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STUDIES ON HYDROPHOBIC INTERACTION ADSORPTION OF BOVINE SERUM ALBUMIN ON POLYPROPYLENE GLYCOL–SEPHAROSE UNDER OVERLOADED CONDITIONS

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ABSTRACT

In this paper, the adsorption of bovine serum albumin on polypropylene glycol–sepharose gel under both the linear and nonlinear hydrophobic interaction chromatography (HIC) conditions is described. Characterization of behavior was pursued through a combination of flow microcalorimetry, linear chromatography, and isotherm measurements. It is shown that these measurements support the strong influences of water release and protein conformation in the HIC process. It has also been shown that the Van't Hoff analysis does not provide reasonable estimates of the heat of adsorption under nonlinear conditions.

Key Words: Hydrophobic interaction chromatography; Flow microcalorimetry; Albumin; Proteins

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INTRODUCTION

Hydrophobic interaction chromatography (HIC) is widely used for separation and purification of proteins. Reports in the literature include the successful isolation of enzymes, purification of therapeutic proteins, and the removal of viruses from human plasma (1–4).

The improvement of the design and operation of adsorptive separation processes has recently gained much attention. A quantitative description of the underlying adsorption process is essential for this purpose. In HIC the adsorption is driven by the increase in entropy that accompanies the reduction in the total hydrophobic surface area (5–7). As the protein mixture flows through a column, hydrophobic patches on the surface of the proteins contact with the hydrophobic ligands on the support. The total wetted area decreases, and water is released. Water molecules in contact with hydrophobic areas, unlike those near polar surfaces or in solution with other water molecules, do not experience a highly favorable enthalpy of interaction. Thus the liberation of water from hydrophobic regions increases the entropy of the system without significantly changing its enthalpy, leading the free energy to decrease on adsorption (6).

Linear HIC has been used as a tool for investigating the mechanisms underlying the hydrophobic interaction process (6,8–12). One approach exploits the well-known Van't Hoff relation. Horváth and co-workers (8,9,12) have used this relation in a study of the HIC retention behavior of amino acids on Spherogel, SynChropak propyl, and TSK-GEL® Butyl-NPR supports. They found that for this systems the Van't Hoff plot was nonlinear, and explained the nonlinearity on the basis of changes in the standard state enthalpy change (ΔH^0), standard state entropy change (ΔS^0), and phase ratio (ϕ), with temperature. As a correction they used Kirchoff's relations, and derived the following equation:

$$\ln k' = \frac{\Delta C_P^0}{R}\left(\frac{T_H}{T} - \ln\frac{T_S}{T} - 1\right) + \ln\phi \tag{1}$$

which is termed the "logarithmic equation". ΔC_P^0 is the standard state heat capacity change, and T_H and T_S are the reference temperatures at which ΔH^0 and ΔS^0 are zero.

In the derivation of Eq. (1) Horváth and co-workers assumed that the heat capacity change does not vary with the temperature, which is a crude assumption, since it was suggested by Privalov and Makhatadze (13–15) that the heat capacity change associated with hydrophobic interactions depends linearly on the temperature and converges to zero at high temperatures. For this reason, Horváth and co-workers (8) also evaluated the thermodynamic

quantities associated with the chromatographic retention process without assuming constant ΔC_P^0. They derived the following parameter expression termed the "quadratic equation":

$$\ln k' = a + \frac{b}{T} + \frac{c}{T^2} + \ln \phi \tag{2}$$

The enthalpy, entropy, and heat capacity changes can be calculated with the parameters in Eq. (2) by using the following expressions:

$$\Delta H^0 = -R \frac{\mathrm{d} \ln k'}{\mathrm{d}(1/T)} = -R\left(b + \frac{2c}{T}\right) \tag{3}$$

$$\Delta S^0 = R(a - c/T^2) \tag{4}$$

and

$$\Delta C_P^0 = 2Rc/T^2 \tag{5}$$

Using Eqs. (1) and (2) Horváth and co-workers concluded that the adsorption of amino acids on the HIC supports studied, both enthalpy and entropy changes were positive at low temperatures and negative at high temperatures (8).

The selectivity of a chromatographic separation process is adjusted by changing salt concentration of the mobile phase. At high salt concentrations, protein adsorption is dominated by hydrophobic interactions, and lowering the salt concentrations causes the protein retention time to decrease. As the salt concentration is lowered further, incomplete shielding of charges on the protein and surface can lead to adsorption by ion-exchange mechanisms. At this point, additional reductions in the salt concentration will cause the retention time to increase (6). An explanation based on the preferential interaction (PI) analysis for the effect of salts on protein adsorption (16) has been proposed by Perkins et al. (6). They used a model-independent thermodynamic analysis to relate the effect of salt on the observed equilibrium constant to the change in the distribution of salt ions and water molecules. This analysis predicts that processes that reduce wetted surface area are favored by solutes that are preferentially excluded from the surfaces. This approach is valid over the full range of solute concentrations (6). The model, when applied to linear chromatography, provides the following equation:

$$\ln k' = c + \frac{(\Delta \nu_+ + \Delta \nu_-)}{g} \ln(m_3) - \frac{n \Delta \nu_1}{m_1 g} m_3 \tag{6}$$

where m is molal concentration. The subscripts 1 and 3 refer to the solvent and solute, respectively. $\Delta\nu_i$ is the stoichiometrically weighted change in the number of species in the local region of products and reactants of the process, n the total number of anions (−) and cations (+) associated with the electrolyte, g a value tabulated for activity coefficients as a function of salt concentration, and c an integration constant.

By plotting Eq. (6), the change in the binding of ions ($\Delta\nu_+ + \Delta\nu_-$) and the number of water molecules released ($\Delta\nu_1$) can be estimated. Perkins et al. (6) applied this equation to a number of HIC systems, and reported that the release of water molecules (100–200) was significantly more than the release of salt ions (0.5–3). This result is consistent with the commonly accepted idea that HIC protein adsorption is an entropically driven process.

In this paper we describe the adsorption behavior of bovine serum albumin (BSA) on polypropylene glycol–sepharose CL-6B gel (PPG–sepharose) in both the linear and nonlinear regions of the isotherm. The utility of PPG–sepharose for the purification of lipases by hydrophobic interaction has been reported previously (17). The heats of adsorption ΔH_{ads}, under HIC conditions were measured by using flow microcalorimetry and linear chromatography. Calorimetric and chromatographic measurements were done as a function of salt concentration and temperature. Adsorption isotherms were also measured to supplement the heat measurements. Using these data in conjunction with the Van't Hoff equation and PI analysis, insight on the complexity of adsorption process was obtained.

EXPERIMENTAL

Material and Methods

The HIC support (Fig. 1) was a sepharose derivative synthesized by covalent immobilization of PPG on sepharose CL-6B according to Sundberg and Porath (18) and described previously by Diogo et al. (17).

Twenty-five grams of suction-dried sepharose CL-6B (Pharmacia, Uppsala, Sweden) were washed on a glass filter-funnel with water and then mixed with 25 mL of PPG-diglycidyl-ether (average number-average molecular

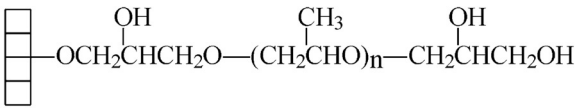

Figure 1. Structure of PPG–sepharose.

mass, M_n, ca. 380) (Aldrich, Milwaukee, WI). Twenty-five milliliters of 0.6 *M* sodium hydroxide solution containing 50 mg of sodium borohydride (Merck, Darmstadt, Germany) were then added. This suspension was mixed by rotation for 8 hr at 25°C and the reaction stopped by washing the gel on a glass filter-funnel with large volumes of water. The PPG–sepharose CL-6B gel thus obtained was then treated with 1 *M* sodium hydroxide overnight at room temperature for the purpose of deactivating the free epoxy groups (17).

The probe protein chosen was BSA. It was purchased from Sigma (St. Louis, MO), and used without further purification. Bovine serum albumin is a globular ellipsoid ($14 \times 4 \times 4$ nm), with a molecular mass of 69,000 Da, and an isoelectric point of 4.7 (11).

A 10 m*M* sodium phosphate buffer was used for all the experiments, and ammonium sulfate (Aldrich, Millwaukee, WI) was used as the modulator.

Isocratic Elutions

The capacity factor measurements for BSA were carried out at 15, 23, 30, and 35°C in a fast protein liquid chromatography system (FPLC) from Pharmacia (Uppsala, Sweden). The gel was packed in a column (4.2×1.0 cm I.D.) and equilibrated with the desired mobile phase (different concentrations of ammonium sulfate) at a flow-rate of 0.24 mL min^{-1}. Elution times (t_r) were obtained by injecting 100 μL of 2.00 ± 0.02 mg mL^{-1} BSA. The elution profile was obtained by continuous measurement of the absorbance at 280 nm. Following the elution, the column was washed with the 10 m*M* phosphate buffer (pH 7).

Isotherm Measurements

The batch method was used to measure the BSA adsorption isotherms at 1.0 and 1.5 *M* ammonium sulfate solutions at 30°C. The procedure has been described in detail elsewhere (19).

Flow Microcalorimetry

The heat of adsorption was measured using a flow microcalorimeter (FMC) (Gilson Instruments, Westerville, OH). The detailed procedure has been described earlier (19). The heats of adsorption measurements of BSA on PPG–sepharose were performed with 1.0 and 1.5 *M* ammonium sulfate at 30°C with a range of BSA concentrations between 20 and 60 mg mL^{-1}.

RESULTS AND DISCUSSION

Analysis of Linear Elution Data

Linear elution data for BSA on the PPG–sepharose were obtained, and are reported in terms of the capacity factor in Fig. 2. This figure summarizes the effects of both temperature and $(NH_4)_2SO_4$ concentration. Within the salt (0.8–1.5 M) and temperature range (15–35°C) studied two trends are evident. The capacity factor increases with an increase in either temperature or salt concentration, as is observed generally for HIC.

The capacity factor data were analyzed within the framework of two models: the Van't Hoff model (8,9) and the PI model (6). According to the Van't Hoff analysis there should be a linear dependence between $\ln k'$ and $1/T$. This is clearly not the case (Fig. 2). Nonlinearity has been reported previously in the literature (8,9). Following Horváth and co-workers approach (9) of incorporating the dependence of ΔH^0, ΔS^0, ϕ, and ΔC_P^0 on temperature, Eq. (2) was used to characterize the data in Fig. 2, using a commercial nonlinear least squares fitting program (20). The best-fit coefficients thus obtained are summarized in Table 1, and the characterizations obtained with these coefficients are shown with the solid curves in Fig. 2.

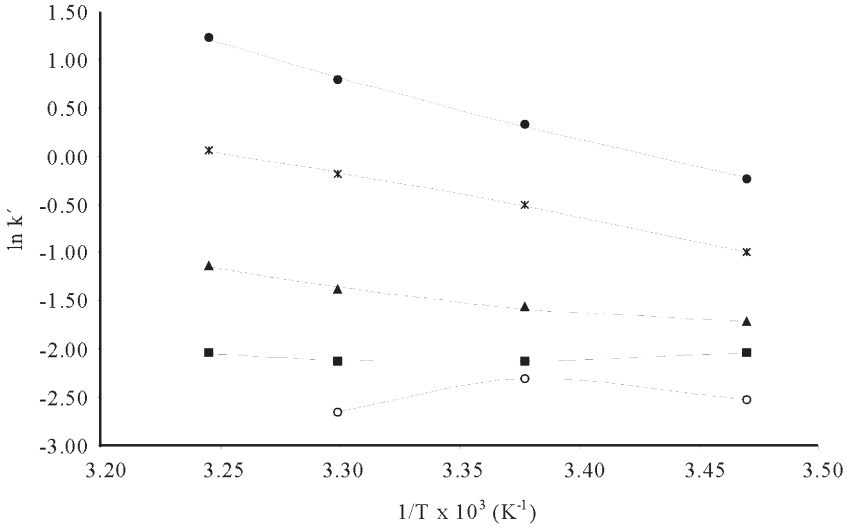

Figure 2. Van't Hoff plot for the retention of BSA on PPG–sepharose at ○—0.80 M; ■—1.00 M; ▲—1.20 M; × —1.40 M, and ●—1.50 M [$(NH_4)_2SO_4$]. The solid curves represent the quadratic equation adjusts.

Table 1. Fit Parameters of Van't Hoff Analysis and Thermodynamic Quantities for the Retention of BSA on PPG–Sepharose

Temperature (°C)	$[(NH_4)_2SO_4]$ (M)	a	b	c	ΔH^0 (kcal mol^{-1})	ΔS^0 (kcal mol^{-1} K^{-1})
15	0.800	−467.1	275,456.6	−4,0578,743.0	12.30	0.0430
23					−2.81	−0.0087
30					−15.38	−0.0506
35					−24.01	−0.0789
15	1.000	80.6	−47,695.8	7,107,114.9	−3.24	−0.0099
23					−0.60	−0.0009
30					1.60	0.0065
35					3.12	0.0114
15	1.200	103.7	−58,601.1	8,356,361.8	1.19	0.0061
23					4.30	0.0168
30					6.89	0.0254
35					8.67	0.0312
15	1.400	−13.4	13,938.3	−2,764,555.4	10.43	0.0396
23					9.40	0.0361
30					8.54	0.0332
35					7.95	0.0313
15	1.500	78.5	−38,536.4	4,788,061.8	10.53	0.0415
23					12.32	0.0476
30					13.80	0.0525
35					14.82	0.0558

The ΔH^0 and ΔS^0, reported in Table 1 were calculated from the best-fit coefficients using the Eqs. (3) and (4). At the higher salt concentrations ($\geq 1.2\,M$) the ΔH^0 values are strongly positive (endothermic), ranging between 1 and 15 kcal mol^{-1}, and indicate an entropically driven process in every case. This is consistent with expectations (5–7). At the lower salt concentrations ($\leq 1\,M$), it is seen that enthalpic driving forces can be significant in some cases. This is probably due to the weaker hydrophobic interactions at these salt conditions, an effect that has been observed previously (6,21,22). What is inconsistent with reports in the literature is the dependence of ΔH^0 on salt concentration and temperature. Using the same analysis technique, it has been previously reported (8,9) (including our recent publication (19)), that the mechanism driving HIC shifts in the direction of a decreasing entropic (or increasing enthalpic) influence as the temperature is increased. This conclusion is based on a consistent observation that ΔH^0 decreases as the temperature increases. In Table 1 it is seen that while the data at 0.8 and 1.4 M do follow the trend reported earlier, the data at 1.0, 1.2, and 1.5 M do not. In these latter cases, ΔH^0 increases with increasing temperature, i.e., the entropic driving force becomes stronger at higher temperatures. Due to this, there are situations where at constant temperature the ΔH^0 can be larger (more endothermic) at a lower salt concentration. For example, at 35°C the value of ΔH^0 at 1.2 M is greater than that at 1.4 M $(NH_4)_2SO_4$. This is inconsistent with the explanation usually put forth that the release of structured water molecules, made possible by the interactions between hydrophobic regions on the protein and support, drives the adsorption. In this case it is to be expected that this driving force would weaken with a decrease in the concentration of a structure creating (protein hydrating) salt such as $(NH_4)_2SO_4$.

A convenient model that has been presented to quantify the number of water molecules released in HIC is the PI model. The analysis is based on Eq. (6). Applying this equation to the linear retention data (Fig. 2), the change in the number of water molecules ($\Delta\nu_1$) and ions ($\Delta\nu_+ + \Delta\nu_-$) in the vicinity of the protein–ligand complex following adsorption was calculated and is shown in Table 2; within the convention used, a negative value indicates a net loss of molecules/ions from the vicinity of the adsorbed complex. It is clear from the results that the adsorption of BSA on PPG–sepharose is accompanied by the release of a large number of water molecules at all temperatures. It is also clear that the number of ions released is significant. Further, in both cases the number of molecules/ions released generally increases with temperature. While the release of a large number of water molecules from the surface is as expected, the substantial ion release observed has not been reported previously in the literature. Following the conclusions of Melander and Horváth (21) for the effects of salt on retention, the data suggest that the adsorption of BSA on the PPG–sepharose gel is substantially influenced by a reduction in the energy of the double layer around the protein upon adsorption. It is therefore conceivable that at lower salt concentrations ($\leq 1\,M$) where hydrophobic interactions are relatively weaker,

Table 2. Estimate of the Release of Water Molecules and Salt Ions for Adsorption of BSA on PPG–Sepharose

Protein	Temperature (°C)	$\left(\frac{-n\times\Delta\nu_1}{m_1\times g}\right)$	$\left(\frac{\Delta\nu_+ + \Delta\nu_-}{g}\right)$	c	$-\Delta\nu_1$	$-(\Delta\nu_+ + \Delta\nu_-)$
BSA	15	7.79	−6.25	−10.10	230.62	10.00
BSA	23	13.44	−12.36	−15.72	397.90	19.78
BSA	30	12.73	−10.39	−15.12	376.88	16.62
BSA	35	19.50	−18.88	−21.58	577.30	30.21

the electrostatic influence may be dominant in some cases, resulting in exothermic heats of adsorption. It should be noted that the double layer around the support is unlikely to be important since both the ligand and the substrate have a negligible charge density.

The trends observed with temperature in the Van't Hoff model appear to be inconsistent with results from the PI analysis. As stated earlier, there is a clear trend for increased release of molecules/ions with temperature. This would imply that ΔS^0 should increase with temperature, which is contrary to what is observed at two salt concentrations (0.8 and 1.4 M).

It has been argued that HIC can be strongly influenced by conformational changes in both the protein (23) and ligand (24) with changes in solution conditions. While it is unlikely that the PPG ligand changes conformation within the range of conditions studied, it is possible that changes in protein conformation may influence the process. There is some indirect evidence that this may be the case. An analysis of the width of the BSA elution peaks has shown that at the lowest temperature (15°C) peak spreading is due solely to axial diffusion; i.e., peak width is proportional to $t^{1/2}$. However, this is not true at higher temperatures. Since the lower temperature data indicate no significant mass-transfer resistance in the gel or solution, and gel structure does not change within the temperature range studied, it is suspected that a change in protein conformation is either altering the diffusion coefficient in solution or the rate of adsorption, leading to the different kinetic response at higher temperature. This change in conformation with temperatures may be a reason for the unusual temperature behavior observed in Table 1. However, it is not clear why this occurs at a particular salt concentration and not at other bracketing concentrations.

Analysis Under Overloaded Conditions

Adsorption behavior under overloaded conditions was also studied for BSA on PPG–sepharose gel. The isotherms for BSA on PPG–sepharose at pH 7 and at

30°C are shown in Fig. 3. A notable characteristic is the multiple plateaus observed. At 1.5 *M* salt, there are three plateaus: approximately at 1.8, 2.8, and 5.5 mmol kg^{-1}. At the lower salt concentration there are two plateaus: one at 1.8 mmol/kg and the other at 3.2 mmol kg^{-1}. The existence of these plateaus suggests that the protein molecules are re-orienting on the surface as the concentration increases. There is evidence that such re-orientations do take place during the adsorption of macromolecules (23,25–27). For example, Lee and Belfort (24) have shown, using a surface force apparatus, that RNase re-orients from side-on to end-on with increasing surface coverage on a mica surface. Also, Tilton et al. (28) have concluded, based on differences in the mobile fraction of E-RNase on a hydrophobic interaction polystyrene surface, that the biomolecule is more densely packed at higher concentrations due to a larger fraction of proteins oriented in the end-on and therefore more mobile position.

The presence of three plateaus at 1.5 *M* as opposed to two plateaus at 1.0 *M* may indicate a difference in conformation of the proteins under these conditions. It appears that at 1.5 *M* the protein has a larger footprint on the PPG–sepharose,

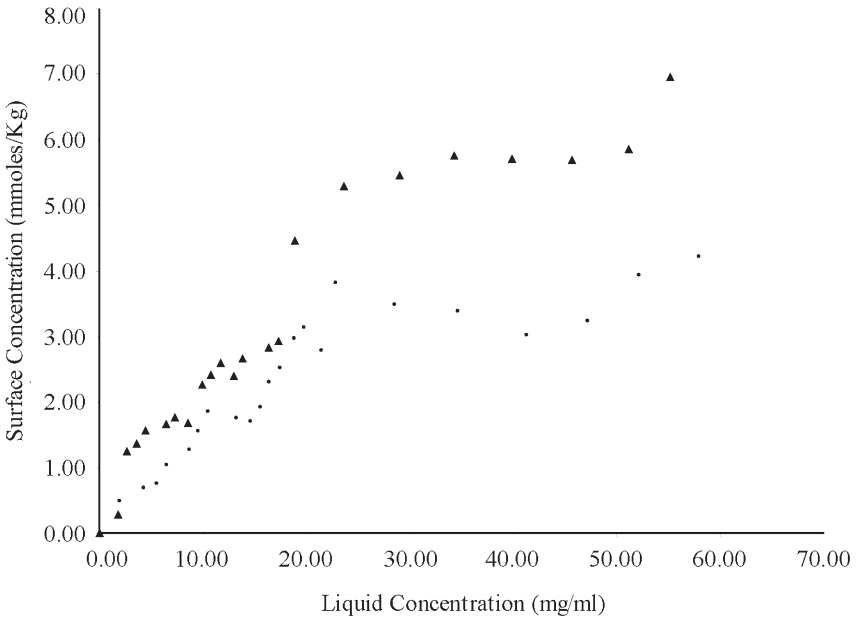

Figure 3. Adsorption isotherms for BSA on PPG–sepharose at ●—1.00 *M* and ▲—1.50 *M* [$(NH_4)_2SO_4$] ($T = 30°C$, pH = 7).

and greater crowding results in a third plateau at solution concentrations greater than approximately 15 mg mL^{-1}.

We have previously reported isotherm data for the adsorption of BSA on another ligand, $-(CH_2)_4-$, at similar salt conditions but at a lower temperature (19). In that case, within the same concentration range (0–60 mg mL^{-1}), single plateaus were defined and there were clear indications that multiple plateaus would form at higher protein solution concentrations; i.e., increases in capacity from the first plateau were observed, though the second plateau was not reached. Significantly, the isotherms at higher salt concentrations showed that the formation of the second plateau occurs at a lower protein solution concentration, consistent with the more open structure postulated at higher salt concentrations.

It has been shown (19) that it is prudent to substantiate results obtained from a Van't Hoff analysis with calorimetric measurements, primarily because the definition of the standard states in the Van't Hoff analysis is obscure, and chromatographic separations are unlikely to be at the standard state. Furthermore, the Van't Hoff analysis is limited to the linear region of the isotherm, and effects present at higher surface coverage cannot be quantified.

In Fig. 4 the heats of adsorption measured with the FMC as a function of protein surface concentration are shown. The measurements were made at the same temperature and salt concentrations as the isotherms in Fig. 3. Upon comparing these values with those obtained from the Van't Hoff analysis, it is

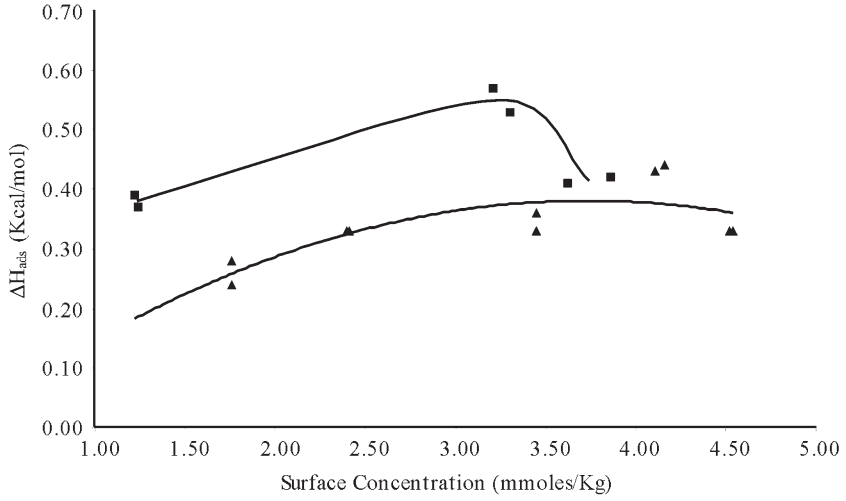

Figure 4. Heat of adsorption of BSA on PPG–sepharose at ■—1.00 *M* and ▲—1.50 *M* $[(NH_4)_2SO_4]$ ($T = 30°C$, pH = 7).

evident that there are large discrepancies. The direct measurements gave heats of adsorption in the range of $0.2-0.6\,\text{kcal mol}^{-1}$, which are more reasonable for hydrophobic interactions than the -3 to $15\,\text{kcal mol}^{-1}$ range calculated in Table 1. It would be expected that the large heats of adsorption obtained from the Van't Hoff analysis would lead to denaturation of the protein. However, in a previous investigation (17) with lipase on the above PPG ligand, there was no evidence for denaturation of the enzyme; virtually all the activity was preserved following chromatographic purification with the PPG–sepharose. The large discrepancy between the calculated and measured values of the enthalpy brings into question the reliability of interpreting thermodynamic processes based on Van't Hoff values. The confusing trends for ΔH^0 with respect to temperature in Table 1 are therefore likely to be an artifact of the analysis, rather than due to mechanisms of adsorption.

A notable feature of the data in Fig. 4 is the maximum defined by each curve. In each case the maximum corresponds to regions on the isotherm (Fig. 3) where rapid increases in capacity are observed to the broad plateau. For the $1.0\,M$ case, this maximum occurs at approximately $3\,\text{mmol kg}^{-1}$, and at $1.5\,M$ it occurs at approximately $4.2\,\text{mmol kg}^{-1}$. One possible explanation is that the re-orientation of the adsorbed molecules on the surface results in enthalpically favorable (attractive) interactions between adsorbed molecules. The process is still driven by an overall increase in entropy, but the favorable lateral interactions between adsorbed proteins reduce the magnitude of the endothermic heat. It is postulated that the favorable interactions between the adsorbed molecules stabilize the adsorbed layer from further changes in orientation leading to the broad plateaus observed in Fig. 3.

A second important feature of the calorimetric data is the generally lower endothermic heat at the higher salt concentration. As was discussed earlier, if the adsorption process was driven solely by the release of water molecules, an increase in the concentration of $(NH_4)_2SO_4$ should give a larger endothermic heat, opposite to the experimental observation. The experimental trend can be explained with the inclusion of attractive interactions between adsorbed BSA molecules. We have previously shown (19) for the adsorption of BSA on the $-(CH_2)_4-$ ligand that at high salt concentrations the FMC thermogram includes two peaks: an endothermic peak followed in time by an exothermic peak. It was concluded that the exothermic peak resulted from attractive lateral interactions between adsorbed BSA molecules. It was further shown that these interactions, though present, are not initially evident because of the dominance of the entropically driven hydrophobic interactions. Following adsorption the biomolecules re-orient, and this is accompanied by strong attractive interactions between the re-oriented molecules. Under certain conditions these attractive forces have been shown to be of the same order of magnitude as the primary hydrophobic interaction. It was further found that the attractive interactions

between adsorbed molecules was a function of the salt concentration, and this was attributed to differences in conformation, with the conformation at the lower salt concentrations leading to weaker interactions. Also, since the thermograms at high salt concentration indicate that the attractive interactions exist from the beginning of the adsorption process, it is expected that attractive interactions, though weaker, are present at lower salt concentrations as well, but are not manifested as a separate exothermic peak. Instead, the exothermic and endothermic peak overlap, and the net endothermic heat is observed.

The salt dependence of the BSA adsorption on the PPG–sepharose can be explained within the framework of these conclusions. It is postulated that attractive interactions play a significant role in this case as well, and they reduce the overall magnitude of the endothermic heat. Since the lateral attractions are stronger at higher salt concentrations, possibly due to differences in conformation, the effect is stronger at 1.5 *M*, resulting in a lower observed endothermic heat. Unlike adsorption on the $-(CH_2)_4-$ ligand, however, no separate exothermic peak was obtained on the PPG thermograms. The situation is similar to that of BSA on $-(CH_2)_4-$ at lower salt concentrations (19). The exothermic and endothermic peaks overlap and only the net effect, which is overall endothermic, is experimentally observed. It is postulated that on PPG–sepharose gel re-orientation is more rapid because the ligand density is much lower (17), thus the attractive effect is not displaced in time on the thermogram, even at high surface coverage.

SUMMARY AND CONCLUSIONS

The adsorption behavior of BSA on PPG–sepharose, a HIC support, has been studied under linear and nonlinear conditions. Analysis of data under linear conditions was accomplished with the Van't Hoff and PI approaches. The PI analysis indicates a strong entropic driving force due to the release of a large amount of solvent on adsorption. The analysis also indicates that the adsorption is accompanied by the release of a large number of ions, a characteristic that has not been observed previously. It is speculated that the ion release is due to the substantial influence of the protein double layer on adsorption behavior.

Analysis of the linear elution data with the Van't Hoff analysis gave, in many cases, results that were contrary to expectations. The standard state heat of adsorption obtained with this method showed a temperature dependence that was inconsistent with the results of PI analysis. Furthermore, the values estimated for the heat of adsorption did not correspond to values obtained with direct calorimetric measurements. Based on these observations, it was concluded that the Van't Hoff method was not appropriate for estimating the heats of adsorption for HIC.

The calorimetric data obtained under overloaded conditions confirmed that the adsorption of BSA on the PPG–sepharose is entropically driven within the range of conditions studied. Also, analysis of these data in conjunction with the isotherms indicate that protein conformation on the surface and attractive interactions between adsorbed protein molecules also influence adsorption behavior.

LIST OF SYMBOLS

a, b, c	Parameters evaluated by least squares fitting for nonlinear Van't Hoff analysis
g	Value tabulated for activity coefficients as a function of salt concentration
k'	Capacity factor
m_i	Molal concentration of component i
n	Total number of ions associated with the electrolyte
R	Universal gas constant
T	Temperature
T_H	Reference temperature at which ΔH^0 is zero
T_S	Reference temperature at which ΔS^0 is zero
ΔC_P^0	Standard-state heat capacity change
ΔH^0	Standard-state enthalpy change
ΔH_{ads}	Enthalpy change of adsorption
ΔS^0	Standard-state entropy change
$\Delta \nu_i$	Stoichiometrically weighted change in the number of species in the local region of products and reactants of the process
ϕ	Phase ratio
1	Water or solvent
3	Salt or solute
$+$	Cations
$-$	Anions

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