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X-RAY SCATTERING STUDIES OF A MODEL COMPLEX OF LIPID AND BASIC PROTEIN OF MYELIN

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Low-angle and wide-angle X-ray scattering data from phosphatidylglycerol complexed with myelin basic protein, poly(L-lysine) and calcium ions are analyzed. The results confirm our earlier report (Brady, G.W., Murthy, N.S., Fein, D.B., Wood, D.D. and Moscarello, M.A. (1981) Biophys. J. 34, 345–350) that the basic protein interacts primarily with the polar headgroups of the lipid; and that at high protein concentrations (> 35%) the bilayers aggregate to form multilayers with a repeat period of 68 Å, the single bilayer to multilayer transition being a cooperative process. Polylysine and Ca^{2+} produce multilayers with a smaller repeat of approx. 55 Å. Basic protein and polylysine do not change the fluid-like arrangement of the hydrocarbon chains (diffuse 4.6 Å peak in the wide-angle pattern), whereas Ca^{2+} probably induces a two-dimensional order (4.3 Å and 3.9 Å peak in the wide-angle pattern). Electron density profiles of the lipid and lipid-basic protein vesicles indicate that the basic protein penetrates into the bilayer.

Introduction

Myelin has long been used as a model system for studying the structure of membranes. Two major proteins, one hydrophobic and the other hydrophilic, have been found to constitute most of the unusually low protein content (20–25% by weight) of myelin [1]. The basic protein, which comprises about 35% of the myelin protein, has attracted a great deal of interest because of its possible role in demyelinating disorders such as multiple sclerosis, although recent data have implicated the hydrophobic protein as well [2].

Evidence to date suggests that the basic protein is located at the cytoplasmic surface of the membrane [3–6] and may be responsible for maintaining the lamellar structure of myelin. The mechanism by which basic protein interacts with adjacent bilayer is not known, although both electrostatic and hydrophobic interactions have been implicated. In this paper we analyze the results of X-ray diffraction experiments on mixtures of basic protein and phosphatidylglycerol (PG), poly(L-lysine) and PG, and calcium ions and PG, thus extending the investigations of Brady et al. [7] in which lipid-protein interactions and the localization of basic protein in the bilayer were discussed. The results of the present X-ray diffraction data indicate that the basic protein interacts primarily with the head groups of the lipid bilayer.

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering.

Materials and Methods

Egg PG which had been stored in chloroform at −70°C was taken to dryness under nitrogen and then lyophilized for further 4–6 h. The egg PG was dissolved in 100% freshly redistilled 2-chloroethanol, 5 mg/ml. Myelin was isolated from normal human white matter and basic protein extracted from it by the method of Lowden et al. [8]. The lyophilized myelin basic protein was dissolved in 100% redistilled 2-chloroethanol, 5 mg/ml. The lipid and protein were combined in the desired ratio and then dialyzed against 2 litre of 2 mM Hepes buffer, pH 7.4, containing 10 mM NaCl and 0.1 mM EDTA. The dialysis bath was changed three times. The contents of the dialysis sac were centrifuged at 40000 rpm for 30 min, resuspended in fresh buffer and centrifuged twice more. For analyses, the pellets were suspended in 0.5 ml of vesicle buffer. Measurements were done on samples with the protein to lipid ratio ranging from 0:100 to 45:55. The protein to lipid ratio was determined by amino acid analysis after hydrolysis with 5.7 M HCl, under vacuum for 18 h at 110°C followed by analysis on a Durrum D-500 amino acid analyzer, and by measuring the phosphorus content of the vesicles [9].

The X-ray scattering measurements were done with Cu Kα radiation. Small-angle X-ray scattering (SAXS) data were obtained with a position sensitive detector mounted on a Franks' camera. A pair of delimiting slits were used at right angles to the single focusing mirror to approximate pin-hole collimation. Wide-angle measurements were done using a Philips Powder diffractometer. A 1 mm thick flow cell [10] with 2 mil thick beryllium windows was used. The measurements were done in fixed time mode (200 s) in steps of 0.2° 2θ from 8° to 30° 2θ. Wide-angle data were also obtained using a flat plate camera. All the measurements were performed at 20°C. The background was measured by filling the sample cell with the buffer and was subtracted from the solution scattering curves.

Results and Analysis

The background corrected small-angle X-ray scattering (SAXS) curves for lipid (curve a) and lipid-protein complexes (curve B, 21% protein) are shown in Fig. 1. The intensities have been normalized to the lipid concentration. From these and other curves obtained at various protein concentrations, it was concluded that there were no dramatic changes in the general features of the scattering profiles as the protein to lipid concentration was increased from 0:100 to 35:65. This suggest that the curves a and b can be regarded as primarily arising from monolayers. The small value of intensity at $h = 0$ in curve a for the pure lipid reflects the small net electron density difference between the lipid bilayer and water. This difference increases considerably upon adding protein to the lipid bilayer (curve b).

At protein concentrations, higher than 35%, multilayers with a repeat period of 68 Å were observed (Fig. 2A). The physical appearance of the samples also indicated the formation of multilayers: Lipid and lipid-protein complexes up to 35:65 weight ratio formed homogeneous suspensions; on the other hand, 45:55 sample tended to settle to the bottom, and extra care was necessary during the data collection to prevent the samples from settling and moving out of the X-ray beam. Formation of such multilayers is probably due to the decrease in the electrostatic repulsion between vesicles composed of acidic PG molecules.

In order to mimic the electrostatic effects of the basic protein, after collecting the data from pure

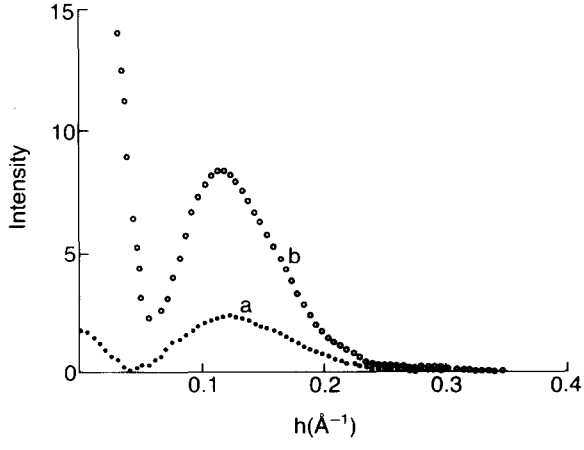

Fig. 1. Observed small-angle X-ray scattering (SAXS) intensities for phosphatidylglycerol (PG and PG + basic protein complexes after subtracting the solvent scattering. (a) Pure lipid (filled circles); (b) 79% lipid and 21% protein (open circles).

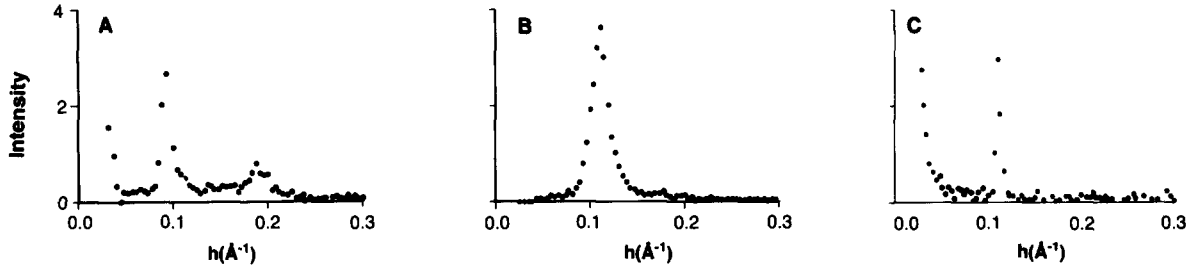

Fig. 2. SAXS curves from stacked lamellae: (A) Basic protein-phosphatidylglycerol (PG) complex, weight ratio 45:55. (B) PG complexes with Ca^{2+}. (C) PG complexes with poly(L-lysine).

PG (Fig. 1a) the sample was dialyzed against 10 mM $CaCl_2$ in Hepes buffer pH 7.4 (10 mM Ca^{2+} has been shown to be sufficient to cause compaction of myelin [11]). The resulting diffraction pattern (Fig. 2B) clearly indicates the formation of stacked lamellae. The repeat period for this sample is approx. 55 Å and is smaller than the 68 Å obtained with basic protein. By dialyzing this Ca^{2+} complexed lipid against Hepes buffer into which EDTA was added as chelating agent, it was found that the aggregation was completely reversible. In other experiments, the PG vesicles were prepared from 2-chloroethanol by dialysis against 2 mM Hepes buffer pH 7.4 containing 10 mM Ca^{2+}. The diffraction results were similar for the two methods of preparation.

A more striking demonstration of the electrostatic effect is seen in the small angle X-ray pattern obtained when poly(L-lysine) was incorporated into egg PG at a pH of 7.4 at which pH all the lysine residues (pK 10.4) are positively charged (Fig. 2C). The repeat period obtained with poly(L-lysine), approx. 57 Å, is close to that observed with calcium ions. Although poly L-lysine and Ca^{2+} have been shown to have similar effects on the transition temperature of PG bilayers [12], wide-angle X-ray patterns show that unlike Ca^{2+}, poly(L-lysine) does not have any effect on the packing of the hydrocarbon chains.

Wide-angle X-ray scattering data obtained from lipid and lipid-protein mixtures (Fig. 3 and Table I) were used to analyze changes in the organization of the hydrocarbon chains in the bilayer. The 4.6 Å wide-angle peak at $h = 1.37$ Å^{-1}, $h = (4\pi \sin\theta)/\lambda$, θ being the Bragg angle, arises from the side to side arrangement of the hydrocarbon

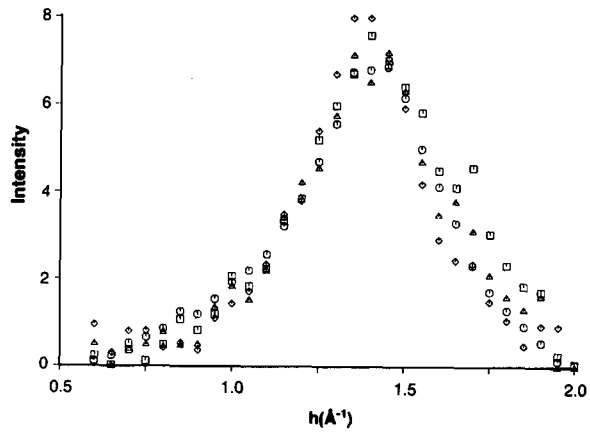

Fig. 3. Wide-angle diffraction patterns from various lipid-protein complexes. Net intensity, obtained after subtracting the background due to the solvent, is shown in the figure. Protein to lipid weight ratios for the four curves are: Circles, 0:100, triangles, 20:80; squares, 35:65, diamonds, 45:55.

TABLE I

X-RAY DIFFRACTION DATA IN THE FOR OF *d*-SPACINGS (Å) FROM SMALL-ANGLE (SAXS) AND WIDE-ANGLE (WAXS) MEASUREMENTS OF THE VARIOUS PHOSPHATIDYLGLYCEROL (PG) COMPLEXES

The *d*-spacings are obtained using the Bragg's relation $\lambda = 2d \sin\theta$. Lys, poly(L-lysine); BP, basic protein.

Sample	*d* spacing (Å)	
	SAXS [a]	WAXS
PG	50.9[d]	4.6
PG + Ca^{2+}	55.5[1], 28.9[2], 18.7[3]	4.3, 3.9
PG + Lys	55.5[1], 27.6[2], 18.4[3]	4.6
PG + < 21% BP	54.3[d]	4.6
PG + > 35% BP	68.0[1], 34.0[2]	4.6

[a] Superscripts: [1, 2, 3], are order of reflection; [d], indicates a diffuse band.

chains packed with a liquid-like disorder. As can be seen from curves in Fig. 3, there are no systematic differences in the intensity profiles of the samples with and without the basic protein. This confirms our earlier conclusion [7] that the basic protein interacts primarily with the polar headgroups and its effect on the lipid chain interactions in the hydrocarbon interior of the bilayer are minimal. This is in contrast with the effect of the hydrophobic myelin proteolipid protein, lipophilin, which has been found to make the hydrocarbon interior of phosphatidylcholine bilayers more fluid [13].

Table I shows that poly(L-lysine) which causes the vesicles to form multilayers, but with a smaller repeat of 57 Å compared to lipid-basic protein (> 35%) complex, gives rise to the same 4.6 Å reflection suggesting fluid-like arrangement of the hydrocarbon chains. Ca^{2+} however, gives rise to two sharp reflections at 4.3 and 3.9 Å. This might be due to the two-dimensional ordering of the hydrocarbon chains [14] and possibly to the tilting of the hydrocarbon chains with respect to the plane of the bilayers [15].

We can analyze the effect of the basic protein on the lipids in more detail by calculating the electron density profiles of the PG and PG + basic protein vesicles from their SAXS profiles. A pure lipid bilayer is symmetric, and because of the manner in which lipid-protein complexes are prepared, the latter are also symmetric. One can therefore carry out a cosine Fourier transform on the observed amplitudes to obtain the electron density profiles. The electron density distribution was obtained from the relation

$$\rho(x) = \int_{h_{min}}^{h_{max}} (\pm) h\sqrt{I(h)}\, \cos(hx)\mathrm{d}h \qquad (1)$$

where $\rho(x)$ is the electron density along x, and $h = (4\pi \sin\theta)/\lambda$. Since we are interested in the electron density profile perpendicular to the plane of the bilayer, the amplitude $\sqrt{I(h)}$ from the vesicle, which in effect is a randomly oriented bilayer, will have to be multiplied by h [16]. This is valid for $h > (S_{min})^{-1/2}$, where S_{min} is the smallest surface area of the lamellae [17]. Since the radius of our vesicles is approx. 1000 Å, if we ignore the curvature of the vesicle, the Eqn. 1 will be valid or $h > 0.5 \cdot 10^{-3}$ Å^{-1}. h_{min} used in our calculation was $1 \cdot 10^{-3}$ Å^{-1}. By setting $h_{max} \approx 0.4$ Å^{-1} only the first lobe of the intensity curve was transformed. The Fourier transform was carried out by assigning positive phases to the amplitudes at $h \leqslant 0.05$ (i.e., the first minima) in the intensity curve, the negative values to the amplitudes between the first and the second minima ($0.05 \leqslant h \leqslant 0.4$) [16]. In order to place the electron density profiles of lipid and the lipid-basic protein complex on the same scale, the intensity data were collected under identical conditions in the same X-ray capillary and normalized to the measured concentration of the lipid. Since the electron density outside the bilayer is due only to the buffer, this was assigned a value of 0.334 e/Å^3. These two factors are sufficient to scale the two profiles. Although this scaling procedure may not be rigorous (for example, it does not take into account the changes in the electron density fluctuations due to the presence of the protein), it is adequate for the purposes of this report.

The electron density profiles scaled using these approximations are shown in Fig. 4. The electron density scale was established by assuming the electron density at the middle of the bilayer at our resolution to be 0.27 e/Å^3 [11,18] and by assigning a value of 0.334 e/Å^3 for the electron density of the buffer outside the bilayer. Since other lipid complexes (basic protein > 35%, polylysine and Ca^{2+}) are multilayers and give rise to discrete reflections, it is difficult to compare the electron

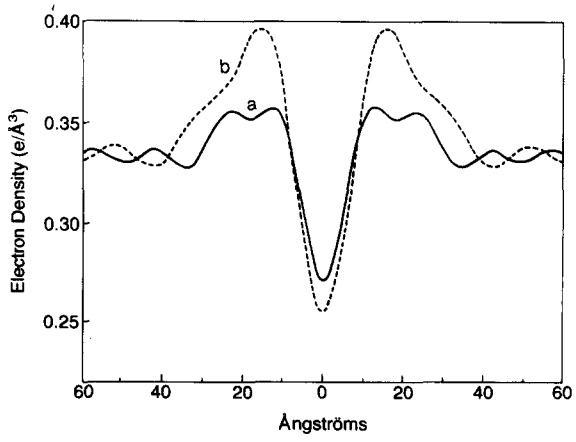

Fig. 4. Electron density profiles: (a) phosphatidylglycerol (PG) bilayer (full line); (b) PG + 21% basic protein of myelin (dashed line).

density profiles from these samples to that derived from the continuous transforms obtained form pure lipid and the lipid vesicle with 21% basic protein. The electron density profiles in Fig. 4 show that the basic protein does not significantly alter the electron density levels in the interior of the bilayer. This is consistent with the wide-angle results which show little change in the organization of the hydrocarbon chains due to the presence of the basic protein. Fig. 4 shows that the protein penetrates into the bilayer and also increases the width of the bilayer.

Discussion

The influence of the basic protein on a bilayer has been shown to depend on the lipid composition of the membrane [19–23]. The interaction and penetration of the basic protein was shown to be greater in phosphatidylglycerol (PG) bilayers than in phosphatidic acid, phosphatidylserine, cerebroside sulfate, or phosphatidylethanolamine [22,23]. Therefore, the basic protein-PG system was considered most suitable for X-ray analysis.

The basic protein is a highly charged molecule ($pI = 10.8$) and has 25% basic amino acids distributed throughout the molecule [21]. It readily binds to acidic lipids [20,22–25] and its binding can be inhibited by high salt concentrations [21]. Therefore, as reported in our earlier publication [7], the basic protein interacts with lipids primarily through electrostatic interactions. Similarities in the formation of multilayers on addition of calcium ions, poly(L-lysine) and the basic protein (Fig. 2) show that the basic protein can induce the formation of multilayers by just neutralizing the charges on the surface of the bilayer and without any specific binding sites being involved in the process. The electron density profiles (Fig. 4) show that the basic protein does penetrate into the headgroups of the bilayer. Our observations that no dramatic changes occur in the scattering curves at low protein concentrations and the formation of multilayers at about 45 : 55 protein to lipid ratio, suggests that the process of cross-linking by the basic protein should be a cooperative phenomena. Our data however, cannot distinguish between cross-linking by pairs of protein molecules [26] or by a single molecule.

The above evidence suggest that a reduction in electrostatic repulsion between the bilayers brought about by basic protein binding to the head groups is probably responsible for the formation of multilayers. But several workers [12,19,20,23,27,28] have found that certain parts of the basic protein, which contains 52% apolar amino acids, penetrate into the bilayer where these segments interact hydrophobically with the lipid fatty acids. Although electron density profiles (Fig. 4) cannot be used to infer that the basic protein interacts hydrophobically with the lipid fatty acids, it does suggest that the basic protein penetrates the bilayer.

Since the basic protein has also been found to increase the permeability of lipid vesicles to glucose [29], to decrease the enthalpy and temperature of lipid phase transition of acidic lipids [12,21,30], and when allowed to interact with monolayers increase the surface pressure of acidic lipids [20], it follows that in addition to the electrostatic interaction with the headgroups, basic protein might also disrupt the hydrophobic interaction between the hydrocarbon chains. Smith and McDonald [26] have implied that the hydrophobic interactions might play a role in the cross-linking of the bilayers by basic protein. However, our wide-angle results (Fig. 3) show that the protein does not significantly perturb the organization of the hydrocarbon chains. The electron density profiles (Fig. 4) only show that the basic protein penetrates the bilayer. The relative role of the hydrophobic and electrostatic interactions between the basic protein and the bilayer is still to be resolved. Since there have been reports [30,31] showing a correlation between the increase in the permeability of the membrane and the ability of the basic protein to partially penetrate the bilayer, it is possible that the basic protein might not be just a structural protein, but might regulate the membrane permeability in vivo.

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