Adsorption Equilibrium of α-Amylase in Aqueous Solutions

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The influence of pH, ionic strength and temperature on the equilibrium of adsorption of α-amylase from Aspergillus oryzae on a hydrophobic (Duolite XAD-761) and an anion-exchange (Duolite A-568) polymer-based adsorbent was studied by moment analysis of the chromatographic peak responses in HPLC. The adsorption isotherms were also measured in batch experiments at different temperatures. Both systems show a nonlinear equilibrium within the concentration range studied, and the results were fitted to both the Langmuir and Freundlich equations. Enthalpies and entropies of adsorption were estimated from the equilibrium adsorption constant obtained both by moment analysis and by the limit region of the Langmuir isotherm corresponding to Henry's law. The results show that the process of adsorption of α-amylase on the hydrophobic resin was exothermic, while the anion exchange process is endothermic.

Introduction

During the last two decades, advances in biochemical and genetic engineering provided the development of a great number of new biotechnology products and processes as well as the replacement of many of the existing processes carried out by conventional chemical techniques.

Downstream processing of biomolecules obtained from fermentation broths is a fundamental step in process biotechnology, since it often represents the major manufacturing cost. The economic viability of a biochemical process depends not only on the innovations achieved in production due to advances in molecular biology, immunology, microbiology, and so on, but also to innovations and optimization of downstream processes (Wheelwright, 1987).

Chromatographic processes are widely used as high-performance purification steps in biotechnology. Much of the effort carried out to scale up a process is developed by intermediate pilot-plant studies using empirical or semiempirical methods. To design effective and selective chromatographic separation procedures we require to know the effect of the operating conditions (pH, ionic strength, temperature, concentration of the adsorbate in the inlet stream) on the adsorption processes. Furthermore, the use of phenomenological mathematical models is essential for a rational design, scale-up, and optimization of both new and existing downstream processes. The equilibrium information given by the adsorption isotherm can be used to determine the capacity of the adsorbent. Also, the isotherm, expressed as a mathematical equation, can be implemented in a phenomenological model in order to predict the concentration profile along the column for industrial or preparative purification applications (Aracil and Martínez, 1993). Estimation of the thermodynamic (ΔH^0 and ΔS^0) of the adsorption processes helps in predicting how the retention of adsorbate might vary with temperature. Thermodynamic and equilibrium studies carried out in liquid chromatographic systems for some organics (Ching et al., 1989; Miyabe and Suzuki, 1995), antibiotics (Kirkby et al., 1986), amino acids (Martínez et al., 1994; Grzegorczyk and Carta, 1996), and small peptides (Casillas et al., 1992) have been reported. However, few works concerning large biomolecules have been developed with the aim of increasing the knowledge on the mechanism and the number of chromatographic systems characterized. In particular, Langmuir isotherms have been measured for the adsorption of α-amylase from *Bacillus licheniformis* on cross-linked starch (Somers et al., 1995). Rozie et al. (1991) also studied the effect of temperature on the adsorption of bacterial α-amylase on cross-linked potato starch. They found that the adsorption capacity of the affinity adsorbent increases as

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temperature decreases from 20°C to 4°C, obtaining maximum values of 185 mg/g (*B. subtilis*) and 71 mg/g (*B. licheniformis*). However, cross-linked starch is not appropriate as packing in the design of large columns for downstream processing since it is a very soft adsorbent with a very low mechanical resistance.

The aim of this work is to describe the equilibrium of the adsorption of α-amylase from *Aspergillus oryzae* on two commercial synthetic polymer-based resins: a hydrophobic adsorbent and a weak anion exchanger. The selected approach for this study includes the determination of the effect of pH, ionic strength, and temperature on the retention of the adsorbate. Also, the capacity of both adsorbents in the equilibrium is determined by measuring the corresponding adsorption isotherms. Moment analysis from HPLC experiments was developed to study the variables affecting the adsorption and to estimate the enthalpy and entropy of the process. Also, the adsorption isotherms were measured in batch experiments in order to characterize the equilibrium of adsorption at different temperatures. Thermodynamic results obtained from both experimental systems have been compared.

Experimental Studies

Adsorbate

α-Amylase (α-1,4-glucan-4-glucanohydrolase) from *A. oryzae* was obtained by recrystallization following a method proposed in a previous article (Akabori et al., 1954). Fungamyl 1600 BC-type WF, kindly supplied by Novo Nordisk Bioindustrial S.A. (Madrid, Spain), was used as raw material for the purification just cited. α-Amylase from *A. oryzae* consists of 478 amino acid residues (Toda et al., 1982; Tada et al., 1989) with a molecular weight of 52.6 kDa and an isoelectric point of 4.2 (Vihinen and Mäntsälä, 1989). From the results of X-ray diffraction studies, the molecule can be considered as an ellipsoid of dimensions $80 \times 45 \times 35$ Å (Matsuura et al., 1979, 1980, 1984; Boel et al., 1990; Swift et al., 1991).

Chemicals and buffers

NaH_2PO_4 and Na_2HPO_4 for phosphate buffer, and tris(hydroximethyl aminomethane) and HCl for tris/HCl buffer were reagent-grade chemicals. NaCl was used to adjust the ionic strength of each solution to the desired value. A phosphate buffer of 0.05 M was used for experiments carried out a pH 6.0, 7.0, and 8.0 on the hydrophobic resin and at pH 6.0 on the ion exchanger. A tris/HCl buffer of 0.05 M was used for adsorption experiments on the ion-exchange resin at pH 7.0 and 8.0. The presence of Ca^{2+} ions in α-amylase solutions is necessary for maintaining its enzymatic activity and structural stability (Vallee et al., 1959; Janecek and Baláz, 1992), so 0.4 mM $CaCl_2$ was added to each buffer and α-amylase solution.

Solutions used in pulse experiments in HPLC and in batch equilibrium experiments were filtered through 0.22 μm (ref.: GSWP 047 00) and 0.80 μm (ref.: AAWP 047 00) cellulose esters membranes (Millipore Corp., Bedford, MA), respectively.

Adsorbents

The hydrophobic adsorbent Duolite XAD-761 and the anion exchanger Duolite A-568 were kindly supplied by Rohm and Haas España S.A. (Barcelona, Spain). The physical properties of both resins are summarized in Table 1.

Table 1. Physical Properties of Adsorbents

	Duolite XAD-761	Duolite A-568
Matrix*	Cross-linked phenol-formaldehyde polycondensate	
Functional group*	Phenol (nonionic)	Tertiary amine
Specific gravity [g/cm^3]*	1.08	0.94
Pore volume [cm^3/g]**	0.42	0.53
Skeletal density [g/cm^3]**	1.97	1.89
Porosity**	0.45	0.50
Average pore size [nm]**	22.5	27.3
BET surface area [m^2/g]†	27.2	92.8

*From Duolite XAD-761 and Duolite A-568 Technical Sheets, Rohm and Haas Co.
**Measured by mercury porosimetry.
†Measured by adsorption.

The hydrophobic resin was pretreated sequentially with 1.5 M NaOH, distilled water, 2.0 M HCl, and distilled water again. This procedure was repeated twice, and the resin was then washed with methanol and rinsed with distilled water. For the ion-exchange resin, the same sequential treatment was used, followed by washing with distilled water until the pH was neutral.

Equipment

Moment analysis was achieved from the reponses obtained in HPLC pulse experiments carried out in a Smart System (Pharmacia LKB, Uppsala, Sweden). The system is equipped with two positive displacement pumps of 10-mL capacity, an automatic injection valve fitted with a 5-μL loop, a temperature-controlled column chamber, and variable wavelength UV/vis and conductivity detectors. Data-acquisition hardware and a control workstation complete the system. The empty volume of the equipment, including injector loop and detectors, is 150 μL.

Both adsorbents, previously pretreated, were sieved and the fraction in the range of 25–38 μm used as packing material. Stainless-steel columns (200 mm $\times$ 2.1 mm ID) were packed following the slurry technique described elsewhere (Bautista, 1997).

Adsorption isotherms were determined by batch equilibrium experiments in a 50-mm-diameter spherical stirred glass flask, where the adsorbent particles (0.32–0.50 mm) were contacted with the α-amylase solution. A Heto DT1 CB-8-30e (Heto-Holten, Allerød, Denmark) thermostatic bath, with a stability of ± 0.01°C, was used to develop the experiments at constant temperature. The α-amylase concentration was determined spectrophotometrically in a diode array UV/Vis spectrophotometer HP-8452A (Hewlett-Packard, Avondale, PA) at 280 nm.

Operating procedures

Prior to each HPLC experimental run, the column was eluted overnight with a buffer solution of the chosen pH and ionic strength. The temperature of the chamber where the column was placed was raised to the desired value. About 2 h were allowed for thermal steady state to be attained. Then, 5-μL pulses of a 1.0-mg$\cdot$mL^{-1} α-amylase solution were in-

jected into the column and the responses were monitored by UV absorption at 280 nm for further numeric estimation of first moment.

During batch experiments for the determination of the adsorption isotherms 0.50 g (dry basis) of hydrated adsorbent particles were suspended inside the spherical flask in 50 mL of buffered α-amylase solution of known concentration. This flask was immersed in a thermostatic bath at the selected temperature and the suspension was stirred at 800 rpm. After 12 h, UV absorption at 280 nm of the supernatant solution was measured and the α-amylase concentration was determined by means of a calibration curve. The equilibrium concentration of α-amylase in the adsorbed phase, q, was estimated by mass balance using the following equation:

$$q = \frac{(C_0 - C_e)\cdot V_L}{W}, \tag{1}$$

where C_0 and C_e are the initial and equilibrium concentration of α-amylase in the bulk liquid phase, in $mg\cdot mL^{-1}$; V_L is the volume of solution, in mL; and W is the mass of adsorbent, in g.

Results and Discussion

Moment analysis

Moment analysis of the responses obtained from an input pulse of an adsorbate in a chromatographic column was shown to be a quick and easy tool for evaluating the adsorption process for both gas/solid and liquid/solid systems, involving small and relatively small molecules of adsorbate (Addo-Yobo et al., 1988; Ching and Ruthven, 1988).

Assuming the absence of a chemical reaction, a linear adsorption isotherm, and an instantaneous input pulse, the first moment can be written as follows (Ruthven, 1984):

$$\mu = t_0 + \frac{L}{v}\cdot\left[1 + \left(\frac{1-\epsilon}{\epsilon}\right)\cdot k\right], \tag{2}$$

where t_0 is the system dead time; L the length of the column; v the interstitial velocity of the mobile phase; ϵ the porosity of the column; and k the apparent adsorption constant, which can be expressed as

$$k = \epsilon_p + (1-\epsilon_p)\cdot K_A, \tag{3}$$

where ϵ_p is the particle porosity and K_A is the dimensionless linear adsorption constant.

In order to characterize the porosity of each HPLC column, pulses of nonadsorbable solutes were injected. As tracers, solutes that are not significantly adsorbed ($K_A \approx 0$) and that diffuse freely inside the macroporous structure of the resins must be chosen. In these conditions, and according to Eqs. 2 and 3, column porosity can be determined from the slope of the plot of the first moment and evaluated by numerical integration, which corresponds with the chromatographic peak responses of each tracer vs. L/V. In the present work, 2.0 M NaCl and 0.25 M $NaNO_3$ were used as tracers for the hydrophobic resin and the ion exchanger, respectively. Responses to NaCl pulses were monitored by means of the conductivity detector, and sodium nitrate was monitored by UV absorbance at 280 nm. The values of the column porosity obtained were 0.57 and 0.56 for Duolite XAD-761 and Duolite A-568 respectively.

Table 2. First Moment Analysis for the Systems α-Amlyase/Duolite XAD-761 and α-Amylase/Duolite A-568

Duolite XAD-761				Duolite A-568			
T [°C]	pH	Ionic Strength	K_A	T [°C]	pH	Ionic Strength	K_A
20	6.0	0.10	0.8	20	6.0	0.90	5.8
		0.20	3.5			1.00	5.2
		0.30	8.9			1.10	3.9
		0.35	8.9				
	7.0	0.02	0.2		7.0	0.60	19.1
		0.10	0.3			0.70	12.6
		0.20	1.4			0.80	9.1
		0.30	3.1			0.90	6.6
		0.40	5.4				
	8.0	0.20	0.6		8.0	0.50	11.6
		0.30	0.9			0.60	6.2
		0.40	1.1			0.70	4.4
						0.80	4.7
15	6.0	0.35	9.4	15	7.0	0.60	16.3
17			9.1	17			16.7
23			8.6	23			20.6
25			8.4	25			21.6

First moment analysis for α-amylase experiments was carried out at different conditions of temperature, pH, and ionic strength. In order to determine the experimental error associated with the estimation of first moment, some of the runs were repeated five times. The maximum deviations, which were found from the corresponding average value, were $\pm 6\%$ and $\pm 2\%$ for α-amylase and tracer pulses, respectively.

Since ϵ_p and ϵ are already known, the adsorption constant, K_A, at each operating condition can be determined from the slopes of the corresponding plots (Eqs. 2 and 3). Table 2 shows the results obtained from the moment analysis corresponding to the adsorption of α-amylase on Duolite XAD-761 and Duolite A-568.

Effect of ionic strength

Increasing values of salt concentration have a positive influence on equilibrium length, given by K_A, for the adsorption of α-amylase on the hydrophobic resin. This system shows a marked dependence of ionic strength on K_A at pH 6, moderated at pH 7 and low at pH 8 (Figure 1a). In the experimental pH range studied, the adsorption of α-amylase on Duolite XAD-761 at very low values of ionic strength is nearly negligible. As the salt concentration increases in the mobile phase, the intensity of the hydrophobic interactions between the solute and the hydrophobic ligands attached to the resin matrix also increases. This effect agrees with the theory of retention of an adsorbate modulated by salt concentration (Horváth et al., 1976; Melander and and Horváth, 1977a; Melander et al., 1989), based on the solvophobic theory (Sinanoglu and Abdulnur, 1965; Sinanoglu, 1982).

As expected, the effect of the ionic strength on the ion exchange of α-amylase on Duolite A-568 is opposite to that

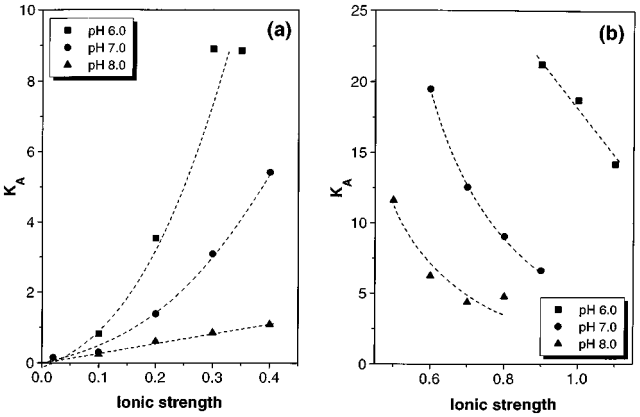

Figure 1. Effect of ionic strength on the adsorption constant for different pH values at 20°C: (a) Duolite XAD-761; (b) Duolite A-568.

found in the hydrophobic system (Figure 1b). The salt anions present in the mobile phase, mainly chloride, compete with the negatively charged groups of α-amylase for the amino groups of the ligands attached to the stationary phase. The equilibrium between α-amylase and the counterion with the stationary phase can be expressed as follows:

$$Z_S E + Z_E \bar{S} \rightleftarrows Z_S \bar{E} + Z_E S, \tag{4}$$

where Z_S and Z_E are the valence of the counterion and the effective electric charge of the enzyme, respectively, and S symbolizes the counterion and E the enzyme. Overbars represent the corresponding molecule linked to the adsorbent by an electrostatic bond. So the equilibrium constant can be written as

$$K_A = \left(\frac{|\bar{E}|}{|E|}\right)^{Z_S} \left(\frac{|S|}{|\bar{S}|}\right)^{Z_E}. \tag{5}$$

It can be directly derived from Eq. 5 that the concentration of α-amylase in the adsorbed phase decreases as the salt concentration in the mobile phase increases.

Effect of pH

The effect of pH on the equilibrium of adsorption in the hydrophobic system has already been partially observed at low ionic strength values. The increment of pH above the isoelectric point of α-amylase produces an increase in the net negative charge of the enzyme. In these conditions, electrostatic interactions between the enzyme and mobile phase become higher than hydrophobic interactions between α-amylase and the hydrophobic resin, reducing the adsorption capacity of the stationary phase.

The influence of pH on the behavior of the ion exchange of α-amylase on Duolite A-568 is qualitatively similar to that found for the hydrophobic interaction adsorption using Duolite XAD-761, that is, the value of K_A increases as pH decreases. This effect does not agree with the theoretical models that predict an exponential increment of the retention factor with increasing pH (Kopaciewicz et al., 1983; Velayudhan and Horváth, 1988) and that the characteristic negative charge of α-amylase slightly increases as pH increases in the experimental range (Bautista, 1997).

The reason for this effect lies in the fact that Duolite A-568 is a weak anion exchanger that progressively increases the degree of ionization of the tertiary amino groups as the pH decreases in the pH range studied (Figure 2), thus creating new adsorption sites on the stationary phase.

Effect of temperature

Operating conditions corresponding to experiments that yield high K_A values were chosen in order to study the effect of temperature on the equilibrium of adsorption. For the α-amylase/Duolite XAD-761 system, a pH of 6.0 and an ionic strength of 0.35 were selected. An ionic strength of 0.60 and a pH value of 7.0 were selected as moderate conditions for the α-amylase/Duolite A-568 system. The effect of temperature on the magnitude of the adsorption constant, K_A, shows qualitatively opposite behavior for both the ion exchange and the hydrophobic systems. The anion exchange of α-amylase on Duolite A-568 is favored at increasing temperatures, while the adsorption of the enzyme on the hydrophobic resin Duolite XAD-761 slightly increases as temperature decreases (Figure 3).

There is a lack of thermodynamic data for the adsorption of proteins both on hydrophobic interaction adsorbents and on ion exchangers. However, the temperature dependence of the dimensionless adsorption constant obtained from the moment analysis of the chromatographic peak responses, K_A, has been used to estimate the thermodynamic parameters of the adsorption process for different compounds, such as β-lactamic antibiotics (Casillas, 1992), aminoacids on hydrophobic (Martínez et al., 1995), silica-based (Uddin et al., 1990), or large pore zeolite adsorbents (Ching et al., 1989) and small peptides (Casillas et al., 1992). The enthalpy of adsorption, ΔH^0, is related to the equilibrium constant through the van't Hoff equation:

$$\frac{\partial \ln K_A}{\partial T} = \frac{\Delta H^0}{RT^2}. \tag{6}$$

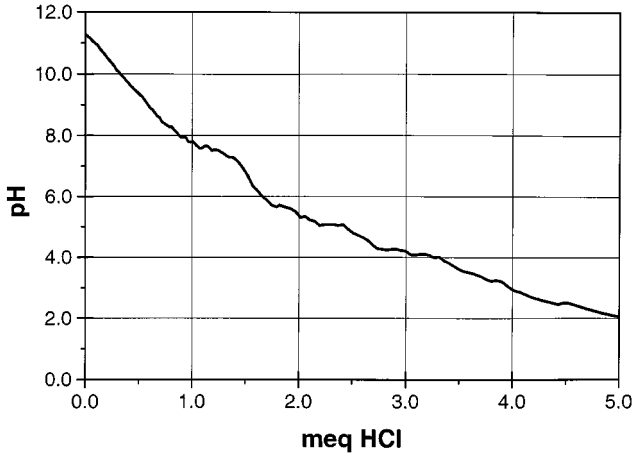

Figure 2. Titration curve for 1.0 g (dry base) of Duolite A-568 in 0.50 M NaCl.

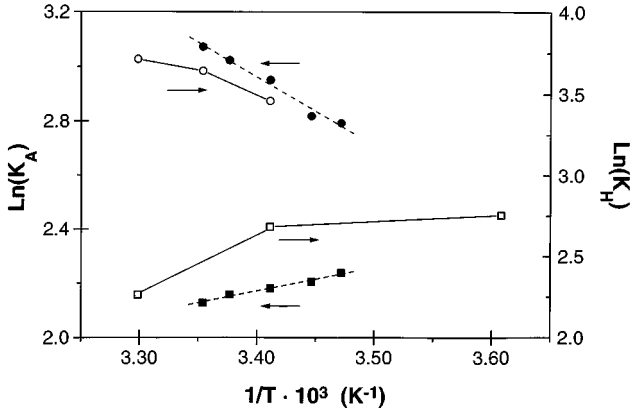

Figure 3. Van't Hoff plots corresponding to both adsorption systems.

Duolite XAD-761: (■) K_A from moment analysis; (□) K_H from Langmuir isotherms.

Duolite A-568: (●) K_A from moment analysis; (○) K_H from Langmuir isotherms.

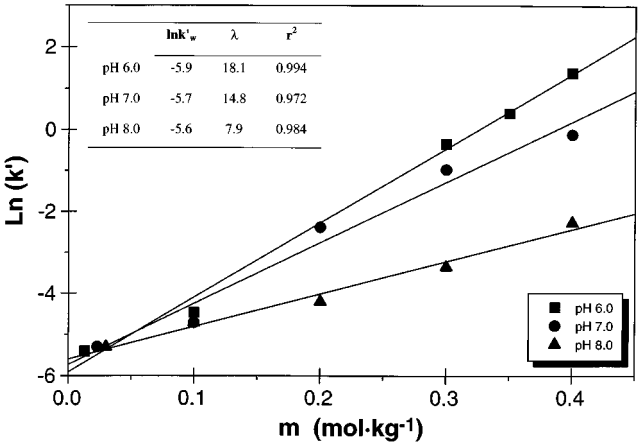

Figure 4. Effect of salt molality on retention factor, k', at three pH values for the system α-amylase/ Duolite XAD-761.

It shows the hydrophobic parameters determined by regression analysis.

The entropy change during the adsorption process, ΔS^0, can be determined by the following equation:

$$\Delta S^0 = \frac{\Delta H^0}{T} + R \cdot \ln K_A. \tag{7}$$

Table 3 shows the thermodynamic parameters obtained for the adsorption of α-amylase on Duolite XAD-761 and Duolite A-568.

The results show a moderate exothermic adsorption of α-amylase on the hydrophobic Duolite XAD-761, accompanied by a decrease in the entropy when the enzyme molecule is adsorbed. Miyabe and Suzuky (1994) described a reduction in the entropy during the adsorption process of different hydrophobic solutes on reversed-phase liquid chromatography operation. This negative change in entropy was larger in absolute value for increasing hydrophobicity in a series of different adsorbate molecules. The degree of hydrophobicity is related to the hydrophobic surface area of the adsorbate in contact with the hydrophobic ligands of the stationary phase, and it is quantified and characterized by the *hydrophobic interaction parameter*, λ (Horváth et al., 1977; Melander and Horváth, 1977b). According to the preceding theory, the retention factor, k', in hydrophobic interaction liquid chromatography varies with salt molality, m, following Eq. 8:

$$\ln\left(\frac{k'}{k'_w}\right) = \lambda \cdot m, \tag{8}$$

where k'_w is the retention factor extrapolated at zero salt concentration in the mobile phase.

In order to characterize the hydrophobicity of the adsorption system α-amylase/Duolite XAD-761, the values of k' were plotted vs. salt molality from HPLC experiments carried out at pH 6.0, 7.0 and 8.0. The intercept, slope, and correlation coefficient determined by linear fit are shown in Figure 4.

The value of λ at pH 6.0 is even larger than those obtained for other hydrophobic proteins such as ovoalbumin and lysozyme in different hydrophobic adsorbents (Szepesy and Rippel, 1994). However, the strength of the hydrophobic interaction, given by the intercept, $\ln(k'_w)$, is very low, which accounts for the mild interaction between the adsorbate and the stationary phase. This effect can be highly interesting for the purification of biological molecules such as enzymes, where not only the purity and yield are important but the enzymatic activity is also a key parameter.

Although both of the adsorption systems studied have opposite thermodynamic behavior in enthalpy and entropy, the overall free-energy change during the adsorption process is negative for the experimental range of temperatures, corresponding to spontaneous processes.

Table 3. Enthalpies and Entropies of Adsorption for α-Amylase on Duolite XAD-761* and Duolite A-568 Based on Moment Analysis [A] and Langmuir Isotherms [B]**

		$-\Delta H^0$ [kJ·mol^{-1}]	ΔS^0 [J·mol^{-1}·K^{-1}]
[A]	Duolite XAD-761	6.5	−4.2
	Duolite A-568	−20.9	95.8
[B]	Duolite XAD-761	12.1	−20.2
	Duolite A-568	−18.9	93.3

*pH 6.0; Ionic Strength 0.35.
**pH 7.0; Ionic Strength 0.60.

Adsorption isotherms

Batch experiments were carried out in a stirred tank in order to make an in-depth study of the equilibrium of the adsorption of α-amylase on both the hydrophobic and the ion-exchange resins used in the present work. The operating conditions, pH, and ionic strength were the same as those used in HPLC pulse experiments to study the effect of temperature.

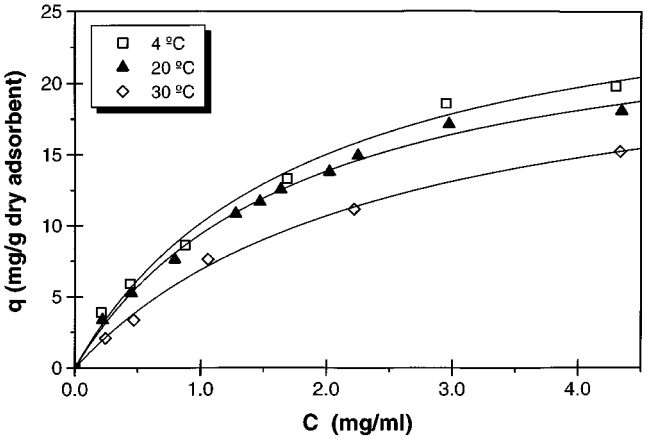

Figure 5. Adsorption isotherms of α-amylase on Duolite XAD-761 (pH 6.0 and ionic strength 0.35) at different temperatures.

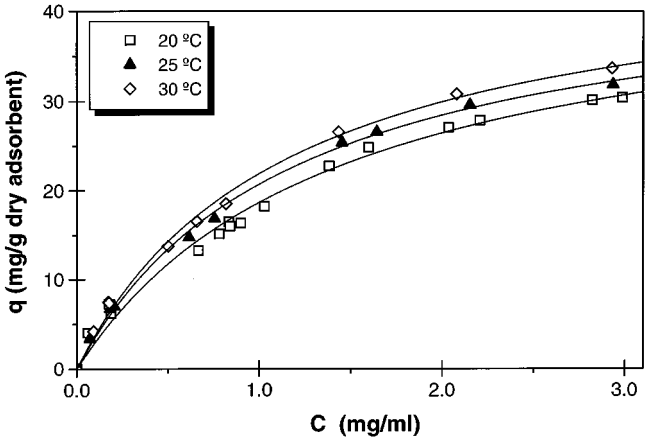

Figure 6. Adsorption isotherms of α-amylase on Duolite A-568 (pH 7.0 and ionic strength 0.60) at different temperatures.

From the results of the equilibrium experiments (Figures 5 and 6) obtained near the saturation capacity, we can see that an increment of 10°C (from 20 to 30°C) yields a shift increment of about 10% in the amount of α-amylase adsorbed on Duolite A-568. On the other hand, on Duolite XAD-761, when the temperature shifts from 4 to 30°C, the enzyme concentration on the adsorbed phase is depleted by approximately 24%. At the three temperatures studied, there is no significant experimental difference in the adsorption level for the anion exchanger at very low α-amylase concentrations in the liquid phase.

As shown in Figures 5 and 6, nonlinear and favorable isotherms are obtained for both systems under study. The experimental results were fitted to both Langmuir and Freundlich equations, Eqs. 9 and 10, respectively:

$$q = \frac{Q_m bC}{1 + bC} \tag{9}$$

$$q = K_F C^{\beta}. \tag{10}$$

The parameters were obtained by nonlinear regression analysis using the Levenberg–Marquardt algorithm. Table 4 contains the parameters corresponding to the adsorption isotherms of α-amylase on Duolite XAD-761 and Duolite A-568, respectively.

In most cases, the correlation between experimental results and fitted equations of the Langmuir isotherm gives better results than the Freundlich expression. The reason for this lies in the fact that the Langmuir isotherm predicts saturation of the adsorbent at the high solute concentration observed in the experimental data. The limiting slope of the Langmuir isotherm at very low concentrations is expressed as

$$\left|\frac{dq}{dC}\right|_{C=0} = Q_m \cdot b = K_H \tag{11}$$

The preceding value is considered to be the Henry's adsorption equilibrium constant, K_H, in mL/g.

Kalghatgi and Horváth (1987) reported enthalpies of lysozyme adsorption in reversed-phase liquid chromatography in isocratic operation at two different concentrations of the organic modifier in the mobile phase estimated by the van't Hoff equation. Grzegorczyk and Carta (1996) checked the adsorption enthalpies obtained by calorimetric measurements and by the van't Hoff equation using the Henry's constant from the Langmuir isotherm for phenol on the polymeric hydrophobic adsorbent Amberlite XAD-4, obtaining reasonably good agreement.

The enthalpies of adsorption, ΔH^0, were calculated (Table 3) by applying Eq. 6 to the discrete experimental values of Henry's constant.

As can be seen, the results corresponding to the adsorption of α-amylase on the ion exchanger agree well with the result obtained by the moment analysis developed from the results obtained in HPLC pulse experiments. The greatest difference observed between the two methods was in the enthalpy of adsorption of α-amylase on the hydrophobic resin. Estimation of thermodynamic parameters in HPLC experiments was carried out in the 15 to 25°C range, while the temperature range for the adsorption isotherms was widened from 4°C to 30°C. The difference in the value of $-\Delta H^0$ could be caused by a change in the intrinsic adsorption mechanism in the 25–30°C range, as plotted in Figure 3 where a change in the corresponding slope is observed. Since the matrix in

Table 4. Fitting Parameters to Both Langmuir and Freundlich Equations for the Adsorption Isotherms of α-Amylase on Duolite XAD-761 [A] and Duolite A-568 [B]

		Langmuir			Freundlich		
	T [°C]	Q_m	b	r^2	K_F	β	r^2
[A]	4	28.8	0.544	0.992	9.7	0.53	0.988
	20	26.3	0.554	0.996	9.3	0.51	0.979
	30	24.1	0.398	0.997	6.5	0.60	0.985
[B]	20	45.5	0.696	0.985	17.9	0.53	0.991
	25	45.4	0.840	0.998	19.2	0.54	0.987
	30	47.2	0.867	0.998	20.2	0.53	0.989

Duolite XAD-761 is hydrophilic, the relative hydrophilic/hydrophobic contribution to the overall adsorption mechanism could change when the temperature increases in the experimental range studied. Wu et al. (1986) studied the adsorption of α-lactalbumin in hydrophobic interaction chromatography, observing nonlinear activity between $\ln(k')$ and $1/T$ due to conformational changes in the protein structure. But in our work, the existence of different peaks or peak distortions corresponding to different folded states of α-amylase has not been observed under any operating conditions studied.

Conclusions

The equilibrium of α-amylase adsorption from *A. oryzae* on a macroporous polymeric hydrophobic adsorbent and weak anion exchanger was studied. The effect of the operating variables, pH, ionic strength, and temperature was studied by means of moment analysis of HPLC pulse experiments. For the hydrophobic system, increasing values of ionic strength yield higher values of the dimensionless adsorption constant, while the effect on the anion exchange system is just the opposite. No significant adsorption was observed at low levels of salt concentration using the hydrophobic resin. In both systems, the adsorption constant increases as pH decreases from 8 to 6. This effect has been explained, for the adsorption of α-amylase on the anion exchanger, in terms of the progressive ionization of the functional amino groups attached to the resin, with the consequent creation of new adsorption sites, as pH decreases in the experimental range studied.

The effect of temperature was also studied in batch stirred-tank experiments, where the adsorption isotherms were determined. In both experimental systems, the equilibrium of adsorption showed a nonlinear behavior that was satisfactorily described by the Langmuir isotherm. The enthalpy and entropy of the process were estimated using the van't Hoff equation from both moment analysis and the Langmuir isotherms, showing them to be exothermic for the adsorption of α-amylase on the hydrophobic resin and endothermic on the anion exchanger.

Acknowledgments

Financial support from the Commission of the European Communities (Brite-EuRam II programme, project BE-8138), DGICYT (project BIO94-1508-CE), and Comunidad Autónoma de Madrid (project AE00265/94) is gratefully acknowledged.

Notation

b = parameter in Eq. 9, $\text{mL}\cdot\text{g}^{-1}$
K_F = parameter in Eq. 10
Q_m = saturation capacity of the adsorbent, $\text{mg}\cdot\text{g}^{-1}$
r = correlation factor
R = gas constant
t_{r0} = retention time of tracer, s
V = velocity of mobile phase defined as $v\cdot\epsilon$, $\text{m}\cdot\text{s}^{-1}$
β = parameter in Eq. 10

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Manuscript received June 23, 1998, and revision received Jan. 13, 1999.