Biochimica et Biophysica Acta, 554 (1979) 133—147

BBA 78402

ASSOCIATION OF MYELIN BASIC PROTEIN WITH DETERGENT MICELLES

ROSS SMITH and BRIAN J. McDONALD
School of Chemistry, University of Sydney, Sydney, N.S.W. 2006 (Australia)
(Received October 11th, 1978)

Key words: Myelin basic protein; Detergent micelle

Summary

Equilibrium measurements of the binding of central nervous system myelin basic protein to sodium dodecyl sulphate, sodium deoxycholate and lysophosphatidylcholine have been obtained by gel permeation chromatography and dialysis. This protein associates with large amounts of each of these surfactants: the apparent saturation weight ratios (surfactant/protein) being 3.58 ± 0.12 and 2.30 ± 0.15 for dodecyl sulphate at ionic strengths 0.30 and 0.10, respectively, 1.34 ± 0.10 for deoxycholate (at 0.12 ionic strength) and 4.0 ± 0.5 for lysophosphatidylcholine. Binding to the ionic surfactants increases markedly close to their critical micelle concentrations. Sedimentation analysis shows that at 0.30 ionic strength in excess dodecyl sulphate the protein is monomeric. It becomes dimeric when the binding ratio falls below 1 at a free detergent concentration of approximately 0.25 mM: below this concentration much of the protein and detergent forms an insoluble complex. The amount of dodecyl sulphate bound at high concentrations and at both above-mentioned ionic strengths corresponds closely to that expected for interaction of a single polypeptide with two micelles. Variability of deoxycholate micelle size on interaction with other molecules precludes a similar analysis for this surfactant. Association was observed only with single micelles of lysophosphatidylcholine. The results provide strong evidence for dual lipid-binding sites on basic protein and indicate that lipid bilayer cross-linking by this protein may be effected by single molecules.

Introduction

Myelin basic protein, a major component of myelin, acts as antigen producing inflammatory demyelination in experimental allergic encephalomyelitis, and has been implicated in several neurological diseases, including

multiple sclerosis. Despite considerable interest in this protein its normal function within myelin has not been unequivocally elucidated. It has recently been shown [1] that the protein may act as a growth factor for astrocytes, but this observation does not appear to explain the comparatively large amounts of the protein that are found in the compact myelin formed by oligodendroglial cells.

On the basis of a demonstration of non-covalent cross-linking of lipid vesicles, a structural role has been suggested for the protein within myelin [2], a role recently advanced also by Braun [3] and by Rumsby [4]. Implied is a more specific function than the earlier proposal that basic protein serves to reduce electrostatic repulsion between apposed bilayers, which on the basis of the overall lipid composition of myelin, are assumed to contain negative lipids (predominantly cerebroside sulphates, phosphatidylserine and phosphatidylinositol).

Although earlier work from this laboratory demonstrated bilayer cross-linking it provided little insight into the molecular mechanism of this bridging. To better define this mechanism studies of association of the protein with detergents were undertaken. Detergent micelles do not as closely mimic the structure of biological membranes as lipid vesicles but they provide a more convenient vehicle for many studies of the protein when it is bound at a hydrophilic-hydrophobic interface. In this instance the detergent binding has proven especially revealing because of the quite atypical behaviour of basic protein in detergent solutions. The results bear not only on the properties and function of basic protein per se, but are relevant to the wider field of the interaction of detergents with proteins and the structural models proposed for these complexes.

Methods

Sodium deoxy[1-^{14}C]cholate was a product of Applied Sciences (State College, PA, U.S.A.): analysis by thin-layer chromatography in our laboratory showed it to be at least 99% pure. Sodium dodecyl sulphate (Pierce, Rockford, U.S.A., 99%), sodium deoxycholate (Merck, Darmstadt, F.R.G., 98.5—101.0%) and egg L-α-lysophosphatidylcholine (Sigma, St. Louis, U.S.A., 98%) were used without further purification. Bovine serum albumin (crystalline), α-chymotrypsinogen (crystalline) and cytochrome *c* (93%) were purchased from Calbiochem. (San Diego, U.S.A.). Myelin basic protein was prepared from bovine brain following the method of Oshiro and Eylar [39]. Dodecyl sulphate gel electrophoresis and equilibrium sedimentation showed the protein to be a single polypeptide of molecular weight 18 400. Other reagents were of analytical grade.

Binding measurements. Binding of the three surfactants was determined by equilibrium dialysis and by column chromatography, the latter technique being used for lysophosphatidylcholine and for all solutions containing free dodecyl sulphate close to, or above, the critical micelle concentration. Attainment of equilibrium was assured by approach to equilibrium with separate protein solutions initially containing an excess and deficiency of detergent.

Equilibrium dialysis was performed in perspex cells with two 3 ml compartments separated by a membrane of dialysis tubing (Visking, Chicago, U.S.A.),

which had been extensively washed in salt solutions and hot distilled water. The cells were constantly rotated.

Gel permeation chromatography utilized a 77 cm × 2.5 cm diameter column of Sepharose 4B (Pharmacia, Sweden) for lysophosphatidylcholine, a 70 cm × 2.5 cm column of Sephadex G-150 (Pharmacia, Sweden) for sodium dodecyl sulphate, and a 80 cm × 2.5 cm column of Sephadex G-100 for deoxycholate studies. For each column the flow rate was in the range 15—30 ml · h^{-1} and 15—20 min fractions were collected. Distribution coefficients (K_d) were calculated from measurements of the column void and included volumes by inclusion of blue dextran (Pharmacia, Sweden) and mercaptoethanol (Fluka, Switzerland, analytically pure) or bromophenol blue (British Drug Houses, U.K.). All binding studies were at 25 ± 2° C.

Protein concentrations were determined spectrophotometrically (assuming $E^{1\%}_{1cm} = 5.44$ at 280 nm, deduced from $E^{1\%}_{1cm} = 5.64$ at 276.4 nm [26]) or colorimetrically [5]. Calibration of the colorimetric assay employed standard basic protein solutions in the same buffer and containing approximately the same detergent concentration as the solutions of unknown protein concentration. The buffer solutions used in some experiments significantly reduced the colour formation in this assay: this effect was substantially reduced by doubling the amount of the alkaline copper solution (reagent C of Lowry et al. [5]) added to the sodium carbonate solution. The absorbance of protein from columns containing dodecyl sulphate in particular was found to be an unreliable index of protein eluate concentration and in general concentration determinations from absorbance measurements at 280 nm were only made for detergent-free solutions.

Dodecyl sulphate concentrations were established following the method of Mukerjee [6], in which the detergent increases the solubility of the dye, methylene blue, in the chloroform phase of a two phase system. Using solutions of known detergent concentration it was found that the presence of basic protein, at least in the concentration range used in these experiments, did not influence the phase distribution of the dye. Phosphate analyses [7] were used to measure concentrations of lysophosphatidylcholine. This lipid is stated to contain primarily palmitic and stearic acids; on this basis a molecular weight of 510 was adopted for subsequent calculations. Deoxycholate concentrations were deduced from liquid scintillation counting of sodium deoxy[1-C^{14}]cholate added to unlabelled deoxycholate (up to 0.002% of radioactive detergent). Samples for counting were carefully weighed into scintillation vials containing 15 ml of toluene-based scintillation fluid [8]: background and water-quenching corrections were applied.

Several buffers were used for these experiments: they are given one-letter designations below to facilitate later reference to them. A: 0.20 M sodium chloride, 2.0 mM EDTA, 2.0 mM sodium azide, 0.05 M phosphate, pH 7.40 ± 0.02. B: as for A but without sodium chloride. C: 0.20 M sodium chloride, 2.0 mM EDTA, 2.0 mM sodium azide, 0.10 M Tris, pH 7.40 ± 0.2. D: 0.02 M Tris, 0.10 M sodium chloride, 2.0 mM sodium azide, pH 9.20 ± 0.02. E: as for D but with 0.20 M sodium chloride.

Precipitation of basic protein at low detergent concentrations. Aliquots of concentrated detergent solutions (typically 25 mg · ml^{-1}) were added to pro-

tein solutions (of the order of 1 mg · ml^{-1}) in the appropriate buffer. After standing overnight at 25°C the solutions were centrifuged at 80 000 × g_{av} (Beckman SW50.1 rotor) for 2 h at 25°C. Samples of the supernatants were taken for protein and detergent analyses.

Critical micelle concentration of dodecyl sulphate. The critical micelle concentration of dodecyl sulphate in the high ionic strength buffer A was measured at 25 ± 2°C by following the rate of dialysis of the detergent. The dialysis cell contained initially a 2 mg · ml^{-1} solution of detergent in one compartment and detergent-free buffer in the other. 200-μl samples of dialysate were taken at various times and the concentration of dodecyl sulphate established.

Determination of the molecular weight of the basic protein-dodecyl sulphate complex. A Beckman Model E analytical ultracentrifuge with interference optics was used for sedimentation equilibrium experiments. Samples (150 μl) of protein in dodecyl sulphate solution (in buffer A), taken from columns or dialysis cells used for binding studies, were placed in one side of a 12 mm double-sector cell with a capillary-type synthetic boundary Al-filled epon centrepiece. Column solvent was placed in the other cell sector. Photographs of the interference pattern were taken on reaching speed (to provide a correction for window distortions) and after 24—30 h at 20—26°C. At low detergent concentrations the temperature had to be kept near 26°C to avoid precipitation of the surfactant. Initial concentrations (in fringes) in the cell were subsequently obtained from synthetic boundary runs in which 200 μl of buffer was added to the protein-free sector and sedimentation carried out at 15 000 rev./min for 0.5—1 h. Photographs were taken at 4—16-min intervals and estimates of the initial concentration gained from extrapolation to zero time of the fringe displacement across the boundary. All photographic plates were measured to an accuracy of ±2 μm on a microcomparator.

Following centrifugation and binding studies the integrity of the protein was examined by gel electrophoresis in the presence of dodecyl sulphate [9].

Results

Binding of dodecyl sulphate

In Fig. 1 data from both dialysis and gel chromatographic measurements of dodecyl sulphate binding are collated. The slow diffusion of dodecyl sulphate micelles through the dialysis membrane precludes binding measurements by dialysis much above the critical micelle concentration, but measurements by both methods near the critical micelle concentration were consistent.

At high ionic strength (0.3 in buffer A) and high free detergent concentration the protein binds unusually large amounts of dodecyl sulphate: the saturation value is 3.58 ± 0.12 g/g of basic protein. (The standard deviation was calculated from the results of six independent experiments). This value was reproducibly obtained over a wide free dodecyl sulphate concentration range above the critical micelle concentration. At each concentration attainment of thermodynamic equilibrium was readily demonstrated by approach to equilibrium from either an initial excess or deficiency of detergent in the protein solution. The Donnan potential, which could lead to spuriously high binding ratios, is expected to be negligible at 0.30 ionic strength: existence of a significant

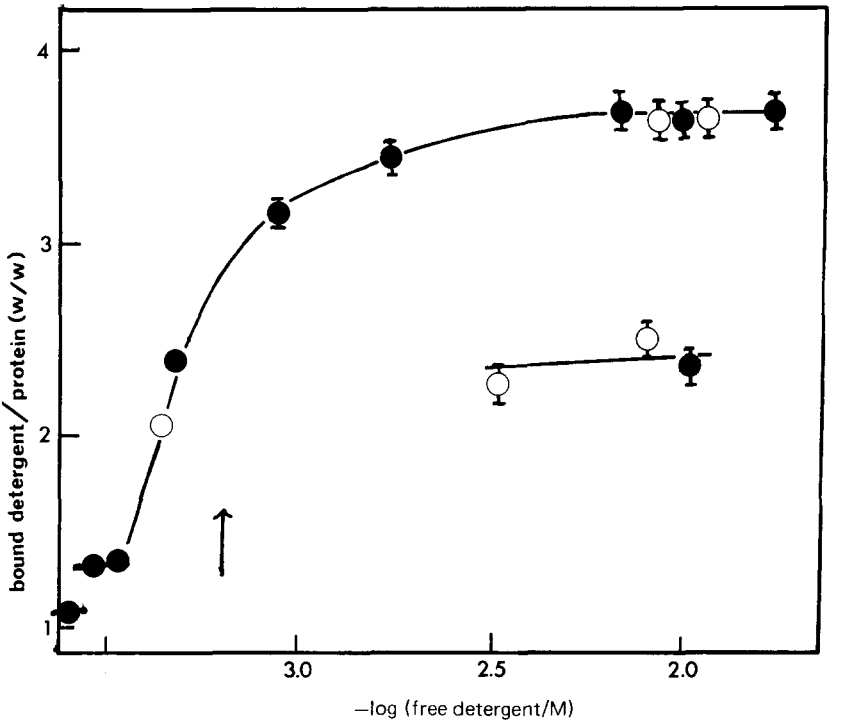

Fig. 1. Isotherms for binding of dodecyl sulphate to bovine myelin basic protein at 25 ± 2°C. Experiments were performed by dialysis (●) or by gel permeation chromatography (●,○). An excess of detergent was initially added to the protein in some experiments (●) and in others the protein solution initially contained less than the equilibrium concentration of detergent (○). Buffer A (ionic strength 0.3) was used for all measurements except three at 0.10 ionic strength (lower right). The measured critical micelle concentration in buffer A is indicated by the arrow.

Donnan effect would also be inconsistent with the observed lower binding at 0.10 ionic stength (see below).

Although the critical micelle concentration of dodecyl sulphate has previously been measured over a wide range of ionic strength in sodium chloride solutions [10,11], the nature of the buffer used in these experiments (which includes both phosphate and EDTA) could not be assumed to be without effect on the critical micelle concentration. The critical micelle concentration was therefore obtained by measuring the rate of dialysis of solutions containing high concentrations of dodecyl sulphate against detergent-free buffer. The detergent concentration in the dialysate increased rapidly (Fig. 2) to a concentration far below that expected for complete equilibrium across the membrane. Continued dialysis resulted in a far slower linear increase over several days, which was attributed to micelle permeation of the membrane. Extrapolation of this line to zero time gave the concentration of detergent that would have been obtained in the dialysate in the absence of micelle permeation, i.e. the critical micelle concentration. In buffer A this value was 0.62 mM, close to previously reported values in 0.30 M sodium chloride [10,12].

Binding of dodecyl sulphate increases rapidly near the critical micelle con-

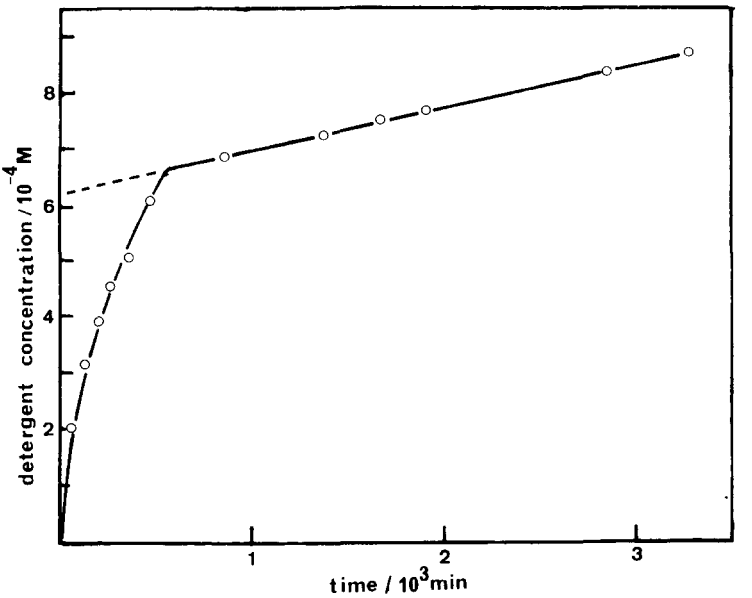

Fig. 2. Measurement of the critical micelle concentration of sodium dodecyl sulphate in buffer A. The slow phase of dialysis was extrapolated to zero time (· · · · · ·) to obtain the critical micelle concentration.

centration though binding persists at lower concentrations. Below a free detergent concentration of approximately 0.25 mM the solutions become turbid. This turbidity is not simply caused by aggregation of the protein at this pH: separate studies (Smith, R., unpublished data) have revealed only limited self-association of the protein under these conditions in the absence of detergent. Experiments performed at fixed protein concentration showed precipitation when the detergent/protein ratio (w/w) in solution fell below 1 (Table I). Below this ratio much of the protein and detergent was lost from solution until at the lowest detergent concentrations employed the protein concentration (but not the detergent concentration) in the supernatant began to rise again. Precipitation was evident over a pH range from 4.18 to 11.57 (Table II).

Jones and Rumsby [13] on adding dodecyl sulphate to basic protein ob-

TABLE I

EFFECT OF DETERGENT CONCENTRATION ON THE FORMATION OF INSOLUBLE DODECYL SULPHATE-BASIC PROTEIN COMPLEX

The experiment was performed in duplicate. The initial protein concentration was in each instance 1.01 mg · ml^{-1}.

Protein concn. in supernatant (mg · ml^{-1})	Initial detergent concn. (mg · ml^{-1})	Final detergent concn. in supernatant (mg · ml^{-1})	Detergent/protein (w/w) in precipitate
1.01 ± 0.03	1.94 ± 0.05	1.89 ± 0.05	—
0.39 ± 0.01	0.97 ± 0.03	0.48 ± 0.01	0.79
0.043 ± 0.009	0.49 ± 0.01	0.050 ± 0.004	0.46
0.056 ± 0.010	0.243 ± 0.007	0.011 ± 0.002	0.24
0.181 ± 0.011	0.121 ± 0.003	0.010 ± 0.002	0.13

TABLE II

EFFECT OF pH ON FORMATION OF INSOLUBLE BASIC PROTEIN-DODECYL SULPHATE COMPLEXES

pH	Initial protein concn. (mg · ml^{-1})	Final protein concn. in supernatant (mg · ml^{-1})	Initial detergent concn. (mg · ml^{-1})	Final detergent concn. in supernatant (mg · ml^{-1})
4.18	0.92 ± 0.03	0.053 ± 0.010	0.231 ± 0.007	0.031 ± 0.003
7.37	0.92 ± 0.03	0.040 ± 0.009	0.231 ± 0.007	0.028 ± 0.003
10.33	0.92 ± 0.03	0.051 ± 0.010	0.231 ± 0.007	0.035 ± 0.003
11.04	0.92 ± 0.03	0.106 ± 0.011	0.231 ± 0.007	0.035 ± 0.003
11.57	0.92 ± 0.03	0.449 ± 0.012	0.231 ± 0.007	0.101 ± 0.008

served an increase in light scattering which decreased over several hours: they attributed this diminution to redissolution of the complex. A similar sequence was noted in the present work but the reduction in light scattering appeared to result from deposition of the complex on the container walls: this observation was verified by analysis of the protein and detergent content of the supernatants obtained by centrifugation.

Changes in the elution position with alteration in detergent concentration were evident in the chromatograms used for evaluation of dodecyl sulphate binding. The distribution coefficient, K_d [14], for dodecyl sulphate in the absence of protein was 0.36 ± 0.02 and for protein at detergent concentrations well above the critical micelle concentration, 0.16 ± 0.02. But at lower detergent concentrations the values of K_d were 0.22 ± 0.02 in 0.87 mM dodecyl sulphate and 0.30 ± 0.02 at a concentration of 0.43 mM. The binding ratios in these two experiments were 3.03 and 1.97, respectively.

Lowering the ionic strength to 0.10 (buffer B) caused a decrease in the binding above the critical micelle concentration. The binding ratio over a free detergent concentration range from 3.3 to 10.2 mM was approximately constant at 2.30 ± 0.15.

Sedimentation analysis in dodecyl sulphate solutions

Sedimentation was generally restricted to low angular velocities to avoid significant sedimentation of free micelles at the higher concentrations. The molecular weight of the protein (excluding bound detergent) was derived following Tanford et al. [15] using an apparent partial specific volume (ϕ') calculated from the known partial specific volumes of the protein (V_p = 0.72 ml · g^{-1}) [16] and detergent (V_d = 0.87 ml · g^{-1}) [15] and the measured binding ratio, δ,

$$(1 - \phi'\rho) = (1 - V_p\rho) + \delta(1 - V_d\rho)$$

where ρ is the solvent density.

The results are summarized in Table III. In the presence of excess micelles the protein appears completely monomeric. A single experiment performed close to the critical micelle concentration showed only monomers at a binding ratio (w/w) near 2.2. But duplicate experiments below the critical micelle concentration (see Discussion), at a binding ratio of 0.52, showed the complex

TABLE III

SEDIMENTATION EQUILIBRIUM MEASUREMENTS ON BASIC PROTEIN IN DODECYL SULPHATE SOLUTIONS

Free detergent concn. (mM)	Detergent bound (g/g protein)	Angular velocity (rev./min)	Molecular weight
0.22	0.52	24 000	35 100 ± 2 000
0.63	2.23	12 000	18 300 ± 1 000
10.4	3.50	10 000	17 700 ± 1 000
10.4	3.56	12 100	22 000 *
10.4	3.58	24 000	18 600 ± 1 000
17.4	3.58	10 000	18 000 ± 1 000

* This sample was taken from a dialysis experiment rather than a gel column and may not have reached thermodynamic equilibrium with the solvent.

contained two polypeptides with minor heterogeneity indicated by a slightly lower molecular weight at the low protein concentrations near the solution meniscus (Table III).

Electrophoresis at high dodecyl sulphate concentrations

Typically gel electrophoresis employs gels and electrode buffer containing 3.5 mM (0.1%) dodecyl sulphate at an ionic strength near 0.10. From the above binding results basic protein under these conditions binds about 2.30 g detergent/g and might be expected to behave anomalously in comparison to standard proteins which have been reported to bind 1.4 g/g. However, electrophoresis in gel systems containing 3.5 mM or 10.4 mM (0.3%) dodecyl sulphate and using bovine serum albumin (M_r = 69 000), α-chymotrypsinogen (M_r = 25 500) and cytochrome *c* (M_r = 12 300) as reference proteins yielded apparent molecular weights of 19 000 ± 10% and 18 400 ± 10%, respectively, at 0.13 ionic strength (0.09 M Na^+).

Mobility in gels has been attributed to hydrodynamic size alone [40], independent of charge. If imposition of an electric field does not affect the amount of detergent bound then the dimensions of the basic protein complex would not a priori be expected to bear a simple relationship to those of proteins binding 1.4 g dodecyl sulphate/g. No clear concensus has, however, emerged on the structure of dodecyl sulphate-protein complexes of globular proteins [40,41] and resolution of this anomaly may be achieved once a clearer understanding of their structures and the principles of gel electrophoresis is obtained.

Association with deoxycholate

Conveniently rapid equilibration across dialysis cell membranes is achieved with the smaller deoxycholate micelles: therefore only dialysis was used for these binding measurements. Large amounts of deoxycholate also bind to basic protein in buffer D at pH 9.2 and, as with dodecyl sulphate, the binding rises sharply near the critical micelle concentration. In the presence of excess micelles the amount bound is constant at 1.34 ± 0.10 g/g (Fig. 3). In contrast to the behaviour in dodecyl sulphate solutions, limited experiments at higher ionic strength (buffer E) showed no significant change in the binding of deoxycholate.

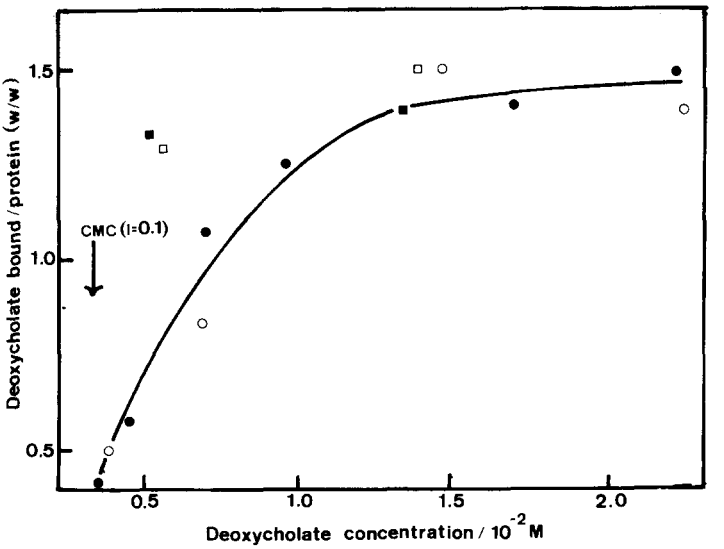

Fig. 3. Sodium deoxycholate binding to basic protein at 25 ± 2°C, measured by dialysis. Experiments utilized buffers of ionic strength 0.12 (in buffer D; ○,●) and 0.22 (buffer E; □,■). Dialysis was initiated with protein solutions containing more than (○,□) or less than (●,■) the equilibrium deoxycholate concentration.

Formation of insoluble complexes was also noted with deoxycholate: as with dodecyl sulphate, this precipitation precluded binding measurements much below the critical micelle concentration even with dilute protein solutions. Precipitation with dodecyl sulphate occurs when few micelles are present but at similar protein concentrations aggregation is still evident well above the critical micelle concentration with deoxycholate, and is markedly increased at high ionic strength. To circumvent this problem the binding curve (Fig. 3) was obtained by using low protein concentrations at low deoxycholate concentrations.

Although deoxycholate binding presents interpretative difficulties because of possible variations in micelle size, the smaller micellar size does permit a measure of the change in gross conformation of the protein on complex formation. Within the limitations of the use of gel chromatography to determine Stokes radii [17], preliminary measurements suggest little change in Stokes radius on binding 1.34 g/g of deoxycholate. This may result from some compaction of tertiary structure, in comparison with the coiled aqueous conformation, on association with this surfactant.

Association with L-α-lysophosphatidylcholine

Even at binding ratio of 1.97 g dodecyl sulphate/g basic protein satisfactory separation of bound and unbound detergent was obtained on Sephadex G-150. But because of the larger micellar size (see Discussion) of the phospholipid a similar separation was not achieved. On Sepharose 4B the distribution coefficient for basic protein without lipid added before chromatography, but in buffer C containing 40 mg · l^{-1} lysophosphatidylcholine, was 0.87 ± 0.04. The value for free micelles was 0.63 ± 0.04 and the value for the lipid-protein com-

plex was experimentally indistinguishable from this, obviating the possibility of demonstrating thermodynamic reversibility of binding by the addition of an initial excess of lipid to the protein. In experiments starting with 2.5—4.3 g lipid/g protein the final ratio in the eluted peak was consistently higher and typically 4.0 ± 0.5. When 5—12 g lipid/g protein was added before chromatography the final ratio was invariably close to the starting ratio, and the distribution coefficient remained 0.63 within the experimental error.

Mercaptoethanol was used as the included volume marker in these experiments as bromophenol blue chromatographed with the lipid-protein complex.

Precipitation was not observed at any mixing ratio from 0 to 12 g lysophosphatidylcholine/g protein (the latter at 1 mg · ml^{-1}) at pH 7.4 or 10.2. At the same concentration the protein did increase the light scattering of a 3.3 mg · ml^{-1} solution of egg L-α-diacylphosphatidylcholine at pH 9.05 [2,18].

Discussion

Myelin basic protein association with dodecyl sulphate and deoxycholate at high concentrations

Dodecyl sulphate binding to basic protein is unusually large. Most proteins have been found to bind about 1.4 g/g protein [19,20] although Nelson [21] has noted that at an ionic strength of 0.4 (cf. maximum 0.30 in the present work) ovalbumin binds up to 2.6 g/g and a similar amount is bound by the apoprotein of human low density lipoprotein at 0.10 ionic strength [22]. High levels of binding are not characteristic of basic proteins in general: histone IV (with charge near +15 at pH 7), histone I, and lysozyme, for example, are associated with a maximum of 1.4 g/g at 0.13 ionic strength and neutral pH [19]. Binding to lysozyme is unchanged at ionic strength 0.26 [19], but some variation with ionic strength has been noted for other proteins [21].

Basic protein also binds an unusually large amount of deoxycholate. Previous studies have shown this detergent to bind only to exposed hydrophobic domains on protein molecules and not to proteins which lack hydrophobic sites in the native state [23].

Binding of dodecyl sulphate to globular proteins is generally a positively cooperative process which begins below the critical micelle concentration and results in denaturation [19,24]. In contrast, myelin basic protein binds little below 0.3 mM and undergoes a marked increase in binding near the critical micelle concentration.

The final conformation of the protein [25,26] in dodecyl sulphate solutions more closely resembles that of the protein associated with lipid bilayers (Keniry, M.A. and Smith, R., unpublished data) than the initial coiled structure, and probably represents a conformational transition towards the native state rather than the usual divergence observed with globular proteins. In contrast to dodecyl sulphate, deoxycholate does not normally cause significant conformational changes on binding to proteins [27].

In Table IV the published micellar weights are used with the binding data from the current work to calculate the number of micelles, with micelle number assumed unchanged on protein binding, bound to basic protein at both ionic strengths studied. The levels of binding correspond closely to those

TABLE IV

ANALYSIS OF DODECYL SULPHATE BINDING TO BASIC PROTEIN

Ionic strength	0.10	0.30
Dodecyl sulphate micellar weight	21 900 *	31 000—34 000 *
Calculated binding ratio (w/w) for two micelles/protein molecule	2.38	3.36—3.70
Observed binding ratio	2.30 ± 0.15	3.58 ± 0.12

* From Refs. 10 and 11.

expected for association of two micelles with each polypeptide. The marked decrease in micelle size which results from a decrease in ionic strength is quantitatively reflected in the binding ratio: the change is in the opposite direction to that which would be expected were binding mediated by coulombic forces between surfactant monomers and the protein [28]. Monomer binding to basic protein, if significant, might also be expected to occur at lower free detergent concentrations than with many proteins, as the open coiled conformation [29] of this protein should ensure ready accessibility of all potential binding sites.

Similar analysis of the association with deoxycholate suggests that at the highest free concentrations of this surfactant several micelles are bound to each protein molecule (Table V). However, even at moderate ionic strengths deoxycholate micelles are small [30] and as the aggregation number is sensitive to interaction with other molecules [31] it cannot be assumed that the micellar weight remains even approximately constant upon interaction with basic protein. An association, similar to that observed with dodecyl sulphate, but with two micelles of deoxycholate which are considerably larger than the free micelles may occur.

Thus several features of the binding indicate that, unlike most proteins, myelin basic protein associates primarily with micelles of both detergents. Micellar interaction has also been proposed for a few other proteins, such as the hydrophobic domain of cytochrome b_5 [32].

Basic protein association with dodecyl sulphate and deoxycholate at low concentrations

Sedimentation analysis shows that close to the critical micelle concentration the protein becomes dimeric, an observation reinforced by the recent demonstration by Golds and Braun [54] that covalently cross-linked dimers can be isolated following reaction of basic protein with bifunctional cross-linking reagents in dodecyl sulphate solutions.

TABLE V

ANALYSIS OF ASSOCIATION OF BASIC PROTEIN WITH DEOXYCHOLATE

Micellar weight in 0.15 M sodium chloride at 20°C, pH 8—9	6290 *
Observed binding rate ratio (w/w) at 0.10 and 0.20 ionic strengths	1.34 ± 0.10
Micelles bound	3.9 ± 0.3 **

* From Ref. 30.

** This calculation assumes no change in micelle size on binding to the protein (see text).

Binding of one micelle/two polypeptide chains might, on the basis of data presented in Table IV, be expected to yield a binding ratio of 0.93 rather than the observed value of 0.5. However, micelle size is approximately constant only well above the critical micelle concentration: close to this concentration the micelle number is expected to vary rapidly in an as yet unpredictable fashion. Indeed, the existence of micelles below the critical micelle concentration is not unexpected as this concentration is not well defined [33]. This possibility is enhanced if the protein can act as a nucleation centre for dodecyl sulphate micelles by lowering the chemical potential of a monomer in the micelle-protein complex below that of a monomer in a protein-free micelle, for example by reducing the electrostatic repulsion between headgroups (see e.g. Ref. 34). This effect would parallel the decrease in critical micelle concentration caused by increased counterion binding to micelles at high ionic strength. The apparently low binding could therefore still derive from association of a single micelle with two basic protein molecules.

Although hydrophobic interactions between proteins and dodecyl sulphate are now well-established the precipitation of other proteins by this detergent has been noted [35] to occur only when the proteins are below their isoelectric points, i.e. with cationic proteins. It was concluded that ionic interactions were dominant in producing the gross aggregation. The precipitation induced by basic protein appears to be less dependent on simple coulombic attaction. Precipitation appears not to be limited to pH values below the isoelectric point, remaining appreciable beyond pH 11.57 (Table II) although the isoelectric point has been reported to be 10.8 * [36]. Further, if the formation of insoluble complexes were dependent only on reduction of the net charge on the polypeptide by bound detergent both peptides produced by proteolytic cleavage at the tryptophan residue (residue 116) would also aggregate at low dodecyl sulphate concentrations. Yet, as noted by Jones and Rumsby [13], these basic peptides show no such precipitation over a wide range of detergent concentration, implying that integrity of the polypeptide is prerequisite.

The precipitate that forms at low dodecyl sulphate concentrations appears not to have fixed stoichiometry. Formation of an extended aggregate of variable composition is readily explicable if basic protein molecules are capable of binding to at least two micelles (as demonstrated in the presence of excess micelles) and each micelle can associate with two or more polypeptides.

Although qualitatively similar, the precipitation observed at low deoxycholate/protein ratios occurs even in the presence of excess micelles.

Basic protein association with lysophosphatidylcholine

Egg lysophosphatidylcholine micelles in water at 25°C have a number-average molecular weight of 98 800 [42] and the corresponding stearoyl lipid has a micellar weight of 65 500 in 0.16 M NaCl. The micelle size of this zwitterionic lipid is expected to show only minor variations with salt con-

* Examination of the published amino acid sequence of the bovine protein [37] suggests an isoelectric point above 12, but this value would be reduced by such in vitro and in vivo modifications as phosphorylation and deamidation [38]. Because of possible variations in these modifications from one preparation to another the value given is necessarily approximate.

centration at moderate ionic strength, hence a value of 65 500 for egg lysophosphatidylcholine (50% stearoyl and 50% palmitoyl) at 0.30 ionic strength has been used in this work. Binding of one micelle/basic protein molecule would correspond to a lipid : protein weight ratio of 3.53. The reasonable correspondence of the calculated and experimental (4.0 ± 0.5) values, and the equal distribution coefficients for the free micelles and the complex indicates that the protein binds to single micelles. Under the conditions employed there was no indication that the protein could simultaneously interact with two micelles. The absence of precipitation at low lipid/protein ratios reinforces this conclusion. Observation that at pH 9 basic protein appears to cross-link vesicles of egg phosphatidylcholine but not lysophosphatidylcholine micelles re-emphasizes the conclusion, drawn from studies of conformational transitions accompanying binding to these lipids (Keniry, M.A. and Smith, R., unpublished observations), that the mode of association differs for these similarly structured lipids.

The nature of the lipid binding sites on basic protein

Basic protein has been shown to interact with negatively charged lipids [46—49]. But the interaction with dodecyl sulphate involves more than electrostatic attraction as demonstrated by studies of tryptophan fluorescence [43], and the observation of selective broadening of ^{1}H NMR resonances associated with aromatic and aliphatic side-chains in the presence of this detergent [26].

Hydrophobic interactions are also evident in the association of basic protein with other lipids, particularly zwitterionic lipids [18,25,44—47]. Similar changes in protein secondary structure are also induced by ionic detergents and lysophosphatidylcholine (Refs. 25, 26 and 50; Keniry, M.A. and Smith, R., unpublished data).

Evidence exists for location of lipid binding sites on both ends of the polypeptide chain. Peptides 1—89 and 90—170 produced by cleavage with cathepsin D bind to phosphatidylserine and phosphatidylcholine to the same extent (on a mol basis) as the intact protein (Keniry, M.A. and Smith, R., unpublished results). London et al. [46] have similarly observed that peptides 1—116 and 117—170 both increase the surface pressure of cerebroside sulphate monolayers, the N-terminal peptide interacting hydrophobically [44,46]. Rumsby [4] have proposed that the C-terminal fragment is ionically bound to lipids. However, this conclusion, and several other reports of purely ionic binding of basic protein to lipids [51—53], was based on studies in biphasic solvent systems which would not necessarily reveal hydrophobic interactions.

We have recently proposed on the basis of interaction with egg phosphatidylcholine, that basic protein can non-covalently cross-link lipid bilayers. The finding that it can also bind two dodecyl sulphate micelles reinforces this conclusion in that it provides evidence for two discrete binding sites on the protein. The earlier evidence did not allow distinction between cross-linking by pairs of basic protein molecules and single molecules (Fig. 4). By demonstrating that each molecule possesses two lipid binding sites the latter is seen to provide a feasible mechanism. Bridging also by pairs of protein molecules (Fig. 4a) cannot be completely discounted, but the behaviour of the protein in

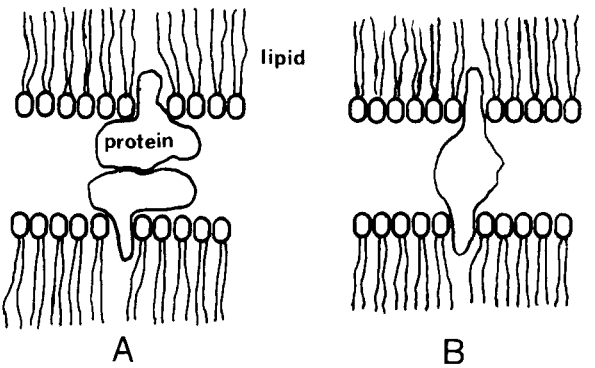

Fig. 4. Schematic illustration of two possible modes of lipid bilayer cross-linking by basic protein. It is not implied that the protein structure or the extent of bilayer penetration by the protein is necessarily as represented here.

detergent solutions at pH 7.4 has yielded no evidence for this mechanism.

Experiments with fragments of basic protein are expected to facilitate further definition of the nature of the interaction of both sites with lipids, and to provide a clearer insight into the molecular mechanism of cross-linking.

Acknowledgements

Financial support from the Australian Research Grants Committee and the Sydney University Research Grants Committee is gratefully acknowledged. We thank Drs. P.E. Braun and M.A. Moscarello for their kindness in allowing us to read several manuscripts prior to publication.

References

1 Sheffield, W.D. and Kim, S.U. (1977) Brain Res. 132, 580—584
2 Smith, R. (1977) Biochim. Biophys. Acta 470, 170—184
3 Braun, P.E. (1977) in Myelin (Morell, P., ed.), pp. 91—115, Plenum Press, New York
4 Rumsby, M.G. (1978) Biochem. Soc. Trans. 6, 448—462
5 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
6 Mukerjee, P. (1956) Anal. Chem. 28, 870—873
7 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
8 Smith, R., Dawson, J.R. and Tanford, C. (1972) J. Biol. Chem. 247, 3376—3381
9 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
10 Emerson, M.F. and Holtzer, A. (1967) J. Phys. Chem. 71, 1898—1907
11 Anacker, E.W., Rush, R.M. and Johnson, J.S. (1964) J. Phys. Chem. 68, 81—93
12 Williams, R.J., Phillips, J.N. and Mysels, K.J. (1955) Trans. Faraday Soc. 51, 728—737
13 Jones, A.J.S. and Rumsby, M.G. (1978) Biochem. J. 169, 281—285
14 Ackers, G.K. (1967) J. Biol. Chem. 242, 3237—3238
15 Tanford, C., Nozaki, Y., Reynolds, J.A. and Makino, S. (1974) Biochemistry 13, 2369—2376
16 Liebes, L.F., Zand, R. and Phillips, W.D. (1975) Biochim. Biophys. Acta 405, 27—39
17 Nozaki, Y., Schechter, N.M., Reynolds, J.A. and Tanford, C. (1976) Biochemistry 15, 3884—3890
18 Smith, R. (1978) in Myelination and Demyelination (Palo, J., ed.) pp. 221—234, Plenum Press, New York
19 Reynolds, J.A. and Tanford, C. (1970) Proc. Natl. Acad. Sci. U.S. 66, 1002—1007
20 Pitt-Rivers, R. and Impiombato, F.S.A. (1968) Biochem. J. 109, 825—830
21 Nelson, C.A. (1971) J. Biol. Chem. 246, 3895—3901
22 Simons, K. and Helenius, A. (1970) FEBS Lett. 7, 59—63

23 Makino, S., Reynolds, J.A. and Tanford, C. (1973) J. Biol. Chem. 248, 4926—4932
24 Takagi, T., Tsujii, K. and Shirahama, K. (1975) J. Biochem. 77, 939—947
25 Anthony, J.S. and Moscarello, M.A. (1971) Biochim. Biophys. Acta 243, 429—433
26 Liebes, L.F., Zand, R. and Phillips, W.D. (1976) Biochim. Biophys. Acta 427, 392—409
27 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—79
28 Steinhardt, J., Stocker, N., Carroll, D. and Birdi, K.S. (1974) Biochemistry 13, 4461—4468
29 Krigbaum, W.R. and Hsu, T.S. (1975) Biochemistry 14, 2542—2546
30 Small, D.M. (1971) in Bile Acids (Nair, P.P. and Kitchevsky, D., eds.), Vol. 1, pp. 249—356, Plenum Press, New York
31 Tanford, C. and Reynolds, J.A. (1976) Biochim. Biophys. Acta 457, 133—170
32 Robinson, N.C. and Tanford, C. (1975) Biochemistry 14, 369—378
33 Tanford, C. (1973) The Hydrophobic Effect, pp. 47—49, Wiley, New York
34 Tanford, C. (1974) J. Phys. Chem. 78, 2469—2479
35 Putnam, F.W. and Neurath, H. (1944) J. Am. Chem. Soc. 66, 692—697
36 Schäfer, R. and Franklin, R.M. (1975) FEBS Lett. 58, 265—268
37 Eylar, E.H., Brostoff, S., Hashim, G., Caccam, J. and Burnett, P. (1971) J. Biol. Chem. 246, 5770—5784
38 Chou, F.C-H., Chou, J., Shapira, R. and Kibler, R.F. (1977) J. Neurochem. 28, 1051—1059
39 Oshiro, Y. and Eylar, E.H. (1970) Arch. Biochem. Biophys. 138, 606—613
40 Shirahama, K., Tsujii, K. and Tagaki, T. (1974) J. Biochem. 75, 309—319
41 Reynolds, J.A. and Tanford, C. (1970) J. Biol. Chem. 245, 5161—5165
42 Kellaway, I.W. and Saunders, L. (1970) Chem. Phys. Lipids 4, 261—268
43 Jones, A.J.S. and Rumsby, M.G. (1975) J. Neurochem. 25, 565—572
44 London, Y. and Vossenberg, F.G.A. (1973) Biochim. Biophys. Acta 307, 478—490
45 Papahadjopoulos, D., Moscarello, M., Eylar, E.H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317—335
46 London, Y., Demel, R.A., Geurts Van Kessel, W.S.M., Vossenberg, F.G.A. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 311, 520—530
47 Demel, R.A., London, Y., Vossenberg, F.G.A., Geurts Van Kessel, W.S.M. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 311, 507—519
48 Boggs, J.M., Moscarello, M.A. and Papahadjopoulos, D. (1977) Biochemistry 16, 5420—5426
49 Mateu, L., Luzzati, V., London, Y., Gould, R.M., Vossenberg, F.G.A. and Olive, J. (1973) J. Mol. Biol. 75, 697—709
50 Smith, R. (1977) Biochim. Biophys. Acta 491, 581—590
51 Jones, A.J.S. and Rumsby, M.G. (1977) Biochem. J. 167, 583—591
52 Steck, A.J., Siegrist, H.P., Zahler, P. and Herschkowitz, N.N. (1976) Biochim. Biophys. Acta 455, 343—352
53 Banik, N.L. and Davison, A.N. (1974) Biochem. J. 143, 39—45
54 Golds, E.E. and Braun, P.E. (1978) J. Biol. Chem. 253, 8171—8177