TABLE I
EFFECT OF TEMPERATURE ON ATPase ACTIVITY AND DPH ANISOTROPY

Detergent	Temperature of discontinuity of Arrhenius plot of ATPase activity (°C)	Temperature of discontinuity in DPH anisotropy (°C)
$C_{12}E_8$	20	18
Triton X-100	25	25
Tween 80	16, 30	16

(5). The structural perturbation possibly represents a rearrangement of polar head groups or a change in the state of aggregation of the micelle since it is highly unlikely that phase transitions occur in the hydrocarbon interior of these micelles (7). Thus, as has been observed for phospholipids, temperature-dependent changes in detergent micelles affect the activity of the ATPase.

The results presented here demonstrate that the study of detergent protein interaction can lend additional insight into the details of protein-lipid interaction. The effects of nonionic detergents on the Ca^{2+}-ATPase from sarcoplasmic reticulum are quite similar to those of phospholipids, and the relatively high c.m.c of detergents allows for direct observation of amphiphile monomer interactions with the protein.

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INTERDIGITATION OF FATTY ACID CHAINS OF DIPALMITOYLPHOSPHATIDYLGLYCEROL DUE TO INTERCALATION OF MYELIN BASIC PROTEIN

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The basic protein (BP) of myelin is water soluble and binds electrostatically to acidic lipids but has several hydrophobic segments which may intercalate partway into the bilayer. This conclusion is based on the perturbing effects of the protein on the bilayer (1–3) as well as evidence for sequestration of some parts of the protein by the lipid (4, 5). Differential scanning calorimetry (DSC) and fatty acid spin labels were used to investigate the dependence of this interaction on the phase state of dipalmitoylphosphatidylglycerol (DPPG).

RESULTS

Intercalation of BP into DPPG decreases the temperature, T_c, and enthalpy, ΔH, of its phase transition (2). The thermograms of DPPG and the DPPG-BP complex for two different sets of samples are shown in Fig. 1. The first set (Fig. 1 *b*, *d–f*) contain 52.4% BP by weight and the second set (Fig. 1 *g*, *j–m*) contains 48% BP. ΔH measurements were made for the second set and are given in the figure caption. BP has a greater effect on the T_c and ΔH on the second and repeated heating scans (Fig. 1 *e* and *k*) than on the first (Fig. 1 *b* and *g*) compared to the pure lipid (Fig. 1 *a* and *h*). This suggests that greater intercalation occurs in the liquid-crystalline phase than in the gel phase (2, 3). Consequently BP has an even greater effect on the cooling scan (Fig. 1 *d* and *j*) compared to the cooling scan of the pure lipid (Fig. 1 *c* and *i*). However, if the sample is reheated from a higher temperature (12–17°C rather than from below 12°C as above) the relative effect on the heating scan (Fig. 1 *f* and *l*) is similar to that on the cooling scan. Most of the thermogram consists of an endothermic peak at 30–35°C. A similar endothermic peak begins in the reheating scans shown in Fig. 1 *e* and *k* but is not completed. An exothermic process occurs

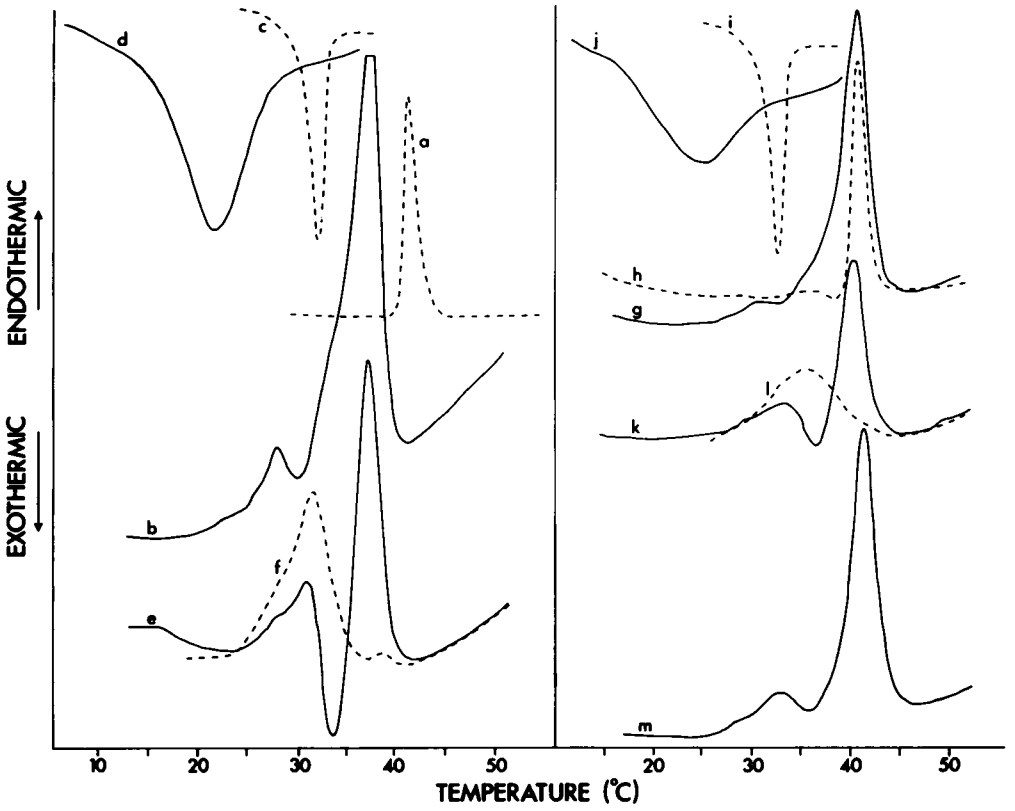
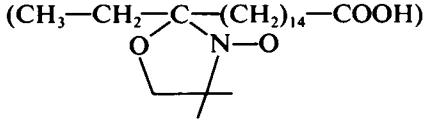


FIGURE 1 DSC thermograms of DPPG alone (*a* and *c*, *i* and *h*, dashed lines) and complexed with 52.4% BP by weight (*b*, *d–e*) or 48% BP (*g*, *j–m*). Scans, *c*, *d*, *i*, and *j* are cooling scans while all others are heating scans: *b* and *g*, first heating scan of sample, heated from −3°C; *e* and *k*, reheating scan, heated from −3°C; *f*, reheating scan, heated from 12°C; *l*, reheating scan, heated from 17°C; *m*, heating scan from −3°C after cycling from 50°→−3°→ 31.5°C and incubation at 31.5°C for 15 min. T_c on cooling scan is normally 7–10°C below T_c on heating scan at heating and cooling rate used, 10°C/min. Samples were prepared in Hepes buffer (10 mM) containing 10 mM NaCl, 0.1 mM EDTA at pH 7.0. ΔH in Kcal/mol was (*g*) 7.4, (*h*) 8.3, (*j*) 5.5, (*k*) 4.4, (*l*) 4.0, (*m*) 7.7.

instead, followed by an endothermic transition at the same temperature as that observed on the first heating scan but of lower ΔH. The exothermic transition, which is more apparent at the higher protein concentration (Fig. 1 *e*), indicates that the phase formed on supercooling to below 12°C is metastable. Incubation of the sample represented in Fig. 1 *k* at a temperature near that of the exothermic transition for 15 min allowed the exothermic process to go to completion. Reheating of the sample then gave a DSC scan (Fig. 1 *m*) which resembled the first heating scan (Fig. 1 *g*) with a T_c and ΔH only slightly reduced from that of the pure lipid. This suggests that the phase formed during the exothermic transition is identical to the gel phase present on the first heating scan.

The nature of this phase was investigated using fatty acid spin labels. BP has a pronounced immobilizing effect on fatty acid motion in the gel phase of DPPG. This is particularly apparent with 16-S-SL



for which BP increases $T_{\parallel}$ from 26 gauss in pure DPPG (Fig. 2 *A*) to 33*G* (Fig. 2 *B*) at 9°. Polylysine, divalent cations, or protonation of the lipid at low pH did not cause this degree of immobilization (7). In the liquid-crystalline phase BP has a smaller motion restricting effect, increasing τ_0 from 0.6 to 1.1 ns (6, 7). Near the temperature of the exothermic transition, 31°, 16-S-SL in the DPPG-BP complex (Fig. 2 *D*, solid line) is initially more mobile than in pure DPPG (Fig. 2 *C*). However, incubation at 31° causes it to become more immobilized than in pure DPPG (Fig. 2 *D*, dashed line). The rate of this change depends on the temperature used within the range 29–34°C and was nearly complete in 3 min at 33°. After this treatment 16-S-SL is even more immobilized at 9°C (Fig. 2 *B*, dashed line) than it was on cooling directly to 9°C from above T_c (Fig. 2 *B*, solid line) and resembles the spectrum obtained at 9°C before heating the sample (not shown).

DISCUSSION

The high degree of immobilization of 16-S-SL in DPPG-BP below the T_c indicates a restriction in motion of the terminal methyl groups of the lipid fatty acids comparable to that which occurs near the polar head group. An

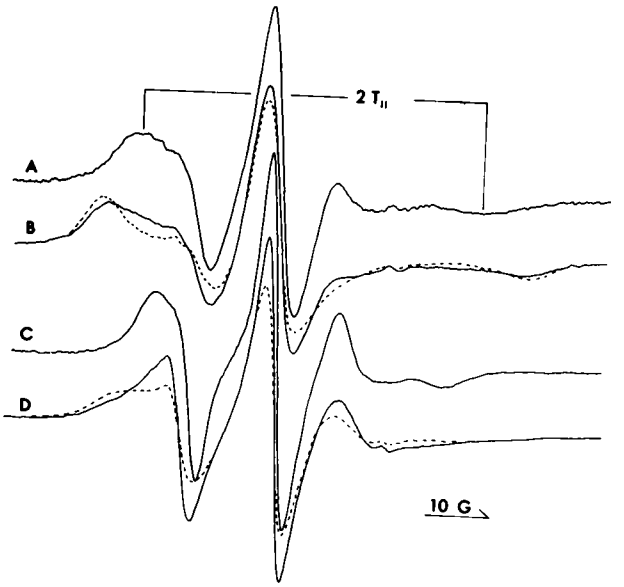


FIGURE 2 ESR spectra of 16-S-SL in (*A*) DPPG at 9°C; (*B*) DPPG-52.4% BP complex by weight at 9°C on cooling directly from 59°C (solid line) and after cycling through 59°→9°→31°C and incubation at 31°C for 45 min (dashed line); (*C*) DPPG at 31°C; (*D*) DPPG-52.4% BP complex at 31°C after cycling through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