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Interactions of Free and Immobilized Myelin Basic Protein with Anionic Detergents†

Peter F. Burns,‡ Celia W. Campagnoni, Irwin M. Chaiken, and Anthony T. Campagnoni*

ABSTRACT: The interaction of free and immobilized myelin basic protein (MBP) with sodium deoxycholate (DOC) and sodium dodecyl sulfate ($NaDodSO_4$) was studied under a variety of conditions. Free MBP formed insoluble complexes with both detergents. Analysis of the insoluble complexes revealed that the molar ratio of detergent/MBP in the precipitate increased in a systematic fashion with increasing detergent concentration until the complex became soluble. At pH 4.8, equilibrium dialysis studies indicated that ~15 mol of $NaDodSO_4$ could bind to the protein without precipitation occurring. Regardless of the surfactant, however, minimum protein solubility occurred when the net charge on the protein–detergent complex was between +18 and −9. Complete equilibrium binding isotherms of both detergents to the protein were obtained by using MBP immobilized on agarose. The bulk of the binding of both detergents was highly cooperative and occurred at or above the critical micelle concentration. At $I = 0.1$, saturation levels of 2.09 ± 0.15 g of $NaDodSO_4$/g of protein and 1.03 ± 0.40 g of DOC/g of protein were obtained. Below pH 7.0 the $NaDodSO_4$ binding isotherms revealed an additional cooperative transition corresponding to the binding of 15–20 mol of $NaDodSO_4$/mol of protein. Affinity chromatography studies indicated that, in the presence of $NaDodSO_4$ (but not in its absence), [^{125}I]MBP interacted with agarose-immobilized histone, lysozyme, and MBP but did not interact with ovalbumin–agarose. These data support a model in which the detergent cross-links and causes precipitation of MBP–anionic detergent complexes. Cross-linking may occur through hydrophobic interaction between detergents electrostatically bound to different MBP molecules.

The myelin basic protein (MBP)[1] is a low molecular weight, highly charged, peripheral membrane protein which constitutes ~30% of the protein of the myelin sheath in the central nervous system (Eylar et al., 1969; Eylar, 1972). In aqueous solution it exists as a highly flexible molecule with an axial ratio of approximately 10:1 (Eylar & Thompson, 1969; Epand et al., 1974). The basic protein has been reported to bind

† From the Department of Chemistry, University of Maryland, College Park, Maryland 20742 (P.F.B., C.W.C., and A.T.C.), and the Laboratory of Chemical Biology, National Institutes of Health, Bethesda, Maryland 20205 (I.M.C.). *Received June 4, 1980.* This work was supported in part by National Institutes of Health Grant NS-13934 to A.T.C.

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[1] Abbreviations used: CD, circular dichroism; cmc, critical micelle concentration; DOC, sodium deoxycholate; MBP, myelin basic protein; ORD, optical rotary dispersion; $NaDodSO_4$, sodium dodecyl sulfate; CM, carboxymethyl; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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electrostatically to a number of acidic lipids (Banik & Davison, 1974; Jones & Rumbsby, 1977). Palmer & Dawson (1969) determined that the protein could bind ~23 mol of phosphatidylinositol (one negative charge) and proportionately fewer moles of phospholipids bearing greater numbers of negative charges. Steck et al. (1976) reported that this binding could be reduced by acylating a number of the lysyl residues on the protein, presumably by reducing its net positive charge. Evidence that portions of the basic protein could extend into a lipid bilayer also has been presented (Boggs & Moscarello, 1978). These and other studies (London & Vossenberg, 1973; Demel et al., 1973; London et al., 1973; Smith, 1977a) support the notion that MBP can interact with both the hydrophobic and hydrophilic portions of lipid bilayers.

The MBP also has been shown to interact with anionic detergents such as $NaDodSO_4$ and DOC (Jones & Rumsby, 1975, 1978; Smith, 1977b). Both detergents increase the apparent α helicity of the protein as measured by ORD and/or CD (Anthony & Moscarello, 1971; Smith, 1977b; Liebes et al., 1976). Recently Smith & McDonald (1979) have obtained partial binding isotherms of these detergents to the basic protein. Their equilibrium dialysis and gel permeation chromatography data indicate that at saturation, the MBP binds unusually large amounts of $NaDodSO_4$ and DOC. However, because the basic protein precipitates at low molar ratios of either $NaDodSO_4$ or DOC to protein at pH values below its p*I*, a detailed examination of the binding of these amphiphiles to the protein has not yet been made. We report here the results of such an investigation in which we have obtained complete binding isotherms for $NaDodSO_4$ and DOC to the basic protein by using MBP immobilized on agarose. In addition, we have correlated this detergent binding with the concomitant precipitation of the basic protein.

Experimental Procedures

Materials

[^{3}H]Deoxycholic acid (4 Ci/mmol), sodium dodecyl [^{35}S]sulfate (413 mCi/mmol), and [^{3}H]glucose (34 Ci/mmol) were obtained from the New England Nuclear Corp., Boston, MA. Radiolabeled $NaDodSO_4$ was diluted with unlabeled $NaDodSO_4$ (>99% ^{12}C alkyl sulfate) obtained from Accurate Chemical and Scientific Corp., Hicksville, NY. Unlabeled DOC was obtained as the sodium salt from Sigma Chemical Co., St. Louis, MO, and was recrystallized 4 to 5 times from 80% (v/v) acetone until the resultant DOC solutions had no detectable absorbance above 255 nm. This preparation was used to dilute the labeled DOC. Bovine serum albumin was purchased from Miles Research Products, Elkhart, IN. Lysozyme, histones (calf thymus), and egg albumin (ovalbumin) were products of the Sigma Chemical Co., St. Louis, MO.

Methods

Isolation of Bovine MBP. Myelin basic protein was isolated from defatted bovine brain by methods described previously (Campagnoni et al., 1978a,b). MBP preparations obtained from CM-cellulose chromatography of pH 3 extracts of defatted tissue were purified by chromatography on a Sephacryl S-200 superfine column (2.5 × 90 cm) in 0.5 M sodium acetate, pH 4.5. The purified protein migrated as a single electrophoretic band in both acetic acid–urea (Panyim & Chalkley, 1969) and $NaDodSO_4$ (pH 6.8)–polyacrylamide gels (Weber & Osborne, 1969).

Iodination of Bovine MBP. Radioiodinations were performed according to the method of Cohen et al. (1975) with the following modifications. The iodinated MBP was separated from free radioactive iodine by a Sephadex G-75 column (0.9 × 30 cm) equilibrated in 0.5 M sodium acetate buffer, pH 4.5, containing 1 mg/mL calf thymus histones. The iodinated protein was stored at 4 °C in 0.5 M sodium acetate buffer (pH 4.5) containing 1 mg/mL histones.

Buffer Conditions. The following buffers were used for the precipitation and binding studies: 0.20 M sodium acetate buffer, pH 4.8 (I = 0.1); 0.075 M sodium phosphate buffer, pH 6.5 (I = 0.1); 0.15 M Tris-HCl buffer, pH 7.8 (I = 0.1); 0.35 M Tris-HCl buffer, pH 8.5 (I = 0.1); 0.086 M sodium carbonate buffer, pH 9.2 (I = 0.1); 0.047 M sodium carbonate buffer, pH 9.2 (I = 0.055); 0.0086 M sodium carbonate buffer, pH 9.2 (I = 0.01); 0.028 M sodium phosphate buffer, pH 11.6 (I = 0.1).

Precipitation of MBP in the Presence of Detergents. A constant volume (42.5% of the total) of MBP solution was mixed with a constant volume of radiolabeled detergent solution containing 8.8×10^{-3} μCi of [^{35}S]$NaDodSO_4$ or 2.6×10^{-2} μCi of [^{3}H]DOC such that the final detergent concentration ranged between 1×10^{-5} and 1×10^{-1} M. Depending on the experiment, the total assay volume was either 300 or 400 μL. The stock protein solution and labeled and unlabeled detergent solutions were made up in the respective buffers at the desired pH and ionic strength. Following incubation for 30 min at 20 ± 2 °C, the tubes were centrifuged for 20 min at maximum setting in a desk-top clinical centrifuge (International Equipment Co., Model CL-17415M-1, Boston, MA). The amount of precipitate was found to be constant over a 20–300-min range of incubation time and a 10–40-min range of centrifugation time. After centrifugation, aliquots of the supernatant were removed for protein and detergent analyses. Protein concentration was determined by the method of Lowry et al. (1951) using albumin as the standard. Neither of the detergents was found to interfere with the Lowry analysis, and standard curves employing MBP alone or MBP in various detergent concentrations were virtually indistinguishable from those using albumin alone. Detergent concentration was determined by radioactivity measurement. Supernatant aliquots of 20 μL were mixed with 100 μL of Protosol (New England Nuclear) and counted in an Intertechnique (Model SL-30) liquid scintillation spectrometer at 30–40% efficiency for ^{3}H and >90% efficiency for ^{35}S as determined by the automatic external standard method.

Immobilization of Proteins on Sepharose 4B. The method described by Cuatrecasas & Anfinsen (1971) was used to couple MBP, lysozyme, histones, and ovalbumin to Sepharose 4B. Activation of the agarose was accomplished by adding BrCN (160 mg/mL packed gel) to a 1:2 (v/v) suspension of Sepharose 4B in water. Coupling was performed in 0.2 M sodium citrate buffer (pH 6.3) with 2 mg of protein/mL of packed agarose. In the case of the MBP, an acid hydrolysis (6 N HCl, 110 °C, 24 h in vacuo) was performed on each sample of the washed, coupled product to determine the amount of MBP bound to the matrix. Generally, >50% of the input protein was immobilized on the agarose.

Equilibration of MBP–Sepharose with Detergents. The procedure employed by Reed et al. (1975) to measure ligand binding to albumin–agarose was used with slight modification to measure the binding of detergents to the immobilized MBP. Briefly, 200 μL of a 1:2 (v/v) suspension in water of MBP–Sepharose was added to radiolabeled detergent containing 8.8×10^{-3} μCi of [^{35}S]$NaDodSO_4$ or 2.6×10^{-2} μCi of [^{3}H]DOC in 1.5-mL polypropylene capped micro test tubes to give a final volume of 420 μL at the desired pH and ionic strength. The suspensions were incubated at 20 ± 2 °C for 90 min during

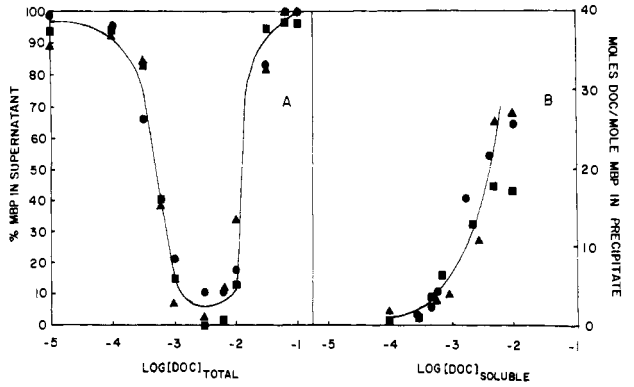

FIGURE 1: Effect of protein concentration on (A) precipitation of MBP in the presence of DOC and (B) composition of insoluble MBP–DOC complexes at pH 9.2, I = 0.10, and 20 °C: (●) [MBP] = 1.77 mg/mL; (■) [MBP] = 1.13 mg/mL; (▲) [MBP] = 0.43 mg/mL.

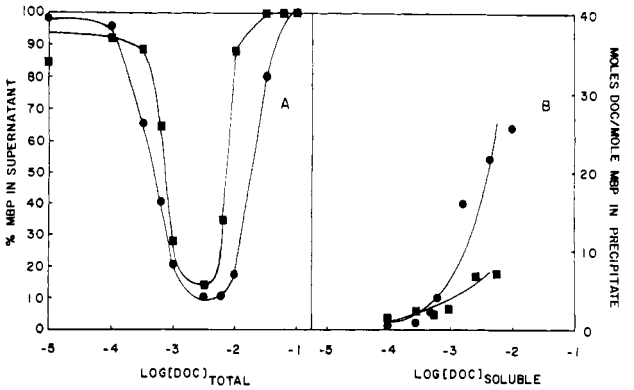

FIGURE 2: Effect of pH at I = 0.10 and 20 °C on (A) DOC-induced precipitation of MBP and (B) composition of the MBP–DOC insoluble complexes. The conditions were as follows: (●) pH 9.2, [MBP] = 1.77 mg/mL; (■) pH 11.6, [MBP] = 1.83 mg/mL.

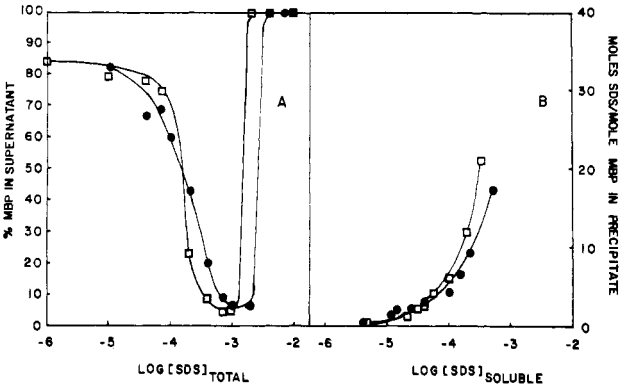

FIGURE 3: Effect of protein concentration at pH 9.2, I = 0.10, and 20 °C on (A) precipitation of MBP in the presence of NaDodSO$_4$ and (B) composition of insoluble MBP–NaDodSO$_4$ complexes: (●) [MBP] = 1.70 mg/mL; (□) [MBP] = 0.80 mg/mL.

which time they were gently mixed by rotation at 15 rpm. After the incubation, the agarose gel was sedimented by centrifugation for 30 min at 1500g. A 100-μL aliquot of each supernatant was removed and analyzed for unbound detergent by liquid scintillation counting. A 200-μL aliquot of each supernatant was removed and discarded, leaving behind the packed gel and excess supernatant. To each tube, 600 μL of Protosol was added, and the resultant suspensions were transferred to separate counting vials. The micro test tubes were washed several times with scintillation fluid and the subsequent washes were placed in the same counting vials as the respective gel suspensions. The samples were counted as previously described. Samples of Sepharose 4B, activated as previously described but with no coupling of protein to the matrix, were used to determine the actual matrix volume and to assess the amount of nonspecific binding of the detergents to the matrix. The matrix volume was obtained by adding a known amount of [^{3}H]glucose to 200 μL of a 1:2 (v/v) suspension of activated–deactivated Sepharose. By comparison of the actual radioactivity of the supernatant with the theoretical value (assuming the matrix occupied no volume), it was determined that a volume of 43.3 μL was occupied by the matrix. This value was independent of glucose concentration (2.4×10^{-5}–2.4×10^{-2} M). Thus, the supernatant volume was 376.7 μL of the 420-μL volume at the onset of the equilibration experiments. On the basis of this, the amount of detergent specifically bound to the MBP was determined by subtracting the amount of detergent present in 76.7 μL of supernatant and the amount of detergent nonspecifically bound to the matrix from the total amount of detergent remaining in the micro test tubes (120 μL) after removal of the 300 μL of supernatant.

Equilibrium Dialysis. Equilibrium dialysis experiments were performed in 200-μL acrylic plastic micro dialysis cells (Technilab Instruments, Pequannock, NJ) by using the dialysis membrane provided with the cells. The cells were rotated gently at 15 rpm for 24 h (20 ± 2 °C). After equilibrium had been reached, aliquots were removed from each dialysis half-cell and analyzed for detergent content by liquid scintillation counting.

Affinity Chromatography of MBP to Immobilized Proteins. Experiments were run on 1-mL affinity columns prepared with agarose-bound MBP, lysozyme, histones, or ovalbumin. The column was preequilibrated with a solution of unlabeled MBP (100 μg/mL) in 0.15 M sodium acetate buffer, pH 4.5, at the desired NaDodSO$_4$ concentration. The presence of MBP in the equilibration buffer minimized nonspecific adsorption of the protein to the matrix and served to define the concentration of MBP in equilibrium with the column. A solution of [^{125}I]MBP (100 μg/mL) was applied to the column and eluted with the same preequilibration buffer. Fractions of 0.2 mL were collected. ^{125}I-Labeled protein elution from the column was determined by counting each fraction in a Packard Model 5023 automatic γ spectrometer.

Results

Precipitation of MBP by DOC. The basic protein was observed to precipitate in the presence of the anionic detergents, NaDodSO$_4$ and DOC, under a wide variety of conditions. Figure 1A shows a precipitation curve of the basic protein in the presence of DOC. Protein precipitation was maximal (90–100%) at $[DOC]_{total} \simeq 3$ mM, and the precipitation curves were relatively insensitive to changes in either protein concentration (Figure 1A) or pH above pH 9.2 (Figure 2A).

Analysis of the precipitates indicated that their compositions varied in a systematic, nonrandom fashion. Figures 1B and 2B show the composition of the insoluble DOC–MBP complexes plotted against the concentration of soluble DOC. Although these curves bear a striking resemblance to the initial portions of binding isotherms, they cannot be interpreted in the same fashion. For example, $[DOC]_{soluble}$ is equivalent to $[DOC]_{free}$ only when complete precipitation of the protein has occurred, because the detergent otherwise could exist as a soluble complex with the protein. As shown in Figure 1B, the composition of the pellet attained a value of 28 mol of DOC/mol of protein before the complex became totally soluble. In addition, the proportion of DOC in the precipitate was sharply reduced when the experiment was carried out at pH 11.6, which is near the pI of the MBP (Figure 2B).

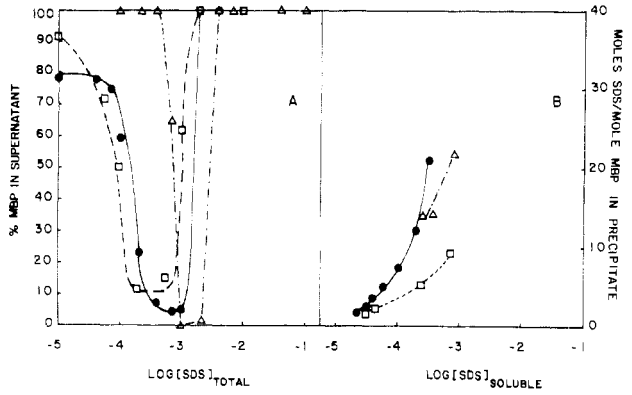

FIGURE 4: Effect of pH on (A) $NaDodSO_4$-induced precipitation of MBP and (B) composition of MBP–$NaDodSO_4$ insoluble complexes at I = 0.10 and 20 °C: (△) pH 4.8, [MBP] = 0.98 mg/mL; (●) pH 9.2, [MBP] = 0.80 mg/mL; (□) pH 11.6, [MBP] = 1.10 mg/mL.

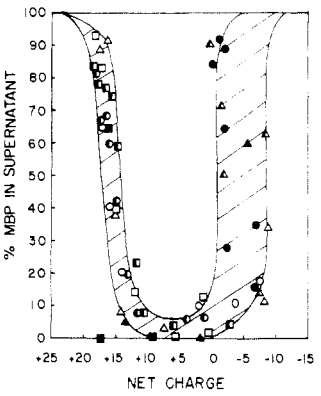

FIGURE 5: Precipitation of MBP as a function of net charge remaining on protein in MBP–detergent insoluble complexes at I = 0.10 and 20 °C in the presence of DOC [(○) pH 9.2, [MBP] = 1.77 mg/mL; (□) pH 9.2, [MBP] = 1.13 mg/mL; (△) pH 9.2, [MBP] = 0.43 mg/mL; (●) pH 11.6, [MBP] = 1.83 mg/mL] and in the presence of $NaDodSO_4$ [(■) pH 4.8, [MBP] = 1.95 mg/mL; (▲) pH 4.8, [MBP] = 0.98 mg/mL; (◐) pH 9.2, [MBP] = 1.70 mg/mL; (◧) pH 9.2, [MBP] = 0.80 mg/mL; (▲) pH 11.6, [MBP] = 1.10 mg/mL]. The limits of the hatched area were drawn by hand and encompass 90% of the experimental points.

Precipitation of MBP by $NaDodSO_4$. Figure 3 shows MBP precipitation and pellet composition curves at pH 9.2 (I = 0.1). These curves are similar in form to those for DOC. The precipitation process was slightly dependent upon protein concentration, with maximal MBP precipitation occurring at higher concentrations of $NaDodSO_4$ as the protein concentration was increased. The effects of pH on the formation and composition of the precipitates were quite pronounced (Figure 4). The $NaDodSO_4$ concentration at which maximal protein precipitation occurred increased as the pH was lowered. In addition, the $NaDodSO_4$ concentration range over which precipitation occurred was much narrower at pH 4.8 than at the two alkaline pH values. As the pH was raised from pH 4.8 to pH 11.6, there was a marked decrease in the proportion of $NaDodSO_4$ found in the precipitate at $[NaDodSO_4]_{soluble}$ > 0.5 mM.

The above data suggested that electrostatic interactions and the net charge on the protein might be important in the formation of insoluble $NaDodSO_4$–MBP aggregates. This interpretation was emphasized by the data shown in Figure 5. This profile represents a composite of data from many precipitation experiments, with both $NaDodSO_4$ and DOC, in which the solubility of the MBP is plotted against the charge on the protein in the insoluble complex. The charge was calculated from data published by Martenson (1978) and from

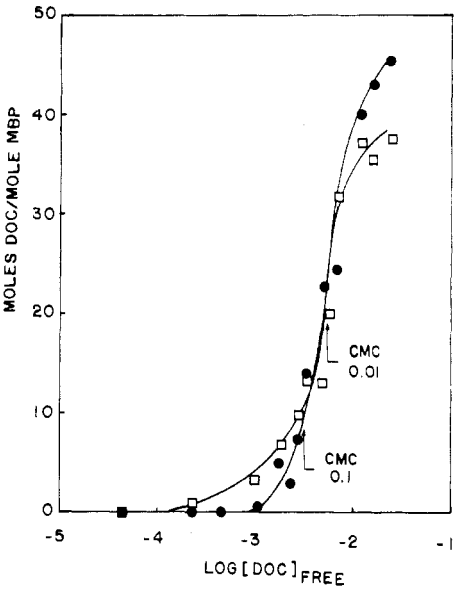

FIGURE 6: Binding isotherms of DOC to agarose-immobilized MBP at pH 9.2 and 20 °C: (●) I = 0.10; (□) I = 0.01. Arrows indicate the cmc of DOC at the indicated ionic strengths in the absence of protein.

our analysis of the composition of the insoluble complexes. In spite of the scatter, the data in the figure define a U-shaped curve in which minimum protein solubility occurs when the net charge on the MBP–detergent complex falls between +18 and −9.

Equilibrium Dialysis Measurements. Since the basic protein did not precipitate in the presence of low $[NaDodSO_4]$ at pH 4.8, it was possible to measure the binding of this detergent to MBP at this pH by equilibrium dialysis. The data indicated that at pH 4.8 each protein molecule can bind up to 15 molecules of $NaDodSO_4$ before precipitation occurs. Analysis of the insoluble MBP–$NaDodSO_4$ complexes under the same conditions of pH, ionic strength, and protein concentration yielded values of 18–38 mol of $NaDodSO_4$/mol of MBP in the precipitate (data not shown). Thus, under these conditions, a basal level of $NaDodSO_4$ binding occurred prior to precipitation.

Binding Isotherms of Anionic Detergents Using Immobilized MBP. Because the precipitation and equilibrium dialysis studies provide only limited binding data, the interaction of $NaDodSO_4$ and DOC was studied by using the basic protein immobilized on agarose. Use of this technique circumvented the precipitation problem and permitted the determination of complete binding isotherms. Figure 6 shows the binding of DOC to immobilized MBP at two different ionic strengths. At pH 9.2 and I = 0.1, significant binding of DOC to the protein was not evident until the cmc of the detergent was reached. Binding continued to increase in a cooperative fashion at DOC concentrations above the cmc until a saturation value of 40–50 mol of DOC/mol of protein was obtained. The precipitation data shown in Figures 1 and 2 indicate that resolubilization of the precipitate occurs when molar ratios of DOC/MBP exceed 28. Taken together, these data suggest that incomplete saturation of the protein causes precipitation of the complex and that further detergent binding results in its resolubilization.

Figure 6 shows the DOC binding isotherm at pH 9.2 and I = 0.01. This curve also indicates strong cooperative binding of DOC to the protein. However, there appears to be more binding below the cmc here than was observed at the higher ionic strength. This suggests that the binding is probably

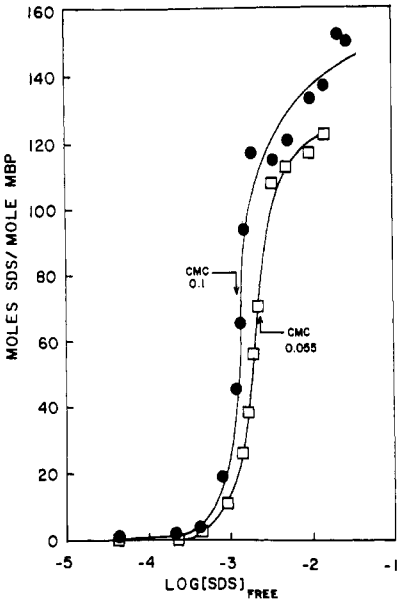

FIGURE 7: Binding isotherms of NaDodSO$_4$ to agarose-immobilized MBP at pH 9.2 and 20 °C: (●) I = 0.10; (□) I = 0.055.

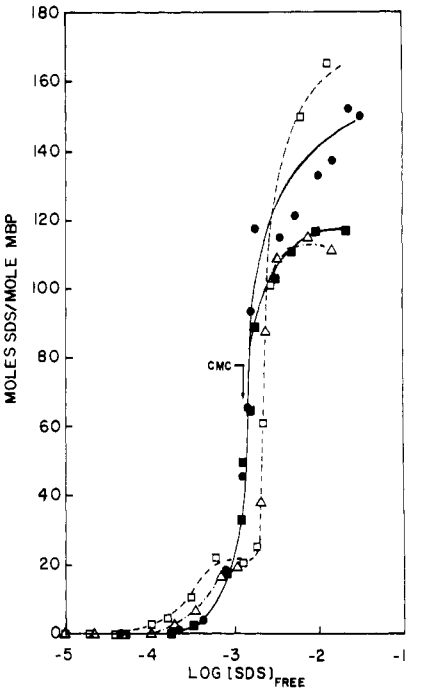

FIGURE 8: Effect of pH on binding of NaDodSO$_4$ to agarose-bound MBP at I = 0.10 and 20 °C: (□) pH 4.8; (△) pH 6.5; (■) pH 7.8; (●) pH 9.2.

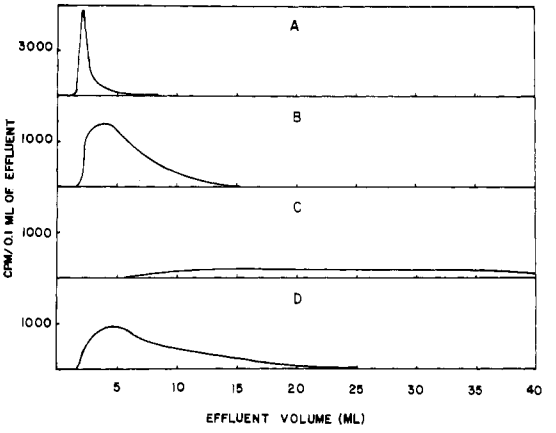

FIGURE 9: Effect of NaDodSO$_4$ concentration on elution of [^{125}I]MBP from immobilized MBP affinity column at pH 4.5 and I = 0.05 at NaDodSO$_4$ concentrations of (A) on NaDodSO$_4$, (B) 5 × 10^{-5} M, (C) 2 × 10^{-3} M, and (D) 1 × 10^{-2} M.

weakly electrostatic and thus largely abolished at the higher ionic strength. As at I = 0.1, DOC binding continues to increase at detergent concentrations above the cmc.

The binding isotherm of NaDodSO$_4$ to immobilized MBP at pH 9.2 and I = 0.1 is shown in Figure 7. Under these conditions, a highly cooperative increase in NaDodSO$_4$ binding was observed as the cmc of the detergent was approached. In addition, the extent of detergent binding continued to increase as the NaDodSO$_4$ concentration was raised above the cmc. As the ionic strength was lowered (I = 0.055), the binding isotherms shifted in accordance with the effects of ionic strength on the cmc of NaDodSO$_4$.

There was a marked dependence of the NaDodSO$_4$ binding isotherms on pH for immobilized MBP. The data obtained at pH 4.8, pH 6.5, pH 7.8, and pH 9.2 are shown in Figure 8. An additional isotherm determined at pH 8.5 is not shown to avoid complicating the figure, but it was nearly identical with that shown for pH 7.8 in Figure 8. The most striking effect of pH was that the binding isotherms were clearly biphasic at pH 4.8 and pH 6.5. At these pH values, a cooperative binding phase was evident at [NaDodSO$_4$]$_{free}$ ≤ 1 mM which plateaued at a binding ratio of 15–20 mol of NaDodSO$_4$/mol of protein. As shown in Figure 8, this cooperative binding phase could be titrated out between pH 6.5 and pH 7.8 such that at pH ≥ 7.8, a monophasic cooperative binding isotherm was obtained with the midpoint of the curve close to the cmc of NaDodSO$_4$. The major cooperative binding phase was shifted to higher [NaDodSO$_4$]$_{free}$ as the pH was lowered. At the two acidic pH values examined, the majority of NaDodSO$_4$ binding occurred well after the cmc of the detergent had been reached.

At all pH values, increased binding occurred at NaDodSO$_4$ concentrations above the cmc, with saturation of the protein with NaDodSO$_4$ becoming evident at ~10 mM free NaDodSO$_4$. An average value of 133 ± 21 mol of NaDodSO$_4$/mol of protein, corresponding to 2.09 ± 0.15 g of NaDodSO$_4$/g of protein, was obtained at saturating levels for the five pH conditions investigated.

Interaction of [^{125}I]MBP with Immobilized Proteins in the Presence of NaDodSO$_4$. In view of the proposals that NaDodSO$_4$ may induce specific MBP–MBP interactions similar to those observed in myelin (Golds & Braun, 1978a,b), the affinity chromatographic behavior of [^{125}I]MBP was studied at pH 4.5 (I = 0.05) in the presence and absence of NaDodSO$_4$. Typical elution profiles of [^{125}I]MBP from a MBP-agarose affinity column in the presence of several NaDodSO$_4$ concentrations are shown in Figure 9. In the absence of NaDodSO$_4$ the radiolabeled protein eluted near the void volume of the column; however, as the detergent concentration was raised, the elution of [^{125}I]MBP was retarded, with the elution volume reaching a maximum at [NaDodSO$_4$] = 2 × 10^{-3} M. Above the cmc ([NaDodSO$_4$] = 1.8 × 10^{-3} M) [^{125}I]MBP retardation was markedly decreased. Precipitation of the MBP in the elution buffer at NaDodSO$_4$ concentrations between 10^{-3} and 10^{-4} M precluded experiments in this concentration range. It should be noted that this interaction was observed in the presence of unlabeled MBP preequilibrated with the column. Even though the soluble unlabeled MBP could act as a competitor for binding of [^{125}I]MBP, adequate binding of the latter was observed. This would indicate that the exchange rate was rapid enough to allow binding of the labeled protein under these conditions.

Table I: Elution Volumes (mL) of [^{125}I]MBP from a Variety of Affinity Columns in the Presence of $NaDodSO_4$[a]

[$NaDodSO_4$] (M)	immobilized protein				
	MBP	histone	lysozyme	ovalbumin	control[b]
0	1.8	1.8	2.0	1.8	ND[c]
5×10^{-5}	2.8	6.3	2.7	2.2	1.8
2×10^{-3}	>30	31.7	30.0	10.7	10.5
10^{-2}	3.8	12.0	18.3	2.6	3.8
10^{-1}	ND	2.4	2.6	ND	ND

[a] Acetate buffer, pH 4.5, $I = 0.05$, 20 °C. Elution volumes were obtained by triangulation. [b] Agarose which had been activated with BrCN and deactivated by reaction with water was used to prepare a control column containing no immobilized protein. [c] ND, not determined.

In Table I, [^{125}I]MBP elution volumes obtained from the MBP affinity column are compared with those obtained from a control column in which the agarose had been BrCN activated and deactivated without coupling protein to it. Some retardation of [^{125}I]MBP was noted on the control column, but not to the extent observed on the MBP affinity column. This indicates that the predominant part of the observed retardation was, indeed, due to interaction of soluble $NaDodSO_4$–MBP complexes with the immobilized protein (or immobilized $NaDodSO_4$–protein complexes).

The specificity of the affinity chromatography interaction was tested by examining the elution of [^{125}I]MBP from columns containing immobilized ovalbumin, histone, and lysozyme. Histone and lysozyme were chosen as examples of proteins with similarly high isoelectric points and relatively comparable molecular weights. Ovalbumin (pI = 4.6) was selected because its interaction with detergents has been well characterized. Ovalbumin does not interact with $NaDodSO_4$ micelles and binds this detergent hydrophobically (Reynolds & Tanford, 1970; Steinhardt et al., 1977; Makino, 1979). As shown in Table I, [^{125}I]MBP was not retarded by the ovalbumin affinity column (relative to the control column). However, in the presence of $NaDodSO_4$, the basic protein was significantly retarded by both the histone and the lysozyme affinity columns.

The chromatographic data support the notion that $NaDodSO_4$ can induce interaction between MBP–detergent complexes. However, in view of the similar results with immobilized lysozyme and histones, it would appear that this interaction is not specific for MBP itself nor does it require site-specific MBP–MPB binding. Rather, the interactions between MBP–detergent complexes may be mediated through the detergent bound to the protein.

Discussion

Studies on the interaction of MBP with anionic detergents have been hampered because the protein precipitates in the presence of these detergents, a phenomenon associated with many proteins at pH values below their isoelectric points (Putnam & Neurath, 1944; Hegg, 1979). The precipitation studies described here indicate, however, that the compositions of the insoluble MBP–detergent complexes formed upon interaction of the protein with $NaDodSO_4$ and DOC varied with detergent concentration in a systematic, nonrandom fashion. While an analysis of the MBP–detergent aggregates might provide information about the general nature of the binding of anionic detergents to the protein which results in precipitation, it would not indicate the nature of detergent binding to the soluble form of the complex. Thus, in order to obtain direct binding data an alternative methodology was needed.

By using MBP coupled to agarose, it was possible to determine such binding isotherms. In many respects the basic protein is well suited for this experimental approach because it is a flexible molecule with relatively little secondary or tertiary structure over a wide pH range in solution; therefore, immobilization might be expected to cause no major changes in its structure. In addition, coupling at pH 6.3 should have minimized the number of covalent linkages between the basic protein and the agarose matrix to provide as few constraints as possible on the flexibility of the immobilized protein (Cuatrecasas & Anfinsen, 1971).

The immobilized MBP was found to bind a maximum of 2.09 ± 0.15 g of $NaDodSO_4$/g of protein and 1.03 ± 0.40 g of DOC/g of protein, values in reasonably good agreement with those of Smith & McDonald (1979), who measured saturation values of 2.30 ± 0.15 g/g for $NaDodSO_4$ and 1.34 ± 0.10 g/g for DOC under similar conditions of ionic strength by equilibrium dialysis and gel permeation chromatography. Although the average binding values for both detergents are slightly lower when using the immobilized protein, the values obtained in the two studies lie with experimental error. Thus, it is likely that detergent binding to immobilized MBP is similar to that for the free protein.

The binding isotherms obtained in this study show little evidence for discrete, noncooperative detergent binding sites such as those reported for albumin (Makino et al., 1973). Instead, at pH values above pH 7, the DOC and $NaDodSO_4$ isotherms are characterized by a single, highly cooperative increase in detergent binding, while for $NaDodSO_4$ at pH values below pH 7, the binding curves are biphasic consisting of two highly cooperative regions. Regardless of pH, the bulk of both DOC and $NaDodSO_4$ binding occurs around the cmc of the detergent. For both detergents the saturation levels of binding were quite high. The value of 2.09 g of $NaDodSO_4$/g of MBP is 40% more than the 1.2–1.5 g/g Reynolds & Tanford (1970) ascribed to the average protein while 1.03 g of DOC/g of MBP is much larger than the approximately 0.04 and 0.09 g/g reported for ovalbumin and bovine serum albumin, respectively (Makino et al., 1973).

The MBP has been reported to bind electrostatically to a number of acidic lipids (Palmer & Dawson, 1969; Steck et al., 1976), and several of the experiments reported here are consistent with the view that electrostatic interactions are also important in the binding of anionic detergents to the protein. For example, at $[DOC]_{free} <$ cmc, the binding of monomeric DOC to the MBP is reduced when the ionic strength of the buffer is increased (Figure 6). Raising of the pH from 9.2 to 11.6 (close to the pI of the MBP) significantly reduces the number of moles of DOC per mole of MBP in the precipitated complex under conditions of constant ionic strength and constant soluble DOC concentration (Figure 2). Binding of $NaDodSO_4$ monomers is pH dependent (Figure 8), and, insofar as the data allow comparison in Figure 4, there is a reduction in the number of moles of $NaDodSO_4$ precipitated per mole of MBP with increasing pH under conditions of constant ionic strength and constant soluble $NaDodSO_4$ concentration. While these data indicate that electrostatic interactions are important in the binding of anionic detergents to MBP, they do not exclude the possibility that hydrophobic MBP–detergent interactions also occur.

What causes MBP to precipitate in the presence of $NaDodSO_4$? The relationship between the charge on the MBP in the insoluble complex and the solubility of MBP (as shown in Figure 5) suggests that when the overall charge on the MBP–detergent complexes falls between +18 and −9, Cou-

lombic repulsions between the complexes are minimized and interactions leading to aggregation prevail. Since MBP is soluble in the absence of detergent at most pH values where it bears a net charge between +18 and −9, it would appear that the charge on the complex alone is not the only factor that governs precipitation and that the anionic detergents participate in the precipitation process.

Several possible mechanisms could account for the precipitation of MBP by NaDodSO$_4$. The detergent may induce specific MBP–MBP interactions (Golds & Braun, 1978b) which result in precipitation of MBP–NaDodSO$_4$ complexes. The affinity chromatographic studies, however, suggest that this mechanism is unlikely since, in the presence of NaDodSO$_4$, the MBP interacts with other basic proteins (e.g., lysozyme and histone). Alternatively, NaDodSO$_4$ monomers could bind electrostatically to one protein molecule and hydrophobically to another such that several MBP molecules could become cross-linked and precipitate (Jones & Rumsby, 1978). Again, the affinity chromatographic results do not support this possibility. Since ovalbumin has been reported to bind NaDodSO$_4$ monomers hydrophobically (Reynolds & Tanford, 1970; Steinhardt et al., 1977; Makino, 1979), then radiolabeled MBP, in the presence of NaDodSO$_4$, would have been expected to be retarded by the ovalbumin affinity column. As seen in Table I, no such retardation was observed over that of the control column.

The affinity chromatographic data are consistent, however, with a model whereby (at least at pH 4.8) a substantial portion of the NaDodSO$_4$ could bind electrostatically to MBP, with the resultant NaDodSO$_4$ monomer–MBP complexes interacting hydrophobically through clusters of NaDodSO$_4$ hydrocarbon tails on the surfaces of soluble and immobilized protein. This hydrophobic interaction would increase with increasing amounts of NaDodSO$_4$ until at the cmc, it could possibly become or include micellar bridging. When the MBP is saturated with NaDodSO$_4$ the protein–detergent complexes no longer interact.

It should be noted that in the absence of NaDodSO$_4$, the MBP is essentially monomeric at acidic pH (Smith, 1980). Thus, immobilization of the MBP at pH 6.3 would ensure conjugation of monomeric MBP to the matrix. In addition, the affinity chromatographic data were obtained at pH 4.8 such that in the absence of NaDodSO$_4$, dimerization of the MBP (Smith, 1980) was minimized. The results from the affinity columns suggest that the observed retardations in the presence of NaDodSO$_4$ were due to the detergent. We believe the proposed model should also be valid for the NaDodSO$_4$-induced precipitation at higher pH values where the MBP may exist as self-associated dimers (Smith, 1980). Precipitation of MBP–NaDodSO$_4$ complexes should be independent of the self-association of the protein since precipitatin is presumably mediated by the electrostatically bound detergent. Thus, we suggest that association of MBP–anionic detergent complexes may be viewed, in general terms, as occurring through hydrophobic interactions of the detergents which are electrostatically bound to the protein molecules.

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