Journal of Chromatography, 522 (1990) 23–36
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 707

Measurement of the heats of adsorption of chiral isomers on an enantioselective stationary phase

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(Received May 23rd, 1990)

ABSTRACT

The adsorption isotherms of the N-benzoyl-D- and L-alanine were measured at different temperatures, and the enthalpy of adsorption and the isosteric heat of adsorption were extracted from the data. These thermodynamic functions provide further evidence that a bimodal retention mechanism is present for the separation of enantiomeric pairs on a bovine serum albumin stationary phase. The first mode of interaction is associated with the chiral selective properties of the column whereas the second is associated with the non-chiral selective properties.

INTRODUCTION

The separation of enantiomeric pairs by chromatography has become a topic of substantial interest in the past decade [1,2]. The choice of the proper stationary phase is crucial in order to achieve separation, and currently a number of different types are available. How each stationary phase effects separations has not been fully elucidated for many of them, especially those where a protein is immobilized on the surface of porous silica particles. One such phase is the bovine serum albumin (BSA) immobilized on a solid support, which was developed by Allenmark and co-workers [3–9]. This phase is extremely efficient in resolving the L- and D-isomers of benzoyl derivatives of a number of amino acids.

Much of the previous work has been concerned with the resolution of enantiomers under linear conditions [1–9]. Although the presence of multiple binding sites on the immobilized BSA stationary phase has been recognized [9], little has been done to study the fate of these adsorption sites at high concentrations. Commonly, in linear, reversed-phase chromatography the values of the capacity factor are used to determine the thermodynamic quantities [10–12]. Only the thermodynamic functions at infinite dilution can be determined by this method, however. Under these conditions, it is difficult to separate the contributions of several interaction sites. Most often, the chiral recognition mechanism involves the formation of hydrogen bonds between the enantiomers and the substrate, to stabilize a short-lived but well defined

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complex. This involves interaction (*e.g.*, adsorption) sites on the stationary phase which have a high interaction energy.

Interactions between an immobilized BSA stationary phase and an N-benzoylamino acid involves a large number of possible configurations which can be classified as chiral selective and chiral non-selective. The former include essentially those interactions between an amino acid residue of the protein and the alanine residue of the enantiomeric derivatives in which the relative configuration between the two amino acid residues permits the formation of two hydrogen bonds [1,2]. The column saturation capacity for these sites should be comparable to the number of proteinic amino acid residues fixed in the column, but actually is less because of possible steric hindrance and the inaccessibility of some residues. The latter include all the other interactions, mainly dominated by Van der Waals forces and polar interactions. If we compare these two types of interactions, we can expect the selective type to correspond to a higher energy, hence to vary more rapidly with temperature and to contribute more to the retention under linear conditions, but also to saturate at lower concentrations than the non-selective type.

Thus, a comparison could be drawn between what is called an "active site", which plays an important role in retention in analytical chromatography, and a chiral selective site which is instrumental in the separation of optical isomers. However, the interaction energy with an active site is usually higher than with a chiral selective site. The density of the active sites is usually very low and, consequently, they saturate for small sample sizes, causing peak tailing [13]. It would be more correct to describe the surface of the stationary phase as being covered by two site distributions than by two different but well defined sites, the chiral recognition active site and the non-selective site. The non-selective site should behave identically with both isomers because the two antipodes are chemically identical in an achiral environment. The chiral recogition site should interact strongly, but differently, with the two enantiomers.

Important information regarding these interactions can thus be derived from the determination of the equilibrium isotherms. By measuring the adsorption isotherms at different temperatures, several thermodynamic functions of adsorption can be extracted including the enthalpy of adsorption and the isosteric heat of adsorption. Because of the presence of multiple binding sites on the BSA stationary phase [9], the non-linear effects must be accurately accounted for by the appropriate model. If two site distributions are assumed to exist for the simplest case in a multiple site model and to be non-cooperative, a bi-Langmuir expression is one such avenue [13–15]. This two-site model has been shown to describe precisely both the adsorption isotherms and the band profiles of the enantiomers of N-benzoylalanine [16].

One of the most important applications of phase equilibrium isotherms is in the accurate prediction of individual chromatographic band profiles. The tailing in the elution profile of small or moderate sample sizes attributed to the existence of active (or high-energy) sites is well depicted by the two-term Langmuir isotherm [13]. For preparative work where the recovery of concentrated, pure fractions of optical isomers is desired, the operational concentration range extends into the non-linear region of the adsorption isotherm. One problem with protein-based chiral separations is that the site responsible for the enantiospecific interactions is easily saturated and, as a result, the preparative potential for these stationary phases is limited unless more information about the exact adsorption process is available. If more is known, optimization of the

chiral selectivity of the stationary phase could broaden the preparative applications of these stationary phases.

The purpose of this paper is to investigate further the evidence previously reported [16] that more than one site accounts for the interaction of the optical isomers with the BSA stationary phase, and that a two-site model properly accounts for what can be described as a distribution of selective sites and non-selective sites of adsorption. We studied the influence of temperature on the adsorption isotherms of N-benzoyl-L- and D-alanine on immobilized BSA and determined the thermodynamic functions of the interaction between these compounds and the stationary phase.

THEORY

Adsorption isotherm

If for each of the two isomers the rate of adsorption is proportional to its concentration in the mobile phase and to the number of empty sites available for its adsorption on the stationary phase, and if the rate of desorption is proportional to its concentration in the stationary phase, a first-order Langmuir model can be constructed. While this one-site Langmuir isotherm fails to fit the experimental data accurately, a two-site bi-Langmuir model was found to fit them correctly under the chosen experimental conditions [16].

In the case of the N-benzoyl derivatives of D- and L-alanine, the first site is analogous to the chiral recognition site attributed to the interaction between the alanine residue and the amino acid residues of BSA that is bound to the surface of the silica support. The numerical coefficients for this isotherm were found to differ markedly for the enantiomers [16]. The second of the two sites accounts for the various interaction modes of the solute with a non-chiral selective site, *e.g.*, unreacted silanol groups and peptide bonds, or of the N-benzoyl hydrophobic group with the various residues of the protein. Both isomers adsorb equally to this non-selective site, contributing nothing to the enhancement of the separation between the two isomers [16]. Moreover, the selective site provides a higher energy of adsorption, covers a smaller fraction of the available adsorption surface and, unfortunately, has a much lower saturation capacity than the non-selective site. For the bi-Langmuir adsorption isotherm, the adsorption process for one of the enantiomers at either of the two types of sites must be considered independent, *i.e.*, non-cooperative.

The appropriate kinetic equation for each isomer at each site under these conditions can be described as follows [17,18]:

$$\frac{\partial q_{x,y}}{\partial t} = k_{a,x,y} C_x (q_{s,x,y} - q_{x,y}) - k_{d,x,y} q_{x,y} \tag{1}$$

where q and q_s are the number of adsorption sites occupied and the total number of adsorption sites available (adsorbent saturation capacity), respectively, t is the time, k_a and k_d are the rate constants for the adsorption and for the desorption processes, respectively, C is the concentration of the solute in the mobile phase, x = L or D, corresponding to the L-isomer and D-isomer, respectively, and $y = 1$ or 2, representing the selective and the non-selective sites of adsorption, respectively.

At equilibrium eqn. 1 is set equal to zero and, following rearrangement, the familiar Langmuir adsorption isotherm equation results [19]:

$$\frac{q_{x,y}}{q_{s,x,y}} = \frac{b_{x,y}C_x}{1+b_{x,y}C_x} \tag{2}$$

where $b_{x,y} = k_{a,x,y}/k_{d,x,y}$.

At high mobile phase concentration, a monolayer coverage of the solute on the stationary phase can be expressed in terms of the Langmuir adsorption isotherm coefficients, $a_{x,y}$ and $b_{x,y}$. The concentration of the solute in the stationary phase approaches a limiting value known as the saturation capacity, which is defined as

$$q_{s,x,y} = \frac{a_{x,y}}{b_{x,y}} \tag{3}$$

Combining eqns. 2 and 3, the Langmuir adsorption isotherm for each of two isomers at each of the two sites can be written as

$$q_{x,y} = \frac{a_{x,y}C_x}{1+b_{x,y}C_x} \tag{4}$$

As the total amount adsorbed, q_{tot}, equals the amount adsorbed on the selective site plus the amount adsorbed on the non-selective site, then

$$q_{tot,x} = q_{x,1} + q_{x,2} \tag{5}$$

Finally, the bi-Langmuir isotherm for the total amount adsorbed for each isomer is accounted for by the following expression [14]:

$$q_{tot,x} = \frac{a_{x,1}C_x}{1+b_{x,1}C_x} + \frac{a_{x,2}C_x}{1+b_{x,2}C_x} \tag{6}$$

Enthalpy of adsorption

As the b coefficients of the bi-Langmuir isotherm are equal to the equilibrium constants of the adsorption processes involving both the selective and non-selective sites, the classical thermodynamic functions of these equilibria can be derived, assuming that the system is in equilibrium. First, the enthalpy of adsorption is ascertained by calculating the slope of a Van 't Hoff plot for a first-order approximation for the selective and non-selective sites. This first-order approximation assumes that the adsorption enthalpy is independent of temperature, *i.e.*, neglects the difference between the heat capacities of the solute in the two phases.

Starting with the Gibbs–Helmholtz equation, which is expressed in terms of the enthalpy, the temperature dependence of the Gibbs function is given by [20]

$$\frac{\partial\left(\frac{\Delta G}{T}\right)}{\partial\left(\frac{1}{T}\right)} = \Delta H \tag{7}$$

where ΔG is the Gibbs free energy at temperature T and ΔH is the enthalpy at T. Again, assuming that the system is in equilibrium, then [21]

$$\Delta G^\circ = -RT \ln K = -RT \ln b \tag{8}$$

where ΔG° is the molar Gibbs free energy at the standard state, R is the gas constant and $K = b = k_a/k_d$ is the thermodynamic equilibrium constant.

Inserting eqn. 8 into eqn. 7, the following expression permits the determination of the molar enthalpy of adsorption at standard state, ΔH_a°, by plotting ln b against $1/T$:

$$\frac{\partial(\ln b)}{\partial\left(\frac{1}{T}\right)} = -\frac{\Delta H_a^\circ}{R} \tag{9}$$

Having determined both ΔG° and ΔH_a°, the molar entropy of adsorption, ΔS°, is easily calculated by [21]

$$\Delta S^\circ = -\frac{\Delta G^\circ - \Delta H_a^\circ}{T} \tag{10}$$

Isosteric heat of adsorption

Another thermodynamic measure is the heat of adsorption taken for a constant amount of solute adsorbed on the stationary phase. As seen above in eqn. 7, the relationship between the Gibbs free energy and the standard enthalpy at temperature T is the starting point. At equilibrium, the chemical potential of the solute in the liquid phase is equal to the chemical potential in the stationary phase [21]:

$$\mu_s = \mu_l \tag{11}$$

where μ_s and μ_l are the chemical potentials of the solute in the stationary and mobile phase, respectively. These chemical potentials are defined as

$$\mu_l = \mu_l^\circ + RT \ln a_l \tag{12}$$

and

$$\mu_s = \mu_s^\circ + RT \ln a_s \tag{13}$$

where μ_s° and μ_l° are the standard chemical potentials of the solute in the stationary and mobile phase, respectively, and are equal to the molar free energy of the pure solute (*i.e.*, activity equal to unity) in the corresponding phase, and a_l and a_s are the activities of the solute in the mobile and stationary phase, respectively.

Equating eqns. 12 and 13, the expression for ΔG° can be written as

$$\Delta G^\circ = \mu_s^\circ - \mu_l^\circ = RT \ln \left(\frac{a_l}{a_s}\right) \tag{14}$$

Two assumptions are now necessary. First, the activity of the solute on the stationary phase, a_s, remains constant when the temperature changes if the amount adsorbed is kept constant, and second, for a dilute solute concentration in the mobile phase, the activity of the solute is equal to the concentration of the solute in the liquid phase, *i.e.*, $a_l = C$. Following the substitution of eqn. 14 into eqn. 7 and implementing the previous assumptions, the following equation demonstrates a function for the isosteric heat of adsorption [22–24]:

$$\frac{\partial(\ln C)}{\partial\left(\frac{1}{T}\right)} = \frac{\Delta H_{st}^{\circ}}{R} \tag{15}$$

where ΔH_{st}° is the isosteric heat of adsorption.

By plotting ln C *versus* $1/T$ at constant q, the isosteric heat of adsorption for the first-order approximation (*i.e.*, assuming that ΔH_{st}° is independent of the temperature) is extracted from the slope of the best linear fit of the data points.

EXPERIMENTAL

Equipment and materials

The chromatographic experiments were performed on an HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with a diode-array UV detector, a computer data acquisition system and a multi-solvent delivery system. A Haake (Karlsruhe, F.R.G.) A81 circulating water-bath was used to control the temperature of the column during the entire analysis.

Column. A Resolvosil-BSA-7 column (Alltech, Deerfield, IL, U.S.A.) was used, with dimensions 150 mm × 4 mm I.D.

Chemicals. N-Benzoyl D- and L-alanine (Sigma, St. Louis, MO, U.S.A.) and 1-propanol were used without further purification.

Mobile phase. For all chromatographic runs, the mobile phase was a 0.1 *M* aqueous phosphate buffer solution with a constant concentration of organic modifier, 3% (v/v) 1-propanol, of pH 6.8. The flow-rate of the mobile phase was 1 ml/min.

Procedures

The adsorption isotherm profiles of the two isomers were obtained by frontal analysis [25–27]. Because the HP 1090 chromatograph is not equipped with a large enough sample loop to handle profile measurements by frontal analysis in the standard fashion, the multi-solvent delivery system was modified for the procedure as described in a previous paper [25]. As a broad concentration range was undertaken for the analysis, the adsorption profile for each isomer at each temperature was carefully divided into three smaller concentration ranges. The column was submerged in the circulating water-bath to control the temperature of the column within 0.1°C for the six temperatures measured, 0, 5, 10, 20, 30 and 40°C. Before switching to a new temperature range, the column was cleaned by reversing the direction of flow through the column and by flushing with phosphate buffer containing 10% (v/v) 1-propanol. After subjecting the column to such high concentrations of solute, this procedure was

necessary to obtain accurate retention times at the lower concentrations in the subsequent temperature range. Also, wavelengths from the diode-array detector of 240 and 254 nm were employed, depending on the concentration range under consideration, and the dead time of the column was 92.4 s at a flow-rate of 1 ml/min.

The best-fit bi-Langmuir isotherm parameters were calculated using a simplex algorithm developed in the laboratory. The procedure fitted the q_{tot} *versus* C data weighing each data point equally. Twenty-six points for each profile were fitted with all four parameters being optimized simultaneously, allowing 22 degrees of freedom. Linear regressions were carried out using a standard method.

RESULTS AND DISCUSSION

The temperature range was limited because an aqueous solution was used and, therefore, in spite of the buffer and the organic modifier used, the lower limit could not extend much below 273 K. The upper limit of 313 K was set because a protein is involved and raising the temperature beyond this point might damage the column, resulting in drifting retention times and irreproducible data.

Figs. 1 and 2 show the adsorption isotherm curves for the six temperatures measured in the experiments, for the L- (Fig. 1) and the D- (Fig. 2) isomers, respectively. For both isomers the curve on top represents the data at the lowest temperature measured in the study, and the amount adsorbed at each mobile phase concentration decreases with increasing temperature. The values of the a and b coefficients of the isotherms of the two enantiomers on both types of sites (eqn. 6) are reported in Table

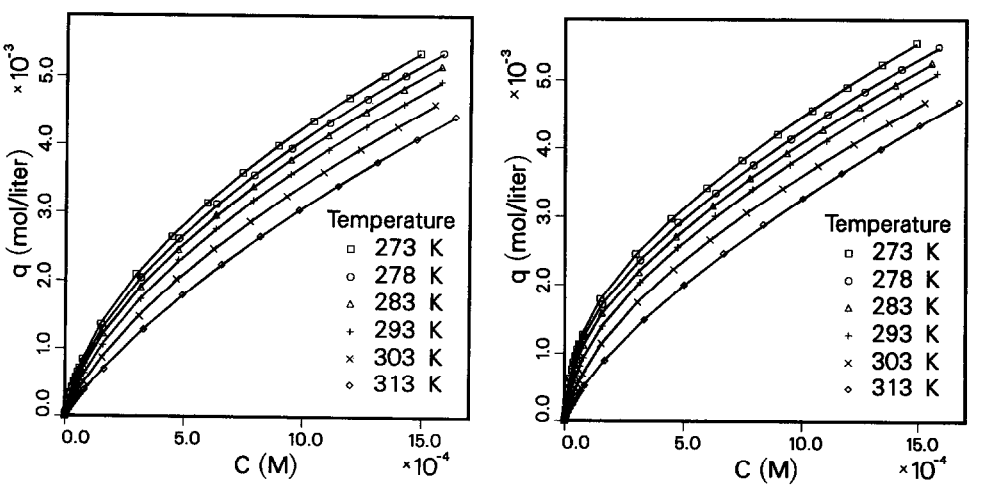

Fig. 1. Adsorption isotherms for N-benzoyl-L-alanine at increasing temperatures. Experimental data (symbols) and best bi-Langmuir isotherms (solid lines). See eqn. 6 for isotherm equation and Table I for isotherm coefficients. Column, 150 mm × 4 mm I.D.; stationary phase, immobilized BSA on silica, mobile phase, 3% (v/v) 1-propanol in 0.1 *M* phosphate buffer (pH 6.8); flow-rate, 1 ml/min.

Fig. 2. Adsorption isotherms for N-benzoyl-D-alanine at increasing temperatures. Experimental data (symbols) and best bi-Langmuir isotherms (solid lines). See eqn. 6 for isotherm equation and Table I for isotherm coefficients. Conditions as in Fig. 1.

I for each temperature, together with the value of the adsorbent saturation capacities. The adsorbent saturation capacity, q_s, and the coefficient b are reported in units of mol/l and l/mol, respectively, owing to the fact that the position of the Gibbs adsorption surface plane is difficult to define.

At low mobile phase concentrations, the disparity in the isotherm coefficients between the isomers at the selective site (Table I) indicates the selectivity of BSA as a chiral recognizer. However, the adsorption isotherms for both isomers are similar for mobile phase concentrations above $2 \cdot 10^{-4}$ M. This is as expected, because the selective sites are easily saturated and the only available sites of adsorption at high concentrations are the non-selective sites [16]. Because the two antipodes behave identically with the non-selective sites, the slopes of the second isotherm components at each temperature should be nearly identical, as previously reported [16]. As seen with the coefficients of the isotherms for the non-selective sites, the a and b parameters (Table I) are almost equal for each of the isomers at each temperature. This maintains the initial understanding that a non-selective site contributes significantly at high concentrations when the chiral selective sites become saturated.

It is remarkable that the saturation capacities of the chiral selective site are nearly the same for both enantiomers at all temperatures (Table I). Only the ratio of the rates of formation and dissociation of the chiral recognition constant is different

TABLE I

ISOTHERM PARAMETERS

Site	Isomer	T (K)	a	b (l/mol)	q_s (mol/l)
Selective	L-	273	13.09	8830	0.00148
		278	11.36	8020	0.00142
		283	9.78	7110	0.00138
		293	7.26	5550	0.00131
		303	4.82	4180	0.00115
		313	2.57	2590	0.00099
	D-	273	36.80	22720	0.00161
		278	33.34	21210	0.00157
		283	27.68	18920	0.00146
		293	17.79	12650	0.00140
		303	10.24	7740	0.00132
		313	5.06	5240	0.00097
Non-selective	L-	273	3.65	249	0.0146
		278	3.46	229	0.0151
		283	3.24	200	0.0161
		293	3.05	181	0.0168
		303	2.85	151	0.0189
		313	2.65	124	0.0213
	D-	273	3.61	248	0.0146
		278	3.39	228	0.0150
		283	3.27	210	0.0155
		293	3.08	187	0.0165
		303	2.83	163	0.0174
		313	2.76	127	0.0218

for the two enantiomers favoring retention of the D-isomer. The saturation capacities for the chiral selective site decrease with increasing temperature, by *ca.* 33% for a 40°C increase in column temperature. On the other hand, the ratio of the saturation capacities of the chiral site for the D- and L-enantiomers seems to decrease only very slowly, if at all, with increasing temperature. If the point at 313 K is eliminated, the average saturation capacity ratio is 1.09 with a standard deviation of only 0.035. At the same time, the selectivity for the D-enantiomer decreases, the ratio $a_{D,1}/a_{L,1}$ decreasing from 2.81 to 1.97. The decrease in saturation capacity with increasing temperature can be attributed to thermal changes in the conformation of the protein, especially in the dimension of the cavity within the structure of the BSA [28], which is in part responsible for the chiral recognition.

The parameters of the non-selective term of the isotherm are the same for the two enantiomers, as expected and as previously reported [16]. The average values of $a_{D,2}/a_{L,2}$ and $b_{D,2}/b_{L,2}$ are 1.003 and 1.029, respectively, with standard deviations of 0.021 and 0.030. This gives the precision of the parameters reported here. The saturation capacity of the non-selective sites increases with increasing temperature, by *ca.* 50% for a temperature increase of 40°C. With the parallel drop of the saturation capacity of the selective sites due to steric changes in the BSA, the non-selective contribution to the retention and column saturation capacity increases. The extent of the increase is sharp considering the relative change in the saturation capacity of the selective site. The saturation capacity of the chiral selective sites falls from 10 to 5% of the total adsorbent saturation capacity when the column temperature increases from 0 to 40°C.

In Fig. 3 the Van 't Hoff plot gives a good estimate of the energies of each of the sites for each compound. By plotting ln *b versus* $1/T$, the energy of adsorption can be determined. The values for the heat of adsorption can be seen in Table II. The top two curves of the plot in Fig. 3 represent the data corresponding to the D- and L-enantiomers at the selective sites, respectively.

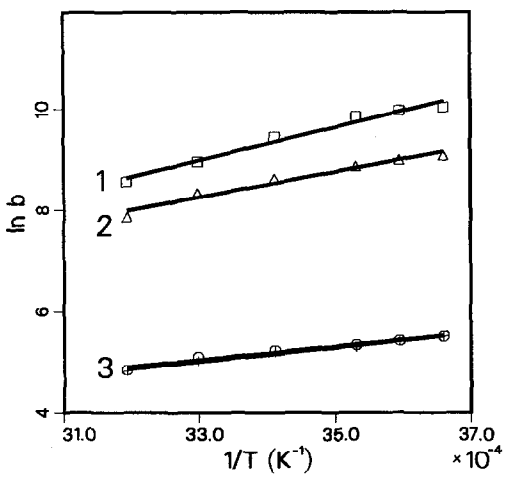

Fig. 3. Van 't Hoff plot for the determination of the enthalpy of adsorption. Calculated values (symbols) and best linear fit (solid lines). (1) D-isomer for the selective site; (2) L-isomer for the selective site; (3) D- and L-isomers for the non-selective site.

TABLE II

THERMODYNAMIC FUNCTIONS OF ADSORPTION

Site	Isomer	T (K)	ΔG° (kcal/mol)	ΔH_a° (kcal/mol)	ΔS° (cal/mol · K)
Selective	L-	273	−4.93	−5.01	−0.30
		278	−4.97	−5.01	−0.16
		283	−4.99	−5.01	−0.09
		293	−5.02	−5.01	0.03
		303	−5.02	−5.01	0.03
		313	−4.89	−5.01	−0.39
	D-	273	−5.44	−6.51	−3.90
		278	−5.51	−6.51	−3.61
		283	−5.54	−6.51	−3.42
		293	−5.50	−6.51	−3.44
		303	−5.39	−6.51	−3.68
		313	−5.33	−6.51	−3.77
Non-selective	L-	273	−2.99	−2.86	0.51
		278	−3.00	−2.86	0.53
		283	−2.98	−2.86	0.45
		293	−3.03	−2.86	0.59
		303	−3.02	−2.86	0.55
		313	−3.00	−2.86	0.47
	D-	273	−2.99	−2.68	1.16
		278	−2.99	−2.68	1.17
		283	−3.01	−2.68	1.18
		293	−3.04	−2.68	1.27
		303	−3.07	−2.68	1.30
		313	−3.01	−2.68	1.08

The more retained compound, the D-isomer, has a higher absolute value of the enthalpy of adsorption than the L-isomer, *i.e.*, 6.5 compared with 5.0 kcal/mol. However, the interaction energy of the less retained L-isomer is still much higher than the average interaction energy of either isomer at the non-selective site, 2.77 kcal/mol. Hence the L-isomer meets some of the requirements for stereoselective interaction with the chiral selective sites, but not all of them as does the D-isomer. The chiral recognition complex appears to form faster and/or dissociate more slowly with the D- than with the L-isomer. Assuming a classical three-point interaction mechanism for chiral recognition, we conclude that the L-isomer can interact with two of the three points, but sterically cannot interact with the third point of the site as well as the D-isomer.

The difference observed between the interaction enthalpies of the two isomers with the non-selective sites (*ca.* 4%) is hardly significant. The value of this enthalpy is reasonable for that kind of reversed-phase system and shows that the interaction of the enantiomers with the non-selective sites is dominated by non-hydrogen bonding contributions. The slight curvature of the plot observed in Fig. 3 is probably a consequence of the wide distribution of interaction configurations, each with its own temperature dependence, and of possible changes in the degree of solvation of the protein residues and of the solutes. A more precise fit of the enthalpy of adsorption could not be obtained utilizing the classical temperature dependence of the enthalpy

due to the difference between the molar heat capacities of the solute in the two phases. The curvature is too strong and the numbers obtained had no physical meaning, showing that another effect is responsible for this curvature.

In Table II, the thermodynamic functions of adsorption are calculated for both isomers at each of the sites for all of the temperatures. The Gibbs free energy for each of the groups of data are essentially constant and are within experimental error. The values for the D-isomer at the selective sites should and are the lowest numbers generated, guaranteeing that the most energetically favorable interactions occur there, as expected. The adsorption entropies calculated from these previous values also explain why the D-isomer is the more retained enantiomer, because the formation of the corresponding complex increases the degree of order the most in comparison with the other three groups of data. The set of numbers with the second greatest order corresponds to the L-isomer at the selective site. This implies that these molecules undergo more ordering than when interacting with the low-energy non-selective sites, but not as much as the D-isomer at the selective sites.

Figs. 4 and 5 depict the determination of the isosteric heats of adsorption for the two isomers at both sites. To determine the isosteric heat of adsorption, the amount adsorbed must be held constant, and the corresponding value C of the mobile phase concentration is determined for each temperature. Then ln C is plotted against $1/T$ and the slope is proportional to the isosteric heat of adsorption. For both of these plots the greatest amounts adsorbed are the lines at the top (line 1) and the least amounts adsorbed are the lines at the bottom (line 4). In Fig. 4 (selective sites) the data points for the L-isomer are higher than those for the D-isomer, for an identical amount adsorbed. To adsorb the same amount of D- or L-isomer, a lower concentration of the more retained D-isomer in the mobile phase is needed. In Fig. 5 the lines for the two isomers coincide, providing further evidence that no special consideration is given to either

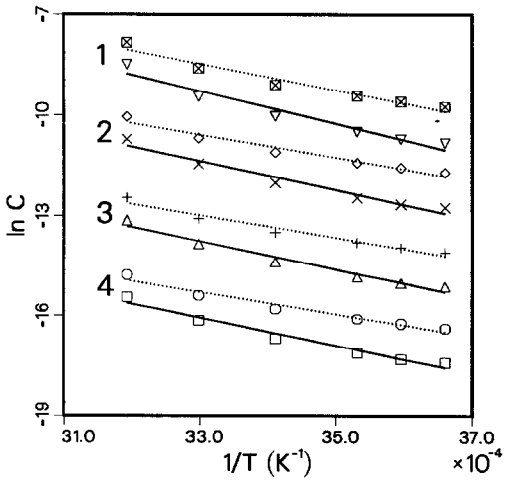

Fig. 4. Plots for the determination of the isosteric heat of adsorption for the selective site. Calculated values (symbols), best linear fits for the L-isomer (dotted lines) and best linear fits for the D-isomer (solid lines). Constant amounts adsorbed: (1) q = 0.00025 mol/l (loading factor, 19%); (2) q = 0.0001 mol/l (loading factor, 8%); (3) q = 0.00001 mol/l (loading factor, 0.8%); (4) q = 0.000001 mol/l (loading factor, 0.08%).

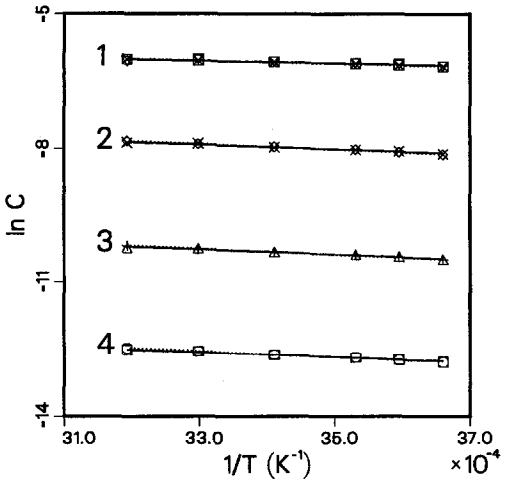

Fig. 5. Plot for the determination of the isosteric heat of adsorption for the non-selective site. Calculated values (symbols), best linear fits for the L-isomer (dotted lines) and best linear fits for the D-isomer (solid lines). Constant amounts adsorbed: (1) q = 0.003 mol/l (loading factor, 17%); (2) q = 0.001 mol/l (loading factor, 6%); (3) q = 0.0001 mol/l (loading factor, 0.6%); (4) q = 0.00001 mol/l (loading factor, 0.06%).

isomer. The energies differ slightly owing to experimental error in determining the adsorption isotherms, as reported and discussed above. As the constant surface concentration at which the determination is made decreases, the isosteric heat approaches a limiting value which is recorded for each isomer at each site in Table III.

In Fig. 6 the isosteric heat of adsorption is plotted against the percentage loading of the column in an attempt to normalize the isosteric heat of adsorption between the selective and non-selective sites. The percentage loading is the fraction of the amount injected divided by the saturation capacity of the adsorption site. Here, the saturation capacities of the selective sites are averaged, and a value of 0.00131 mol/l is used as the mean for this site for both the L- and D-isomers. Also, the saturation capacities of the non-selective sites were averaged to obtain a value of 0.0174 mol/l. The average saturation capacity of the non-selective site is *ca.* thirteen times that of the selective site.

For the selective site a slight decrease in the isosteric heat of adsorption is seen as the percent loading increases, and conversely, for the non-selective site a slight increase

TABLE III

ISOSTERIC HEATS OF ADSORPTION

$q = 10^{-7}$ mol/l.

Site	Isomer	ΔH°_{st} (kcal/mol)
Selective	L-	−6.63
	D-	−8.40
Non-selective	L-	−1.31
	D-	−1.14

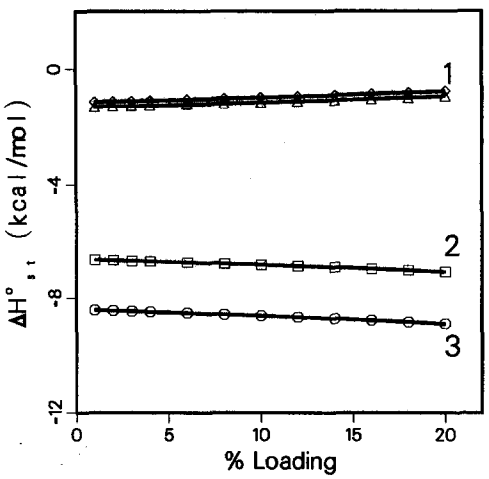

Fig. 6. Plot of the isosteric heat of adsorption for increasing loading factor (%). Calculated values (symbols). (1) D- and L-isomers for the non-selective site; (2) L-isomer for the selective site; (3) D-isomer for the selective site.

is seen as the temperature increases. The value calculated for the isosteric heat is different from the value of the enthalpy of adsorption. This occurs because the isosteric heat not only reflects the enthalpy of adsorption but also several other factors such as the energy associated with rearrangement of adsorbed molecules which are not directly attached to the surface and the heat of adsorption of the solvent. Unlike the enthalpy of adsorption, the isosteric heat can increase or decrease with a change in the surface coverage due to these factors.

However, the data in Fig. 6 do not give the exact value of the isosteric heat, as two other errors are introduced because of the assumptions made in order to extract the isosteric heat. The first assumption is that the solute is present in a low concentration in the mobile phase and the activity is proportional to the concentration. The second is that the activity of the solute on the stationary phase is independent of the temperature. Both of these assumptions are no longer valid once the mobile phase concentration is increased above linear chromatographic conditions, *e.g.*, for loading factors above 0.1%. As the concentration of the amount adsorbed increases, the activity of the solute changes because interactions between adjacent solute molecules must be included.

CONCLUSION

The results of this work verify the assumption that at least two types of sites exist when considering the separation of chiral isomers on an immobilized protein and probably on a number of other chiral selective phases. For the sake of simplicity, the type of sites were broken down into two groups: chiral selective sites and achiral, non-selective sites. The former sites have a higher interaction energy and lower saturation capacity than the latter. This result was established by considering the values of the enthalpy of adsorption and the isosteric heat of adsorption whereby the energies were calculated for each type of site.

ACKNOWLEDGEMENTS

We thank Irving W. Wainer (St. Jude's Medical Center, Memphis, TN, U.S.A.) for useful discussions. The gift of the Resolvosil-BSA-7 column by Alltech (Deerfield, IL, U.S.A.) is gratefully appreciated. We acknowledge the continuous support of our computational effort by the University of Tennessee Computing Center. This work was supported in part by grant CHE-8901382 from the National Science Foundation and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory.

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