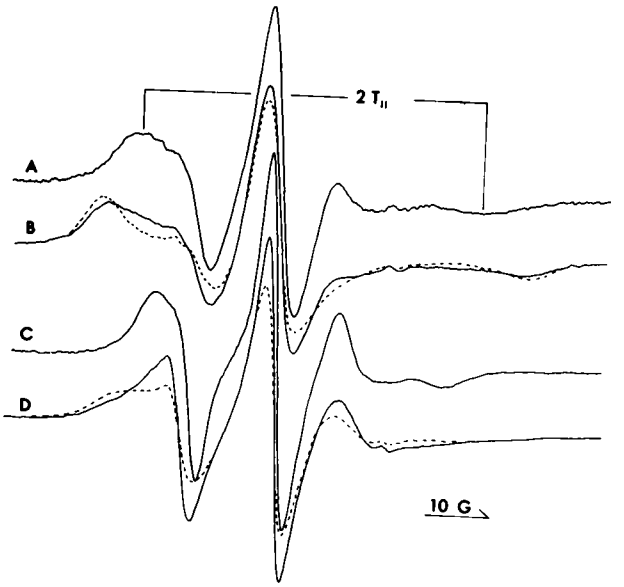

FIGURE 2 ESR spectra of 16-S-SL in (*A*) DPPG at 9°C; (*B*) DPPG-52.4% BP complex by weight at 9°C on cooling directly from 59°C (solid line) and after cycling through 59°→9°→31°C and incubation at 31°C for 45 min (dashed line); (*C*) DPPG at 31°C; (*D*) DPPG-52.4% BP complex at 31°C after cycling through 59°→9°→31°C, immediately after equilibration at 31°C (solid line) and after incubation at 31°C for 45 min (dashed line).

explanation for this immobilization is that interdigitation of the fatty acid chains occurs in the gel phase of the DPPG-BP complex (8). Interdigitation would compensate for the lipid expansion caused by intercalation of the protein partway into the bilayer and would stabilize the complex by allowing greater van der Waals interactions between the fatty acid chains below T_c. Above T_c greater intercalation and correspondingly less interdigitation probably occurs. The fully interdigitated state does not recur on cooling and the complex freezes into an unstable state. Reheating, as in the scans shown in Fig. 1 *e* and *k*, allows molecular rearrangement as the sample partially melts and further interdigitation in the temperature range 29–34°C. This restricts the motion of 16-S-SL and causes the lipid to refreeze, releasing heat. The complex in its stable interdigitated state then melts with a T_c and ΔH only slightly less than the pure lipid.

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INTERACTION OF BASIC PROTEIN AND PERIPHERAL NERVE P2 PROTEIN WITH LIPIDS

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The basic protein (BP) of central nervous system myelin and the P2 protein of peripheral nerve myelin are concerned in cell-mediated autoimmune diseases such as experimental allergic encephalitis (EAE) (1) and experimental allergic neuritis (EAN) (2). The conformations of these proteins in the myelin membrane differ markedly from their conformations in aqueous media. Thus information on conformations in the presence of various lipids is pertinent to the role of the proteins in the structure of myelin and the mechanisms of their antigenicity. This paper summarizes results from our laboratory obtained during the past several years.

MATERIALS AND METHODS

Myelin basic protein (BP) was prepared from fresh white matter of bovine brain by the procedure of Eylar et al. (3). Peripheral nerve P2 protein was prepared from myelin obtained from bovine intradural dorsal roots by the method of Brostoff et al. (4). Lysophosphatidyl choline was a

product of Sigma Chemical Co., St. Louis, MO. Ganglioside G_{m1} was separated from bovine brain ganglioside mixture and kindly supplied by R. W. Ledeen (5). All chemicals were lyophilized at least once from D_2O before use. The lipids dispersed readily in D_2O to form small micelles at 2–10 mg/ml. ^1H NMR spectra were obtained on a Bruker HX-270 spectrometer (Bruker Instruments, Inc., Billerica, MA) at the National NMR Centre, Canberra, Australia. The protein concentrations were 5–10 mg/ml at pH 4.5–6.5. The HDO peak was suppressed by the gated-decoupling method (6).

RESULTS AND DISCUSSION

NMR and circular dichroism data indicate that BP exists in aqueous solution as an extended coil with regions of local structure. Denaturation in 8 M guanadinium chloride caused changes in chemical shifts indicating that the coil structure of the native protein in aqueous solution is not random. The proton NMR data indicate, for example, that aromatic side chains and one methionine occur in structured regions, but there is no evidence of interaction of the solitary methylarginine (Me-R) residue with phenylalanine rings, a mechanism that had been suggested for hydrophobic stabilization of a bend in the structure at its central Pro-Pro-Pro sequence (7). The protein binds to lipids, such as ganglioside-G_{M1}, which form small micelles, and a particularly strong interaction of the ganglioside with the methyl arginine residue is observed. Fong et al. (8) had noted that myelin of both the central nervous system (CNS) and the peripheral nervous system (PNS) contained gangliosides and BP in approximately equimolar amounts. This observation led to a surmise that gangliosides might bind specifically to BP in the organization of myelin membranes. The NMR evidence is consistent with such a structure, but it is of course far from definitive.

The most unusual result of the lipid-binding studies of Littlemore and Ledeen (9) was that the central region of the BP structure must be intact in order for the Me-R residue to bind lipid. When the protein was split enzymatically between the phenylalanine residues 89 and 90, thereby preventing the suggested hydrophobic interaction between these residues and the Me-R at 107, the Me-R no longer interacted strongly with lipid. Circular dichroism (CD) measurements by Smith and Keniry (10) showed that the central region of the protein must be intact to give the increased α-helicity that occurs on lipid binding. The net result of these studies, therefore, is to suggest a model in which a bend occurs at the central Pro-Pro-Pro sequence of BP, which promotes an ordered structure, partially at least α-helix, with enhanced lipid-binding properties. We can speculate that this "hydrophobic plug" enters the lipid bilayer of myelin membranes, leaving the terminal region of the protein free to interact, possibly with similar structures across the intracellular apposition of the double bilayers.

The P2 protein of PNS myelin has predominantly a β-sheet conformation in aqueous media (11). Its antigenicity in causing EAN in Lewis rats is strongly dependent upon the particular lipid with which it is complexed. The lipid binding causes an increase in α-helical conformation at the expense of the β-sheet, as can be seen from CD data. The NMR data indicate that the protein in aqueous media has a highly structured region and a smaller more floppy region (12). It undergoes a stepwise denaturation in increasing concentration of urea, and part of the structure, believed to include the S-S bridge at the C-terminal end, is very resistant to denaturation.

The NMR spectrum of the P2 protein at 20 mg/ml was examined at increasing concentrations of lysophosphatidyl choline from 1 to 70 mg/ml at pH 5. Even at the lowest lipid concentration, the aromatic region of the spectrum and the methionine peaks were markedly altered. The upfield methyl peaks were less affected, even up to 10 mg/ml lipid. This binding may be associated with the shift from β-sheet to α-helical structure observed in the CD spectra, but this interpretation has not yet been proved. An alternative possibility is that the regions of the protein that are unstructured in aqueous solution acquire α-helicity and an increased affinity for lipids.

As yet we can say little about the role of P2 in the structure of peripheral myelin. BP is essential for the organized structure of CNS myelin but it is not essential for PNS myelin. The fact that in many species PNS myelin contains little P2 suggests that it is not essential to the organization of the membrane. The role of P2 in acute and chronic peripheral neuropathies presents many problems of great interest and importance, which are just beginning to be investigated.

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INTERACTION OF BASIC PROTEINS WITH CHARGED PHOSPHOLIPIDS FOLLOWED BY FLUORESCENCE, DSC, AND RAMAN SPECTROSCOPY

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Melittin (Mel) and cardiotoxins (CTX) are basic proteins which act on membranes as direct lytic factors. Mel binds to both negative and zwitterionic phospholipids, but it exhibits higher affinity toward the former (1). CTX interacts only with charged lipids (2). Studies on the perturbations of the bilayers induced by such toxins have been restricted to the effects of melittin on phosphatidylcholines (3–5). Here we report on the perturbations of Mel and CTX on bilayers of pure negative lipids or of binary mixtures as followed by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), calorimetry (DSC), and Raman spectroscopy.

RESULTS

Melittin-Phospholipid Interactions

According to their behavior in the presence of Mel, acidic phospholipids can be divided in two groups. Group one is constituted by mono- and divalent lipids in C_{14} ($DMPS^-$, $DMPG^-$, $DMPA^{--}$), divalent lipids in C_{16} ($DPPA^{--}$) and natural lipids such as PS^-. In this case, Mel leads to a progressive disappearance of the transition without any shift in temperature. For the second group of lipids, which includes monovalent lipids in C_{16} ($DPPG^-$, $DPPS^-$, $DPPA^-$) and also a monovalent C_{14} lipid ($DMPA^-$), two well-defined transitions are detected. One occurs at the same temperature as that of the pure lipid; the second one is 10–15°C lower. By increasing the amount of Mel, the high temperature transition progressively disappears while the lower one increases in amplitude (see Fig. 1).

Cardiotoxin-Phospholipid Interactions

For all the lipids used, CTX induces the same effects as those of Mel on group one lipids. The Raman spectra in the C–C stretching region (Fig. 2) for CTX-DMPA complexes show that even in the gel phase CTX induces formation of *gauche* conformers. At $R_i = 7.5$, the population of *gauche* rotamers is almost as high as that of pure DMPA in the liquid-crystalline state at 60°C. The C–H stretching region parameters indicate that before the occurrence of a significant disordering ($R_i > 30$), CTX induces a change in the packing of the aliphatic chains.

Interaction of Mel and CTX with Binary Mixtures of Lipids

In the presence of toxins the transition temperature as detected by fluorescence polarization for 1:1 mixtures PC-PA and PC-PS is always shifted toward that of the PC component. For example, the transition temperature of a DMPC-DMPA mixture is shifted downward by 13°C whereas in the case of DMPC-natural PA, an upward shift of 6°C is observed, the final transition being very close to that of pure DMPC (23°C) in both cases.

CONCLUSIONS

The presence of two well-defined transitions for complexes of group two lipids and Mel demonstrates that the latter induces phase separation between pure lipid regions and a peptide-lipid complex which melts at 10–15°C below the pure lipid. This phase separation could also occur for Mel and group one lipids as well as for CTX and all the lipids investigated in this study if the complexes formed do not give rise to a cooperative transition. We thus believe that Mel does not disrupt the bilayer structure formed by the group two lipids, which display stronger intermolecular interactions than group one lipids because the aliphatic chains are longer and/or the polar headgroups bear only one negative charge. The perturbations induced by Mel on group one lipids, which form less stable bilayers, and by CTX on all the lipids investigated are such that a cooperative transition can no longer occur.

The results obtained with mixtures indicate that Mel and CTX are able to induce phase separation between PC-rich regions that still give a transition and domains