Dependence of Boundary Lipid on Fatty Acid Chain Length in Phosphatidylcholine Vesicles Containing a Hydrophobic Protein from Myelin Proteolipid†

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ABSTRACT: Lipophilin, a hydrophobic protein fraction, purified, and delipidated from the proteolipid of human myelin, possesses a layer of boundary lipid surrounding it when incorporated into lipid vesicles. The protein reduces the energy absorbed during the lipid phase transition, indicating that the boundary lipid does not go through the phase transition. The amount of boundary lipid was estimated by plotting the enthalpy of the transition against the protein to lipid mole ratio and extrapolating to $\Delta H = 0$ for a number of synthetic phosphatidylcholines, to determine the ability of fatty acid chains of varying length to interact with the protein. The amount of boundary lipid was found to be similar, 21–25 molecules per molecule of lipophilin, for fatty acid chains of length 14–18 carbons but somewhat less, 16 molecules of lipid per molecule of protein, for a fatty acid chain length of 12 or for one with a trans double bond ($18{:}1_{tr}$). No preferential interaction was observed with a lipid containing a particular fatty acid chain length when the protein was incorporated into a mixture of these lipids. These results suggest that the binding of lipids to the boundary layer of other membrane proteins and enzymes may not depend significantly on lipid fatty acid chain length.

Membranes contain a mixture of lipids with different fatty acid chain lengths as well as different polar head groups. The activity of membrane enzymes has been shown to depend on both lipid polar head group and on fatty acid chain length (Kimmelberg, 1977). The dependence on fatty acid chain length could be due to the effect of fatty acid chain length on average membrane fluidity or to the stability of a specific interaction of the enzyme with a boundary layer or annulus of lipid. The activity of the Ca^{2+}, Mg^{2+} dependent ATPase from sarcoplasmic reticulum has been shown to depend to a greater extent on the fluidity of the annular lipid than the bulk lipid (Hesketh et al., 1976). It is possible that the stability of the interaction of a protein with a boundary layer of lipid might depend on lipid fatty acid chain length as well as polar head group and therefore a membrane protein might be able to select out those lipids having a polar head group or particular fatty acid chain length with which it can form the most energetically stable complex. An enzyme might be able to control its activity in this way even if the average membrane lipid composition changes.

We have extensively studied the interactions of lipophilin, a protein fraction purified and delipidated from the proteolipid of human CNS myelin, with lipid. Although this protein has no known enzymatic activity or other dynamic function, it is a hydrophobic membrane protein of the intrinsic type and studies of the interaction of this protein with lipid are relevant to other membrane proteins. Lipophilin has been shown by ESR spectroscopy to possess a layer of boundary lipid when incorporated into lipid vesicles (Boggs et al., 1976). The ESR spectrum of a fatty acid spin-label incorporated into these vesicles possessed two components, one due to immobilized lipid indicating the presence of a boundary layer of lipid as seen for cytochrome oxidase (Jost et al., 1973), and the other component characteristic of a lamellar phase but slightly more ordered than in the absence of protein. This protein was also shown by differential scanning calorimetry to decrease the enthalpy of the lipid gel to liquid crystalline phase transition without altering the transition temperature (Papahadjopoulos et al., 1975) suggesting that the boundary layer of lipid did not participate in the phase transition. The amount of boundary lipid was estimated from the decrease in enthalpy in dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol and was found to be similar for these neutral and acidic lipids. However, we demonstrated that when neutral and acidic lipids were mixed this protein could cause lipid phase separation by binding the acidic lipid preferentially to its boundary layer (Boggs et al., 1977), indicating that it can interact electrostatically with lipid polar head groups as well as hydrophobically with lipid fatty acids. The present study was performed to investigate the dependence of the amount of boundary lipid on fatty acid chain length over the range C-12 to C-18 in a neutral lipid, phosphatidylcholine. Mixtures of these synthetic phosphatidylcholines were also studied to determine whether the protein can interact preferentially with one fatty acid chain length when a choice is available.

Materials and Methods

Preparation of Lipophilin. Myelin was isolated from normal human white matter by the method of Lowden et al. (1966). Lipophilin (previously called N-2) was extracted and purified by chromatography on Sephadex LH-20 in chloroform/methanol (1:1, v/v) containing 5% of 0.1 M HCl, by the method of Gagnon et al. (1971) and stored in the lyophilized form.

Lipids. Dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were purchased from either Serdary Research Laboratory (London, Ontario) or Sigma Chemical Co. Dilauroylphosphatidylcholine (DLPC) and distearoylphosphatidylcholine (DSPC) were purchased from Supelco. Dielaidoylphosphatidylcholine (DEPC) was a gift from Dr. D. Papahadjopoulos. All lipids were stored at −50 °C under nitrogen and were chromatographically pure with

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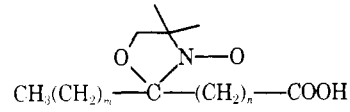
the exception of DLPC which had a small contaminant of lyso compound which resulted in an extra thermotropic peak when examined by DSC. The amount of this impurity is probably less than 5% since addition of 5% lyso-PC to a pure phosphatidylcholine produces a similar higher melting peak. Attempts at purifying this lipid by preparative TLC resulted in greater contamination so the lipid was used as purchased. 2-Chloroethanol was obtained from BDH, redistilled periodically, and stored in the dark at 4 °C. *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes)[1] was obtained from Calbiochem. 5-Doxylstearate I (12, 3) and 16-doxylstearate I (1, 14) were purchased from Syva.



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Preparation of Vesicles. Lipid vesicles with or without lipophilin were prepared by dialysis from 2-chloroethanol against aqueous buffer as described previously (Boggs et al., 1976). The buffer contained NaCl (100 mM) and Hepes (2 mM) adjusted to pH 7.4. Dialysis was carried out in a temperature controlled bath at a temperature above the lipid phase transition temperature. The temperature used for all the lipids except DSPC was 45 °C to minimize the effect of temperature on the protein. However, it was necessary to use a higher temperature, 62 °C, for DSPC. Dialysis was carried out against a high salt concentration, 100 mM NaCl, to allow precipitation of the multilayered vesicles. Dialysis against a low salt concentration results in single layered vesicles in the case of high protein concentrations which are difficult to sediment out. Centrifugation on a sucrose gradient indicated that there was little or no lipid or protein outside of the complex, which formed a single band, and therefore most of the samples used for DSC were not routinely purified on sucrose gradients. The suspensions were centrifuged at 12 000 rpm for 5 min in an Eppendorf microcentrifuge. The lipid–protein ratio of the pellets was analyzed by measuring phospholipid content in an aliquot by a modified Bartlett procedure (1959) and protein content by hydrolyzing another aliquot and measuring amino acid content on a Durrum D-500 amino acid analyzer.

Differential Scanning Calorimetry. The temperature and heat content of the thermotropic transition of the vesicle preparations with and without protein were determined with a differential scanning calorimeter (Perkin-Elmer DSC-2) using a scanning rate of 5 °C/min unless specified otherwise. All samples used for enthalpy determination were scanned at 5 °C/min at a fast chart speed of 80 mm/min. Vesicle pellets were taken up in micropipets and transferred to the sample pans. The reference pan contained buffer. Four pans were filled and measured for each sample. The area of the peaks was measured by Xeroxing several copies, cutting out, and weighing. In the case of DLPC where an extra higher melting peak was present due to an impurity, the higher melting peak was ignored in measuring the area. The pans were opened by sonication in 1 mL of distilled water, and aliquots were taken for phosphate analysis to determine the total amount of phospholipid in the pan. The calorimeter was standardized against indium.

[1] Abbreviations used: DSC, differential scanning calorimetry; DLPC, dilauroylphosphatidylcholine; DEPC, dielaidoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2'-ethanesulfonic acid; ESR, electron spin resonance spectroscopy.

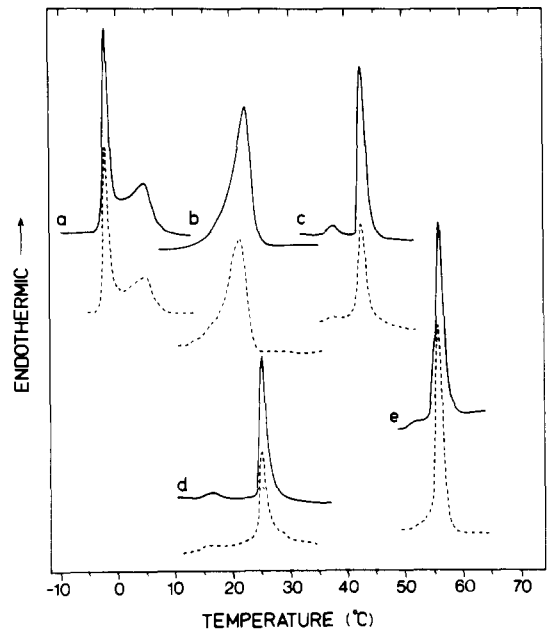

FIGURE 1: Differential scanning calorimetry thermograms for lipid vesicles in the presence (- - -) and absence (—) of lipophilin. (a) DLPC ± 54% lipophilin; the higher melting peak is due to an impurity in the lipid used; (b) DEPC ± 31.9% lipophilin; (c) DPPC ± 40% lipophilin; (d) DMPC ± 31% lipophilin; (e) DSPC ± 30.1% lipophilin. The thermograms shown were obtained by scanning at 10 °C/min; however, the appearance was identical at 1.25 °C/min. All samples used for ΔH determination were scanned at 5 °C/min at a fast chart speed, 80 mm/s.

Electron Spin Resonance Measurements. Vesicles were labeled with a fatty acid spin label by evaporating a chloroform solution of the spin label in a test tube and adding the vesicle suspension. Spin-labeled suspensions were taken up in 50 μL disposable micropipets and centrifuged at 2000 rpm for 10 min to obtain a concentrated sample for ESR measurements. Spectra were obtained on a Varian E-4 spectrophotometer with a Varian temperature control accessory. The microwave power used was 10 mW.

Results

DSC scans showing the effect of lipophilin on the thermotropic transition of each of the lipids are shown in Figure 1. In agreement with earlier results by Papahadjopoulos et al. (1975), lipophilin has no effect on the midpoint of the phase transition but broadens the transition slightly at high concentrations. It does not abolish the premelt until concentrations over 40%. However, it causes a decrease in the heat absorbed proportional to the amount of protein present. The scans shown in Figure 1 were run at 10 °C/min but have a similar appearance at heating rates down to 1.25 °C/min. However, background noise is higher. The scans were completely reversible when cooled and reheated at least five times. The protein itself does not absorb any detectable energy from 0 to 70 °C and CD studies have shown that temperature-induced changes in the CD spectrum up to 60 °C are not great, the ellipticity at 222 nm increasing by 14%. The presence of lipid was also found to decrease the rate and extent of thermal denaturation and increases the reversibility of changes in the CD spectrum (Cockle et al., 1978).

The enthalpy of the transition is plotted against the mole ratio of lipophilin to lipid in Figure 2 assuming a monomer molecular weight of 25 000 g/mol for lipophilin (Moscarello et al., 1973). The standard deviations for enthalpy values are low indicating that random errors involved are low. However, the enthalpy values for the pure lipids are higher than those

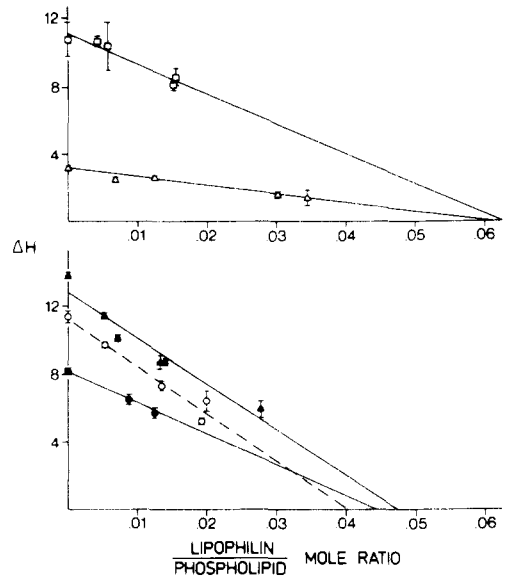

FIGURE 2: Enthalpy of transition in kcal/mol for the lipid phase transition in vesicles containing various concentrations of lipophilin for DEPC (□), DLPC (△), DSPC (▲), DPPC (○), and DMPC (●). The protein to lipid mole ratio was calculated from the weight percentage using a monomer molecular weight of 25 000 for lipophilin. Each point is an average of four determinations and the standard deviation is indicated.

TABLE I: Boundary Lipid Surrounding Lipophilin in Phosphatidylcholine Containing Various Fatty Acids.

lipid	boundary lipid (molecules/ molecule of lipophilin)	corr[b] coeff
DLPC (C-12:0)	16.6 ± 1.0[a]	−0.977
DMPC (C-14:0)	22.6 ± 0.25	−0.998
DPPC (C-16:0)	24.9 ± 1.3	−0.978
DSPC (C-18:0)	21.1 ± 1.2	−0.966
DEPC (C-18:1_{tr})	15.9 ± 0.6	−0.962

[a] Standard deviation calculated for x intercept from deviation of best straight line from data points. [b] Correlation coefficient for fit of line to points by linear regression.

reported elsewhere (de Kruyff et al., 1974). This may be due to a different calibration of the instrument but does not affect comparison of the enthalpy of various samples. The best straight line was fitted to the data using linear regression, and the x intercept, the protein to lipid mole ratio at zero enthalpy, was calculated. This value represents the amount of lipid which does not go through the phase transition and is defined as the boundary lipid. The amount of boundary lipid for each lipid and the standard deviations and correlation coefficients for fit of the best straight line are shown in Table I. These results indicate that the amount of boundary lipid is similar for DMPC, DPPC, and DSPC (21–25 molecules lipid/molecule of protein), while it is somewhat less for DLPC and DEPC (approximately 16). This difference is statistically significant ($P < 0.01$) by a two-tailed student's t test. However, the impurity in DLPC may affect the boundary lipid so that little significance can be attributed to the lower amount of boundary lipid obtained for this lipid.

Although the amount of boundary lipid is similar for lipids containing fatty acid chains 14–18 carbons in length when only a single lipid is present, it was thought possible that the protein might bind one type of fatty acid preferentially when a mixture of lipids was used. Therefore we studied the ability of the

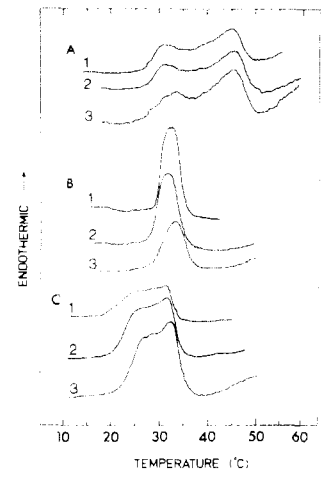

FIGURE 3: DSC thermograms scanned at 5 °C/min for (A) an equimolar mixture of DMPC and DSPC prepared by dialysis at 62 °C (1) with lipid only, (2) with 15% lipophilin, (3) with 36% lipophilin; (B) an equimolar mixture of DMPC and DPPC prepared by dialysis at 45 °C (1) with lipid only, (2) with 15% lipophilin, (3) with 29% lipophilin; (C) an equimolar mixture of DEPC and DPPC prepared by dialysis at 45 °C (1) with lipid only, (2) with 13% lipophilin, (3) with 32% lipophilin.

protein to cause lipid phase separation in a cocrystallizing mixture of lipids or to interact preferentially with one species in a mixture which does not cocrystallize. If preferential binding of lipophilin occurs, it can be detected from a decrease in height of one peak relative to the other, and/or a shift in phase transition temperature of one or both peaks where some mixing occurs.

DMPC and DSPC, whose fatty acids differ by 3 carbons in length, do not cocrystallize completely and an equimolar mixture has two transitions at temperatures higher and lower than that of the pure compounds as indicated in Figure 3A. Lipophilin decreases the height of the lower melting peak slightly and increases the temperature of the lower melting peak by 2.5 °C at a concentration of 36% (Figure 3A, 2 and 3) and Table II. This indicates that it may bind preferentially to the lower melting component which contains primarily DMPC and some DSPC and also that it may bind DMPC preferentially, increasing the DSPC concentration of the lower melting component. The effect of lipophilin on the transition of an equimolar mixture of DMPC and DPPC is shown in Figure 3B. These lipids whose fatty acid chains differ in length by only 2 carbons, cocrystallize giving a single peak. Lipophilin does not alter the transition temperature significantly (Table II); a small decrease is seen for 15% protein while there is a small increase for 29% protein. This indicates that in DMPC–DPPC, lipophilin does not bind one lipid preferentially to its boundary layer to a significant extent.

An equimolar mixture of DEPC and DPPC, which also does not cocrystallize completely, gives a broad peak with two components (Figure 3C). Lipophilin increases the temperature at which melting begins by 3.3 °C (Table II) and also increases the temperature at which melting finishes slightly. It also decreases the height of the lower melting component relative to the higher one slightly indicating that lipophilin binds preferentially to the lower melting component, DEPC, to some extent even though it binds more DPPC to its boundary layer than DEPC when only a single lipid is present.

A mixture of DLPC and DSPC whose fatty acid chains differ by 4 carbons does not cocrystallize very much; it gives two peaks, the lower at a temperature similar to that of DLPC, while the upper is less than that of DSPC indicating that the component melting at the upper temperature contains some

TABLE II: Phase-Transition Temperatures of a Variety of Samples in the Presence and Absence of Lipophilin.

sample	heating rate (°C/min)	T_c[a] (°C) lower	T_c[a] (°C) upper	ratio of heights of lower temp peak to higher temp peak
DMPC	5	23.8		
DSPC	5	54.3		
DMPC:DSPC 1:1	5	31	45	0.72
DMPC:DSPC + 15% protein	5	31.5	45.1	0.65
DMPC:DSPC + 36% protein	5	33.5	45.2	0.60
DMPC:DPPC 1:1	5	32.3		
DMPC:DPPC + 15% protein	5	31.8		
DMPC:DPPC + 29% protein	5	33.2		
DLPC	10	−3		
DLPC:DSPC 1:1	10	−3	43.6	0.74
DLPC:DSPC + 11% protein	10	−2.5	43.9	0.93
DLPC:DSPC + 28% protein	10	−2.9	43.9	1.07
DEPC	10	22.0		
DEPC:DPPC 1:1	10	18.7[b]	33.9	0.84
DEPC:DPPC + 13% protein	10	20.7[b]	35	0.82
DEPC:DPPC + 32% protein	10	22.0[b]	35.8	0.80

[a] T_c is the temperature of the peak midpoint of each component except where noted. [b] Temperatures are for the onset and completion of heating obtained by extrapolating the slope of the peak to the base line.

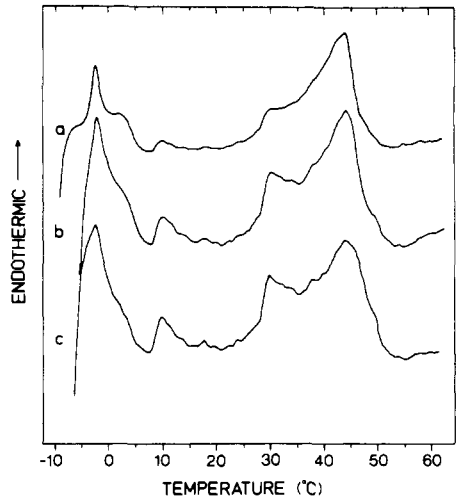

FIGURE 4: DSC thermograms scanned at 10 °C/min for (a) an equimolar mixture of DLPC and DSPC prepared by dialysis at 62 °C; (b) containing 12% lipophilin; (c) containing 28% lipophilin. Samples were recorded at high sensitivity and background noise is high.

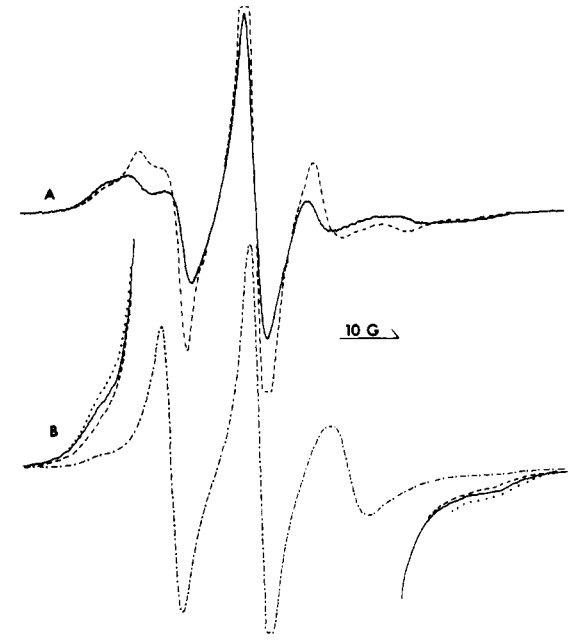

FIGURE 5: ESR spectra of (a) 5-doxylstearate in vesicles of DMPC containing 35% lipophilin at 30 (—) and 50 °C (- - -); and (b) 16-doxylstearate in the same vesicles recorded at low sensitivity at 22 °C (— · — ·) and recorded at five times higher sensitivity at 22 (· · ·), 30 (—), and 50 °C (- - -).

DLPC as well as DSPC (Figure 4a). In this mixture, the ratio of lower to higher melting component cannot be measured very accurately because of the impurity in DLPC and background noise, but it appears to increase in the presence of lipophilin (Table II, Figure 4b,c) indicating that in this case the protein may bind to the higher melting component preferentially. This is consistent with the greater amount of boundary lipid for DSPC compared with DLPC.

These results suggest that in general there may be a slight preferential interaction with the lower melting component in most mixtures of lipids with the exception of DLPC and DSPC. However, the effect is not large and lipophilin interacts to a nearly equal extent with both lipids in the mixtures used.

The amount of boundary lipid as determined by DSC is the amount which exists at the lipid phase transition. As reported earlier, the amount of immobilized probe in egg phosphatidylcholine at 37 °C, a temperature well above its phase transition, indicated that the amount of boundary lipid is considerable. ESR spectra for the fatty acid spin labels, 5-doxylstearate and 16-doxylstearate in DMPC vesicles containing lipophilin at different temperatures are shown in Figure 5. There is a small decrease in height of the immobilized component when the temperature is raised from 30 to 50 °C. This can be seen more clearly for 16-doxylstearate (Figure 5B) where there is less overlap between the immobilized component and the fluid component. The amount of boundary lipid cannot be quantitated by determining the amount of immobilized spin label as this depends on the partition coefficient of the probe in the immobilized and fluid phases and probably depends on the probe used as well as temperature. Although the amount of boundary lipid may decrease somewhat with increase in temperature, these results indicate that there is still a considerable amount of boundary lipid at 50 °C, well above the phase transition temperature of DMPC.

Discussion

Lipophilin was found to have a similar effect on all of the lipids studied. It decreased the enthalpy of the lipid phase transition but did not alter the phase transition temperature in agreement with an earlier study using DPPC (Papahadjopoulos et al., 1975). In contrast, Curatolo et al. (1977) recently reported that bovine myelin proteolipid increased the phase transition temperature of DMPC by 2 °C. The difference between their results and the earlier report by Papahadjopoulos et al. (1975) was attributed to the heating rates used. However, even when we examined vesicles containing lipophilin at the slow heating rate used by Curatolo et al. (1977), 1.25 °C/min, in the present study or by monitoring the TEMPO partition coefficient by ESR spectroscopy (Boggs et al., 1976), we did not observe a higher melting peak. The protein used here is a fraction of the apoprotein from the total proteolipid from human myelin and represents at least half of the proteolipid apoprotein (Gagnon et al., 1971), while Curatolo et al. (1977) used the unfractionated apoprotein from the total proteolipid of bovine myelin. Lipophilin is also purified and delipidated by different methods than used by Curatolo et al. Although it is expected that both proteins should behave similarly, it is possible that because of these differences the results cannot be compared. However, observation of this higher melting peak led Curatolo et al. (1977) to define the lipid which melted at the higher temperature as boundary lipid. In our studies we interpret the decrease in enthalpy to indicate that the boundary lipid does not go through a phase transition. Indeed there is a significant amount of boundary lipid at temperatures well above the phase transition as monitored by the immobilization of a fatty acid spin label (Boggs et al., 1976; Figure 5). The boundary lipid surrounding the Ca^{2+}, Mg^{2+}-ATPase of sarcoplasmic reticulum also does not appear to go through a phase transition (Hesketh et al., 1976).

Although the fatty acid chains of the boundary lipid are immobilized by interactions with the protein this does not mean that they are in the gel state or more ordered. They may in fact become more disordered in order to conform to the irregular surface of the protein and provide an interface between the protein and the fatty acid chains of the lamellar lipid. A recent Raman spectroscopic study by Curatolo et al. (1978) on the total proteolipid which showed that this protein increased the gauche character of the lipid fatty acid chains below the phase transition temperature is consistent with an increase in disorder of the boundary layer relative to gel phase lipid.

Since the boundary layer of lipid surrounding lipophilin does not go through the phase transition, the amount of boundary lipid can be estimated by extrapolating to $\Delta H = 0$ in Figure 2. This requires the assumption that the enthalpy of the lamellar lipid remains unchanged, which may not be true, since the protein has been shown to increase the order of the lamellar lipid slightly (Boggs et al., 1976) and the DSC peaks are broadened slightly. However, the effect of this on the enthalpy is probably small and is probably similar for all the lipids so that the boundary lipid estimates for different lipids can be compared.

Assuming a monomer molecular weight for lipophilin of 25 000 g/mol, the amount of boundary lipid was found to be 21–25 molecules of lipid per molecule of protein for DMPC, DPPC, and DSPC and thus does not depend on fatty acid chain length over the range C-14 to C-18. However, the amount of boundary lipid was somewhat less for DLPC and DEPC at a value of about 16 molecules of lipid/molecule of protein. This indicates that a short chain fatty acid and an unsaturated trans fatty acid may not be able to interact as well with the protein. The effect of cis double bonds was not examined since these lipids melt below 0 °C. However, most membrane lipids have fatty acids longer than C-12 and have one saturated fatty acid and thus it can be concluded that their interaction with lipophilin would probably not depend significantly on fatty acid content but would depend on the presence of an acidic polar head group as shown previously (Boggs et al., 1977).

Even in a mixture of lipids with different fatty acid chain length, no significant preferential interaction with one particular lipid could be demonstrated, regardless of whether the lipids cocrystallize or not. This may be in contrast to other membrane proteins which have been shown by freeze fracture electron microscopy to interact with the lipid which melts first in lipid mixtures and to aggregate as they are squeezed out of gel phase lipid as for glycophorin (Grant & McConnell, 1974) and the sarcoplasmic reticulum ATPase (Kleemann & McConnell, 1976). Lipophilin can be incorporated into lipid below its phase transition temperature (Boggs et al., 1976) and freeze-fracture results indicate that the protein does not aggregate when the lipid is in the gel phase (Papahadjopoulos et al., 1975).

The amount of boundary lipid found for this protein is comparable to that found for other proteins, approximately 30 for the sarcoplasmic reticulum Ca^{2+}, Mg^{2+}-ATPase (Warren et al., 1975) and 47 for cytochrome oxidase (Jost et al., 1973). However, these latter two proteins have a much higher molecular weight than lipophilin. The radius of lipophilin in vesicles has been estimated from X-ray diffraction as 54 Å (Brady et al., 1978). It would thus have a circumference of 339 Å which would allow room for 70 phospholipid molecules around it if both fatty acid chains of the phospholipid are interacting with the protein, assuming a diameter of each fatty acid chain at 4.8 Å (Engelman, 1971). The oligomeric state of lipophilin in the bilayer is not known; it exists as a monomer in organic solvents and a trimer or higher oligomers in water depending on how it is prepared (Moscarello et al., 1973; Cockle et al., 1978). The amount of boundary lipid was calculated per monomer of protein. The amount per trimer, assuming a molecular weight of 75 000 g/mol, would be 63–75 molecules of lipid. Since a sphere of 54 Å radius has room for 70 molecules of phospholipid, this suggests that the protein may exist as a trimer in the bilayer.

Two recent theoretical studies predicted that membrane proteins should perturb several layers of lipid beyond the boundary layer (Marcelja, 1976; Owicki et al., 1978). We showed earlier that lipophilin increases the order of the lamellar phase lipid as well as immobilizing a layer of boundary lipid (Boggs et al., 1976). Although it seems reasonable that there be a boundary gradient between the 1st layer of boundary lipid and the lamellar phase lipid, the techniques used to date are not sufficient to detect it. Longmiur et al. (1977) also suggested that cytochrome oxidase can perturb lipid outside the boundary layer, although they could not resolve the boundary lipid from the remaining lipid. Curatolo et al. (1977) interpreted the increase in phase transition temperature of DMPC induced by the proteolipid as evidence for a perturbing effect of the protein on three or four layers of lipid around the protein. However, we do not observe this higher melting peak with our purified protein. They also observed an increase in trans character of the fatty acid chains above the lipid phase transition by Raman spectroscopy, and interpreted this as belonging to the boundary lipid, but it is also consistent with the increased order which we observed in the lamellar lipid outside the boundary layer.

The boundary lipid determined by DSC is the amount which exists at the phase transition temperature. Theoretical studies

(Marcelja, 1976; Owicki et al., 1978) predicted a decrease in the amount of boundary lipid with an increase in temperature. Dahlquist et al. (1977) presented evidence that the amount of boundary lipid around cytochrome oxidase decreases with increasing temperature. However, the effect of temperature on the height of the peak in vesicles containing lipophilin indicates that, although some decrease in the amount of boundary lipid may occur, there is still a significant amount at temperatures well about the phase transition.

The interaction of lipophilin with lipids thus depends on polar head group but not on fatty acid chain length. It can interact with both neutral and acidic lipids but in a mixture of lipids it preferentially binds acidic lipids to its boundary layer (Boggs et al., 1977). However, it can interact equally well with fatty acid chains over the range C(14)–C(18). There is evidence that the sarcoplasmic reticulum ATPase also does not bind one lipid preferentially to its boundary layer in mixtures of DMPC–DOPC or DPPC–DOPC (Warren et al., 1975) since the activity, which is high in dioleoylphosphatidylcholine (DOPC) and low in DPPC or DMPC, decreases linearly with increasing concentration of DMPC or DPPC in mixtures with DOPC. Whether other membrane proteins and enzymes behave similarly or interact more specifically with certain lipids remains to be determined.

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