BRIEF COMMUNICATION

THE EFFECT OF BASIC MYELIN PROTEIN ON MULTILAYER MEMBRANE FORMATION

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The myelin membrane is composed of 75–80% lipids and 20–25% proteins. The lipids are organized into regular multilayers, with the protein incorporated in them. The protein fraction has been found to be relatively simple in comparison with that of plasma membranes, in that two types of protein make up 70–85% of the total membrane protein (Boggs and Moscarello, 1978*a*). The first of these, the basic protein, makes up about 35% of the total protein, whereas the second, the proteolipid, makes up about 50%. The proteolipid consists mainly of lipophilin with lesser amounts of Wolfgram proteins, some glycoprotein, and a few enzymes.

We have been engaged in a series of studies on the interaction of these two types of protein with phospholipid, using liquid x-ray diffraction techniques. The first of these, on the lipophilin (N-2)-phosphatidyl choline system (Brady et al., 1979*a,b,c*) showed that this protein was incorporated into the interior of the bilayer in highly associated form, and that as more protein was incorporated, the multilayer structure was broken down to give way to single bilayers. Because the myelin membrane in its native form is a highly organized multilayer structure, it is of interest to see how the incorporation of the hydrophilic basic protein affects the multilayer structure and whether it counterbalances the disrupting effect of the lipophilin. It is also important to determine whether the basic protein exists in extended form in the membranes or whether it too is associated. In this brief communication, we present results of some studies on the interaction of basic protein with phospholipid, using egg phosphatidyl glycerol (PG) as a prototype acidic lipid. PG was used because it is easily obtained and interacts well with basic protein to form stable liposomes. The interaction is similar to that of other acidic lipids, including nerve myelin (phosphatidyl serine, cerebroside sulphate) which has been extensively studied by DSC, ESR and NMR. The results shed light on the two questions raised above.

Basic protein was purified from normal white matter (Lowden et al., 1966). Vesicles containing the protein were prepared according to Boggs and Moscarello (1978*b*). Egg PG was dissolved in 100% redistilled 2-chloroethanol, 5 mg/ml. Lyophilized myelin basic protein was dissolved in 100% redistilled 2-chloroethanol, 5 mg/ml. The lipid and protein solutions

were combined, giving a total volume of 5 ml and dialyzed against 2 liters of 2 mM Hepes buffer, pH 7.4. The contents of the dialysis bag were centrifuged at 35,000 rpm (Beckman ultracentrifuge, type K rotor Beckman Instruments, Spinco Div., Palo Alto, Calif.) for 30 min. The pellets obtained were suspended in the above buffer. Intensities were measured at 23°C with a position-sensitive detector and corrected for background by subtracting the scattering from the cell filled with buffer. These background-corrected curves are shown in Fig. 1. The lipid concentrations were in the range 50–60 mg/ml. The protein content of each sample is listed in the legend to Fig. 1.

We discuss first the scattering at the lowest angles, roughly the range $s < 0.06$. The fact that the pure lipid curve 1 is minimal in this region serves conveniently to isolate the protein-dependent part of the curve from the rest of the pattern. On addition of protein there is a marked increase in the scattering (this effect was also noted in the earlier work on the hydropholic protein, Brady et al., 1979). The relative intensities of this inner scattering in curves 2, 3, 4, and 5 are in the ratios of 1:2.0:3.8:7.7, agreeing within experimental error with the corresponding ratios of the protein concentrations, 1:2.0:4.0:7.5. Scattering of this type, concentrated around the zero angle is identified in liquid systems with the scattering arising from an extended region of scattering matter such as a particle. From the protein dependence we identify the particle with a protein molecule, an identification reinforced by the fact that the shape of these zero-angle curves is invariant with respect to the protein concentration, as would be expected for an increasing number of nonassociated protein molecules. The identification is further strengthened by the plots in Fig. 2. There is a Guinier plot of the

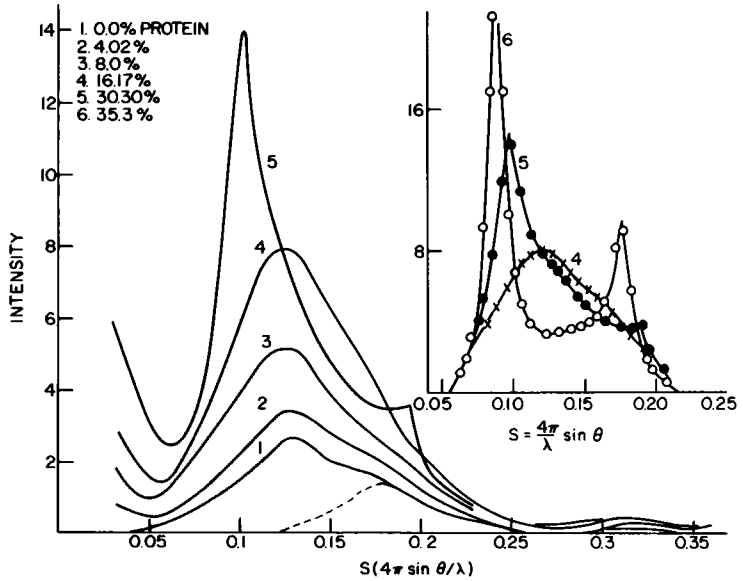

FIGURE 1 X-ray scattering curves of the membrane samples, normalized to the same lipid concentration. The protein concentrations are in weight percent. The inset shows the curves for the higher protein concentration. The dashed line sketched in curve 1 is an approximate resolution of the left-hand side of the bilayer peak contribution. The abscissa is $s = 4\pi/\lambda \sin\theta$, where λ is the wavelength and θ is one half the scattering angle.

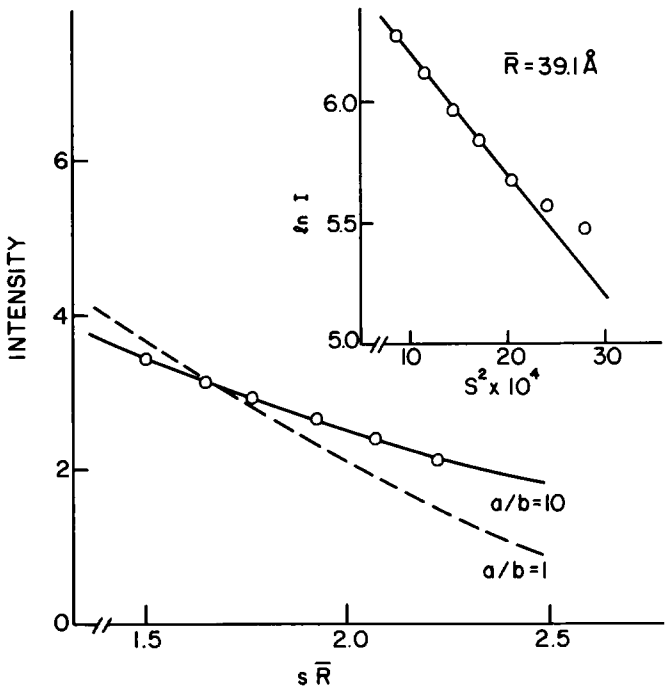

FIGURE 2 The inner portion of the scattering curves of Fig. 1, plotted as a function of $s\bar{R}$, where $\bar{R}$ = 39Å, is the radius of gyration; a/b = 1.1 is the theoretical curve for a sphere and a/b = 10 is for a prolate ellipsoid (or a cylinder) of axial ratio 1:10. The inset shows a Guinier plot of the innermost angles. The intensity data used are the mean of the values determined from all the experiments on the protein-containing membranes. *I*, intensity.

innermost data points of the inner scattering shown in the insert, from whose slope a radius of gyration of 39.1 Å is found. Using this value of $\bar{R}$, the intensities of the outermost data points of the inner region are plotted against $s\bar{R}$ in the main part of the graph. A plot of this type is more descriptive than the usual ln I vs. s^2 Guinier plot in that the dependence on particle shape is specifically included in it. Also shown in the Fig. 2 are the scattering curves for particles corresponding to the same radius of gyration with axial ratios of 1:1 and 1:10 (Guinier, 1955). It can be seen that within experimental error the data fit the 1:10 curve over the whole scattering range. The radius of gyration and axial ratio agree with the values that were determined for the isolated basic protein molecule by Epand et al. (1974) using small-angle scattering and electron microscope techniques. These values show that, in sharp contrast to the lipophilin particle, basic myelin protein exists in these model membranes in nonassociated form. The intensity that is scattered by the basic protein is at least one order of magnitude less than that scattered by lipophilin at the same concentration, again pointing to the contrasting states of association of the two protein types.

In the outer region of the scattering curves in Fig. 1 between $s = 0.06$ and $s \simeq 0.25$, the most noticeable feature is the presence of a broad diffuse peak in the region of $s \simeq 0.13$. Unlike the inner scattering, this peak occurs in both the pure phospholipid and protein-containing curves, but as shown in curves 2–5, it is also sensitive to the presence of protein. With small to

moderate amounts of protein (curves 2–4) there is a marked increase in intensity of the broad peak; with still higher amounts the peak sharpens significantly and shifts to smaller angle (curves 5–6).

The broad peaks at $s = 0.13$ in curves 1–4 are markedly asymmetrical, indicating that they are composed of two overlapping components. The first and major component has its maximum centered at $s = 0.127$; the second appears in curves 1 and 2 as a pronounced shoulder with a maximum at $s \simeq 0.18$. It is still evident in curve 4, although it is obscured by the main peak, which has now become much more intense. The presence of two peaks follows directly from theory. Curves 1–4 are of the type characteristic of liquids and for such systems the scattered intensity is given by the Debye equations:

$$I = \sum_i \sum_j \rho_i \rho_j \frac{\sin s r_{ij}}{s r_{ij}},$$

where the double sum is over all distances r_{ij} between pairs of points (i,j) with local electron densities ρ_i and ρ_j. Liquids have no long-range order. Accordingly, the intensities scattered by all pairs of points are summed, in contrast to the amplitudes scattered from planes, as in the crystallographic case, in which the scattering is essentially given by a lattice function that samples the bilayer (unit cell) scattering at angles corresponding to the multilayer repeat. In a liquidlike system of irregular bilayers loosely organized into multilayers, distances r_{ij} both between point pairs within each bilayer and between point pairs in neighboring bilayers contribute to the Debye equations. Thus the double sum would be expected to have two peaks, one corresponding to the mean separation between bilayers and one corresponding to the mean bilayer thickness. The maxima occur at $sr_{ij} = 7.72$ (the first maximum of the $\sin sr_{ij}/sr_{ij}$ function). Inserting the two values of s found above for the position of the maxima, we get 60.8 Å and 42.9 Å for the two corresponding correlation distances. The first distance can be identified at once with the distance between adjacent bilayers. We note that in agreement with freeze-fracture studies (Mateu et al., 1973),[1] which show an increase in the extent of multilayer ordering, this peak also increases in magnitude with increasing protein content. The second distance, 42.9 Å, is consistent with the bilayer thickness. The inter-bilayer distance is probably accurate to 1%; the error in the inter-head group bilayer distance is $\sim \pm 2$ Å, because it appears as a shoulder on the other peak. Because of the extensive peak overlap, a resolution of the two peak areas could not be accomplished to an accuracy 15–20% and was not attempted (the bilayer peak sketched in Fig. 1 is for illustrative purposes only).

The marked tendency of the protein to promote the multilayer formation, already evident in curves 1–4 in Fig. 1, is even more strikingly apparent in the curves for the higher protein concentrations (see insert). The multilayer scattering manifested in curve 4 (16% protein) by the broad maximum centered at $s \simeq 0.127$ sharpens in curve 5 (30.3%) into a definite peak shifted inwards to $s \simeq 0.096$, with a second-order peak appearing at $s \simeq 0.19$. Above 30.3% a regular repeat pattern is seen, with two sharp reflections at $s = 0.09$ and $s = 0.18$. The inward shift of the peak is not necessarily indicative of an increasing distance between the bilayers. Alternatively, the broad bands characteristic of the liquid state, whose maxima are specified

[1]Boggs, J. M. 1980. Personal communication.

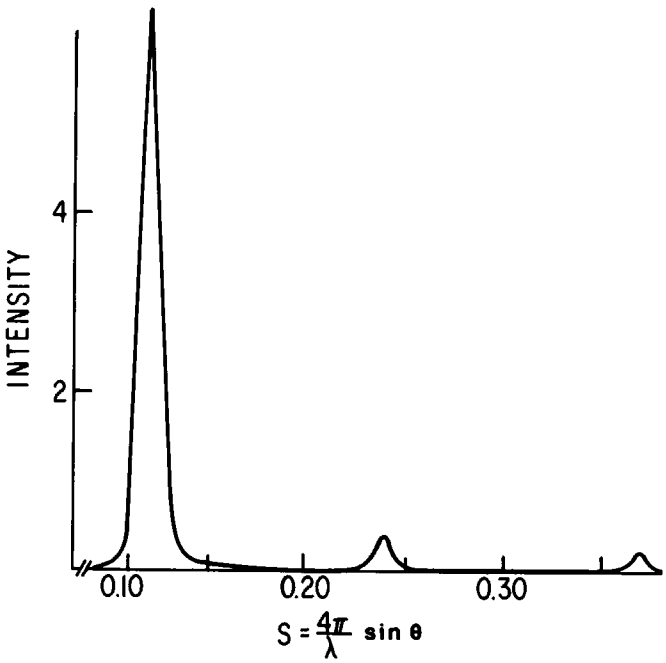

FIGURE 3 Intensity pattern of egg PG into which poly-L-lysine has been incorporated.

by the relation $s = 7.72/r$, may have given way to peaks more characteristic of a crystalline ordering obeying the Bragg's law relation $s = 2\pi/r$. The transformation is reminiscent of a phase transition. Attempts to determine whether there was a critical protein concentration above which the membranes transformed into the highly ordered form were not conclusive. On occasion, 30% protein samples, after standing for a few days, gave scattering curves that differed from those of freshly prepared samples and resembled those of samples of higher (>30%) concentration. It thus appears that there are time effects, and these must be accounted for before deciding whether the transition is abrupt or gradual with respect to the protein content.

Our results strongly suggest that one of the primary roles of the basic protein in the myelin membrane is to produce and maintain the highly ordered spiral pattern found in the natural system. The detailed mechanisms by which the basic protein exerts its effects is not clear. Freeze-fracture micrographs (Mateu et al., 1973)[1] show no evidence of intermembrane particles, so the protein must be incorporated into the surface region of the membrane. That some neutralization of the surface PG group is involved is strikingly demonstrated by the fact that, as shown in Fig. 3, a perfect repeat pattern is obtained when poly-L-lysine instead of the basic protein is incorporated into the egg PG. At pH 6.8, all of the lysine residues of poly-L-lysine, pK = 10.4, are positively charged.

Lipophilin, on the other hand, produces unilamellar vesicles when incorporated into either neutral phosphatidyl choline (PC) or acidic phosphatidyl serine (PS) bilayers (Papahadjopoulos et al., 1975; Vail et al., 1974). PS, similar in its net negative charge, is completely analogous to PG. Significantly, micrographs of these vesicles show the presence of intermembrane particles.

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