The Adsorption of Proteins and Protein–Dodecyl Sulphate Complexes on *N*-(3-Carboxypropionyl)aminododecyl-Sepharose

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Equilibrium and kinetic aspects of the binding of several proteins to *N*-(3-carboxypropionyl)aminododecyl-Sepharose, an amphiphilic ampholytic adsorbent, were studied at 22°C, pH 7.0, *I* 0.10–0.12. In the absence of detergents Scatchard plots are linear for human haemoglobin and soya-bean trypsin inhibitor, but non-linear for bovine serum albumin, which is also adsorbed more tightly than the other two proteins. The introduction of 3.5 mM-sodium dodecyl sulphate causes dramatic increases in the amounts and affinities of serum albumin and haemoglobin adsorbed, but has relatively little effect on the trypsin inhibitor. At concentrations of sodium dodecyl sulphate greater than about 10 mM there is a fall in the binding of all proteins, owing to competition from the detergent for binding sites on the adsorbent, and a tendency towards more uniform behaviour by different proteins. Kinetic experiments suggest that in the absence of the detergent haemoglobin and serum albumin are adsorbed initially by mainly ionic forces, but that subsequently hydrophobic forces become dominant. Addition of 3.5 mM-sodium dodecyl sulphate causes pronounced changes in the time course of adsorption of haemoglobin and serum albumin, the nature of the changes being different for each protein. The significance of these results is discussed.

In recent years the column chromatography of proteins on stationary phases that contain non-polar groups attached to polysaccharide matrices has attracted increasing attention, and some attempts have been made to understand the underlying interactions, of which chromatographic behaviour is an expression [see reviews by Shaltiel (1978), Hjerten (1976), Hofstee (1976) and Yon (1978)]. In some studies (Porath *et al.*, 1973; Hjerten, 1973, 1976) these interactions were judged to be almost solely hydrophobic in nature. In most cases, however, hydrophobic interactions have been accompanied by electrostatic effects of varying intensity, such as the attractive effects due to the positive isourea group introduced into the stationary phase by the CNBr-coupling technique of Axen *et al.* (1967). Adsorption to stationary phases that contain hydrophobic and positively charged groups appear to involve multiple-point attachments (Hjerten, 1973; Jennissen, 1976), heterogeneous binding sites (Hofstee, 1974) and a substantial electrostatic component of binding (Wilchek & Miron, 1976).

More complex interactions may be expected when the stationary phase contains hydrophobic posi-

tively charged and negatively charged groups. Such stationary phases are produced, for example, by CNBr coupling of the 'hydrophobic' amino acids (valine etc.) to polysaccharide matrices (Rimerman & Hatfield, 1973; Doellgast & Fishman, 1974), or by succinylation (3-carboxypropionylation) of CNBr-coupled aliphatic diamines (Cuatrecasas, 1970; Yon, 1972). These adsorbents have been collectively referred to as 'imphilytes', an abbreviation of immobilized amphiphilic ampholytes (Yon, 1977). One of these adsorbents, CPAD-Sepharose, has been the subject of several studies, including its interactions with enzymes (Yon, 1974, 1977; Yon & Simmonds, 1975) and with the proteins of the human erythrocyte membrane (Simmonds & Yon, 1976, 1977). Some of the latter studies were carried out in the presence of the anionic detergent SDS, to aid in solubilizing the membrane proteins. The detergent was found to enhance the binding of several proteins when it was present at moderately low concentrations. However, the major membrane glycoprotein, glycoporphin, behaved sufficiently differently from other proteins in this respect to facilitate its convenient purification (Simmonds & Yon, 1977).

To gain further insight into the chromatography of proteins on CPAD-Sepharose, we have studied equilibrium and kinetic aspects of the binding of several commercially available proteins to the

Abbreviations used: CPAD-Sepharose, *N*-(3-carboxypropionyl)aminododecyl-Sepharose; SDS, sodium dodecyl sulphate.

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adsorbent, and of the effects of SDS on this binding. These studies are reported in the present paper.

Experimental

Materials

Bovine serum albumin (Cohn fraction IV), human haemoglobin and soya-bean trypsin inhibitor were from Sigma, Kingston upon Thames, Surrey, U.K. The sources of all other chemicals have been given in Simmonds & Yon (1976).

Preparation of CPAD-Sepharose

The adsorbent is prepared essentially as described by Cuatrecasas (1970), and involves CNBr coupling of 1,10-diaminodecane to Sepharose 4B, followed by succinylation of the modified gel with succinic anhydride; details are given in Yon & Simmonds (1975). In more recent studies we have taken considerable care to ensure a charge-ratio close to 1.0. Charge-ratio is defined as the ratio:

$$\frac{\mu\text{mol of OH}^- \text{ required to titrate the gel from pH 3.0 to 6.0}}{\mu\text{mol of OH}^- \text{ required to titrate the gel from pH 8.0 to 11.0}}$$

by using the titration procedure described in Yon & Simmonds (1975). This is assumed to reflect the ratio of negative to positive charge on the adsorbent at pH values close to 7. To achieve a charge-ratio of 1 the diamine-Sepharose conjugate is treated with small portions of succinic anhydride at pH 6.0, each followed by extensive washing and titration, until the required ratio is reached. The main adsorbent used in the studies reported in this paper was from a single batch containing 17 μmol of anionic groups/ml of settled gel, at a charge-ratio of between 1.0 and 1.1. Batches of similar charge-ratio, but different density

of substitution (used for the data of Table 1), were prepared by varying the amount of CNBr used in the coupling step. Where we have referred to the 'settled volume' of the gel, this was determined by leaving the gel to settle overnight under gravity in a measuring cylinder, after equilibrating the gel in the appropriate buffer.

In previous publications we have assumed the structure shown in Fig. 1(a) for the adsorbent groups of CPAD-Sepharose, with a charge-ratio no greater than 1. However, occasional batches of the gel had charge-ratios well in excess of 1. This is in agreement with the work of Wilchek & Miron (1976) and Werber (1976), who showed that the isourea group is as good a nucleophile as the amino group and would therefore also be acylated by succinic anhydride. We now view the succinylated gel as a mixture predominantly of the two structures shown in Fig. 1, together with small amounts of the unacylated and doubly acylated groups.

Assay procedures

Protein concentrations were determined by the method of Lowry *et al.* (1951), which was independently calibrated for each different protein, in the absence and in the presence (3.5 and 10.5 mM) of SDS. The concentration of SDS in the experiment reported in Fig. 4 was determined by a modification of the method of Mukerjee (1956). Portions (0.2 ml) of the test solutions were each transferred into 10 ml of an aqueous solution of Methylene Blue (54 mg/litre) and vigorously mixed for 10 s. A portion (0.5 ml) of this mixture was transferred into 10 ml of chloroform and the mixture was vigorously shaken for 10 s. The organic phase was separated and its A_{650} read. SDS facilitates the partition of the dye into the organic phase. The method was calibrated for SDS concentrations up to 17.5 mM. All glassware was washed

Fig. 1. Charge-bearing groups on a diaminodecane-Sepharose conjugate after treatment with succinic anhydride. The charge state is shown as at pH 7.0.

thoroughly to remove traces of detergents before use.

Equilibrium binding studies

All binding experiments were performed at 20–22°C. Unless otherwise stated, the buffer used was 0.05M- NaH_2PO_4 adjusted with 10M- NaOH to pH 7.0. All solutions and gel suspensions were made up in this buffer. Samples (5.0ml) of protein solutions (in the range 0–3.0mg/ml) were placed into 20ml test tubes. A well-stirred suspension of CPAD-Sepharose containing 0.5ml (settled volume) of the gel in 1.0 ml of suspension was added to each tube. The tubes were securely sealed with Parafilm and the contents gently shaken for 16h by slow tumbling. Kinetic experiments showed that equilibrium was reached within 16h by haemoglobin and bovine serum albumin; this was assumed to be the case for soya-bean trypsin inhibitor also. The tubes were then left for 2h, after which samples of the clear supernatants were taken for protein assay. The amount of adsorbed protein was obtained by difference. When SDS was included, the appropriate protein/SDS solution was prepared approx. 1h before addition of the adsorbent. Trial experiments showed that significantly different results were obtained when protein was added to adsorbent/SDS mixtures.

Binding data are presented in terms of the variables P (the concentration of unadsorbed protein in equilibrium with the gel, expressed in mg/ml of solution) and r (mg of protein adsorbed/ml of settled gel). We have avoided the use of molarities, since (1) the adsorption sites are very probably heterogeneous, and 1 ml of gel therefore contains different numbers of binding sites for different proteins (this is certainly the case for proteins of greatly different size, owing to molecular filtration effects in Sepharose), and (2) with a multi-subunit protein such as haemoglobin it is uncertain whether the adsorbed molecular unit is the aggregate protein or its subunit, especially when SDS is introduced. Results are presented both as binding isotherms (r versus P) and as Scatchard plots (r versus r/P) (left and right of Fig. 2 respectively). Scatchard plots were used to determine the maximum protein uptake (intercept on the r axis) and the binding affinity (reciprocal of the slope). Where the plot was non-linear, two sets of these parameters were measured, corresponding to the tangents drawn to the extremes of the curve.

Kinetic studies

The time course of uptake of protein from solution was studied at 20–22°C in the same sodium phosphate buffer as described above. The buffered protein solution (100ml) containing protein at 1.5mg/ml was gently stirred with a magnetic follower bar. If SDS was included, this was added to the protein at

least 1h before addition of the adsorbent. At zero time, 20ml of a buffered suspension containing 10ml (settled volume) of CPAD-Sepharose was added. The stirring was sufficiently vigorous to ensure a uniform distribution of the gel. At timed intervals 2.0ml samples of the suspension were immediately and rapidly filtered under pressure through glass wool to remove as much as possible of the solution from the gel grains. The gel was resuspended without delay in 5.0ml of 0.5M-trisodium phosphate, pH 12, shaken for 10min and filtered again. Protein concentrations in the two filtrates were determined separately. Protein in the first filtrate enabled the total adsorbed protein to be calculated by difference. A small correction is applied to the protein in the second filtrate to allow for the small amount of unadsorbed protein in the internal volume of the gel; the corrected value is that part of the adsorbed protein that is desorbable by a high-pH high-ionic-strength pulse. This protein is taken to have been bound by mainly ionic forces. The remaining adsorbed protein is assumed to be bound by mainly hydrophobic forces.

Results

Effects of pH and degree of substitution of the adsorbent

The effects of these variables on the binding of bovine serum albumin were investigated. The binding of the protein at pH 4.0 (0.05M-citric acid/ NaOH), pH 7.0 and pH 10.0 (0.05M-glycine/ NaOH) were compared. Adsorption isotherms showed that the maximum protein uptake increased in the order $\text{pH } 4 > \text{pH } 7 > \text{pH } 10$. Since the isoelectric points of the protein and the adsorbent are 4.8 and approx. 7 respectively (Young, 1963; Yon & Simmonds, 1975), the binding order for pH 7 and 10 was as expected, but that for pH 4 was at variance with the prediction (Yon, 1972) that binding would become increasingly weaker at low pH owing to repulsion between positive charges. This anomaly has not been further investigated. In the same buffer systems, unmodified Sepharose 4B did not adsorb bovine serum albumin at pH 4, 7 or 10.

The binding of bovine albumin at pH 7.0 to several batches of CPAD-Sepharose that varied in degree of substitution are shown in Table 1. As expected, the maximum protein uptake increased with the degree of substitution. However, it is clear that a minimum threshold occurs, since there is no protein binding below a substitution density of $6\mu\text{mol}$ of anionic groups/ml of settled gel. This suggests strongly that binding involves multiple bonds between the protein molecule and the site of adsorption on the gel, in support of the findings of Hjerten (1973), Schmidt & Raftery (1973) and Jennissen (1976).

Table 1. *Effect of degree of substitution on the adsorption of bovine serum albumin*

Equilibrium binding of bovine serum albumin at pH7.0 was performed as described in the Experimental section.

Degree of substitution (μ mol of anionic groups/ml of settled gel)	Charge ratio	Protein adsorbed (mg/ml of settled gel)
17	1.2	4.8
10	1.2	3.0
6	1.0	0

Equilibrium binding of proteins at pH7 in the absence of SDS

The results of binding experiments for three proteins, serum albumin, haemoglobin and soya-bean trypsin inhibitor, are presented in Fig. 2; data obtained in the absence of SDS are plotted as filled circles, and are presented both as binding isotherms (left) and Scatchard plots (right).

Over the assayable range of protein concentrations the data for haemoglobin and trypsin inhibitor could be fitted quite well to straight-line Scatchard plots,

Fig. 2. *Equilibrium adsorption to CPAD-Sepharose of (a) bovine serum albumin, (b) human haemoglobin and (c) soya-bean trypsin inhibitor*

Binding isotherms (left) and Scatchard plots (right) are presented. Data were collected in the absence of SDS (●) and in the presence of SDS at the following concentrations: 3.5 mM (○), 7.0 mM (■) and 10.5 mM (□). Data points at the extreme right of the Scatchard plots correspond to values of r/P greater than 400.

suggesting that the binding sites for each protein are very similar in binding affinity. Moreover, the binding affinity was nearly the same for the two proteins (2.0ml/mg), but the maximum protein uptake was nearly twice as great for trypsin inhibitor as for haemoglobin (16.5 and 8.5mg/ml of settled gel respectively), showing that the two proteins were not simply binding to the same set of sites. The difference is thought to reflect a gel-permeation effect because of the smaller size of the trypsin inhibitor which has access to a considerably larger fraction of the internal volume of the gel.

The Scatchard data for bovine serum albumin were fitted to a curved line suggesting predominantly two types of binding site on the adsorbent, a high-affinity set (binding affinity 20ml/mg, maximum protein uptake 6mg/ml of settled gel) and a low-affinity set (binding affinity 6ml/mg, maximum protein uptake 1.5mg/ml of settled gel). The high-affinity set has a binding affinity that is 10 times that of the other two proteins, and binding of serum albumin to this set may be, in part at least, an interaction involving the known binding sites on the protein for long-chain fatty acids and detergents (Reynolds *et al.*, 1967). Adsorbents resembling CPAD-Sepharose have, in fact, been used for the affinity chromatography of bovine serum albumin (Peters *et al.*, 1973).

Equilibrium binding of proteins at pH7 in the presence of SDS

Fig. 2 also shows the binding of proteins in the presence of 3.5, 7.0 and 10.5mM-SDS. Additional data were obtained for these proteins and for ovalbumin and yeast hexokinase for SDS concentrations of 14 and 17.5mM-SDS, but are not shown in Fig. 2.

In the presence of 3.5mM-SDS both haemoglobin and serum albumin showed dramatic changes in binding to CPAD-Sepharose, the maximum protein uptake increasing several fold in comparison with binding in the absence of SDS. In each case the Scatchard plot was non-linear when account was taken of the point on the extreme right, corresponding to a value of r/P estimated to be at least 400 and probably higher (in fact the free protein concentration, P , for this point was undetectable by the Lowry assay). This non-linear Scatchard plot was interpreted in terms of two sets of binding sites. The high-affinity set had a binding affinity in excess of 160ml/mg; the binding affinity could not be measured accurately over the range of protein concentrations that was accessible to our assay method. This set is responsible for the apparent intercept on the vertical axis of the binding isotherms on the left of Figs. 2(a) and 2(b). This high-affinity set may contain more than one subset, which we cannot therefore detect. Both proteins interacted with a second lower-affinity set of adsorption sites over the accessible range of protein

Fig. 3. Effects of different concentrations of SDS on the equilibrium adsorption of proteins at a constant total concentration

Binding experiments were performed as described in the text, starting with 5 ml of a solution containing the protein at 1.5 mg/ml and various amounts of SDS to which 0.5 ml (settled volume) of the adsorbent were added. The proteins were bovine serum albumin (●), haemoglobin (■) and soya-bean trypsin inhibitor (○).

Fig. 4. Adsorption of SDS by CPAD-Sepharose. Binding experiments were performed as described in the text, but with omission of proteins and by using various starting concentrations of SDS. Concentrations of free SDS at equilibrium were determined by the method of Mukerjee (1956) as modified in the text.

concentrations. By comparison, SDS at 3.5 mM produced only small increases in binding affinity and maximum protein uptake for soya-bean trypsin inhibitor. The effects of the detergent are emphasized in Fig. 3, where the three proteins are compared at a single concentration of total protein and various SDS concentrations. This figure emphasizes that although both haemoglobin and serum albumin show the sharp rise and fall in adsorption with increasing SDS concentration, their behaviour is not identical; the three proteins therefore each exhibit a unique type of interaction at moderately low concentrations of SDS.

As the concentration of SDS is raised much above 3.5 mM there is a decrease in the maximum protein

uptake in every case. This fall appears to be due to increasing competition from SDS itself for binding sites on the adsorbent. The binding of SDS to CPAD-Sepharose is shown in Fig. 4, which shows that when the free SDS is at 3.5 mM the adsorbent is already approaching saturation with the detergent. At first glance this appears to contradict the substantial adsorption of all proteins observed at 3.5 mM-SDS. However, the SDS concentrations quoted in Figs. 2 and 3 are total concentrations; in the presence of protein most of the detergent will be protein-bound and the free detergent will be well below saturation concentrations, as indicated by Fig. 4.

At 14 and 17.5 mM-SDS there was only weak binding by all proteins. This binding showed considerable uniformity for all proteins studied (the three shown in Figs. 2 and 3 as well as ovalbumin and yeast hexokinase); maximum protein uptakes were all between 1.5 and 2.2 mg/ml of settled gel, and binding constants between 0.5 and 1.0 ml/mg. A possible reason for this uniformity at higher SDS concentrations could be that all proteins are tending to the same physical state, i.e. the highly negatively charged extended conformation known to be adopted at high concentrations of SDS (see Tanford, 1973).

Kinetics of adsorption at pH 7 in the absence of SDS

The uptake of protein as a function of time was studied for bovine serum albumin and for haemoglobin. Results obtained in the absence of SDS are given in Figs. 5(a) and 6(a), which show the total protein adsorbed (■), the part of the adsorbed protein that could be desorbed by 0.5 M- Na_3PO_4 , pH 12 (○) and the remainder of the adsorbed protein (●). The protein desorbed by the high-ionic-strength high-pH pulse is assumed to have been adsorbed by mainly ionic forces; the remainder is assumed to be bound by mainly hydrophobic forces. The total protein uptake for the first 40 min is also shown in Figs. 5(c) and 6(c) (Δ) as reaction-order plots.

Bovine serum albumin (Fig. 5a) is rapidly taken up by an apparently first-order process with a half-time of 1.2 min. Initially a high proportion (70%) of the adsorbed protein could be desorbed by a high-ionic-strength high-pH pulse; however, this proportion falls to about 35% during the ensuing 1 h, although there is no further change in the total protein uptake after the first 10 min. This behaviour suggests that the protein is taken up at the outset by essentially ionic forces, but that subsequently the main binding is taken over by hydrophobic forces. We envisage that after an initial adsorption event involving the surface groups on the protein and charged groups on the adsorbent, thermal agitation causes the hydrophobic groups on the adsorbent to become increasingly enmeshed with the more hydrophobic interior of the protein.

Fig. 5. Kinetics of adsorption of bovine serum albumin on CPAD-Sepharose

(a) Adsorption in the absence of SDS: (■) total protein adsorbed, (○) protein desorbed by a high-pH high-ionic-strength pulse, and (●) non-desorbed protein. (b) Adsorption in the presence of 3.5 mM-SDS: symbols as for (a). (c) Reaction-order plots for total protein adsorbed in the absence of SDS (Δ, left-hand ordinate axis) and in the presence of 3.5 mM-SDS (▲, right-hand ordinate axis). c_{eq} , Absorbed protein (mg/ml of settled gel) at equilibrium; c , adsorbed protein during approach to equilibrium.

Haemoglobin (Fig. 6a) is also taken up by a rapid initial first-order process (half-time = 0.5 min), but this is followed by a second first-order process (half-time = 13.5 min) as shown in Fig. 6(c). In the first few minutes all of the bound protein could be desorbed by the high-ionic-strength, high-pH pulse, but the proportion fell to approx. 50% over the next 12 h. Thus although the times involved differ, the events in the adsorption of haemoglobin and serum albumin appear to be qualitatively similar.

Kinetics of adsorption at pH 7 in the presence of 3.5 mM-SDS

Equilibrium binding studies (described above) showed a dramatic increase in the binding of bovine serum albumin and of haemoglobin in the presence of 3.5 mM-SDS. The kinetics of this adsorption are reported in Figs. 5(b) and 6(b), where the symbols

Fig. 6. Kinetics of adsorption of human haemoglobin on CPAD-Sepharose. Details are as for Fig. 5.

have the same meaning as in Figs. 5(a) and 6(a). Order-of-reaction plots for the uptake of protein are given in Figs. 5(c) and 6(c) (Δ).

The uptake of bovine serum albumin in the presence of 3.5 mM-SDS did not fit an integral-order plot; as shown in Fig. 5(c), a good fit was obtained to a plot of order 1.6. The reaction took 4 min to reach 50% of completion and 40 min to reach 90%. By comparison, the proportion of adsorbed protein that could be desorbed by the high-ionic-strength high-pH pulse reached a nearly constant value of approx. 30% after only 10 min, well before cessation of the total protein uptake; subsequent protein uptake was therefore increasingly non-desorbable, and assumed to be mainly by hydrophobic interaction. This suggests that, from the outset, some molecules of the protein are adsorbed by mainly ionic forces, whereas others are adsorbed by mainly hydrophobic forces, the hydrophobic uptake being the slower process. Such a complex adsorption behaviour would account for the non-integral reaction order. Over a

long period, there was a slight fall in the desorbable protein to approx. 25% of the total adsorbed protein.

The uptake of haemoglobin in the presence of 3.5 mM-SDS could be fitted quite well to a second-order plot. In the first few minutes nearly all of the adsorbed protein could be desorbed by the high-ionic-strength, high-pH pulse, but with increasing time up to about 20 min, the proportion of desorbable protein fell (to about 65% of the total adsorbed protein), in common with all the other kinetic results. Surprisingly, after this the proportion of desorbable protein rose again slowly, until after 12 h it was 80% of the total adsorbed protein. This was the only result where the major component of binding at equilibrium appeared to be ionic.

Discussion

Protein-adsorbent interactions in the absence of SDS

Of the three proteins studied, only bovine serum albumin gave evidence of heterogeneity in binding sites on the basis of non-linear Scatchard plots. However, evidence from the present study and from the work of others (Hjerten, 1973; Jennissen, 1976; Hofstee, 1976) suggests strongly that adsorption involves multiple contacts between adsorbent and protein. Since there are probably many different spatial distributions of the hydrophobic, positive and negative groups on the adsorbent that can interact with complementary distributions on the surface of the protein, it seems reasonable to expect considerable heterogeneity in adsorption sites; indeed, we consider that heterogeneity is likely to be the rule rather than the exception. Our failure to show more instances of heterogeneous binding is probably due to two factors: (1) many of the spatially different binding sites will have similar free energies of interaction with the protein and will not therefore be resolved in a Scatchard plot, and (2) our data were collected over a relatively limited range of protein concentrations (about 0.01–2.0 mg/ml) and it is possible that heterogeneity would be evident outside of this range. Heterogeneity has been demonstrated in related adsorbents by Hofstee (1974, 1976), who used adsorbents with hydrophobic and positively charged groups and showed multiple peaks for a single pure protein when frontally eluted by changes in eluent composition, and by Caldwell *et al.* (1976), who used a neutral hydrophobic adsorbent at a very high degree of substitution. Jennissen (1976) obtained non-linear Scatchard plots for the adsorption of phosphorylase *b* to an adsorbent with hydrophobic and positively charged groups, but argued in favour of negative co-operativity in protein adsorption in preference to heterogeneous adsorption sites.

Kinetic studies of the binding of haemoglobin and bovine serum albumin to CPAD-Sepharose suggest that the initial binding event is electrostatic, rein-

forced on a longer time scale by hydrophobic interactions. This order of events in the binding of a protein to an amphiphilic adsorbent agrees with a speculation by Wilchek & Miron (1976). Since the adsorbent may be viewed as an immobilized ionic detergent, these results provide presumptive support for the view that proteins interact with non-micellar ionic detergent molecules first through the ionic head-group and then with the hydrocarbon 'tail' (Burkhard & Stolzenberg, 1972).

Most studies of the adsorption of proteins to adsorbents with hydrophobic groups have used either neutral adsorbents or positively charged adsorbents. The occurrence of both positive and negative charges, as in CPAD-Sepharose, may confer important additional properties on the adsorbent. Porath & Fryklund (1970) pointed out that the ion-dipoles characteristic of such immobilized ampholytes have electric field-strengths that decline rapidly with distance from the dipole. Thus unlike ion-exchangers, these adsorbents exert very short-range electrostatic effects. Since the protein surface must approach closely for effective interaction, a considerable steric and electronic 'fit' may be expected between the juxtaposed protein and adsorbent. Such close matching of the surfaces of the adsorbent and protein may account for the perturbation of the binding of several enzymes by their biospecific ligands (Yon, 1977).

Protein-adsorbent interactions in the presence of SDS

These studies show that for some, but not all, proteins, SDS may cause dramatic increases in adsorption to adsorbents with hydrophobic groups, probably by the exposure of additional hydrophobic or ionic sites on the protein. It would be interesting and potentially useful to see whether similar induced binding effects are produced by milder detergents such as deoxycholate. This detergent at 0.2% (w/v) was effective in decreasing the binding affinity of wheat-germ aspartate transcarbamoylase to CPAD-Sepharose (Yon & Simmonds, 1975); however, its effects at lower concentrations were not investigated. Triton X-100, Brij 35 and poly(ethylene glycol) did not decrease the binding affinity of this enzyme, but, again, other effects were not investigated.

The studies of Reynolds, Tanford and their collaborators (see Tanford, 1973) on the interactions of SDS with bovine serum albumin and other proteins showed that several kinds of protein-SDS complexes, of increasing saturation with SDS, can exist. It would be of interest to be able to discuss the effects of SDS shown in this paper in terms of such complexes. However, the equilibrium mixture of SDS (in both non-micellar and micellar states), protein and adsorbent contains a large number of complexes resulting from interactions among these components in all possible combinations and with largely un-

known stoichiometries. At the present time, therefore, it is not possible to describe the compositions of such equilibrium mixtures adequately.

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