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Gibbs free energy of adsorption for biomolecules in ion-exchange systems

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Abstract

The Gibbs free energy of adsorption (ΔG^0_{ads}) was estimated for several amino acids, peptides and proteins in a cation-exchange system. Using the steric mass action formalism that describes biomolecular adsorption in ion-exchange systems, the ΔG^0_{ads} was chromatographically determined under infinitely dilute conditions. The ΔG^0_{ads} measured for seven globular proteins ranged from -5.7 to -13.9 kcal/mol. The average bond energy (defined as ΔG^0_{ads} divided by the number of bonds formed between the protein and the surface) for these proteins varied from -1.1 to -1.7 kcal/mol. These bond energies were found to be comparable to the bond energies for lysine and arginine (-1.1 and -1.5 kcal/mol, respectively), the amino acids which primarily contribute to the cation-exchange of proteins. In contrast, an elevated average bond energy of -2.6 kcal/mol was observed for two peptides and protamine (a polypeptide) suggesting that synergistic binding may play a role for unstructured macromolecules, but not for globular proteins.

Keywords: Protein adsorption; Interaction energy; Ion-exchange

1. Introduction

Several researchers have recently attempted to model protein adsorption using fundamental molecular interactions. Stahlberg et al. [1] treated the adsorption of proteins onto a charged stationary phase as an electrostatic interaction between two charged surfaces in the presence of a buffered salt solution. Using a linearized Poisson–Boltzmann equation to describe the interaction between charged surfaces, they determined interaction energies as a function of separation distance, mobile phase ionic strength and stationary phase charge density. In a recent paper, they have extended this approach to include van der Waals interactions [2] and showed that Coulombic forces dominate at low ionic strengths while the importance of van der Waals interactions increases with high ionic strengths and short separation distances.

Prieve and Ruckenstein [3] examined the inter-

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action energy between a charged sphere and a charged surface. In their analysis, they examined van der Waals attraction, Bohr repulsion and electrostatic interactions. They calculated relatively short optimum separation distances of 1–2 Å between the sphere and surface. The energy of interaction at this distance was found to be approximately −13 kcal/mol.

Haggerty and Lenhoff [4] have explored the relationship between protein charge and chromatographic ion-exchange behavior. Using the non-linear Poisson–Boltzmann equation (as implemented in the Del Phi program of Honig and co-workers [5–8]), they calculated the mean surface potential for seven proteins at 0.01 M ionic strength. This value correlated well with the retention time for these proteins on a strong cation-exchange resin when eluted with a linear sodium chloride gradient from 0.01 to 0.75 M [9]. The correlation with net charge was not nearly as good. This result is striking and suggests that protein chromatographic behavior may be understood and predicted from structural and electrostatic considerations. However, as Haggerty and Lenhoff [4] themselves point out, this type of correlation is difficult to extend to a more detailed, mechanistic analysis.

Yoon and Lenhoff [10] have investigated protein orientation upon adsorption to a charged surface. Concentrating on electrostatic interactions and using a linearized Poisson–Boltzmann equation to describe the potential in the mobile phase, they explored the interaction of a protein with a charged, planar surface. For ribonuclease A, they determined that the protein has a preferred binding orientation (−3.67 kcal/mol) with the active site facing the stationary phase. In addition, depending on the orientation, there can actually be repulsion (0.95 kcal/mol) between the net positively charged ribonuclease A and the negatively charged surface. Lysozyme, on the other hand, experienced attraction at all orientations examined (−2.0 to −4.3 kcal/mol). Continuing this work, Roth and Lenhoff [11] included van der Waals contributions in the calculation of protein adsorption equilibria. They also showed that the protein could be simulated as a sphere with its charge distribution represented by its monopole moment placed at the center of the sphere. This simplification reduced the computation time required for simulations with minimal adverse effect on model predictions.

Reports of experimentally derived interaction energies in chromatographic systems have been limited mainly to reversed-phase supports. Purcell et al. [12] examined the adsorption of three peptides onto *n*-octadecyl silica and *n*-butyl silica adsorbents. The Gibbs free energy of adsorption was found to range from −1.0 to −3.8 kcal/mol depending on solute and temperature. Seidel-Morgenstern and Guiochon [13] report on the adsorption of Tröger's base enantiomers onto cellulose triacetate using ethanol as a mobile phase. The interaction energies varied from −0.37 to −1.22 kcal/mol. Roush et al. [14] have reported enthalpies of adsorption (ΔH_{ads}) in the range of 5.22 to 10.56 kcal/mol, depending on sodium chloride concentration, for the adsorption of recombinant rat cytochrome b_5 onto an anion-exchange surface.

In this paper, we report values of the interaction energies for the adsorption of biomolecules onto a cation-exchange chromatographic adsorbent. Important comparisons can be made between the experimental values deduced from the steric mass action theory and other theoretical approaches which model adsorption in ion-exchange systems.

2. Materials and methods

Protein-Pak SP-8HR (100 × 5 mm I.D.) column was a gift from Waters Chromatography Division of Millipore (Milford, MA, USA). Sodium phosphate dibasic, phosphoric acid, sodium chloride, arginine, lysine, bovine α-chymotrypsinogen A, bovine α-chymotrypsin, bovine trypsin, bovine lactoferrin, equine cytochrome C, bovine ribonuclease A and chicken egg white lysozyme were purchased from Sigma (St. Louis, MO, USA). Protamine sulfate was obtained from ICN Biomedicals (Costa Mesa, CA, USA). The peptides benzoyl-ArgPheTrp-LysThrPhe and benzoyl-ArgArgArgTyr were gifts from Carl Biotech (Copenhagen, Denmark).

2.1. Analytical chromatograph

The system employed for analysis consisted of an LKB 2150 HPLC pump (Pharmacia-LKB Biotechnology, Uppsala, Sweden), connected to the column through a Rheodyne 7125 injector fitted with a 20 μl sample loop (Rheodyne, Cotati, CA, USA). The column effluent was monitored using a Spectroflow 757 UV–VIS detector (Applied Biosystems, Ramsey, NJ, USA) with data acquisition and chromatographic analysis performed using a Maxima 820 chromatography workstation (Waters Chromatography Division of Millipore, Milford, MA, USA).

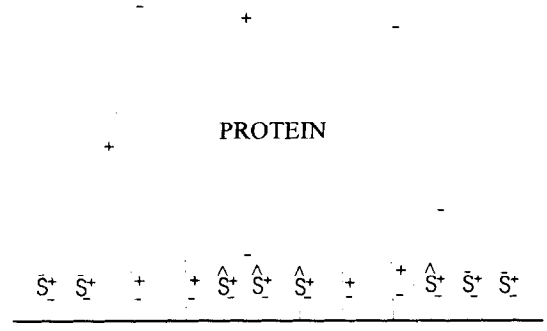

Fig. 1. Schematic representation of protein adsorption in ion-exchange systems. Salt counter-ions available for exchange denoted by $\bar{S}^+$; Sterically hindered salt counter-ion denoted by $\hat{S}^+$. Interactions contributing to the characteristic charge are shown as rectangles.

2.2. Capacity factor vs sodium concentration

The mobile phase for each sodium concentration was prepared using 12.5 mM sodium phosphate, dibasic (25 mM Na^+) with an appropriate amount of sodium chloride to obtain the desired sodium concentration. The pH was then adjusted to 6.0 using phosphoric acid. Stock solutions (1–2 mg/ml) were prepared for each biomolecule using 12.5 mM sodium phosphate, dibasic (pH 6.0). Feed solutions for each biomolecule were obtained by diluting the stock solution 10 fold with mobile phase. Multiple injections of each biomolecule were made at each sodium concentration to confirm the chromatographic retention time.

3. Theory

3.1. Steric mass action formalism

The steric mass action (SMA) model of Brooks and Cramer [15] explicitly accounts for salt effects in multi-component protein equilibria and is able to predict complex behavior in ion-exchange chromatographic systems. In the SMA model, the adsorption of proteins in ion-exchange systems is modeled using the following stoichiometric exchange reaction

$$C_{\text{protein}} + \nu\bar{Q}_{\text{salt}} \rightleftharpoons Q_{\text{protein}} + \nu C_{\text{salt}}, \qquad (1)$$

where Q_i and C_i denote the stationary and mobile phase concentrations of component i, respectively. The overbar '$\bar{\ }$', denotes bound salt counter-ions available for exchange with the protein. The number of adsorption sites that the protein interacts with upon binding is termed the characteristic charge and is denoted by ν in Eq. (1).

The unique feature of the SMA formalism as compared to other stoichiometric exchange models [9,16–22] is the incorporation of the steric factor which represents the sterically hindered exchange sites as illustrated in Fig. 1. The number of exchangeable sites ($\bar{Q}_{\text{salt}}$) is determined through an electroneutrality constraint on the adsorbent surface

$$\bar{Q}_{\text{salt}} = \Lambda - (\nu + \sigma)Q_{\text{protein}}, \qquad (2)$$

where Λ is the ion capacity of the adsorbent phase. From this, the equilibrium constant is defined as

$$K = \left(\frac{Q_{\text{protein}}}{C_{\text{protein}}}\right)\left(\frac{C_{\text{salt}}}{\bar{Q}_{\text{salt}}}\right)^{\nu}. \qquad (3)$$

In this study, we will focus on the adsorption behavior of biomolecules at concentrations (< 0.2 mg/ml) which correspond to the linear region of the adsorption isotherm. Under these conditions, the SMA model reduces to the mass action formalism of biomolecular adsorption presented

previously [9,16–22]. The dilute biomolecule concentrations used in this study should also minimize any protein–protein interactions which might occur in solution or on the adsorptive surface. At high biomolecular concentrations it may be necessary to explicitly account for protein–protein interactions in order to obtain accurate results. Nevertheless, it is interesting to note that the SMA formalism (which excludes protein–protein interactions) accurately predicts the displacement chromatographic separation of proteins at elevated concentrations ($\approx$ 30 mg/ml) [23,24].

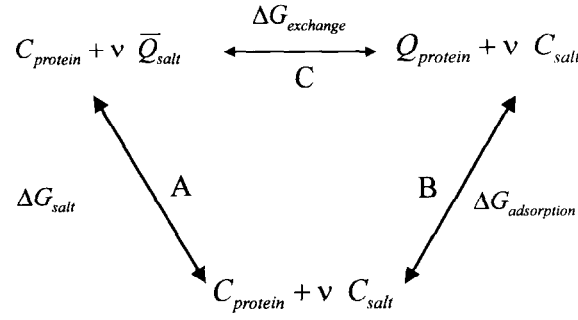

Fig. 2. Constituent reactions for exchange reaction of proteins with ion-exchange adsorbents.

3.2. Development of linear equilibrium constant

The change in Gibbs free energy for a reaction is related to the equilibrium constant in the following manner

$$\Delta G^0 = -RT \ln K. \tag{4}$$

For the exchange reaction of Eq. (1), the change in the Gibbs free energy of exchange, ΔG^0_{exc}, is defined as

$$\Delta G^0_{exc} = \mu^0_{Q_{protein}} + \nu\mu^0_{C_{salt}} - \mu^0_{C_{protein}} - \nu\mu^0_{\bar{Q}_{salt}}, \tag{5}$$

where μ^0_i is the chemical potential of species i at its standard state. Thus, the change in Gibbs free energy calculated using the equilibrium constant of Eq. (3) includes the change in free energy for all species (protein and salt ions) involved in the exchange reaction. However, in the present work, we are less interested in the energetic state of the salt ions and would like to focus on the interaction energy between the protein and the charged chromatographic surface. This interaction energy is determined from the change in protein free energy which occurs during the adsorption process. Thus, the Gibbs free energy of adsorption (ΔG^0_{ads}) of a protein is defined as

$$\Delta G^0_{ads} = \mu^0_{Q_{protein}} - \mu^0_{C_{protein}}, \tag{6}$$

This change in free energy describes the energetics of the following adsorption reaction

$$C_{protein} \rightleftharpoons Q_{protein}. \tag{7}$$

Since the Gibbs energy is a state function, the adsorption reaction can be visualized to occur by breaking the exchange reaction of Eq. (1) into constituent reactions as is shown in Fig. 2.

$$\Delta G^0_{exc} = \Delta G^0_{ads} + \Delta G^0_{salt} \tag{8}$$

Thus, the overall exchange reaction (Reaction C, Fig. 2) can be assumed to proceed by first desorbing the appropriate number of bound salt ions (reaction A, Fig. 2). The next step is an unbound protein adsorbs to the stationary phase sites vacated by the salt ions (reaction B, Fig. 2). Following the analysis of Velayudhan and Horvath [22], an additional reaction describing co-ion interactions with the biomolecule was not included as the co-ions remain thermodynamically unchanged during this reaction. The change in Gibbs free energy for protein adsorption can be calculated from the equilibrium constant of reaction B,

$$K^* = Q_{protein}/C_{protein}. \tag{9}$$

For dilute protein concentrations, the protein stationary phase concentration ($Q_{protein}$) approaches zero and the number of available exchange sites ($\bar{Q}_{salt}$) can be accurately represented as the total ion capacity of the adsorbent phase, Λ. With this approximation and using the SMA equilibrium constant of Eq. (3), rearranging Eq. (9) one obtains

$$K^* = K\left(\frac{\Lambda}{C_{salt}}\right)^{\nu} = \frac{Q_{protein}}{C_{protein}}. \tag{10}$$

The Gibbs free energy of adsorption, ΔG^0_{ads}, can now be calculated as a function of mobile phase salt concentration using the SMA parameters;

equilibrium constant K, characteristic charge ν and the ion capacity of the column Λ,

$$\Delta G^0_{ads} = -RT \ln K = -RT \ln\left[K\left(\frac{\Lambda}{C_{salt}}\right)^{\nu}\right]. \quad (11)$$

The linear equilibrium constant of Eq. (10) is related to the chromatographic capacity factor (k') using the column phase ratio, β,

$$K^* = \frac{k'}{\beta}, \quad (12)$$

with $k' = V_r - V_o/V_o$ and $\beta = V_s/V_o$, where V_r is the chromatographic retention volume of the solute, V_o is the void volume and V_s is the stationary phase volume of the column.
and thus Eq. (11) could also be written as

$$\Delta G^0_{ads} = -RT \ln\left(\frac{k'}{\beta}\right). \quad (13)$$

It is well established that the adsorption behavior of proteins and other polyelectrolytes exhibits a strong dependence on mobile phase salt concentration. Using the SMA model parameters in Eq. (11), one can readily study the ΔG^0_{ads} of biomolecules with significantly different retention behavior. In contrast, Eq. (13) is limited in use to biomolecules which exhibit similar adsorption behavior and thus has marginal utility.

3.3. Measurement of SMA parameters

The SMA parameters are determined from a series of linear elution experiments performed at various salt concentrations. Starting with the capacity factor, k', which can also be defined as

$$k' = \beta\left(\frac{Q_{protein}}{C_{protein}}\right), \quad (14)$$

where β is the phase ratio of the column. Combining with Eq. (3) yields

$$k' = \beta K\left(\frac{\Lambda}{C_{salt}}\right)^{\nu}. \quad (15)$$

Taking the logarithm of Eq. (15)

$$\log k' = \log(\beta K \Lambda^{\nu}) - \nu \log C_{salt}. \quad (16)$$

Plotting Eq. (16) for changing C_{salt} yields a straight line with

$$\text{slope} = -\nu \quad (17a)$$

$$\text{intercept} = \log(K\Lambda^{\nu}\beta), \quad (17b)$$

from which the equilibrium constant is calculated as

$$K = \frac{10^{\text{intercept}}}{\beta\Lambda^{\nu}}. \quad (18)$$

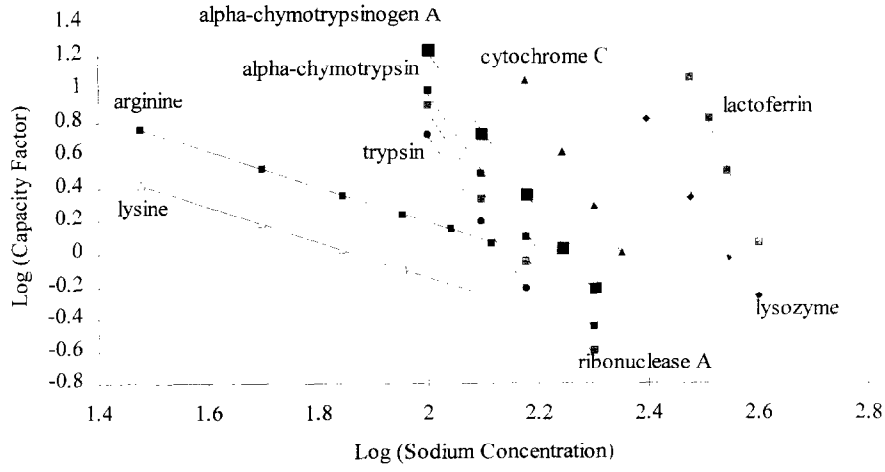

Fig. 3. Plot of log(capacity factor) versus log(sodium concentration) for several biomolecules.

4. Results and discussion

4.1. Energetics of adsorption

Table 1 presents the net charge, characteristic charge (ν), equilibrium constant (K) for the exchange reaction (Eq. (3)), as well as the Gibbs free energy of exchange, ΔG^0_{exc} for a variety of biomolecules. The net charge ranged from 1 for the amino acids lysine and arginine up to 21 for the protamine.

At the pH (pH 6) of this study arginine and lysine possess three charges; a negatively charged α-carboxyl group, a positively charged α-amino group and a positively charged side chain group. As seen in the table, for these amino acids, the characteristic charge is similar to the net charge on the molecule. Wang et al. [25] have also reported that the characteristic charge of amino acids is similar to the net charge of the molecule. For larger biomolecules, the characteristic charge is not determined solely by the net charge.

Neither is the characteristic charge an accurate measure of protein affinity in ion-exchange systems. For example, even though lysozyme and trypsin have identical characteristic charges, the equilibrium constant for lysozyme is much greater than that for trypsin. Moreover, while the peptides examined possess characteristic charges of only 1–2, they exhibit a greater affinity (as measured by the equilibrium constant) for the adsorbent phase than any of the proteins. Clearly, affinity in ion-exchange systems is determined by many more physio-chemical phenomena than simply the number of interactions between the biomolecule and the surface.

The Gibbs free energy of exchange ranges from 4.0 to −8.2 kcal/mol. While the peptides and protamine have favorable energetics, the other biomolecules examined in this study exhibited positive Gibbs free energies for the exchange reaction as written in Eq. (1). The positive value of ΔG^0_{exc} indicates that the equilibrium for this reaction, will lie to the left for these compounds. Thus, these results would indicate that the thermodynamically favorable standard state is for the salt rather than the protein to be bound to the solid phase. In fact, the adsorption of these proteins to the solid phase is not actually disfavored.

The values of the linear equilibrium constant, the Gibbs free energy of adsorption, and the average bond energy for these biomolecules are presented in Table 2. In contrast to the results in Table 1, when the Gibbs free energy is written in terms of the adsorption reaction, all compounds exhibit favorable energetics. The negative values of ΔG^0_{ads} (Table 2) indicate that, in the absence of salt effects, the bound state for the biomolecule is

Table 1
Characteristic charge, equilibrium constant and free energy of exchange for several biomolecules

Biomolecule	Net charge [a]	Characteristic charge (ν) [b]	Equilibrium constant (K) [b]	Gibbs free energy of exchange (ΔG^0_{exc}) (kcal/mol)
α-chymotrypsinogen A	4	4.8	8.9×10^{-3}	2.8
cytochrome C	9	5.9	9.3×10^{-3}	2.8
lysozyme	8	5.3	1.8×10^{-4}	1.0
ribonuclease A	4	4.9	3.1×10^{-3}	3.4
lactoferrin	10	8.1	1.5×10^{-1}	1.1
α-chymotrypsin	3	4.8	5.4×10^{-3}	3.1
trypsin	6	5.3	1.2×10^{-3}	3.9
arginine	1	1.1	5.6×10^{-4}	0.34
lysine	1	1.1	2.6×10^{-4}	0.80
bz-ArgPheTrpLysThrPhe	1	0.9	3.4×10^{0}	−0.71
bz-ArgArgArgTyr	2	1.9	9.3×10^{0}	−1.3
protamine	21	10.2	1.0×10^{6}	−8.2

[a] Estimated by assuming aspartic acid, glutamic acid, lysine and arginine are completely ionized and other amino acids are not.
[b] From data. Fig. 3.

Table 2
Linear equilibrium constant and free energy of adsorption for several biomolecules

Biomolecule	K^* $A = 570$ mM, $Na^+ = 25$ mM	Gibbs free energy of adsorption (ΔG^0_{ads}) (kcal/mol)	Average bond energy $\Delta G^0_{ads}/\nu$ (kcal/mol)
α-chymotrypsinogen A	3.1×10^4	−6.1	−1.3
cytochrome C	1.1×10^6	−8.2	−1.4
lysozyme	3.0×10^6	−8.8	−1.7
ribonuclease A	1.7×10^4	−5.7	−1.2
lactoferrin	1.7×10^1	−13.9	−1.7
α-chymotrypsin	1.7×10^4	−5.7	−1.2
trypsin	1.9×10^4	−5.8	−1.1
arginine	1.6×10^1	−1.6	−1.5
lysine	7.5×10^0	−1.2	−1.1
bz-ArgPheTrpLysThrPhe	6.2×10^1	−2.4	−2.6
bz-ArgArgArgTyr	3.7×10^3	−4.8	−2.5
Protamine	6.6×10^{19}	−26.9	−2.6

preferred. In the presence of salt, significant protein adsorption occurs only when the system is pulled to the right of Eq. (1) by operating at relatively low mobile phase salt concentrations.

As seen in Table 2, the average bond energy of the globular proteins is similar to the bond energies for the positively charged amino acids, arginine and lysine. These results seem to indicate that each charge moiety on the protein surface acts independently and that synergistic or facilitated binding (often observed in polymer adsorption [26,27]) is not occurring to a large extent in these proteins. Except for lysozyme and lactoferrin, the positive charge on these proteins is supplied primarily by lysine. Using the assumption of no synergistic binding, the average bond energy of the lysine rich proteins should approach the bond energy of lysine. In fact, this is what is observed for trypsin, α-chymotrypsin and ribonuclease A. The other lysine rich proteins, α-chymotrypsinogen A and cytochrome C, have slightly higher average bond energies. For lysozyme and lactoferrin, the average bond energy is essentially equal to the bond energy of arginine as would be predicted from the large arginine content of these proteins.

In contrast to the results for the globular proteins, the average bond energy for the peptides and protamine is larger than the bond energy of either lysine or arginine. One plausible explanation for the higher average bond energy is the lack of negatively charged amino acids in these peptides and protamine. Since the proteins contain negatively charged amino acids, there may be repulsion between these negative groups and the negative surface (Fig. 1). This would result in longer bond distances and lower bond energies for molecules with both positive and negative charges as compared to a molecule with only positive charges. On the other hand, an equally possible explanation is that synergistic binding could be occurring in linear molecules due to the added degrees of freedom that charges on a linear molecule possess as compared to the charges on a semi-rigid, globular proteins.

One final observation concerning the magnitude of the ΔG^0_{ads} for the proteins: the low value of ΔG^0_{ads} may explain why ion-exchange is a mild mode of chromatography with respect to protein denaturation. The Gibbs energy of denaturation for three proteins is listed in Table 3. For all three proteins, the energy of denaturation is considerably more than the SMA Gibbs free energy of adsorption. Thus, it would appear that electrostatic interactions in ion-exchange chromatogra-

Table 3
Change in Gibbs energy for protein unfolding

Protein	ΔG (kcal/mol)
lysozyme	14.2
α-chymotrypsin	8.3
ribonuclease A	9.7

From Privalov [28].

phy are small enough to avoid protein denaturation.

4.2. Comparison to theoretical predictions

Yoon and Lenhoff examined the adsorption of ribonuclease A and lysozyme to a charged surface in the presence of a 100 mM 1:1 electrolyte at pH 7. The ΔG^0_{ads} they obtained are compared to values calculated using the SMA method at a sodium concentration of 100 mM, pH 6.0 (Table 4). As seen in the table, the ΔG^0_{ads} calculated using this experimental approach compare favorably to the theoretical values obtained by Yoon and Lenhoff [10]. In subsequent work, Roth and Lenhoff [11] report a slightly lower ΔG^0_{ads} for lysozyme ranging from -0.2 to -1.6 kcal/mol depending on orientation and separation distance. In this latter work the authors used a surface charge density of -2.2 $\mu C/cm^2$ as compared to -10 $\mu C/cm^2$ employed by Yoon and Lenhoff. Clearly, the accurate description of the surface is critical in order to obtain realistic modeling of the adsorption process.

Stalberg et al. [1,2] modeled the adsorption of proteins as the interaction between two infinite surfaces of opposite charge. They report interaction energies in terms of mJ/m^2, thus in order to compare their values to those reported here, a protein was assumed to have a circular projection onto a surface with a diameter of 30 Å. For this scenario, the ΔG^0_{ads} calculated using their data is between -1.5 and -4.0 kcal/mol, depending on ionic strength.

The similarity of the values of ΔG^0_{ads} obtained from such radically different approaches adds credibility to the measured values and to the theoretical frameworks used in their calculation. This is especially true since the magnitude of ΔG^0_{ads} is much lower than might be expected. The average bond energies listed in Table 2 are less than that of hydrogen bonds which are on the order of 2.4–10 kcal/mol, and much less than covalent and ionic bonds, with bond energies of 90–120 kcal/mol [29].

Table 4
Comparison of experimental and theoretical Gibbs free energy of adsorption for lysozyme and ribonuclease A

Protein	ΔG_{ads} (kcal/mol) (SMA)	ΔG_{ads} (kcal/mol) (Ref. [10])
lysozyme	−4.5	−4.4
ribonuclease A	−1.7	−3.7

4.3. Other factors influencing adsorption

The SMA model is the simplest model that is consistent with our data. It ignores some phenomena that may be important under some conditions: protein–protein interactions in solution, protein–protein interactions while adsorbed and binding of ions by protein.

Under conditions of dilute protein solutions, for proteins that are not prone to aggregate, protein–protein interactions in solution will probably be of little consequence. While these conditions exist for the present experiments, they will not always apply in actual preparative situations. Protein–protein interactions while absorbed may be of greater significance because of the elevated local protein concentration on the surface of the stationary phase. For complex protein mixtures typically found in biotechnology feed stocks, such interactions may indeed be significant.

Velayudhan and Horvath [22] have shown that the ion-exchange chromatographic behavior of proteins can be accurately modeled without including the effect of co-ions on protein adsorption. Similarly, our results to date suggest that ion-exchange chromatography of proteins can be understood without explicitly accounting for co-ion binding to the proteins.

Nevertheless, binding of co-ions by proteins should be considered in detail, particularly at elevated salt concentrations such as those encountered in step-gradient chromatography. Monovalent ions also play a role in protein conformation. It is well known that surface charge pH and ion concentrations can have a profound effect on catalytic activity of enzymes [30]. Monovalent ion binding that affects catalytic activity has also been linked to conformational changes [31].

Although the above considerations are not to be dismissed lightly,they seem not to dominate

the behavior of the chromatographic system under consideration. Nevertheless, application of the SMA model to more complex systems may require attention to some of the above interactions and effects.

5. Conclusions

In this work we have demonstrated that the average bond energy (i.e. Gibbs free energy of adsorption divided by the number of bonds formed between the protein and the surface) for globular proteins on ion exchange surfaces is comparable to the bond energies for the positively charged amino acids, arginine and lysine, involved in protein cation-exchange. These results seem to indicate that each charge moiety on the protein surface acts independently and that synergistic or facilitated binding is not occurring to a large extent in these proteins. In contrast, the average bond energy for the peptides and protamine is larger than the bond energy of either lysine or arginine.

The ΔG^0_{ads} calculated using the experimental approach described in this manuscript compare favorably to the theoretical values obtained from various investigators. The similarity of the values of ΔG^0_{ads} obtained from such radically different approaches adds credibility to the measured values and to the theoretical frameworks used in their calculation. In future work we will attempt to relate the SMA parameters to more fundamental physicochemical characteristics of the biomolecules and chromatographic surface.

6. List of symbols

Q_i = stationary phase concentration of species i (mM)

$\overline{Q}_i$ = stationary phase concentration of salt ions available for exchange (mM)

C_i = mobile phase concentration of species i(mM)

K = equilibrium constant (dimensionless)

K^* = linear equilibrium constant (dimensionless)

k' = capacity factor (dimensionless)

ΔG^0_{ads} = Gibbs free energy of adsorption (kcal/mol)

ΔG^0_{exc} = Gibbs free energy of exchange (kcal/mol)

ν = characteristic charge (dimensionless)

σ = steric effect (dimensionless)

Λ = total ion capacity of stationary phase (mM)

β = column phase ratio (dimensionless)

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