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An Analysis of the Interactions of BSA with an Anion-Exchange Surface Under Linear and Non-Linear Conditions

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Abstract. The interactions of BSA with an anion-exchange adsorbent have been studied to aid in the understanding of protein adsorption in ion-exchange chromatography. Linear chromatography, flow microcalorimetry and isotherm measurements were used to analyze adsorption energetics in the linear and overloaded regions of the equilibrium isotherm. The effects of salt type, salt and protein concentration, and temperature are reported. It was observed that under all conditions studied the adsorption process was entropically driven. This was contrary to expectations, since at the pH selected ion exchange is expected to dominate. A major driving force for the adsorption of BSA on the anion exchanger was concluded to be the increase in entropy from the release of water due to interactions between hydrophobic regions on the protein and adsorbent. The data further suggest that the conformational entropy change accompanying protein adsorption on the ion exchanger may also be significant.

Keywords: protein adsorption, BSA, isotherms, ion-exchange, heat of adsorption, zeta potential, water-release

1. Introduction

Ion-exchange chromatography (IEC) is a popular technique that is commonly used for downstream purification of biomolecules. Its popularity stems primarily from the mild conditions in which separations take place. There are numerous reports in the literature that demonstrate the applicability of this technique for protein purification (Thrash Jr. and Pinto, 2000). In IEC, attractive electrostatic forces between ionic moieties on the adsorbate (biomolecule) and fixed charged groups (ligands) on a solid support are considered to be primarily responsible for adsorption. Separations are achieved through selectivity differences in this reversible adsorption. Though IEC is widely used for the purification of proteins, separation characteristics often cannot be predicted under chromatographic (salt gradient) conditions. Preparative applications are further complicated by the non-ideal interactions associated with protein adsorption under overloaded conditions.

A favorable interaction (i.e., release of energy) is expected between two oppositely charged surfaces. In some IEC applications, calorimetry has given results contrary to this expectation. Bowen and Hughs (1993) conducted direct calorimetric measurements for the adsorption of BSA on two polymeric anion-exchange adsorbents. The heat of adsorption was observed to be mostly endothermic, although exothermic heats were recorded under certain experimental conditions. The heat of adsorption was influenced by both the salt concentration and the adsorbent type. It was suggested that in cases where the enthalpy change was unfavorable, the increase in entropy due to the release and/or rearrangement of water molecules is driving adsorption. Gill et al. (1994) measured the change in enthalpy of adsorption (ΔH) of recombinant cytochrome b5 onto a polymeric anion-exchange surface at different temperatures. The ΔH was positive in all cases, implying that adsorption was entropically driven. This was attributed

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to the release of ions and water of hydration from the surface of the protein and the adsorbent. Raje and Pinto (1998) also reported endothermic adsorptive heats for BSA adsorbing onto a siliceous anion-exchange adsorbent. In addition to dehydration and ion release, it was postulated that structural rearrangements may contribute to the entropic force driving adsorption. These results underscore the point that adsorption of an ionized protein on charged surfaces can be influenced by more than electrostatic attraction or repulsion, and the controlling thermodynamic force may be an entropy increase. Dragan et al. (2003a) have argued that the association of AT-hook peptides with DNA is an entropically driven process in which the dehydration of polar and apolar moieties are key entropic events. The same research group (Dragan et al., 2003b) has also shown that the release of water plays a central role in the association of HMG-D protein with DNA. Salvay et al. (2003) have discussed the influence of dehydration on the kinetics of oxygen binding to hemoglobin. Dias-Cabral et al. (2003) have also discussed the impact of water-release as it relates to protein binding on hydrophobic adsorbents.

All of the studies cited conclude that water release is a key component of the entropic driving force underlying protein adsorption/binding to charged or uncharged surfaces/ligands. This is significant because the entropy change of water release has been estimated to lower the adsorptive free energy by as much as 4–12 mJ/m^2 (Norde, 1998; Nemethy and Scheraga, 1962); the magnitude of the free energy reduction depends on whether the contact surface of the protein consists of aromatic or aliphatic moieties. In addition to the effects of water release, it has been shown (McKay and Fernandez, 2002) that structural changes associated with protein adsorption on mildly hydrophobic surfaces can be significant.

In this study, the adsorption of BSA on a weak anion-exchange support was used to evaluate the significance of non-electrostatic effects in ion exchange. Protein retention data in the linear chromatographic mode were obtained as a function of temperature and salt concentration and analyzed with the Van't Hoff relation (Esquibel-King et al., 1999; Perkins et al., 1997; Lee and Cho, 1993a, 1993b),

$$\ln(k') = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln(\Phi) \qquad (1)$$

where: k' is the chromatographic retention factor, ΔH° is the standard-state enthalpy change for transfer from the solution phase to the adsorbent phase, ΔS° is the corresponding standard-state entropy change, T is the temperature, R is the universal gas constant, and Φ is the phase ratio. ΔH° and ΔS° were calculated from this equation.

Linear chromatographic retention data were also analyzed with the Preferential Interaction (PI) model (Perkine et al., 1997), to estimate the water release from the contact surface of the protein and the adsorbent. The defining relationship for this model is:

$$\ln(k') = c + \frac{(\Delta\upsilon_+ + \Delta\upsilon_-)}{g}\ln(m_3) - \frac{n\Delta\upsilon_1}{m_1 g}m_3 \qquad (2)$$

c is a constant of integration, $\Delta\nu_1$ is the net displacement of water molecules from the adsorbent surface and the protein contact surface per molecule of protein adsorbed, $(\Delta\nu_+ + \Delta\nu_-)$ is the net quantity of modulator ions released from the adsorbent surface and the protein contact surface per molecule of protein adsorbed, m_3 is the molal modulator concentration, m_1 is the molal water concentration, n is the total number of cations and anions per formula unit of the modulator, and g is the ratio $(\partial\ln(m_3)/\partial\ln(a_3))_{T,P}$, where a is the activity of the modulator. For the temperature range used in this study, g was assumed to be constant. Although this model was initially developed for calculating water release for HIC, it is a general model applicable to other forms of chromatography such as IEC and RPC (Esquibel-King et al., 1999). By correlating the retention factor to the salt concentration, as per Eq. (2), the water release and counter-ion release parameters were determined from a non-linear regression analysis that employs the Leventhal-Marquardt method.

To study equilibrium behavior under overloaded conditions, BSA adsorption isotherms were measured in the presence of three different 1:1 modulators (salts) at two different temperatures. Zeta potential measurements were made to characterize the electrical potential of BSA at equilibrium solution conditions. The energetics of adsorption under overloaded (non-linear) conditions was studied with flow microcalorimetry.

2. Experimental

2.1. Materials and Apparatus

Bovine Serum Albumin (BSA) was purchased from Sigma (St. Louis, MO, USA) and used without further purification. The IEC support used (PEI-1000-10)

is siliceous-based with a mean particle diameter of 10 μm and an average pore size of 1000 Å. The macroporous surface area is approximately 30 m^2/g (Esquibel-King et al., 1999), and is activated with cross-linked polyethylenimine ligands. The IEC support was purchased from the Millipore Corporation (Bedford MA., USA).

The carrier fluid for the IEC experiments was 10 mM piperazine (pH = 6.2). Sodium chloride, potassium chloride and lithium chloride were used as modulators for all IEC experiments. The salts were purchased from the Fisher Scientific Company (Hanover Park, IL., USA). Piperazine was purchased from the Eastman Company (Kingsport TN, USA).

2.2. *Flow Microcalorimetry*

The Flow MicroCalorimeter (FMC) (Gilson Instruments, Westerville, OH., USA) is operated similar to a liquid chromatograph. The column (or cell) volume is 0.171 ml and is interfaced with two highly sensitive thermisters. The FMC is capable of detecting small temperature changes within the cell that are associated with the adsorption of an analyte onto the surface of a particular adsorbent. The flow rate through the cell is controlled by precision syringe micropumps. A block heater is used to monitor and control the cell temperature. As in a chromatograph, the FMC is equipped with a configurable injection loop to accommodate different injection volumes. The effluent was collected and analyzed with a UV Spectrophotometer (Milton Roy, Rochester NY).

The FMC is initially filled with a specified volume of adsorbent. The next step is the evacuation of the cell. The evacuation process to a vacuum pressure of 30 in Hg usually requires 24 hrs. The purpose of this step is to remove all air from the resin surface. Once the cell has been successfully evacuated, the contents are "wetted" with the carrier fluid. Following wetting the syringe pumps are turned on and the adsorbent is equilibrated with the carrier solution at a flow rate of 3.3 ml/hr. When the system has reached thermal equilibrium, the sample (20 mg/ml of protein dissolved in the carrier fluid) is loaded into a 1 ml injection loop, and introduced into the cell by switching a multiport valve. The adsorption of the sample onto an adsorbent surface causes a change in cell temperature, which is converted to a heat signal by the FMC through an experimentally determined calibration factor. (The calibration factor was obtained using the 50 μm PEI-1000 particles.) Once the mass in the effluent is quantified with the spectrophotometer, a simple mass balance is performed to determine the quantity of sample adsorbed. From these data the specific heat of adsorption is calculated.

2.3. *Isocratic Elutions*

Capacity factor data were obtained on an HP1100 chromatograph unit with a 25.0 × 0.46 cm I.D. column at 25, 33 and 40°C at pH 6.2 (piperazine buffer). The column was equilibrated with buffer at different concentrations of salt and a flow-rate of 1.0 ml/min. Elution times were measured for the injection of 3 μl of 2.0 mg/ml protein solution. For Van't Hoff plots the salt range was 300–500 mM salt, and for analysis with the PI model it was 300–1000 mM salt. All responses were monitored at 280 nm.

2.4. *Isotherms*

BSA isotherms were measured at selected salt concentrations in 10 mM piperazine buffer at 25°C (pH 6.2) by the batch method. PEI-1000-10 was weighed into test tubes, and protein solution of a known concentration was pipetted into the tube. The test tubes were sealed with parafilm, placed in a shaker, and agitated at 200 rpm for 24 hrs at 25°C. Preliminary experiments have established that equilibrium is effectively reached in 8–10 hrs. After equilibration, the slurry solution was allowed to stand for 1 h before filtering with a 0.45-μm filter. The absorbance of the filtrate was measured at 280 nm to obtain the equilibrium solution concentration. The equilibrium distribution was calculated from a mass balance.

2.5. *Zeta Potential Measurements*

Zeta potential measurements were made on a Zeta-Pals instrument (Brookhaven Instruments). A 10 mg/ml protein solution was added to a cuvette, which was inserted into a temperature-controlled chamber. An electrode in the cuvette creates an electric field that induces protein movement. When the electrode is energized, a laser beam is fired through the solution. The magnitude and direction of the shift in the beam is proportional to the mobility of the protein. Once the mobility is known, the zeta potential is calculated using the Smoluchowski Equation (Hunter, 1981).

3. Results and Discussion

The Van't Hoff plots for the interaction of BSA with PEI in the presence of LiCl, NaCl and KCl are shown in Figs. 1(a), (b) and (c), respectively. The BSA retention is longest in the presence of KCl and shortest in LiCl, at 25, 33 and 40°C. Hearn et al. (1980) have explained this trend on the size of the hydrated cation

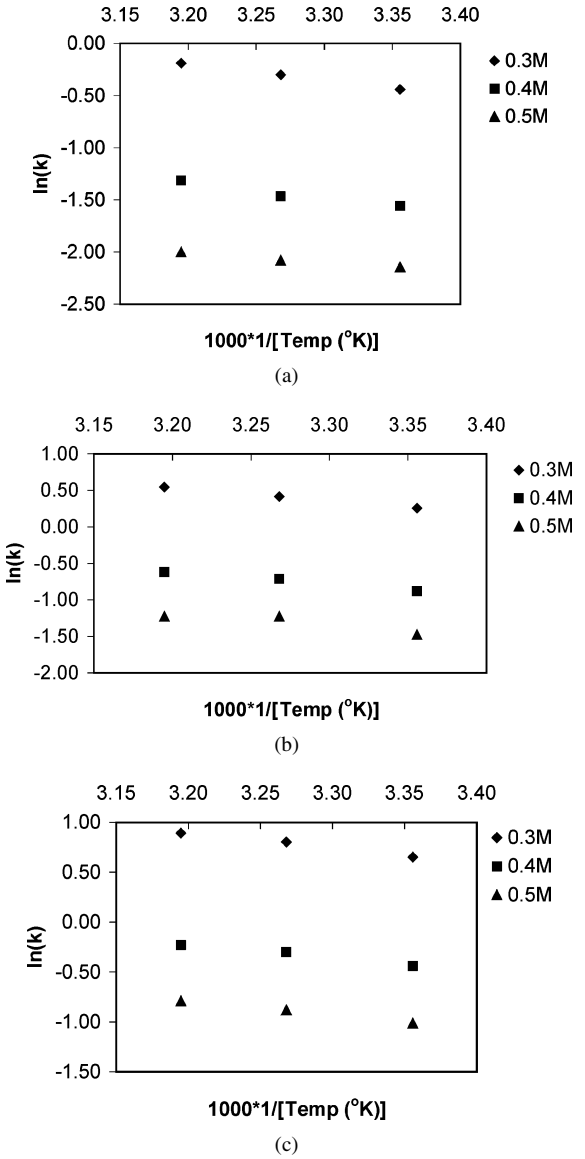

Figure 1. (a) Van't Hoff plot for BSA retention in the presence of LiCl. (b) Van't Hoff plot for BSA retention in the presence of NaCl. (c) Van't Hoff plot for BSA retention in the presence of KCl.

and its effect on the interaction between the adsorbate and the adsorbent. The plots in Fig. 1 are close to linear, suggesting that the adsorption mechanism is not changing significantly with temperature. The negative slope indicates that the standard-state enthalpy change (ΔH^o) is endothermic in each case.

The values of ΔH^o and ΔS^o calculated by applying Eq. (1) to Fig. 1 are summarized in Table 1. The data indicate that an increase in entropy is driving adsorption under linear conditions. No clear correlation is discernable between ΔH^o and modulator type; however, the ΔH^o does noticeably decrease with increasing salt concentration. ΔS^o also generally decreases with increasing salt concentration. Though entropy is the driving force, in some cases ΔS^o is not significantly different in the presence of KCl and NaCl, though the corresponding differences in BSA retention are significant. This suggests that the balance of thermodynamic forces (entropy/enthalpy) shifts with salt concentration.

It has been argued that the release of water from the contact surface of the protein and adsorbent may significantly contribute to the adsorptive driving force in ion-exchange chromatography. Estimates of water release, calculated from Eq. (2), are shown in Table 2. As expected, the ion-exchange term ($\Delta\nu_+ + \Delta\nu_-$) is large, however, the amount of water release ($\Delta\nu_1$) is also significant. Most noticeably, the amount of water released appears to depend on the cation present in solution. Since there is no significant temperature dependence in Table 2, the water release numbers were averaged over the temperature range for each salt for ease of comparison and are also presented. Water release is largest in the presence of KCl and is significantly lower in the presence of LiCl. If this is the major contributor to entropy change, the largest entropy change can be expected for KCl and the smallest for LiCl. Significant water release would also explain why retention increases with temperature. It has been shown (Norde, 1998) that the free energy reduction arising from water release becomes more negative with increasing temperature. Therefore as water release becomes more favorable, the interaction between the protein and surface should increase. This will be discussed further later in this section.

Differences in water release can arise from conformational or orientational differences between adsorbed proteins. Protein conformational changes have been shown to produce endothermic heats, and in these cases a significant entropy increase is associated with the structural change (Andrade, 1985). It is, however, unlikely that conformational changes underlie the

Table 1. Van't Hoff estimates of enthalpy and entropy changes for adsorption of BSA on anion exchanger PAE-1000-10 (0.3–0.5 M Salt).

	298 K			306 K			313 K		
Modulator	ΔH^o kcal/mole	$T\Delta S^o$ kcal/mole	ΔG^o kcal/mole	ΔH^o kcal/mole	$T\Delta S^o$ kcal/mole	ΔG^o kcal/mole	ΔH^o kcal/mole	$T\Delta S^o$ kcal/mole	ΔG^o kcal/mole
0.3 M LiCl	3.1	6.6	−3.5	3.1	6.8	−3.7	3.1	6.9	−3.8
0.4 M LiCl	2.9	5.8	−2.6	2.9	5.9	−3.0	2.9	6.1	−3.2
0.5 M LiCl	1.7	4.3	−2.6	1.7	4.4	−2.2	1.7	4.5	−2.8
0.3 M NaCl	3.6	7.5	−3.9	3.6	7.7	−4.1	3.6	7.9	−4.3
0.4 M NaCl	3.2	6.5	−3.3	3.2	6.7	−3.5	3.2	6.8	−3.6
0.5 M NaCl	3.1	6.0	−2.9	3.1	6.2	−3.1	3.1	6.3	−3.2
0.3 M KCl	3.0	7.1	−4.1	3.0	7.3	−4.3	3.0	7.5	−4.5
0.4 M KCl	2.6	6.1	−3.5	2.6	6.3	−3.7	2.6	6.4	−3.8
0.5 M KCl	2.8	5.9	−3.1	2.8	6.1	−3.3	2.8	6.2	−3.4

Table 2. Preferential interaction estimate of water release accompanying adsorption of BSA on anion exchanger PAE-1000-10 (0.3–1.0 M salt).

Modulator	Temp (°K)	$\Delta\upsilon_1$ (mol/mol)	Avg. water release (mol/mol)	$(\Delta\upsilon_+ + \Delta\upsilon_-)$ (mol/mol)
LiCl	298	57	60	3
	306	60		3
	313	63		3
NaCl	298	70	80	5
	306	80		5
	313	89		5
KCl	298	111	108	5
	306	107		5
	313	108		5

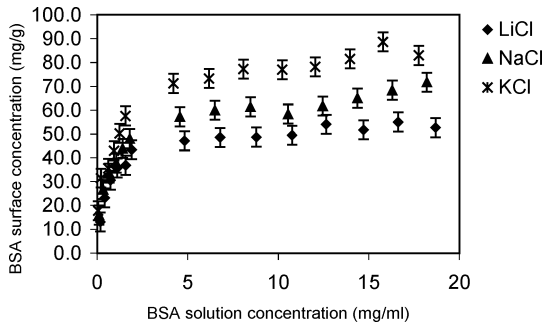

Figure 2. Effect of salt on BSA adsorption isotherms on anion exchanger PEI-1000-10 at 25°C. The concentration of each salt was 100 mM.

differences observed, since there is no evidence in the literature that alkali ions cause such changes in BSA in solution. Another possibility is that the protein adsorbs onto the surface in different orientations due to the influence of the salt cation. Since BSA is an ellipsoid (Squire et al., 1968), it can adsorb to the surface in an orientation between "end-on" and "side-on". In the presence of potassium and sodium the adsorbed protein may be predominantly side-on giving a higher specific water release, while in the presence of lithium the end-on position predominates.

To study adsorption behavior under overloaded conditions, isotherm measurements were made at 100 mM salt (Fig. 2). Type I (Langmuir) isotherms were observed, and, as expected, a significantly greater capacity was measured in the absence of salt. For ion-exchange systems, this behavior is generally explained as the result of competition between protein and salt for the ion-exchange sites, or due to a reduction in the electrostatic potential of the adsorbent and/or the protein (Thrash Jr. and Pinto, 2002). It is also significant, for this anion-exchange system with very similar *cations*, that at high protein concentrations the capacity is a strong function of the cation type; KCl gives the highest capacity and LiCl the lowest

Heats of adsorption were measured by calorimetry under overloaded (non-linear) conditions and the results obtained at 25°C are shown in Fig. 3. The experimental conditions are such that $\Delta H = Q$. There are a number of significant characteristics in the data. First, in all cases the heat of adsorption is endothermic, with significantly larger values than estimated by the Van't Hoff method under linear conditions. Secondly, the heat of adsorption is strongly influenced by the surface coverage. As the protein surface coverage increases,

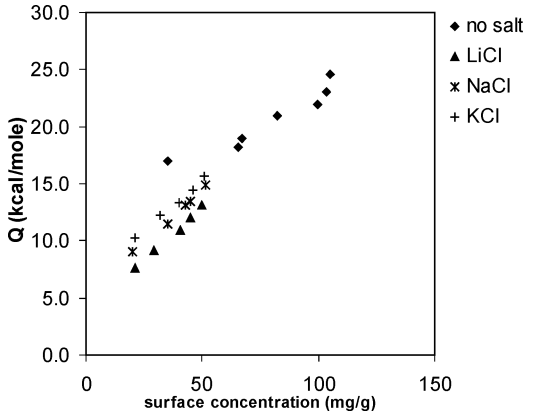

Figure 3. Effect of salt and protein concentration on heat of adsorption data for BSA on PEI 1000–10 at 25°C. The concentration of each salt was 100 mM.

the endothermic heat increases. Also, the magnitude of the endothermic heat is largest in the absence of salt. It is clear that electrostatic forces are not driving the adsorption of BSA onto this anion-exchange support. If they were, the net heat of adsorption would be exothermic. Also, the usual explanation for the effect of salt on capacity (previous paragraph, Fig. 2), which is derived solely from an analysis of isotherm data, is clearly inadequate. These results stress the importance of measuring heats of adsorption calorimetrically, rather than estimating them from retention or isotherm data.

Endothermic heats of adsorption for protein ion-exchange systems have been reported the literature (Bowen and Hughes, 1993; Raje and Pinto, 1998; Thrash Jr. and Pinto, 2002; Lin et al., 2000). The net driving force in such systems is an increase in the entropy. The increase in endothermic heat of adsorption with increasing surface coverage can be explained by repulsive interactions between surface proteins (Bowen and Hughes, 1993; Raje and Pinto, 1998; Nemethy and Scheraga, 1962). The heat of adsorption measurements are predominantly at a high fractional coverage (Fig. 2), and repulsive interactions between the similarly charged proteins can be significant. The repulsive energy is seen to increase approximately linearly with increasing protein surface concentration. This is observed both in the presence and absence of salt. Higher endothermic heats are observed in the absence of salt, because the salt screening effect between adsorbed proteins is not present.

To investigate the origin of the entropic driving force, an analysis was performed to determine if entropy increase due to water release, as estimated by the PI model, is significant enough to counter the unfavorable enthalpic contribution directly measured from calorimetry. At 25°C the release of water upon adsorption can reduce the adsorptive free energy by approximately 12 mJ/m^2 of dehydrated surface, if the contact surface of the protein and the adsorbent are primarily aliphatic in nature, as is the case for BSA and the PEI adsorbent. The free energy of water release from an aliphatic surface was calculated from Eq. (3) (Nemethy and Scheraga, 1962).

$$\Delta G = 957 - 6.08T + 0.00824T^2 \qquad (3)$$

Here ΔG is the free energy of water release in calories/(mole of water), and T is the temperature in degrees Kelvin. This reduction in free energy arises primarily from the increase in entropy associated with the release of water from the first hydrated layer around the protein surface.

The entropic contribution ($T\,\Delta S_{\mathrm{wr}}$) of water release was estimated from Eq. (3), using the average number of water molecules released (Table 2), and the measured ΔH averaged over the protein surface concentration range studied. The measured ΔH is the net sum of all enthalpic events associated with adsorption: repulsive interactions (ΔH_{rep}), electrostatic protein-surface interactions (ΔH_{ps}), van der Waals interactions (ΔH_{vdw}), the release of water (ΔH_{wr}), and structural changes (ΔH_{conf}) on the adsorbent surface. It was assumed that half of the water molecules released are from the monolayer on the contact-surface of the protein and the other half are from a monolayer on the adsorbent. Each water molecule was assumed to have a radius of 1.5 Å (Perkins et al., 1997) with an effective surface area of 7.1×10^{-20} m^2.

The estimated entropic contribution ($\Delta S_{\mathrm{wr}}/R$) of water release at 25°C is reported in Table 3. The variation in the magnitude of ΔS_{wr} with salt is

Table 3. Comparison of enthalpy and entropy changes for BSA adsorption on anion exchanger PAE-1000-10 under overloaded conditions.

	Average ΔH_{expt}/RT (dimensionless)		$\Delta S_{\mathrm{wr}}/R$ (dimensionless)	
Salt concentration	25°C	37°C	25°C	37°C
100 mM KCl	19.8	32.1	63.6	52.9
100 mM NaCl	17.9	32.9	47.1	39.2
100 mM LiCl	16.6	31.5	35.3	29.4

consistent with the corresponding trend in adsorption capacities in Fig. 2. In each case the entropic portion of the Gibbs free energy is large enough to overcome the unfavorable enthalpic contribution associated with adsorption at 25°C.

The free energy reduction resulting from water release at 37°C, calculated from Eq. (3), is approximately 13.3 mJ/m^2 of dehydrated surface. Since this is larger than at 25°C, adsorption capacity should be higher at 37°C, if the water release effect is significant. Figures 4(a), (b) and (c) compare experimental isotherms for BSA on PEI in the presence of LiCl, NaCl and KCl, respectively, at 25°C and 37°C. These figures show that at the higher temperature there is a small but consistently larger capacity in the linear portion of the isotherm. However, it is unclear from these plots if there is a significant difference in adsorption capacity in the non-linear regions of the isotherms.

Heat of adsorption experiments were performed at 37°C to better understand the effects of temperature. The results are shown in Fig. 5. As at 25°C, the heat of adsorption is endothermic and there is a noticeable difference in magnitude in the absence presence of salt. Significantly, the magnitude of the endothermic heat is consistently higher at 37°C.

It has been previously suggested (Thrash Jr. and Pinto, 2002) that the endothermic adsorptive heats arise primarily from repulsive interactions between adsorbed proteins possessing the same charge. If the potential around the protein significantly increases with temperature, then an increase in repulsion would explain the higher endothermic heats at 37°C. To investigate this, the zeta potential for BSA in the presence of each salt was measured at 25°C and 40°C. As seen in Table 4, the change in zeta potential with temperature is negligible. Thus, repulsive forces are not significantly contributing to the increased magnitude of the endothermic adsorptive heats at the higher temperature.

The effect of a change in temperature on ΔH_{wr} (enthalpy change of water release) was estimated to establish its contribution to the overall enthalpy change. ΔH_{wr} was calculated by adding the entropic contribution, $T\Delta S_{\mathrm{wr}}$, estimated using the method of Nemethy and Scheraga (1962), to the free energy given by Eq. (3). These calculations show that the enthalpic contribution of water release is more favorable at the higher temperature, and therefore cannot be a contributor to the increased endothermic heats observed experimentally.

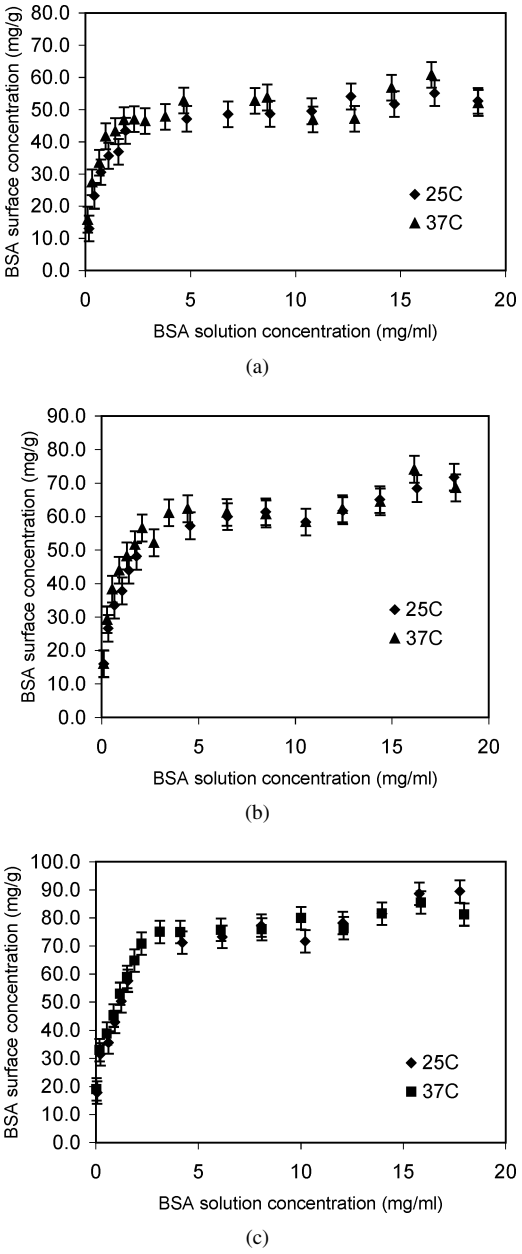

Figure 4. (a) Effect of temperature of BSA isotherms in the presence of 100 mM LiCl. (b) Effect of temperature on BSA isotherms in the presence of 100 mM NaCl. (c) Effect of temperature on BSA isotherms in the presence of 100 mM KCl.

An evaluation was performed at 37°C, identical to that at 25°C, to determine if the $T\Delta S_{\mathrm{wr}}$ can counter the larger unfavorable enthalpic contribution at this temperature. The results (Table 4) show that the entropy increase from water release exceeds the unfavorable

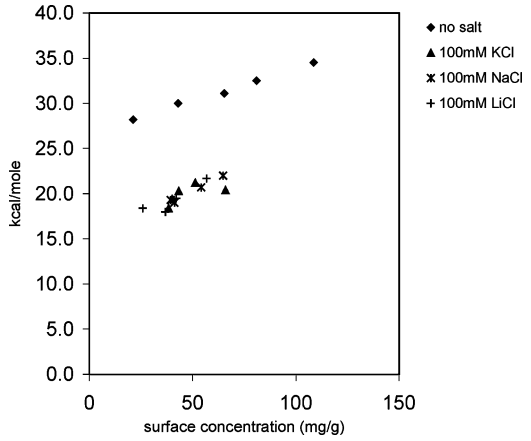

Figure 5. Effect of salt and protein surface concentration on heat of adsorption data for BSA on anion exchanger PEI 1000-10 at 37°C.

Table 4. Influence of salt and temperature on zeta potential of BSA.

Salt concentration	25°C	40°C
0 mM	−8.3 mV	−8.3 mV
100 mM LiCl	−4.41 mV	−4.77 mV
100 mM NaCl	−3.41 mV	−2.80 mV
100 mM KCl	−4.25 mV	−2.97 mV

enthalpy change for the KCl and NaCl cases. For LiCl, however, this is not the case. Since significant adsorption of protein does occur in the presence of LiCl at 37°C, the overall ΔG is negative. Moreover, the BSA adsorption capacity at 37°C in the presence of KCl and NaCl is similar to the corresponding adsorption capacity at 25°C. These results suggest that additional entropic effects, beyond that of water release considered for the estimates in Tables 4 and 5, must be present, at least at the higher temperature.

If the protein upon adsorption rearranges its structure into a more thermodynamically favorable conformation, the endothermic heat of adsorption will increase due to the energy requirement for rearrangement. The change in the differential heat capacity of adsorption $\Delta_{\text{ads}}C_p$ has been suggested (Haynes and Norde, 1995) as a measure of a change in conformation of the protein with temperature. An increase in this value is thought to indicate a loss of order in the secondary structure of a protein or be due to the transfer of nonpolar moieties to a more polar environment. Since the latter is not significant for the present case, the change in the differential heat capacity can be used to probe for a change in conformation with temperature. The differential heat capacity of adsorption was calculated from (Haynes and Norde, 1995):

$$\Delta_{\text{ads}}C_p = \left(\frac{\partial \Delta_{\text{ads}}H}{\partial T}\right)_{P,\text{pH}} \approx \left(\frac{\Delta H}{\Delta T}\right)_{P,\text{pH}} \quad (4)$$

using the data in Table 3. The results in Table 5 show an increase in the $\Delta H_{\text{ads}}C_p$ in all cases. Therefore, changes in the protein's secondary structure may underlie the larger endothermic heats observed at the higher temperature, and explain why adsorption occurs in the presence of LiCl at this temperature, despite the absence of a sufficient driving force from water release. It also suggests that the contribution of conformational changes to the overall thermodynamic force may be significant under conditions that appear to be dominated by the release of water.

Table 5. Differential heat capacities of adsorption ($\Delta_{\text{ads}}C_p$) for BSA on anion exchanger PAE-1000-10.

Salt concentration	$\Delta_{\text{ads}}C_p$ kcal/(mole °K)
100 mM NaCl	0.7333
100 mM KCl	0.975
100 mM LiCl	1.05

4. Conclusions

Protein adsorption under non-linear chromatographic conditions is clearly a complicated phenomenon. For the system studied (BSA-PEI 1000-10) Van't Hoff data and direct calorimetric data show that adsorption is endothermic in all cases, indicating that protein adsorption is entropically driven. Results from the preferential interaction model show that water release accompanying adsorption onto of BSA on the anion exchanger is significant, and of the same order of magnitude as water release accompanying BSA adsorption onto hydrophobic surfaces. For the particular case studied, it has been shown that the release of water is large enough to overcome the unfavorable enthalpic energy change at 25°C. At 37°C the free energy reduction from water release was not always sufficient to overcome unfavorable enthalpic effects. At the higher temperature it is suggested that the entropy increase associated with structural rearrangement is also contributing to the entropic driving force responsible for BSA adsorption.

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Nomenclature

g	$(\partial \ln(m_3)/\partial \ln(a_3))_{T,P}$
ΔH	Heat of adsorption (kcal/mole)
k	Boltzman constant
$\ln(k')$	Natural log of retention factor
m_1	Molal concentration of water (55.51 moles/Kg)
m_3	Molal modulator concentration (moles/Kg)
n	Number of atoms comprising salt
R	Universal gas constant
ΔS	Entropy of adsorption (kcal/(mole-^{o}K))
Φ	Column phase ratio (volume of active surface/volume of liquid)
$(\Delta\nu_+ + \Delta\nu_-)$	Net release of ions from the contact surface of the protein and the contact surface of the adsorbent per molecule of adsorbed protein.
$\Delta\nu_1$	Net release of water molecules from the contact surface of the protein and the contact surface of the adsorbent per molecule of adsorbed protein.

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