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Adsorption of BSA on DEAE-Dextran: Equilibria

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ABSTRACT

Equilibrium isotherms for adsorption of BSA on DEAE-dextran have been determined experimentally. They were little affected by the initial concentration of BSA but were considerably affected by pH. They were correlated by the Langmuir equation when pH $\geq$ 5.05 and by the Freundlich equation at pH 4.8 ($\cong$ pI). The maximum amounts of inorganic anion exchanged for BSA were 1 and 0.4% of the exchange capacity of the ion exchanger at pH 6.9 and 4.8, respectively. When NaCl coexisted in the solution, the shape of the isotherm was similar to the Langmuir isotherm but was shifted to the right. When the concentration of NaCl was 200 mol/m^3, BSA was not adsorbed on the resin. When BSA was dissolved in pure water, the saturation capacity of BSA on HPO_4^{2-}-form resin was about 3.5 times larger than that with buffer (pH 6.9). The amount of BSA adsorbed on univalent-form resin decreased when the liquid-phase equilibrium concentration of BSA was increased.

Key Words. Adsorption; Ion exchange; Equilibrium; DEAE; BSA

INTRODUCTION

The chromatographic separation of proteins is important not only for analysis but also in such large-scale industries as the food industry and

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the drug industry. A number of ion exchangers for protein separation have been developed to use for analysis, and a large number of chromatograms have been presented to show that they are useful for protein separation. When these ion exchangers are applied to large-scale chromatographic separation, it is necessary to investigate the equilibria, kinetics, and dynamics in detail.

Yamamoto et al. (1) showed that the adsorption of BSA on DEAE (a weakly basic dextran-type ion exchanger) decreases with decreasing pH. Tsou and Graham (2) showed the effect of the concentration of NaCl (C_E) in the BSA solution on the isotherm for adsorption of BSA on DEAE at pH 6.9. They showed that the isotherm for $C_E = 0$ is much more favorable than that for $C_E = 1\%$. However, these reports are fragmentary, and systematic experimental investigations for the adsorption isotherms of protein have not been reported.

We presented experimental equilibrium isotherms for the adsorption of BSA on QAE (a strongly basic dextran-type ion exchanger) [Yoshida et al. (3)]. We showed the ways in which the ionic form of the resin, the pH of the solution, the initial concentration of BSA, and the concentration of NaCl affected the equilibrium isotherm. The data were correlated by the Langmuir equation or the Freundlich equation, which were not used as the theoretical equations for adsorption of BSA on QAE but were applied as empirical expressions that were useful for fitting the experimental data. Using the fitting results, we extracted some ideas for the adsorption mechanism of a protein on QAE. It has not been proved experimentally or theoretically that protein is adsorbed by electrostatic attraction, ion exchange, hydrophobic interaction, and/or hydrogen bonding. We determined the amount of inorganic anion exchanged for BSA experimentally, and we proved that the contribution of ion exchange to the sorption of BSA on the ion exchanger is small.

In this work we present detailed experimental equilibrium isotherms for the adsorption of BSA on DEAE-dextran ion exchanger. We discuss the adsorption mechanism of a protein on the DEAE-dextran by comparing the results for QAE-dextran (3).

MATERIALS AND METHODS

We used DEAE Sephadex A-50 (Pharmacia Fine Chemicals), hereafter called DEAE, which is a weakly basic dextran-type ion exchanger. Before measuring the equilibrium isotherm, the resin particles (dry, Cl^- form, about 10 g) were soaked and thoroughly washed with 200 mol/m^3 HCl aqueous solution. The Cl^--form resin particles were transformed to the OH^- form by using a 200-mol/m^3 NaOH aqueous solution. The OH^- form resin particles were transformed to the Cl^- and phosphate forms by using a 200-mol/m^3 HCl aqueous solution and a 6.975 eq/m^3 phosphate buffer, respectively. Four different phosphate-form resins, which had been in equilibrium with phosphate buffers of pH 4.8, 5.05, 6.9, and 8.79, respectively, were prepared: HPO_4^{2-}-form resin, which was used for measuring the equilibrium isotherm at pH 8.79; two different (HPO_4^{2-} + $H_2PO_4^{2-}$)-form resins, which were used for pH 6.9 and 5.05, respectively; and $H_2PO_4^-$-form resin, which was used for measurement of the equilibrium isotherm at pH 4.8. These resin particles were thoroughly washed successive with deionized distilled water, ethanol, and ether. Thereafter they were dried at room temperature for 2 days.

We measured equilibrium isotherms for the adsorption of BSA by the batch method. The pH of the BSA solution was adjusted by using the phosphate buffer, as mentioned earlier. The resin particles were contacted with the BSA solution and gently mixed. We measured the amount of BSA adsorbed on the resin after 2, 3, and 6 days, and confirmed that there was no difference among them. Then we contacted the resin and BSA solution for 3 days in this experimental study. We analyzed the solutions for BSA with a Shimadzu UV-visible recording spectrophotometer UV-260 (λ = 278.0 nm). The adsorbed phase concentration of BSA was calculated with

$$q = \frac{V(C_0 - C)}{W} \tag{1}$$

where C_0 and C are the initial and equilibrium concentrations of BSA in the liquid phase (kg/m^3), respectively, q denotes the resin-phase concentration of BSA (kg/m^3 wet resin), and V and W are the volume (m^3) of the solution and resin particles, respectively.

The concentration of Na^+ was analyzed by plasma spectroscopy (SMI Spectraspan III) and frame analysis. The concentration of phosphorus was analyzed by plasma spectroscopy.

All experiments were carried out at 298 K.

RESULTS AND DISCUSSION

Physical Properties of the Resin

Table 1 shows the experimental properties of DEAE. The diameter of a wet particle is about 2.78 times that of a dry particle. The concentration of the diethylaminoethyl group in the resin phase, Q (equiv/m^3), was determined by measuring the equilibrium isotherms for two different ion-exchange systems:

$$R{\cdot}Cl^- + H_2PO_4^- \overset{K_1}{\rightleftharpoons} R{\cdot}H_2PO_4^- + Cl^- \tag{2}$$

TABLE 1
Experimental Physical Properties of DEAE Sephadex A-50 and QAE Sephadex A-50

	DEAE	QAE (3)
Diameter in water[a] (mm):		
Free	0.2364	0.2330
Saturated by BSA	0.2182	0.2187
Diameter of dry particle[a] (mm)	0.08508	0.08822
Water content (wt%)	94.1	95.2
Density:		
True ($kg\cdot m^{-3}$)	1690	1615
Apparent ($kg\cdot m^{-3}$)	604.0	716.8
Porosity	0.963	0.957
Concentration of quaternary ammonium group (ion-exchange capacity, Q) ($equiv\cdot m^{-3}$ wet resin):		
Eq. (2)	188 (K_1 = 0.49)	227 (K_1 = 0.36)
Eq. (3)	186 (K_2 = 0.025)	196 (K_2 = 0.11)

[a] Average value of 50 particles.

$$2R\cdot Cl^- + HPO_4^{2-} \overset{K_2}{\rightleftharpoons} R_2\cdot HPO_4^{2-} + 2Cl^- \tag{3}$$

Applying the mass action law to Eqs. (2) and (3), Eqs. (4) and (5) are derived, respectively:

$$K_1 = \frac{q_1(C_0 - C_1)}{(Q - q_1)C_1} \tag{4}$$

$$K_2 = \frac{q_2(C_0 - C_2)^2}{(Q - q_2)^2 C_2} \tag{5}$$

where subscripts 1 and 2 denote $H_2PO_4^-$ and HPO_4^{2-}, respectively. Equations (4) and (5) are transformed to Eqs. (6) and (7), respectively:

$$q_1 = Q - \frac{1}{K_1}\frac{(C_0 - C_1)q_1}{C_1} \tag{6}$$

$$q_2 = Q - \frac{1}{\sqrt{K_2}}\frac{(C_0 - C_2)\sqrt{q_2}}{\sqrt{C_2}} \tag{7}$$

We plotted q_1 versus $(C_0 - C_1)q_1/C_1$ and q_2 versus $(C_0 - C_2)\sqrt{q_2/C_2}$ for the ion-exchange systems of Eqs. (2) and (3), respectively, in Fig. 1. The data for both systems were correlated by straight lines. The equilibrium constant K and the exchange capacity Q were determined from the slope

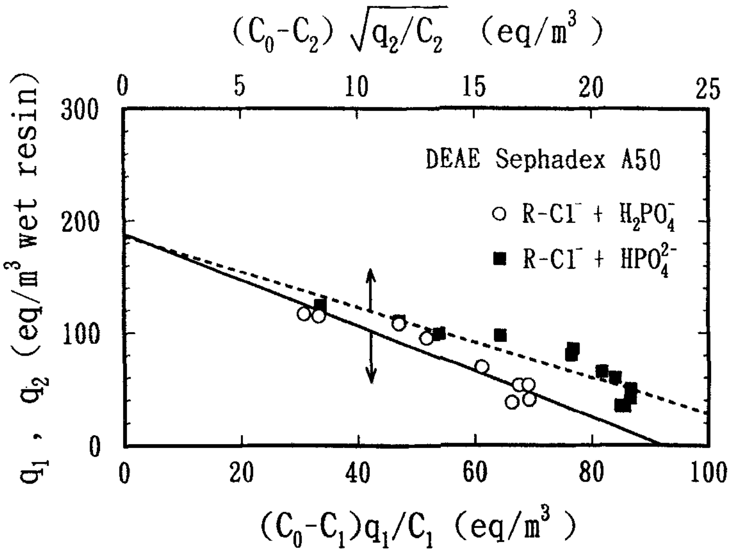

FIG. 1 Ion-exchange equilibria for (R—Cl$^-$ + $H_2PO_4^-$) and (R—Cl$^-$ + HPO_4^{2-}) systems. C_0 = 5 mol/m^3: (—) Eq. (4); (- - -) Eq. (5).

and the intercept of the plot, respectively, and they are listed in Table 1. The exchange capacities determined from the ion-exchange systems of Eqs. (2) and (3) are close.

Table 1 shows that the physical properties of DEAE are close to those of QAE Sephadex A-50 (hereafter called QAE) (3).

Equilibrium Isotherm of BSA

Figure 2 shows the effect of the initial concentration of BSA (C_0) on the equilibrium isotherm for phosphate-form DEAE. The pH of the solution was adjusted to 6.9 by using a phosphate buffer (5 mol/m^3, 6.975 eq/m^3). Equilibrium isotherms for the adsorption of proteins are often described by the Langmuir equation [ion exchanger: Tsou and Graham (2), Yoshida et al. (3); affinity chromatography: Chase (4), Arnold et al. (5, 6), Arve and Liapis (7), McCoy and Liapis (8)]. We also applied the Langmuir equation:

$$q = \frac{Kq_0C}{1 + KC} \tag{8}$$

The solid lines in Fig. 2 show the Langmuir isotherm. The data correlate well with the Langmuir equation. The equilibrium constant K and the

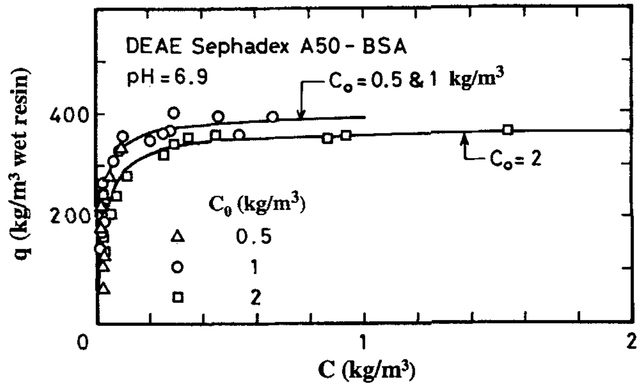

FIG. 2 Effect of initial concentration of BSA on equilibrium isotherm for phosphate-form DEAE (pH 6.9, C_{Buf} = 6.975 equiv/m³): (—) Eq. (8).

saturation capacity q_0 are listed in Table 2 (Nos. 6–8). They were determined by the following equation to which Eq. (8) was transformed:

$$C = -\frac{1}{K} + q_0\left(\frac{C}{q}\right) \tag{9}$$

Figure 3 shows the relation between C and C/q for phosphate-form DEAE at pH 6.9. The data measured for three different C_0 correlate well by straight lines without scattering. The values of K and q_0 were determined from the intercept and slope of the line, respectively. The values of K and q_0 of the other systems shown in Table 2 were determined in the same way. The relationship between C and C/q for the other system correlate well by straight line except for the equilibrium data at pH 4.8 (see below). The equilibrium isotherms for phosphate-form DEAE are independent of the initial concentration of BSA (C_0) when the values of C_0 are 0.5 and 1 kg/m³, but the values of K and q_0 for C_0 = 2 kg/m³ are smaller than those for C_0 = 0.5 and 1 kg/m³ as shown in Table 2. These results suggest that when $C_0 \leq 1$ kg/m³, BSA is adsorbed on the phosphate-form resin by the following Langmuir-type reaction which may be caused by electrostatic attraction between the positively charged fixed group of DEAE and the negatively charged BSA:

$$\mathrm{R} + \mathrm{BSA} \rightleftharpoons \mathrm{R{\cdot}BSA} \tag{10}$$

When $C_0 > 1$ kg/m³, other reactions, such as the ion-exchange reaction, may contribute to adsorption, although Eq. (10) is a main reaction.

Figure 4 shows the effect of C_0 on the equilibrium isotherm for Cl^--

form DEAE. The pH of the solution was adjusted to 6.9 by using a phosphate buffer whose concentration was the same as that in Fig. 2 (5 mol/m^3). The effect of C_0 on the isotherm is larger than for phosphate-form DEAE (Fig. 2). Table 2 (Nos. 10–12) shows that the equilibrium constant K decreases with increasing C_0. The saturation capacity q_0 for $C_0 = 0.3$ kg/m^3 is larger than those for $C_0 = 0.5$ and 1 kg/m^3. q_0 for $C_0 = 0.5$ kg/m^3 is close to that for $C_0 = 1$ kg/m^3. q_0 and K must be independent of C_0 when Langmuir-type adsorption is applicable [Ruthven (9)]. The results for Cl^--form DEAE imply that BSA is adsorbed not only by Eq. (10) but also by the other reactions, although Eq. (10) may be a main reaction. The equilibrium constant for phosphate-form DEAE is larger than that for Cl^--form DEAE, and the saturation capacity for phosphate-form DEAE is close to that for Cl^--form DEAE. Figure 5 shows the equilibrium isotherms for adsorption of BSA on Cl^--form DEAE at pH 6.9, which is the same as in Fig. 4, but the concentration of the buffer was 50 mol/m^3. The isotherms are peculiar in that they do not start from $q = 0$ and $C = 0$ but from $q = 0$ and $C > 0$. The amount of BSA adsorbed on the resin is much smaller than for the case of Fig. 4. Similar behavior occurred when NaCl existed in the BSA solution, as mentioned below. Since the isotherms in Fig. 5 are not good for the adsorption of protein, the concentration of the buffer should be determined carefully. Figures 4 and 5 shows that the lower the concentration of the buffer is, the better.

Figure 6 shows the effect of pH on the equilibrium isotherm for adsorption of BSA on phosphate-form DEAE. The initial concentration of BSA was 1 kg/m^3. The equilibrium isotherm is strongly affected by the pH. The effect of pH on the isotherm is strong around pH = pI [$\cong$ 4.8, Wallevik (10), Evanson and Deutsch (11)] and becomes small when pH $\geq$ 6.9. The solid lines were calculated according to the Langmuir equation (Eq. 8) using the coefficients shown in Table 2. The data for pH $\geq$ 5.05 correlate reasonably well with the Langmuir equation. Figure 7 shows the effect of pH on q_0 and K for DEAE and QAE (3). In spite of the close values of the exchange capacities of both resins (Table 1), the value of q_0 of DEAE is smaller than that of QAE. This is because the basicity of the fixed groups of DEAE is weaker than that of QAE. q_0 decreases with increasing pH for both ion exchangers. Since the pI of BSA is approximately 4.8, the BSA molecules would be negatively charged at pH $\geq$ 5.05 and may be adsorbed by electrostatic attraction between the negatively charged BSA and the positively charged fixed groups of the resin. As the negative charges of the BSA molecule increase with increasing pH of the solution, the electrostatic attraction becomes stronger with an increase of pH. Since when the negative charges of the BSA molecule increase, the number of fixed groups needed to neutralize the negative charges of

TABLE 2

Experimental Langmuir Coefficients and Freundlich Coefficients

No.	pH	C_0 (kg·m^{-3})	C_E (mol·m^3)	q_0 (kg·m^{-3})	K (m^3·kg^{-1})	k	n
DEAE Sephadex A-50: With buffer[a] system: Phosphate form:							
1	4.8	1	0			517	0.391
2	5.05	0.5	0	584	2.44		
3		1	0	480	6.04		
4		2	0	576	3.24		
5		3	0	552	3.03		
6	6.9	0.5	0	401	52.3		
7		1	0	401	52.3		
8		2	0	369	35.8		
9	8.79	1	0	360	25.0		
Cl^- form:							
10	6.9	0.3	0	504	28.8		
11		0.5	0	408	21.5		
12		1	0	405	15.5		
Without buffer system: (HPO_4^{2-} + $H_2PO_4^-$) form:							
13		1	0	800	89.0		

HPO_4^{2-} form:							
14		1	0	1400	12.0		
DEAE Sephadex A-50 (2):							
With buffer[a] system:							
15	6.9	1	0	326	32.7		
16	6.5	1	170	14	2.26		
QAE Sephadex A-50 (3):							
With buffer[a] system:							
Phosphate form:							
17	4.8	1	0			645	0.543
18	5.05	1	0	721	2.76		
19	6.9	1	0	555	9.05		
20	8.79	1	0	491	16.4		
Without buffer system:							
(HPO_4^{2-} + $H_2PO_4^-$) form:							
21		1	0	12.1	73.8		
HPO_4^{2-} form:							
22		1	0	973	7.6		

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[a] 6.975 equiv/m^3 phosphate buffer.

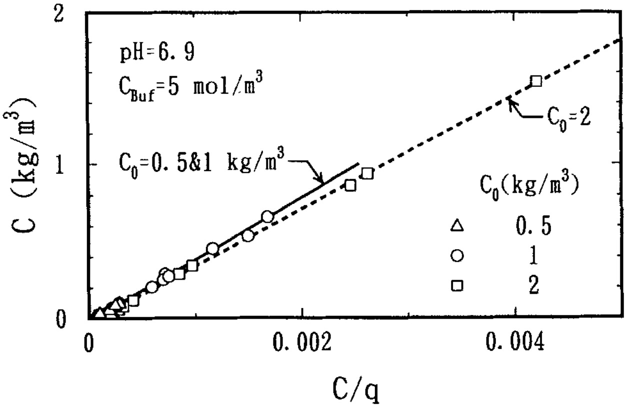

FIG. 3 Langmuir plots of equilibrium data for adsorption of BSA on phosphate-form DEAE [pH 6.9, $C_{\mathrm{Buf}} = 6.975$ equiv/m³ (5 mol/m³)].

one BSA molecule increases, q_0 may decrease with increasing pH. Because the dissociation of anionic groups of the BSA molecule is completed at a pH value in the alkaline region, the values of q_0 tend to converge to a constant value. In the case of QAE, the value of K increases with increasing pH (3). However, the value of K in DEAE shows a maximum. This difference may be caused by the different functional groups of the resins: QAE is a strongly basic resin and DEAE is a weakly basic resin.

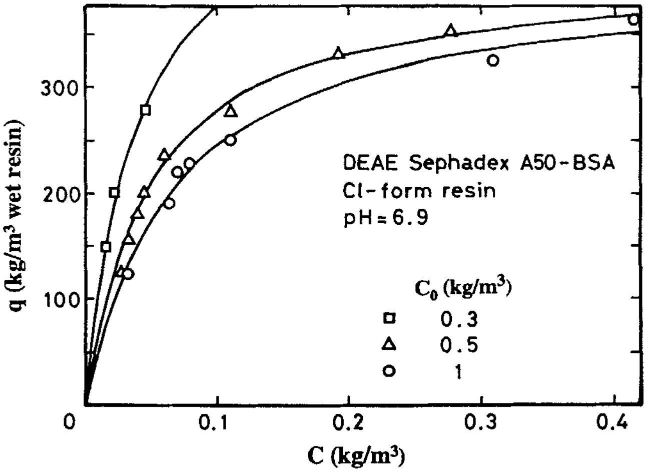

FIG. 4 Effect of initial concentration of BSA on equilibrium isotherm for Cl⁻-form DEAE (pH 6.9, $C_{\mathrm{Buf}} = 5$ mol/m³): (—) Eq. (8).

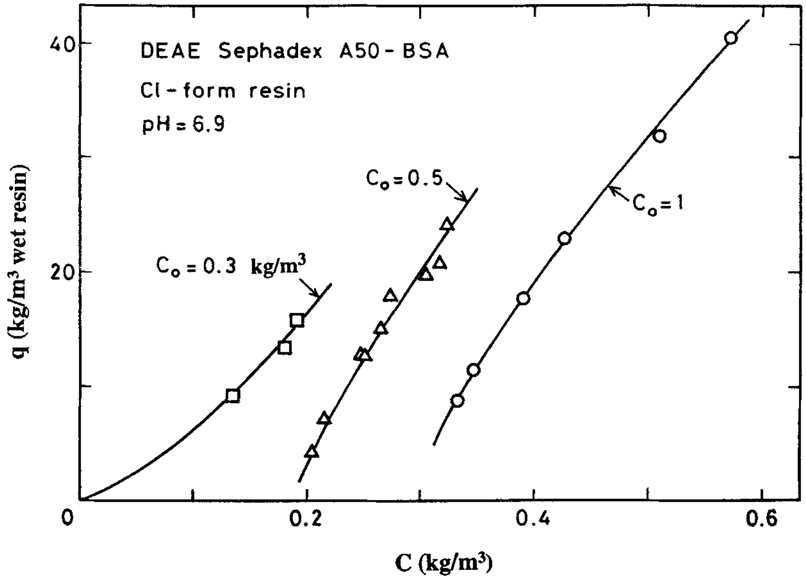

FIG. 5 Effect of initial concentration of BSA on equilibrium isotherm for Cl^--form DEAE (pH 6.9, C_{Buf} = 50 mol/m³): (—) Eq. (8).

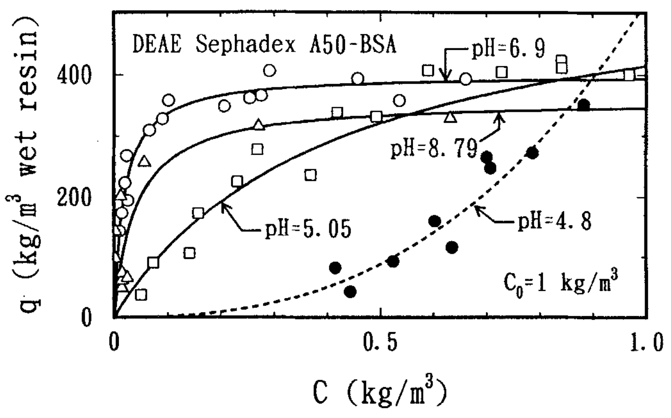

FIG. 6 Effect of pH on equilibrium isotherm for adsorption of BSA on phosphate-form DEAE. C_0 = 1 kg/m³, C_{Buf} = 6.975 equiv/m³: (—) Eq. (8); (- - -) Eq. (11); (●) pH 4.8; (□) pH 5.05; (○) pH 6.9; (△) pH 8.79.

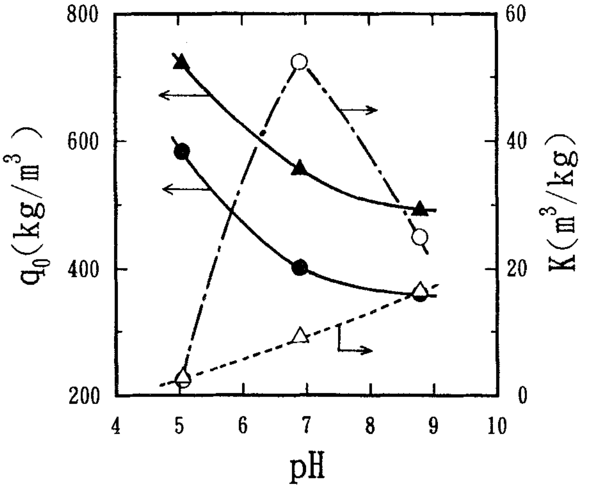

FIG. 7 Effect of pH on saturation capacity and equilibrium constant for Langmuir isotherm: (●, ○) DEAE; (▲, △) QAE.

When pH 4.8, which is close to the pI of BSA (≅ 4.8), the isotherm is unfavorable and the data are not correlated by the Langmuir equation. Figure 8 shows Freundlich plots of the equilibrium data for DEAE at pH 4.8. The data are correlated by the straight line. The solid line gave the Freundlich parameters *k* and *n* expressed by Eq. (11) and listed in Table 2 (No. 1):

$$q = kC^{1/n} \qquad (11)$$

QAE showed the same behavior as DEAE at pH 4.8 (No. 17 in Table 2). The dotted line in Figure 6 shows the Freundlich isotherm calculated by using the values of *k* and *n* shown in Table 2. The equation correlates the data well. The Freundlich equation is often considered to be an empirical equation. Suzuki (12) showed that it is possible to interpret this equation theoretically in terms of adsorption on an energetically heterogeneous surface. He proved that the Freundlich equation corresponds to the Dubinin–Astakov equation [Dubinin and Astakov (13)] for the case where the ratio of the pore diameter to the adsorbate diameter is larger than 5. The adsorption takes place by an attractive force from the pore wall, and the adsorbate molecules start to fill the micropore volumetrically. Since at pH 4.8 the BSA molecule is almost neutral, electrostatic attraction may not contribute to adsorption. According to Suzuki's discussion on the Freundlich equation, the adsorption mechanism for pH ≅ pI may be ex-

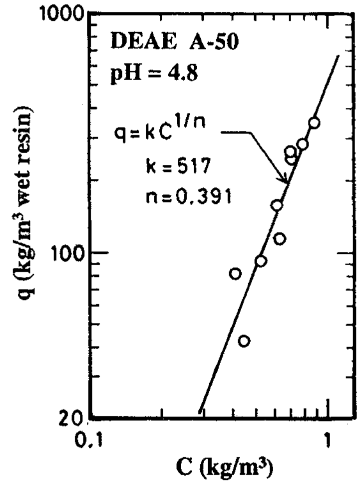

FIG. 8 Freundlich plots of equilibrium data for adsorption of BSA on phosphate-form DEAE at pH 4.8.

plained by the pore-filling-type adsorption caused by hydrophobic interaction or hydrogen bonding.

The ion exchanger which had been in equilibrium with the phosphate buffer solution was used to adjust the pH of the BSA solution. Therefore, if BSA is adsorbed by ion exchange, the concentration of phosphorus in the liquid phase must increase after reaching equilibrium. We determined experimentally the amount of phosphorus exchanged for BSA, q_P (mol/m^3 wet resin), which was calculated from

$$q_P = \frac{V(C_P - C_{P,0})}{W} \tag{12}$$

The maximum value of the ratio of q_P to the exchange capacity of the ion exchanger gives the contribution of ion exchange to the sorption of BSA on the ion exchanger. Figure 9 shows the experimental results for DEAE at pH 4.8 and 6.9. When pH = 6.9, equilibrium is relatively favorable but the maximum q_P is about 1% of the exchange capacity of resin Q shown in Table 1. Therefore, when pH = 6.9, the effect of ion exchange on the sorption of BSA may be small and BSA may be mainly adsorbed by electrostatic attraction between positively charged fixed groups and negatively charged BSA. When pH = 4.8, equilibrium is very unfavorable and the maximum q_P is about 0.4% of the exchange capacity of the resin. This means that the amount of BSA adsorbed by ion exchange may be

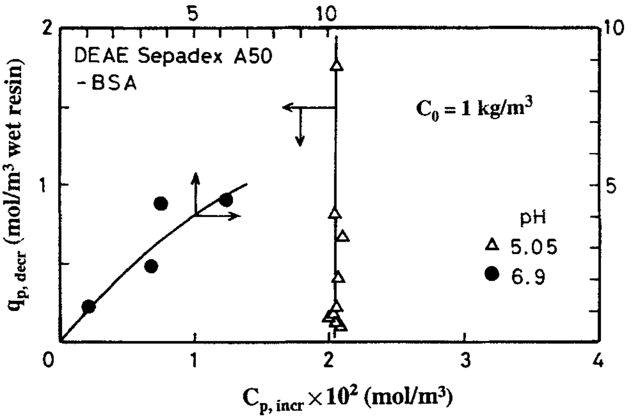

FIG. 9 Amount of phosphorus exchanged for BSA. Resin: DEAE; C_0 = 1 kg/m^3.

negligibly small. Since pH = 4.8 is very close to pI (≅ 4.8) of BSA, almost all BSA molecules are neutral and they may be mainly adsorbed by hydrophobic interaction and/or hydrogen bonding, as discussed earlier. QAE Sephadex A-50 showed almost the same behavior as that shown in Fig. 9.

Figures 10 and 11 show the effect of NaCl on the equilibrium isotherm for phosphate-form and Cl^--form resins, respectively. The pH of the solution was 6.9. The experimental equilibrium isotherms are peculiar. The shapes are similar to the Langmuir isotherm but are shifted to the right. Although the reason for this is not clear, we do not think it is caused by experimental error. Tsou and Graham (2) presented a similar result for adsorption of BSA on DEAE when NaCl coexisted in the BSA solution, although they neglected the shifted data and correlated by using the Langmuir equation approximately. We presented similar isotherms for adsorption of BSA on QAE (3). Since BSA is not adsorbed on the resin at all when the concentration of NaCl is 200 mol/m^3, an NaCl aqueous solution of about 200 mol/m^3 can be used as an eluant of protein.

Figure 12 shows experimental equilibrium isotherms for the case when no inorganic electrolytes exist in the BSA solution (BSA dissolved in pure water). The data for the ($H_2PO_4^-$ + HPO_4^{2-})-form DEAE, which was in equilibrium with a phosphate buffer of pH 6.9, and the HPO_4^{2-}-form DEAE were correlated by the Langmuir equation. The equilibrium coefficients are shown in Table 2. The saturation capacity for the HPO_4^{2-}-form DEAE + BSA solution without a buffer system (No. 14 in Table 2) is about 3.9 and 3.5 times larger than that for the HPO_4^{2-} form with buffer (pH 8.79, No. 9 in Table 2) and the ($H_2PO_4^-$ + HPO_4^{2-}) form with buffer

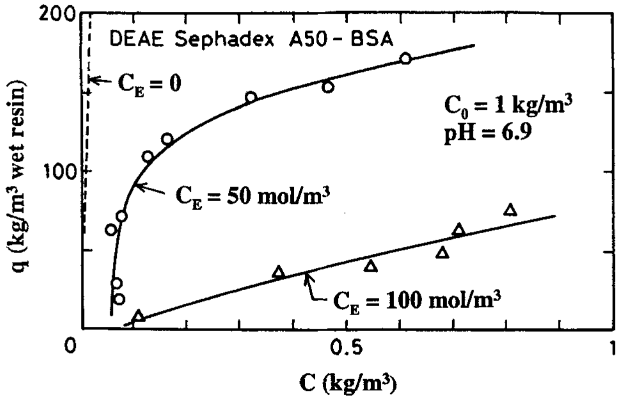

FIG. 10 Effect of NaCl on equilibrium isotherm of BSA for phosphate-form DEAE (pH 6.9, C_{Buf} = 6.975 equiv/m³).

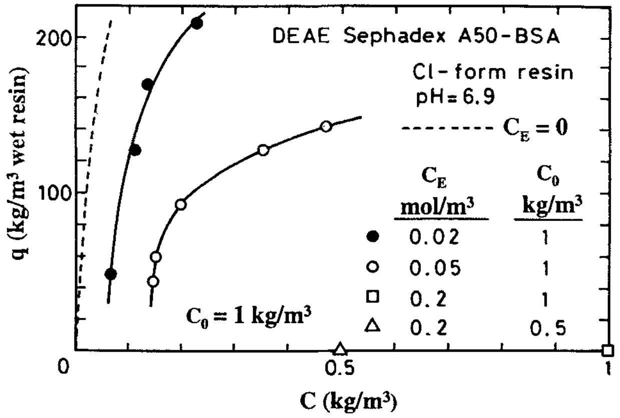

FIG. 11 Effect of NaCl on equilibrium isotherm of BSA for Cl⁻-form DEAE (pH 6.9, C_{Buf} = 6.975 equiv/m³).

(pH 6.9, No. 7 in Table 2), respectively. In the case of QAE, the saturation capacity did not increase as much (Nos. 19, 20, and 22 in Table 2) (3). The saturation capacity of BSA for the ($H_2PO_4^-$ + HPO_4^{2-})-form DEAE + BSA solution without a buffer system (No. 7 in Table 2) is twice that for the same DEAE + BSA solution with a buffer system (pH 6.9, No. 13 in Table 2). This is totally different from QAE; that is, the saturation capacity of BSA for the ($H_2PO_4^-$ + HPO_4^{2-})-form QAE + BSA solution without a buffer system (No. 21 in Table 2) is much smaller than that for

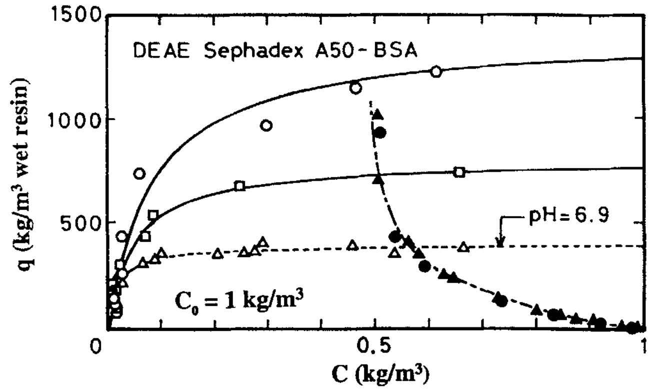

FIG. 12 Equilibrium isotherm of BSA for adsorption of BSA dissolved in pure water. Without buffer system: (○) HPO_4^{2-}-form DEAE; (□) ($H_2PO_4^-$ + HPO_4^{2-})-form DEAE which had been in equilibrium with phosphate buffer of pH 6.9 (C_{Buf} = 6.975 equiv/m³; (●) $H_2PO_4^-$-form DEAE; (▲) Cl^--form DEAE. With buffer (pH 6.9, C_{Buf} = 6.975 equiv/m³): (△) ($H_2PO_4^-$ + HPO_4^{2-})-form DEAE (—), (- - -) Eq. (8) using K and q_0 listed in Table 2.

the same QAE + BSA solution with buffer (pH 6.9) system (No. 19 in Table 2) (3). In the case of univalent anion-form resins, which are the Cl^- form and $H_2PO_4^-$ form, the isotherms are peculiar. The adsorbed-phase concentration of BSA decreases with an increase in the liquid-phase equilibrium concentration of BSA, although the reason is not clear. From these results, when a protein dissolved in pure water is recovered, it is not necessary to add buffer to the solution. Bivalent-form DEAE, such as the HPO_4^{2-} form, should be in direct contact with the protein solution.

The equilibrium data presented above give some useful information from the engineering point of view. However, the Langmuir and the Freundlich equations do not explain theoretically all the data presented in this study. Protein molecules are large and complicated, which makes a detailed study of their interaction with a solid surface rather difficult. In order to explain all our data theoretically and consistently, we should know not only the interaction with a solid surface but also the physical properties of BSA in detail. We also have to produce dynamic data to develop equilibrium and dynamic theories as presented by Liapis (14), McCoy and Liapis (8), and McCoy et al. (15).

CONCLUSION

Equilibrium isotherms for the adsorption of BSA on DEAE (weakly basic dextran-type ion exchanger) were determined experimentally and the following conclusions were drawn.

(1) The isotherms for phosphate-form resin were affected little by the initial concentration of BSA, but Cl^--form resin was affected by it more.

(2) The isotherms were considerably affected by pH. The isotherm was correlated by the Langmuir equation when $pH \geq 5.05$. The saturation capacity decreased with increasing pH, and the equilibrium constant of the Langmuir equation showed a maximum with an increase of pH. When $pH = 4.8$ which is close to the pI of BSA, the equilibrium isotherm was correlated by the Freundlich equation.

(3) The contribution of ion exchange to adsorption of BSA on the ion exchanger was small. The maximum amounts of inorganic anion exchanged for BSA were 1 and 0.4% of the exchange capacity of the ion exchanger at pH 6.9 and 4.8, respectively. BSA may be adsorbed by electrostatic attraction when $pH > pI$ and by hydrophobic interaction or hydrogen bonding at $pH \cong pI$.

(4) When NaCl existed in the BSA solution, the shape of the isotherm was similar to the Langmuir isotherm but shifted to the right. When the concentration of NaCl was 200 mol/m^3, BSA was not adsorbed on the resin.

(5) When a protein is adsorbed from pure water, bivalent anion (HPO_4^{2-})-form resin may be much more useful than the other resin forms. In the case without buffer, the saturation capacity of BSA on HPO_4^{2-}-form DEAE was about 3.5 times larger than that for ($H_2PO_4^- + HPO_4^{2-}$)-form DEAE + BSA solution with buffer (pH 6.9). HPO_4^{2-}-form DEAE adsorbed BSA from pure water 1.4 times more than the same QAE form. The amount of BSA adsorbed on univalent anion (Cl^- and $H_2PO_4^-$)-form DEAE decreased when the liquid-phase equilibrium concentration of BSA was increased.

NOTATION

C	liquid-phase equilibrium concentration of BSA ($kg \cdot m^{-3}$)
C_0	liquid-phase initial concentration of BSA ($kg \cdot m^{-3}$)
C_1	liquid-phase equilibrium concentration of $H_2PO_4^-$ ($equiv \cdot m^{-3}$)
C_2	liquid-phase equilibrium concentration of HPO_4^{2-} ($equiv \cdot m^{-3}$)
C_E	liquid-phase concentration of NaCl ($mol \cdot m^{-3}$)
C_{Buf}	liquid-phase concentration of phosphate buffer ($mol \cdot m^{-3}$)
C_P	liquid-phase equilibrium concentration of phosphorus ($mol \cdot m^{-3}$)
$C_{P,0}$	liquid phase initial concentration of phosphorus ($mol \cdot m^{-3}$)
K	Langmuir coefficient ($m^3 \cdot kg^{-1}$)
K_1	equilibrium constant of Eq. (4)
K_2	equilibrium constant of Eq. (5)

k	Freundlich coefficient
n	Freundlich coefficient
Q	concentration of fixed quaternary ammonium group in adsorbent phase (mol·m^{-3})
q	adsorbed-phase equilibrium concentration of BSA (kg·m^{-3} wet resin)
q_0	saturation capacity of BSA (kg·m^{-3} wet resin)
q_1	adsorbed phase equilibrium concentration of $H_2PO_4^-$ (eq·m^{-3} wet resin)
q_2	adsorbed phase equilibrium concentration of HPO_4^{2-} (eq·m^{-3} wet resin)
q_P	amount of phosphorus exchanged for BSA (mol·m^{-3} wet resin)
V	volume of solution (m^{-3})
W	volume of adsorbent (m^{-3})

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