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# Self-Association of Myelin Basic Protein: Enhancement by Detergents and Lipids†

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ABSTRACT: Self-association of basic protein has been proposed to be of functional significance in central nervous system myelin. In aqueous solution this protein self-associates, previous data being consistent with the formation of dimers, which then undergo an indefinite isodesmic self-association [Smith, R. (1980) *Bichemistry 19*, 1826–1831]. As this protein is membrane bound in vivo, we have now examined the effects of amphiphiles on the self-association equilibria. Contrary to the expected effects, at low molar ratios dodecyl sulfate, deoxycholate, Triton X-100, and lysophosphatidylcholine increased protein intermolecular attraction. The anionic detergents led to partial precipitation even at 1:1 protein:detergent molar ratios whereas the zwitterionic lipid and the nonionic detergent exerted less pronounced effects. Sedimentation velocity and equilibrium measurements have been used to define quantitatively the effects of lysophosphatidylcholine. The sedimentation coefficient increases up to a lipid:protein ratio of 4:1 and then remains constant up to a ratio of 12:1. The sedimentation equilibrium data suggest that the mode of protein–protein interaction is the same as in the absence of lipid but with substantially increased association constants. The dimerization constant is increased from $1.20 \times 10^2$ $M^{-1}$ to $1.0 \times 10^3$ $M^{-1}$ and the isodesmic association constant from $3.4 \times 10^4$ $M^{-1}$ to $1.2 \times 10^5$ $M^{-1}$. The effects of detergents on myelin basic protein are compared with the effects on other proteins, and the implications for the state of the protein with myelin are discussed.

Interest in the self-association of the basic protein of myelin has been promoted by the suggestion that dimers of this protein may stabilize the structure of central nervous system myelin (Smith, 1977b, 1978; Braun, 1977; Rumsby, 1978). The self-association data (Smith, 1980) are best described by a scheme involving initial dimer formation followed by an indefinite isodesmic association of dimers. It can, however, readily be argued that studies of the protein in aqueous solution are of limited relevance to its behavior in vivo. For example, one clear objection is that in vivo the protein does not exist alone but in intimate contact with lipids that may influence the properties of the protein, particularly its self-association. This objection does not diminish the force of the thermodynamic argument that a full description of the state of the protein in myelin must take into consideration self-association of free protein molecules but emphasizes that such equilibria may represent only a small perturbation on the behavior of the largely lipid bound protein.

We have previously observed that although the protein is monomeric at high dodecyl sulfate concentrations it is dimeric at lower concentrations, suggesting that specific protein–protein interactions may be retained even in the presence of moderate detergent concentrations (Smith & McDonald, 1979). The self-association equilibria have now been reexamined in the presence of low detergent concentrations. We have found that binding of just one or two amphiphile molecules per protein molecule suffices to markedly increase the association and that the protein–protein interactions persist over a wide range of amphiphile concentrations.

## Materials and Methods

Many of the methods have been outlined in an earlier publication (Smith, 1980): essential details only are set out here. Basic protein was prepared from fresh bovine white matter (Smith, 1977a,b). The protein was fractionated by chromatography on carboxymethylcellulose (Deibler & Martenson, 1973), and only the major peak, which eluted last, was kept. This fraction contained protein that had undergone no posttranslational modification, except for methylation of the arginine residue at position 109.

Sedimentation equilibrium experiments were performed on a Beckman Model E ultracentrifuge with interference optics. Aliquots of 150 μL of solvent and of solution were used in a double-sector cell with a capillary-type synthetic-boundary centerpiece and sapphire windows. In experiments where the meniscus was not depleted at equilibrium the actual concentration in the cell was determined in a subsequent synthetic-boundary experiment. Prior to the sedimentation experiments the protein was dissolved in buffer containing 0.1 M phosphate, 0.2 M sodium chloride, and 2 mM sodium azide and then dialyzed against this buffer for 48 h at 4 °C. The initial protein concentration in the sedimentation equilibrium experiments ranged from 0.3 to 2.5 g $L^{-1}$.

The apparent partial specific volume ($\phi'$) of the protein in detergent solution was calculated by using the equation (1 –

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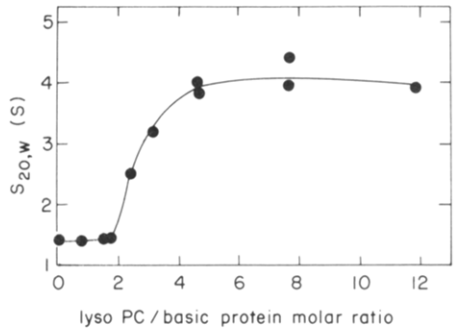


FIGURE 1: Dependence of the sedimentation coefficient of basic protein on the amount of added myristoyllysophosphatidylcholine. All measurements were made at a concentration of 3.0 g $L^{-1}$ at 20.0 °C with an accurately measured rotor speed near 44 700 rpm. The buffer was 0.05 M phosphate, 0.2 M sodium chloride, and 2 mM sodium azide, pH 7.5. The value plotted ($s$ = 3.22) at a molar ratio of 3.1:1 was obtained by using the maximum in the first four photographs of the sedimentation velocity experiment; in the last frame there were two just-resolved peaks.

$\phi'\rho) = (1 - v_p\rho) + \delta(1 - v_d\rho)$, where $\rho$ is the solvent density (1.01 g $cm^{-3}$), $v_\rho$ the partial specific volume of the protein [0.723 $cm^3$ $g^{-1}$ (Smith, 1980)], $v_d$ the partial specific volume of myristoyllysophosphatidylcholine (0.92 $cm^3$ $g^{-1}$) or Triton X-100 [0.908 $cm^3$ $g^{-1}$ (Tanford & Reynolds, 1976)], and $\delta$ the weight ratio of bound detergent to protein. The values assumed for $\delta$ are discussed under Results. Following sedimentation equilibrium experiments the integrity of all basic protein samples was checked by gel electrophoresis in dodecyl sulfate solutions.

Sedimentation velocity experiments were performed at 20.0 °C with a fixed protein concentration of 3 g $L^{-1}$. A double-sector synthetic-boundary centerpiece was used with initially 150 $\mu$L of protein solution in one sector and 350 $\mu$L of solvent in the other. The boundary movement was recorded with a schlieren optical system by using a bar angle of 60° and a rotor speed near 44 700 rpm.

For testing of precipitation by surfactants, 1 g $L^{-1}$ solutions of the basic proteins in buffer (0.05 M phosphate–0.2 M NaCl, pH 7.5) were slowly titrated with concentrated solutions of the surfactants in the same buffer. For solutions containing dodecyl sulfate the samples were left for 1 h after mixing and then centrifuged at 100000$g_{av}$ for 15 min at room temperature. Protein in the supernatant was determined colorimetrically with a final dodecyl sulfate concentration of 1% (w/v) in standards (basic protein) and samples. Similarly, in the colorimetric assay for doecyl sulfate (Mukerjee, 1956) corrections were made for the effect of the protein on the assay.

Ribonuclease (Calbiochem, 5 times crystallized), cytochrome $c$ (Calbiochem, A grade), and lyosozyme (Merck, electrophoretically homogeneous) were used without further purification. Sodium dodecyl sulfate (99%) was obtained from Pierce Chemical Co.; sodium deoxycholate, myristoyllysophosphatidylcholine, and egg lysophosphatidylcholine were from Sigma Chemical Co. (St. Louis, MO); Triton X-100 was from Rohm and Haas; hexadecyltrimethylammonium bromide and decyltrimethylammonium bromide were from K & K Laboratories (Plainview, NY).

## Results

*Association with Lysophosphatidylcholine.* Amphiphiles promote self-association of basic protein. These effects were examined in greatest detail with L-$\alpha$-myristoyllysophosphatidylcholine at concentrations below its critical micelle concentration.

Addition of this lipid below a lipid:protein molar ratio of 2:1 had no effect on the transport properties of the protein. At higher lipid concentrations a second, faster migrating component appeared that increased in proportion with increasing lysophosphatidylcholine:protein ratio (Figures 1 and 2). At ratios of 5 or above the protein again migrated with a single symmetrical boundary, of higher sedimentation rate. These changes in sedimentation coefficient may result not only from protein aggregation but also from alterations in the gross conformation of the protein. Conformational transitions have previously been observed spectroscopically on the addition of lysophosphatidylcholine to solutions of myelin basic protein (Keniry & Smith, 1979, 1981).

Sedimentation equilibrium experiments were performed at pH 7.5 with initial protein concentrations varying from 0.3 to 2.5 g $L^{-1}$ and with equilibrium concentrations varying from 0 g $L^{-1}$ at the meniscus to 5 g $L^{-1}$ at the base of the solution column in different runs. From the protein distributions at equilibrium the monomer concentration was deduced as a function of the total concentration by using the $\Omega(r)$ analysis of Milthorpe et al. (1975). Activity coefficients were taken as unity (Smith, 1980). The $\Omega(r)$ function is given by

$$\Omega(r) = [\bar{c}(r)/\bar{c}(r_F)] \exp[\theta_1 M_1(r_F^2 - r^2)] \quad (1)$$

where $\bar{c}(r)$ and $\bar{c}(r_F)$ are the total weight concentrations of protein at distances $r$ and $r_F$ from the center of rotation, $M_1$ is the monomer molecular weight, and

$$\theta_1 = (1 - \phi'_1\rho)\omega^2/(2RT) \quad (2)$$

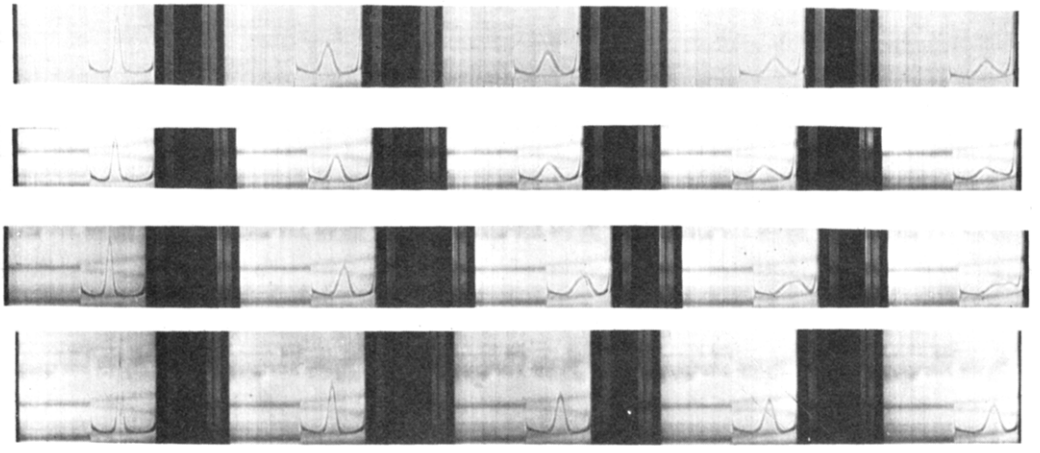

FIGURE 2: Schlieren photographs from sedimentation velocity experiments with bovine myelin basic protein (3 g $L^{-1}$) and varying amounts of lysophosphatidylcholine at pH 7.5. The lipid:protein molar ratios are, from top to bottom, 0.8:1, 1.6:1, 3.1:1, and 11.6:1. The photographs in each set were taken at 15-min intervals, except the last for which the interval was 8 min.

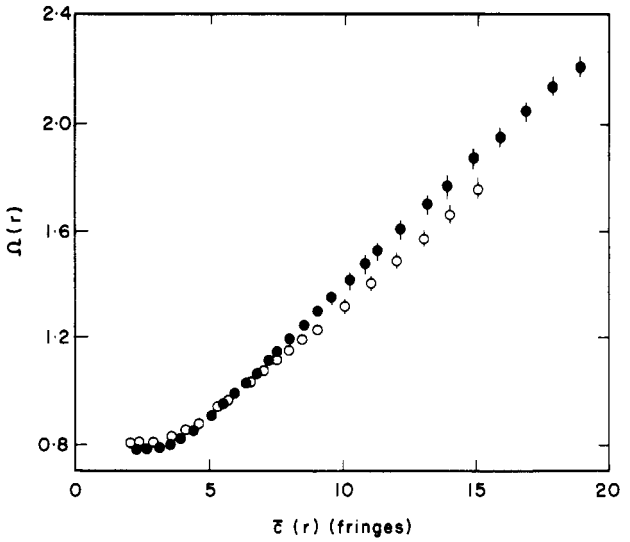


FIGURE 3: Data obtained by sedimentation equilibrium analysis at pH 7.5. The points represent the average values from six experiments performed with a lysophosphatidylcholine:protein molar ratio of 6:1 (●) and from four experiments at a molar ratio of 3:1 (○). The vertical bars show the range of $\Omega(r)$ values obtained at a given protein concentration: where there are no bars the spread of calculated values of $\Omega(r)$ was comparable to the width of the symbol. The omega function was calculated, with the total concentration at the reference point, $\bar{c}(r_F)$ = 6.0 fringes, for 25–30 points through the cell in each experiment.

In eq 2, $\phi'_1$ is the monomer apparent partial specific volume, $\rho$ is the solution density, $\omega$ is the angular velocity, $R$ is the gas constant, and $T$ is the absolute temperature.

$\Omega(r)$ is a measure of the degree of self-association at $r$ relative to that at $r_F$. Assuming no deviation from ideal solution behavior

$$\Omega(r) = \frac{\bar{c}(r)/c_1(r)}{\bar{c}(r_F)/c_1(r_F)} \quad (3)$$

As $\bar{c}(r)$ and $\bar{c}(r_F)$ are obtained experimentally and $\bar{c}(r) \rightarrow c_1(r)$ as $\bar{c}(r) \rightarrow 0$, eq 3 can be used with $\Omega(r)$ from eq 1 to determine the monomer concentration, $c_1$, at any radial distance. In this analysis all calculations were made with concentrations expressed in fringes. The molecular weight of the protein alone was taken as 18 400 and an effective partial specific volume calculated as described under Materials and Methods.

The values of $\Omega(r)$ are dependent on $\phi'_1$ (eq 2) and thus on the amount of lysophosphatidylcholine bound to the protein. Although at the low molar ratios used in this work allowance for bound detergent effects only minor changes in $\Omega(r)$, such corrections were made. Proton nuclear magnetic resonance ($^1$H NMR) experiments (R. Smith, unpublished experiments) have shown that basic protein possesses a small number of high-affinity sites for lysophosphatidylcholine, with almost quantitative binding up to a 6:1 lipid:protein molar ratio. At higher ratios the extent of binding is not significantly increased. With these figures the protein monomer molecular weight was also derived from the conventional sedimentation equilibrium graph of ln concentration against the square of the radial distance. This molecular weight, which is also dependent on $\phi'$, was consistently within 2% of the known value and provided an independent check on these estimates of bound detergent.

The extent of aggregation appears greater at a 6:1 lysophosphatidylcholine:protein ratio than at a ratio of 3:1 (Figure 3): the difference is outside the experimental errors. From several runs at low initial concentrations the limiting value of $\Omega(r)$, at low concentration, was in the range 0.789–0.802: the

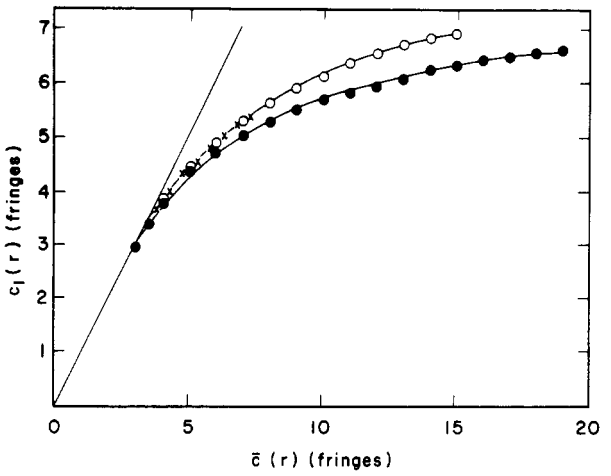


FIGURE 4: Association of bovine myelin basic protein in the presence of a 3:1 (○) and a 6:1 mole ratio (●) of lysophosphatidylcholine:protein and in the presence of Triton X-100 at an 8:1 molar ratio (×). The data for Triton X-100 were obtained from three experiments spanning three different, but overlapping, concentration ranges. The solid curves represent the theoretical lines calculated with the association constants in Table I. The diagonal line at left represents $c_1(r) = \bar{c}(r)$, the relationship expected in the absence of self-association.

Table I: Dimerization ($K_1$) and Indefinite Isodesmic Association ($K_2$) Constants for Bovine Myelin Basic Protein at pH 7.5 in the Presence and Absence of Lysophosphatidylcholine

| | $K_1$ ($M^{-1}$) | $K_2$ ($M^{-1}$) |
|---|---|---|
| in the absence of lipid[a] | $1.28 \times 10^2$ | $3.4 \times 10^4$ |
| at a 3:1 lipid:protein ratio | | |
| including lipid refractive index contribution | $9 \times 10^2$ | $9 \times 10^4$ |
| excluding lipid refractive index contribution | $8 \times 10^2$ | $9 \times 10^4$ |
| at a 6:1 lipid:protein ratio | | |
| including lipid refractive index contribution | $1.0 \times 10^3$ | $1.2 \times 10^5$ |
| excluding lipid refractive index contribution | $8 \times 10^2$ | $1.2 \times 10^5$ |

[a] From Smith (1980).

former value was adopted for all calculations as the experiments yielding this value gave the most accurate figures for the monomer molecular weight.

By use of the limiting value of 0.789 for $\Omega(r)$, the monomer concentration, $c_1(r)$, was calculated as a function of the total concentration, $\bar{c}(r)$, with eq 3 (Figure 4). Attempts were made to fit these data by using several models tested earlier (Smith, 1980). The most satisfactory correspondence was provided by the association scheme that was compatible with the data obtained in the absence of lipid: dimerization followed by an indefinite isodesmic (i.e., equal stepwise equilibrium constants) association. But whereas the onset of aggregation was evident above 3 g $L^{-1}$ in the absence of lysophosphatidylcholine, in its presence self-association was detected below 1 g $L^{-1}$.

The equilibrium constants $K_1$ (for dimerization) and $K_2$ (for indefinite association) were calculated by fitting the data to the equation (Jeffrey et al., 1976)

$$\bar{c}(r) = \frac{M_1 m_1(r)[(1 - K_1K_2[m_1(r)]^2)^2 + 2K_1 m_1(r)]}{(1 - K_1K_2[m_1(r)]^2)^2} \quad (4)$$

where $K_1 = m_2(r)/[m_1(r)]^2$, $K_2 = m_{i+2}(r)/[m_2(r)m_i(r)]$, and $m_i$ is the molarity of the $i$th oligomer.

Table I presents the constants derived for the data obtained at lipid:protein molar ratios of 3:1 and 6:1. Calculation of $K_1$ and $K_2$ was performed in two ways. Initially it was assumed that the bound lipid did not contribute to the refractive index

increment of the protein: the observed concentrations in fringes were converted to *protein* weight concentrations by using a value of 3.94 fringes for a 1 g $L^{-1}$ solution. These figures were then recalculated with a refractive index increment deduced from synthetic-boundary experiments for bound lysophosphatidylcholine: thus the concentrations in fringes were divided by 4.15 (3:1 lysophosphatidylcholine:basic protein) or 4.36 (for 6:1) to derive the *protein* weight concentration in grams per liter. These corrections were found to have only minor effects on the derived association constants (Table I).

In Figure 4 the behavior predicted on the basis of these constants is compared with the experimental results. Although there is some spread in the data obtained at even a single amphiphile:protein ratio, there is little latitude in the values of $K_1$ and $K_2$ that are consistent with the data. At each lipid:protein ratio the most satisfactory fit was provided by the association constants given in Table I. For 6:1 lysophosphatidylcholine:protein samples adequate, but clearly inferior, fits were provided by other values in the range $K_1 = 1.1 \times 10^3$ $M^{-1}$ with $K_2 = 1.1 \times 10^5$ $M^{-1}$ through to $K_1 = 8.0 \times 10^2$ $M^{-1}$ with $K_2 = 1.6 \times 10^5$ $M^{-1}$ (with lipid refractive index corrections). Changing one of the association constants, without a compensating change in the other, caused a marked change in the predicted behavior: thus, with $K_2$ held constant only an 11% change in the value of $K_1$ obtained for a 6:1 ratio is required to fit the data obtained at a 3:1 ratio.

Experiments performed at a 10:1 lysophosphatidylcholine: basic protein molar ratio, spanning a concentration range from two fringes to seven fringes, gave results essentially coincident with those obtained at a 6:1 ratio, consistent with the sedimentation coefficient measurments, which indicated no change in self-association between lipid:protein ratios of 5:1 and 12:1 (Figure 1).

*Association with Triton X-100.* The nonionic detergent Triton X-100 also increased the protein self-association (Figure 4). Again the effect was evident at a 3:1 detergent:protein mole ratio and appeared unchanged at a 10:1 ratio. The self-association, as with lysophosphatidylcholine, was evident above a protein concentration of 1 g $L^{-1}$ and gave similar values for the association constants. The data in Figure 4 were obtained by adding an 8:1 mole ratio of Triton X-100:protein, with an equivalent detergent concentration added to the solvent sector of the double-sector ultracentrifuge cell: under these conditions the refractive index mix–match between the sectors caused by the binding of a small number of molecules of Triton is expected to be small.

*Association with Ionic Detergents.* At low detergent:protein molar ratios dodecyl sulfate caused precipitation of the basic protein. This is not a general phenomenon for basic proteins, as under similar conditions lysozyme precipitated but ribonuclease and cytochrome *c* did not, even at detergent:protein ratios up to 300:1.

Precipitation was also observed with deoxycholate but not with uncharged detergents (see above) nor with the cationic detergents Brij 35, hexadecyltrimethylammonium bromide, and decyltrimethylammonium bromide up to detergent:protein molar ratios of 50:1.

By centrifuging solutions prepared with a range of initial dodecyl sulfate concentrations, it was possible to analyze the composition of the solid phase (Figure 5). At low detergent concentrations the precipitate contained only five to eight detergent molecules per protein molecule, and the complex should still bear a substantial net positive charge. At higher concentrations the proportion of detergent in the precipitate increased, and the amount of protein remaining in solution

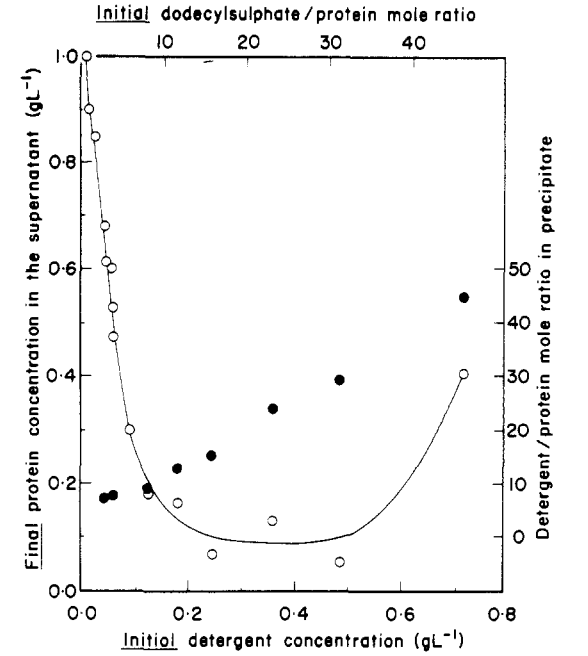


FIGURE 5: Precipitation of bovine myelin basic protein by sodium dodecyl sulfate at pH 7.5. Small aliquots of a concentrated detergent solution were added to 1 mL of a 1 g $L^{-1}$ protein solution at room temperature. The resultant precipitate was removed by centrifugation and the supernatant analyzed for protein (○) and detergent. From these figures the detergent:protein ratio in the precipitate (●) was calculated.

decreased, reaching a minimum at the point where the detergent in the precipitate was almost sufficient to neutralize the charge on the protein (neglecting counterion binding). At higher binding ratios the protein again became soluble. Figure 5 qualitatively follows the solubility curves published for several other proteins (Putnam & Neurath, 1944).

## Discussion

Previously it has been shown that in the absence of detergent myelin basic protein undergoes a weak ($K_1 = 128$ $M^{-1}$) dimerization that appears to result from predominantly hydrophobic interactions (Smith, 1980).[1] The association constant for aggregation of these dimers is appreciably higher, implying that some change occurs in the protein on dimerization that generates the second protein–protein interaction region or enhances a latent attraction. As there is evidence that this protein is able to penetrate the acyl chain region of lipid bilayers (Keniry & Smith, 1980), it is conceivable that within myelin hydrophobic regions on the protein would interact preferentially with lipid rather than with other protein molecules, thereby preventing protein–protein interactions.

The present study was therefore undertaken to assess whether the protein self-association that is observed in lipid-free solutions could occur within myelin. Recombinants with bilayer vesicles would have provided a closer parallel to the in vivo environment of the protein. But the hydrodynamic properties of such recombinants are largely determined by the vesicles themselves, obscuring the properties of attached protein. By using lysophosphatidylcholine at submicellar

[1] An interesting parallel may be drawn with glucagon, a polypeptide that, like myelin basic protein, appears largely unstructured in aqueous solution. At higher concentrations glucagon aggregates with the intermolecular contacts, stabilizing some helical structures. In the crystalline form these contacts, and the helical structure, are increased with the protein intermolecular contact regions being provided by hydrophobic domains (Wagman et al., 1980).

concentrations, it has been possible to follow in some detail the changes in protein properties induced by this amphiphile.

Lysophosphatidylcholine does cause a marked increase in the protein self-association. The effect occurs over a narrow range of detergent concentrations: binding of two molecules of lysophosphatidylcholine induces no change in aggregation, but association with a further two molecules leads to a rapid change in the sedimentation coefficient, which is not further increased by addition of excess detergent (Figure 1). As the equilibrium data in the presence of detergent are satisfactorily fitted by using the same aggregation scheme that describes the detergent-free self-association, the detergent appears to effect quantitative rather than qualitative changes in the mode of aggregation.

Lysophosphatidylcholine is known to promote a coil-to-helix conformational transition in part of the protein molecule (Keniry & Smith, 1979, 1981). It is possible that this transition is linked to the marked enhancement of the dimerization and indefinite associations of the protein by lysophosphatidylcholine (Table I). Whatever the mechanism, it is clear that association with a number of molecules of this zwitterionic detergent does not prevent the protein self-association; indeed, it facilitates it.

As Triton X-100, a nonionic detergent, induces similar changes to lysophosphatidylcholine, the primary effect appears dependent on the hydrophobic moieties of these molecules. The more pronounced aggregation at low dodecyl sulfate:protein ratios presumably results from the additional ability of this anionic molecule to reduce the electrostatic repulsions between the highly cationic protein molecules (see Figure 5). In comparison with uncharged amphiphiles, the situation with dodecyl sulfate may more closely mimic the myelin membrane, with its high content of acidic lipids.

Several mechanisms have been proposed for the precipitation of myelin basic protein by dodecyl sulfate (Jones & Rumsby, 1978; Smith & McDonald, 1979; Burns et al., 1981). Precipitation of other proteins is not dependent solely on reduction of protein net charge by bound detergent. We have, for example, found that lysozyme ($pI \simeq 11$), but not cytochrome *c* ($pI \simeq 11$) nor ribonuclease ($pI \simeq 9.5$), was precipitated by dodecyl sulfate at pH 7.5. Furthermore, dodecyl sulfate causes aggregation of casein even above its isoeletric point (Creamer, 1980). Hence, at low molar ratios this anionic detergent may act in a similar manner to lysophosphatidylcholine and Triton X-100 in promoting self-association of myelin basic protein.

Finally, the observation that basic protein self-association is not eliminated by detergents is consistent with earlier detection of protein dimers in dodecyl sulfate solutions (Smith & McDonald, 1979; Golds & Braun, 1978a) and in myelin (Golds & Braun, 1978b) and lends credence to the concept that these intermolecular interactions are retained, and possibly even enhanced, on binding of the protein to the myelin membrane.

## Acknowledgments

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