + n} = Q_m \quad (16)$$

where Q_m is the adsorption capacity of the adsorbent for a protein.

In a dilute protein solution, the partition coefficient of protein between solid and liquid phases at equilibrium is defined as

$$m = \frac{Q}{C_P} \quad (17)$$

Thus, from Eqs. (15) and (17), we obtain the linearized model equation:

$$m = \frac{K_S K_P \Lambda^n C_S^{\alpha}}{1 + K_S C_S^{\alpha}} \quad (18)$$

Once the ligand density Λ is known, the linear adsorption equilibrium equation described by Eq.

(18) can be used to determine four independent parameters: α, n, K_S and K_P. On the other hand, Eq. (16) can be used to determine the steric factor σ by using the adsorption equilibrium data under an overloaded condition.

Therefore, to estimate the parameters involved in the model, it is needed to estimate the partition coefficient m at first. The partition coefficient can be determined by isocratic elution chromatography experiments [3,18,22]. In this work, we studied the salt effects on protein adsorption equilibrium in a wide range of salt concentration. Since the partition coefficients of bovine serum albumin (BSA) on the Phenyl Sepharose gels at high salt concentrations are very large (100–1500, see below), the retention time of the protein from chromatography column will be extraordinarily long. Therefore, under most experimental conditions, it is impossible to determine the partition coefficient by isocratic elution chromatography. Thus, in the present work, m is estimated from the experimental isotherms obtained by batch adsorption as described by Zhang and Sun [23]. It is observed that the adsorption equilibrium data at high salt concentrations can be well fitted to the Langmuir equation (Fig. 3):

$$Q = \frac{q_m C_P}{K_d + C_P} \tag{19}$$

So we can estimate m from the slope of the linearized Langmuir equation because we have the following relationship as C_P approaches zero:

$$m = \frac{Q}{C_P} = \frac{q_m}{K_d} \quad (C_P \to 0) \tag{20}$$

Thus, once the values of q_m and K_d are estimated by fitting Eq. (19) to the experimental data (Fig. 3), we can calculate m from Eq. (20).

Fig. 4 indicates the relationship between the partition coefficient of BSA adsorption and the salt concentration for each salt and Phenyl Sepharose gel. The model parameters, n, α, K_S, K_P are predicted by least-square fitting Eq. (18) to the partition coefficient data for each salt. Then, the nonlinear parameter, steric factor σ, is estimated from Eq. (16) with the values of Q_m, n and Λ. Here, Q_m is the maximum adsorption capacity reached under the experimental conditions. It is 60.4 mg cm^{-3} (9.02×10^{-4} mol dm^{-3}) for Phenyl Sepharose high sub and 45.1 mg cm^{-3} (6.73×10^{-4} mol dm^{-3}) for Phenyl Sepharose low sub, obtained in the Tris–HCl buffer containing 1.2 mol dm^{-3} Na_2SO_4. Experiments at

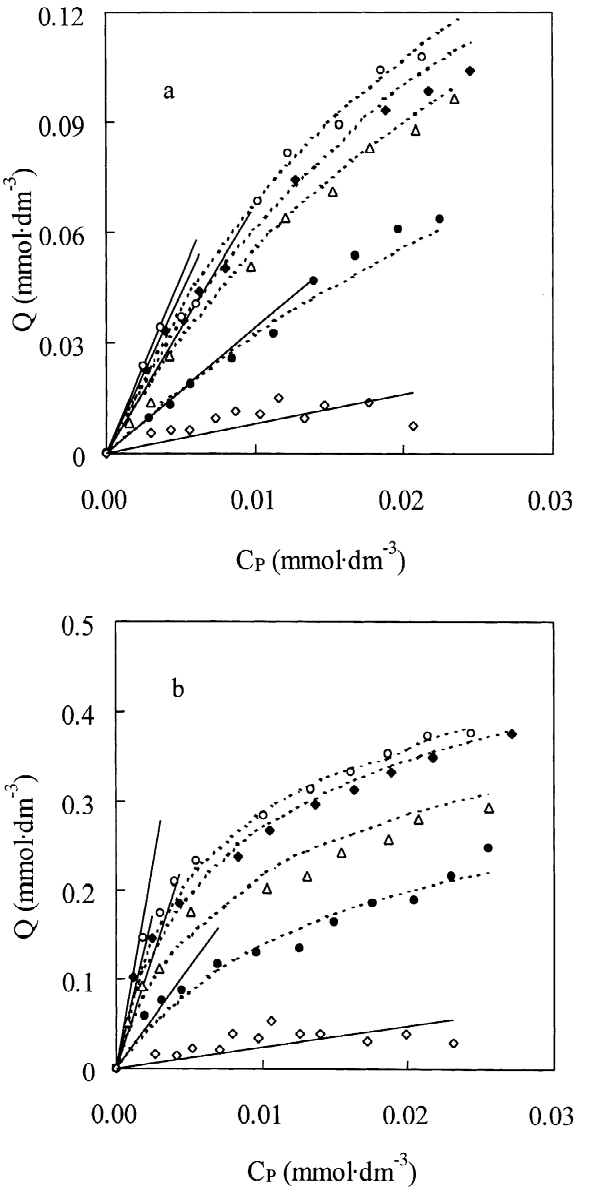

Fig. 3. Examples for the estimation of BSA partition coefficients m on (a) Phenyl Sepharose FF low sub and (b) Phenyl Sepharose FF high sub. NaCl concentrations are (mol dm^{-3}): (◇) 0; (●) 1.0; (△) 1.5; (♦) 2.0; (○) 2.25. Dashed lines are calculated from the Langmuir equation [Eq. (19)], and the straight lines are those with a slope of m (Eq. (20)).

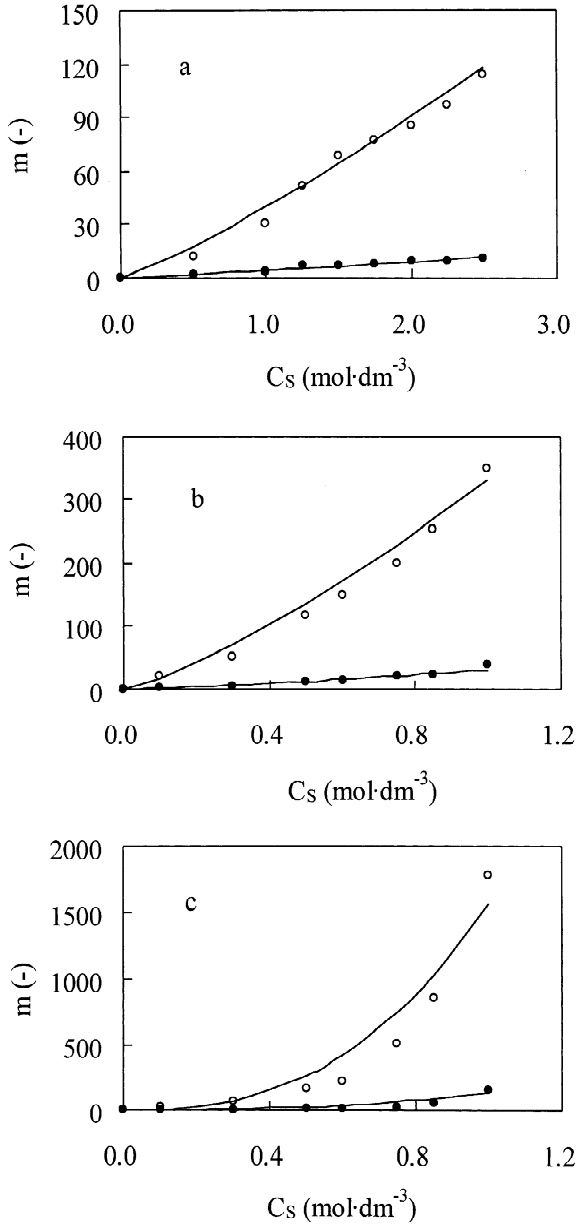

Fig. 4. Plots of partition coefficient of BSA adsorption vs. salt concentration in the presence of (a) NaCl, (b) $(NH_4)_2SO_4$, and (c) Na_2SO_4. Phenyl Sepharose ligand capacity (mol dm^{-3}): (●) 0.02 (low sub); (○) 0.04 (high sub). Solid lines are calculated from Eq. (18).

higher Na_2SO_4 concentration created little rise of the capacity value. The values of n, α, K_S, K_P and σ thus estimated are listed in Table 1.

4.2. Evaluation of the model parameters

To evaluate the validity of the model parameters listed in Table 1, we take the logarithm of both sides of Eq. (18), and obtain the following equation:

$$\ln m = A + n \ln \Lambda \tag{21}$$

where

$$A = \ln \frac{K_S K_P C_S^{\alpha}}{1 + K_S C_S^{\alpha}} \tag{22}$$

According to Eq. (21), the plot of the logarithm of the partition coefficient vs. the logarithm of the hydrophobic ligand density should be a straight line with a slope of n, if the model is valid. We have Phenyl Sepharose media of only two ligand densities to make this plot. Fig. 5 shows the plots for various salts at different salt concentrations. The slope values range from 3.23 to 3.46 for NaCl, from 3.24 to 3.50 for $(NH_4)_2SO_4$, and from 3.40 to 3.78 for Na_2SO_4. It is obvious that the slopes of the lines are almost equivalent with the respective values of n listed in Table 1, indicating the validity of the n values. Moreover, this implies that the value of n is essentially independent of the salt type and concentration. Due to the independence of the n value on salt type, the value of σ thus only depends on the ligand density, as shown by Eq. (16) and in Table 1.

The model validity can be further evaluated by comparison between the experimental data of the adsorbed protein concentration and the theoretical predictions at various conditions. Fig. 6 is the parity plots for this purpose. In the figure, all the experimental data have been used for the determination of the model parameters. The standard deviations (SD) are estimated at 0.013 (Fig. 6a) and 0.030 (Fig. 6b). This indicates that model parameters are satisfactory for describing the hydrophobic adsorption equilibrium data.

Besides, from Eq. (18), the value of m is predicted to be zero when $C_S = 0$. However, the values of m are estimated at 0.8 for Phenyl Sepharose FF low sub (Fig. 3a) and 2.4 for Phenyl Sepharose FF high sub (Fig. 3b). This indicates that there is somewhat hydrophobic interaction between the protein and adsorbent in the buffer system without salt addition. Despite of this, the values of m are so small that the

Table 1
Estimated model parameters for BSA adsorption on Phenyl Sepharose FF gels[a]

Salts	α	K_s	K_p	n	σ(LS)	σ(HS)	$K_{ps}(=K_p\times K_s)$
NaCl	1.18	2.53×10^{-4}	8.90×10^{9}	3.36	26.4	41.0	2.25×10^{6}
$(NH_4)_2SO_4$	1.29	3.81×10^{-4}	5.06×10^{10}	3.41	26.3	40.9	1.93×10^{7}
Na_2SO_4	2.59	3.89×10^{-4}	4.49×10^{11}	3.61	26.1	40.7	1.75×10^{8}

[a] Molarity unit (mol dm^{-3}) is used for all components in determination of the model parameters. LS and HS stand for Phenyl Sepharose FF gels of low and high substitutions, respectively.

assumption of $m=0$ at $C_S=0$ does not produce obvious effect on the model (Fig. 4). This indicates the model validity for description of the hydrophobic adsorption of protein in dilute salt solution, at least for the present system.

4.3. Applications of the model to predict adsorption isotherms

Once the model parameters are determined (Table 1), the adsorption isotherms at given salt concentrations can be predicted using Eq. (12). We performed additional adsorption experiments with NaCl under the conditions different from those used as in Fig. 6. Fig. 7 shows a comparison of the measured and predicted BSA adsorption isotherms. It can be seen that the agreement between the experimental and predicted results is satisfactory at different NaCl concentrations and hydrophobic ligand densities, indicating that the model is promising in predicting protein adsorption isotherms.

5. Discussion

5.1. Effect of salt type

It has been well documented that increasing salt concentration has a positive effect on protein adsorption to hydrophobic adsorbents. This is also obvious in terms of our experimental data shown in Fig. 4, that is, the intensity of the hydrophobic interactions between BSA molecules and the hydrophobic ligands increases with salt concentration. This result, which is described as the ''salt-promoted adsorption'' [24], agrees well with the conclusions based on the solvophobic theory [25–27].

The model parameters listed in Table 1 are helpful to characterize the salt effects on the hydrophobic adsorption of BSA. It has been stated in the Theoretical development section that the salt coefficient α refers to as the number of salt molecules participating in the dehydration of a protein to expose the hydrophobic surfaces of the protein. This coefficient can also be considered as the salt affinity for the protein. Larger salt coefficient means higher salt affinity for the removal of the hydration shell of the protein. It is observed that the affinity of the three salts for the protein is in the order of $\alpha_{Na_2SO_4} > \alpha_{(NH_4)_2SO_4} > \alpha_{NaCl}$ and Na_2SO_4 has much higher affinity than the other two salts do.

Defined as the protein dehydration equilibrium constant, K_S characterizes the ability of a salt to disturb the ordered arrangement of water molecules around the protein and help the protein molecules to expose their hydrophobic surfaces. Because it is assumed that only the dehydrated-state proteins can bind to the hydrophobic ligands, greater value of K_S means stronger ability of the salt to prompt protein adsorption. Na_2SO_4 has the strongest, while NaCl has the weakest, salt-promoted adsorption effect among the three salts. This is consistent with the salt effect on α.

In the hermeneutics based on the solvophobic theory [25], the hydrophobic interactions are associated with the change of the total surface area of proteins upon hydrophobic binding. The other theories [3,16] suggest that the adsorbent property is also an important factor influencing the hydrophobic interactions. It is postulated that the hydrophobicity of ligands may also be affected by salt [3]. Since the ligands have apolar zones as the active interaction sites, the increment of salt concentration can also expose these hydrophobic zones to facilitate their hydrophobic interactions with protein. Different types of salts have different extents of effect on the ligand hydrophobicity. Because the salt effect on the ligand hydrophobicity is not taken into account in the

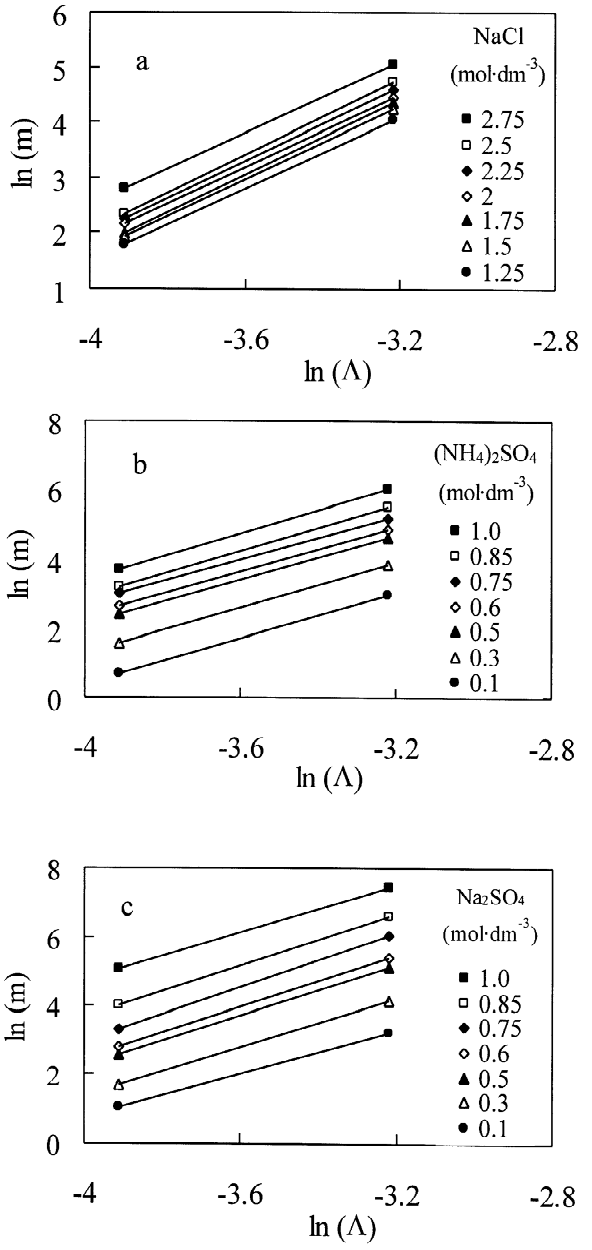

Fig. 5. The logarithm of partition coefficient of BSA adsorption vs. the logarithm of ligand density for the three salts at different salt concentrations. (a) NaCl; (b) $(NH_4)_2SO_4$; (c) Na_2SO_4.

present model, the adsorption equilibrium constant K_P increases in the order of $K_{P,Na_2SO_4} > K_{P,(NH_4)_2SO_4} > K_{P,NaCl}$, similar to α and K_S.

In the presence of salt, the water molecules are

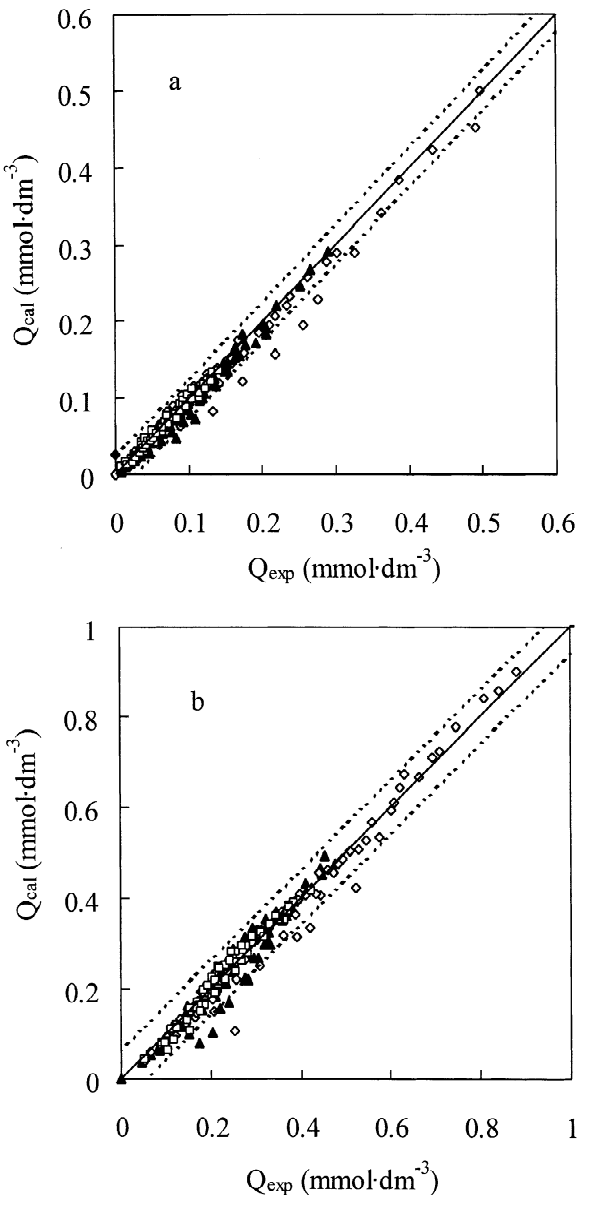

Fig. 6. Parity plots of measured and predicted BSA adsorption concentration from the two-state protein model for (a) Phenyl Sepharose FF low sub and (b) Phenyl Sepharose FF high sub. (□) NaCl concentrations are 1.25–2.75 mol dm^{-3}; (▲) $(NH_4)_2SO_4$ concentrations are 0.1–1.0 mol dm^{-3}; (◇) Na_2SO_4 concentrations are 0.1–1.0 mol dm^{-3}. Dashed lines represent $Q_{cal} = Q_{exp} \pm 2SD$.

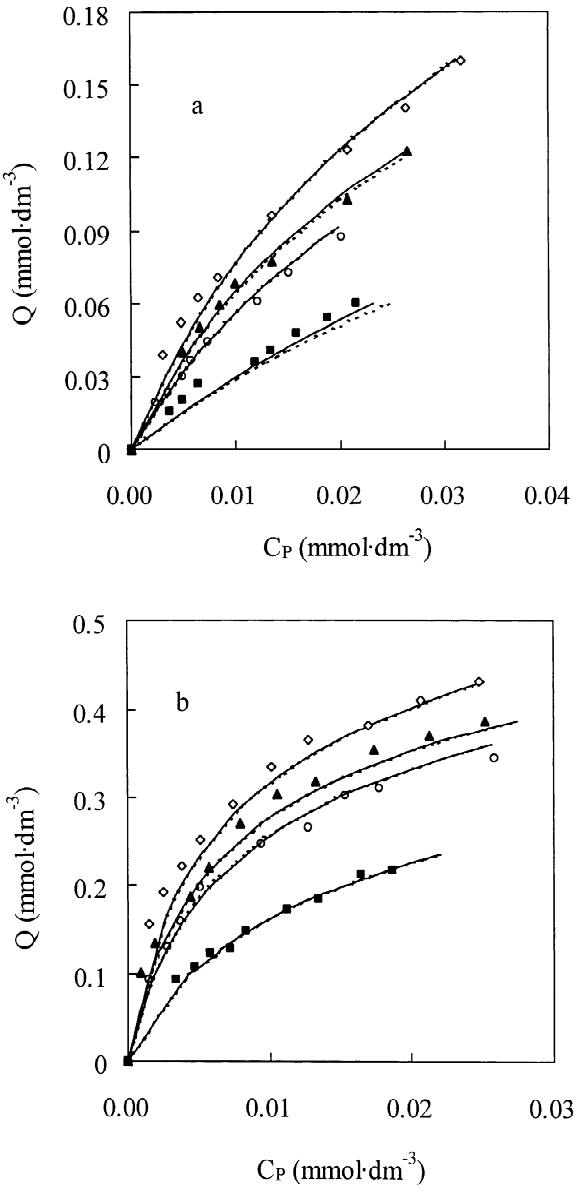

Fig. 7. Comparison of predicted and measured BSA adsorption isotherms on (a) Phenyl Sepharose FF low sub (b) Phenyl Sepharose FF high sub. NaCl concentrations (mol dm^{-3}): (■) 0.9; (○) 1.8; (▲) 1.75; (◇) 2.4. Solid and dashed lines are calculated from Eqs. (12) and (27), respectively.

repulsed from proteins. This results in the exposure of the hydrophobic surfaces and helps the attraction of the protein to the hydrophobic ligands. Therefore, the adsorption will become easier with the increase of salt concentration. Such a salt effect is different for different salts; it can be arranged in the Hofmeister series [28]:

$$\mathrm{NaCl} < \mathrm{BrCl} < \mathrm{NaOAc} < \mathrm{KOAc} < \mathrm{Na_2SO_4} < \mathrm{Na_2HPO_4} < \mathrm{KH_2PO_4} < (\mathrm{NH_4})_2\mathrm{SO_4}$$

The results obtained in this work indicate that Na_2SO_4 has greater effect on the dehydration of BSA than $(NH_4)_2SO_4$, which is different from the Hofmeister series. It may be explained in light of the preferential hydration theory [11], that is, BSA may be more preferentially dehydrated by Na_2SO_4 than by $(NH_4)_2SO_4$. Since the Hofmeister series is a general rule for salt effects on protein hydrophobicity, it may not hold for specific proteins and/or salts. Similar observations have been reported previously [25,29].

Moreover, according to the solvophobic theory, the molar surface tension increment of salt ($\Delta\gamma_s$) can facilitate the hydrophobic interactions [9]. As the salt concentration increases, it is reasonable to see the augmentation of hydrophobic adsorption due to the corresponding increment of the molar surface tension of salt. It has been reported that $\Delta\gamma_s$ is dependent on the type of salt by the order of $\Delta\gamma_{Na_2SO_4} > \Delta\gamma_{(NH_4)_2SO_4} > \Delta\gamma_{NaCl}$ [9]. Therefore, the combination of these effects determine the ability of salts to prompt the hydrophobic adsorption of BSA in the order of $Na_2SO_4 > (NH_4)_2SO_4 > NaCl$.

5.2. Simplification of the model

We find that the values of K_S (Table 1) are so small that the model formulism (Eq. (12)) can be reduced to:

$$\frac{Q}{C_P} = K_S K_P C_S^{\alpha}[\Lambda - (n+\sigma)Q]^n \quad (23)$$

Moreover, it can be seen from Eq. (6) that C_{P-D} is very small due to the small magnitude of K_S. This means that in the two-state protein model there is only a very small fraction of the protein which hydrophobic surfaces can be completely exposed. Thus, C_{P-H_2O} is basically equal to C_P (see Eq. (7)). Combining Eqs. (3) and (5), and replacing C_{P-H_2O} by C_P, we have:

$$C_P + n\overline{L_v} + \alpha C_S \Leftrightarrow Q \quad (24)$$

The adsorption equilibrium constant for the above formula is

$$K_{PS} = \frac{Q}{C_P \overline{L_v}^n C_S^\alpha} \quad (25)$$

It can be seen that

$$K_{PS} = K_P K_S \quad (26)$$

and Eq. (23) can be further reduced to

$$\frac{Q}{C_P} = K_{PS} C_S^\alpha [\Lambda - (n + \sigma)Q]^n \quad (27)$$

Thus, the model is simplified to a four-parameter formulism (Eq. (27)). On the basis of above analysis of salt effects, it is clear that K_{PS} is a lumped parameter related to the salt-induced hydrophobicity increases of both protein and ligand, and protein binding to hydrophobic ligands. Consequently, the simplification has returned the model on the basis of the two-state protein hypothesis to its original mechanism of salt effects on hydrophobic adsorption of protein.

The values of K_{PS} calculated from Eq. (26) are provided in Table 1. With the values of K_{PS}, α, n and σ, the adsorption isotherms under the conditions shown in Fig. 7 are predicted again using Eq. (27). As shown by the dashed lines in the figure, the results from Eq. (27) are nearly the same as those predicted from Eq. (12), indicating that the simplified model also produces satisfactory prediction of the adsorption isotherms.

5.3. Reduction to the Langmuir equation

For a hydrophobic medium with a sufficiently low ligand density, one may have $\sigma = 0$ and $n = 1$. Then, Eq. (27) can be reduced to the following form:

$$Q = \frac{K_{PS} C_S^\alpha \Lambda C_P}{1 + K_{PS} C_S^\alpha C_P} \quad (28)$$

In this case, Eq. (12) can also be reduced to a similar form with Eq. (28). Obviously, Eq. (28) is a Langmuir-type isotherm with salt concentration as a variable. This equation is similar to the exponentially modified Langmuir isotherm [17] (see Eq. (1)), but its coefficients (K_{PS}, α and Λ) have definite physical meanings. Therefore, Eq. (28) may be recommended to replace Eq. (1) as a lumped adsorption equilibrium model in the analysis of hydrophobic adsorption of protein.

6. Conclusions

In this work, a two-state protein hypothesis is made and a predictive model is developed for the salt effects on the adsorption equilibrium of protein to hydrophobic adsorbents. The validity of the model is demonstrated by comparison with experimental data. Analysis of the model parameters has given explanations of the observed effects of the type and concentration of salt. The simplification in terms of the small magnitude of the protein dehydration equilibrium constant has returned the model derived on the basis of the two-state protein hypothesis to its original mechanism of salt effects on the hydrophobic adsorption of protein. This simplified model produces satisfactory results in predicting protein adsorption equilibria. The model can also be reduced to the Langmuir-type isotherm for the adsorbent of a very low ligand density. Compared to the former theoretical approaches, this model is considered useful in the selection of salt as a modulator of HIC to improve separations since salt concentration and salt type-related parameters are involved in the model formulism. Moreover, it can offer a convenient framework for the design and analysis of the gradient elution process of HIC to enhance separation performance.

7. Nomenclature

C_P	protein concentration, mol dm^{-3}
C_{P-D}	dehydrated protein concentration, mol dm^{-3}
C_{P-H_2O}	hydrated protein concentration, mol dm^{-3}
C_s	salt concentration in liquid phase, mol dm^{-3}
K_d	dissociation constant in Langmuir equation (Eq. (19)), mol dm^{-3}
K_P	adsorption equilibrium constant defined by Eq. (9)

K_{PS}	adsorption equilibrium constant defined by Eq. (25)
K_S	protein dehydration equilibrium constant defined by Eq. (6)
$\hat{L}_s$	sterically hindered ligand concentration, mol dm^{-3}
$\overline{L_v}$	accessible ligand concentration, mol dm^{-3}
m	partition coefficient of protein between solid and liquid phases
n	characteristic number of binding sites
q_m	adsorption capacity in Langmuir equation (Eq. (19)), mol dm^{-3}
Q	adsorbed protein concentration on adsorbent, mol dm^{-3}
Q_m	adsorption capacity defined in Eq. (16), mol dm^{-3}
α	salt coefficient
Λ	hydrophobic ligand density, mol dm^{-3}
σ	steric factor

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