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SIMILAR EFFECT OF PROTEOLIPID APOPROTEINS FROM HUMAN MYELIN (LIPOPHILIN) AND BOVINE WHITE MATTER ON THE LIPID PHASE TRANSITION

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Summary

The proteolipid apoprotein from bovine white matter has been reported to increase the phase transition temperature of dimyristoyl phosphatidylcholine, in contrast to a proteolipid apoprotein fraction from human myelin, called lipophilin, which decreases the enthalpy without altering the phase transition temperature. Since these results lead to different conclusions concerning the structure and amount of boundary lipid surrounding these proteins, the effects of the two proteins on the phase transition of dimyristoyl phosphatidylcholine were compared. Neither protein has any effect on the phase transition temperature, regardless of the method of delipidation of the protein, the amount of residual lipid, the method of incorporation into vesicles, or heating rates used for differential scanning calorimetry. However, a higher melting component was observed when decomposition of the lipid to lysophosphatidylcholine had occurred. Addition of as little as 6% of the decomposition products of dimyristoyl phosphatidylcholine, lysodimyristoyl phosphatidylcholine and myristic acid, is enough to produce a higher-temperature peak. The intensity of this peak increases with increasing protein concentration similar to the reported result on the bovine white matter proteolipid.

The question as to whether the protein-induced decrease in enthalpy is due to boundary lipid or entrapment of lipid in protein aggregates was also addressed by studying the appearance of the intramembranous protein particles by freeze-fracture electron microscopy at temperatures above and below the phase transition and between the premelt and main transitions. The protein is randomly dispersed above the phase transition. At low concentrations, below the phase transition, it clusters, forming particle-free and particle-rich areas, but

does not aggregate. At higher concentrations it is randomly dispersed below the premelt and main transition but is clustered between the premelt and main transition. Since the protein is more randomly dispersed above the transition than below, the reduction in enthalpy of the freezing transition was compared to that of the melting transition and was found to be identical, suggesting that the withdrawal of lipid from the phase transition is probably not due to lipid entrapment but due to the formation of a boundary lipid interface between the protein and the bulk lipid.

Introduction

A number of intrinsic membrane proteins have been reported to possess a layer of boundary lipid, as originally observed and defined by Jost et al. [1] for cytochrome oxidase. Recently, conflicting opinions as to the structure and even the existence of boundary lipid have been expressed [2]. In the case of myelin proteolipid, conflicting results regarding its effect on the lipid phase transition have led to different conclusions about the structure and amount of boundary lipid for this protein [3—5].

A pure protein fraction, called lipophilin [6] isolated from the proteolipid of human myelin, has been found to decrease the enthalpy of the lipid gel-to-liquid crystalline phase transition without altering the transition temperature [3,8,9] and to restrict the motion of a population of fatty acid spin labels incorporated into the bilayer [4], indicating that like other intrinsic proteins it induces a boundary layer of lipid which does not go through the phase transition. In two independent studies the amount of boundary lipid was estimated, from the decrease in enthalpy, to be 15 [3] and 21—25 [8] molecules of lipid per monomer of protein (mol. wt. 25 000). This boundary lipid has restricted motion on the electron spin resonance (ESR) time scale but not on the nuclear magnetic resonance (NMR) time scale [7], indicating that it exchanges with bulk lipid at a rate greater than $1 \cdot 10^4$ s^{-1} but less than $1 \cdot 10^7$ s^{-1}. No conclusion regarding the orientation or state of isomerization of the fatty acid chains could be drawn from the ESR spectrum but NMR spectroscopy suggested that the boundary lipid is more disordered than the liquid crystalline phase lipid [7].

The decrease in enthalpy without change in phase transition temperature for lipophilin is not consistent with a differential scanning calorimetry (DSC) study by Curatolo et al. [5] on bovine white matter proteolipid apoprotein. This study indicated that this proteolipid apoprotein induces a new transition 2°C higher than that of the pure lipid. At a molar ratio of lipid-to-protein of 142 : 1, all of the lipid melted at the higher temperature although with a reduced enthalpy. It was concluded that this higher melting lipid represented boundary lipid, thus suggesting that the boundary lipid goes through its own phase transition and includes several layers of lipid surrounding the protein. The higher phase transition temperature and reduction in enthalpy induced by the protein indicated that either the boundary lipid is more ordered above its own phase transition temperature and/or that it is more disordered below its phase transition than unaffected lipid. This was supported by Raman spec-

troscopic data [10], contrary to the NMR results which were similar for the bovine proteolipid apoprotein and lipophilin [7].

Since two theoretical studies [11,12] have considered this observation of a higher-temperature peak in the presence of this protein in their discussion of the origin of boundary lipid, it is important to determine the reason for the discrepancy between the DSC studies on these two proteolipid proteins from bovine white matter and human myelin and to establish the actual effect of this protein on the lipid phase transition.

Human myelin lipophilin, the bovine white matter proteolipid apoprotein as well as a purified proteolipid from rat brain [22] are expected to have similar properties since they have similar amino acid contents, and all possess 2% covalently bound fatty acid [6]. However, since there are differences in the sources of these two proteins, the methods of delipidation and the methods of incorporation into lipid vesicles, we examined whether any of these methodological differences might be the source of the discrepancy in DSC results. It was suggested that the reason for the discrepancy might be the heating rate used for the DSC measurements [5] but we ruled this out in an earlier study [8].

Furthermore, a suggestion has been made that the restriction of motion of fatty acid spin labels and reduction of enthalpy of the phase transition by intrinsic membrane proteins are due to entrapment of lipid between aggregates of these proteins, particularly if the protein is squeezed into aggregates below the phase transition temperature [2]. Therefore, we have also re-examined boundary lipid formation and the arrangement of the protein in the bilayer above and below the phase transition by DSC and freeze-fracture electron microscopy to determine (i) the tendency of the protein to aggregate or remain dispersed below the phase transition and (ii) if the reduction in enthalpy of the exothermic liquid crystalline-to-gel phase transition, where the protein is initially dispersed in the liquid crystalline phase, is similar to the reduction in enthalpy of the gel-to-liquid crystalline phase transition.

Methods

Preparation of human myelin lipophilin. Myelin was isolated from normal human white matter by using the method of Lowden et al. [13]. The proteolipid was extracted and delipidated by chromatography on Sephadex LH-20 in chloroform/methanol (1 : 1, v/v) containing 5% 0.1 N HCl by using the method of Gagnon et al. [14]. The second and major peak (N-2) was taken and chromatographed again to completely delipidate it and stored in the lyophilized form. This major, purified fraction of the proteolipid apoprotein is called lipophilin and contains less than 0.09% phosphorus and 2% covalently bound fatty acid [14,15].

Preparation of bovine white matter proteolipid apoprotein. The proteolipid was extracted from 120 g of bovine white matter according to the procedure of Folch Pi and Stoffyn [16]. It was delipidated by dialysis against chloroform/methanol (1 : 1, v/v) for 3 days followed by dialysis against acidified chloroform/methanol (5% 0.1 N HCl) for 3 days, and then against neutral chloroform/methanol again for 2 days. This preparation (batch I) contained 1.2% phosphorus. Part of batch I was dialysed against acidified chloroform/methanol

for an additional 2 days followed by dialysis against neutral chloroform/methanol (batch II) and part was chromatographed on Sephadex LH-20 in acidified chloroform/methanol (batch III). Batch II contained 0.8% phosphorus while batch III contained less than 0.09% phosphorus.

Other materials. Chromatographically pure dimyristoyl phosphatidylcholine was purchased from Sigma Chemical Co. and stored at −20°C in the dry form. Lysodimyristoyl phosphatidylcholine was purchased from Supelco, myristic acid from Eastman and Hepes from Calbiochem. 2-Chloroethanol was obtained from BDH, redistilled periodically and stored in the dark at 4°C.

Preparation of vesicles. The proteins were incorporated into dimyristoyl phosphatidylcholine by three methods: (i) by dialysis from 2-chloroethanol against 2 mM Hepes buffer (pH 7.4) containing 10 mM NaCl as described [4]; (ii) by evaporation of a chloroform/methanol/water (10 : 5 : 1, v/v) solution of the lipid and protein and suspension of the dry material in buffer by vortexing at room temperature as described [3]; and (iii) by evaporation of the chloroform/methanol/water solution directly into aluminum Perkin-Elmer DSC pans, dessication under vacuum for 10 h, hydration with distilled water, sealing and equilibration at 37°C for 6 h [5]. The vesicle suspensions were pelleted by centrifugation at 12 000 rev./min in an Eppendorf microcentrifuge. The lipid-protein ratio of the pellets was analyzed as described previously [8].

Lysodimyristoyl phosphatidylcholine and myristic acid were added to vesicles containing 20% of batch II proteolipid apoprotein or lipophilin by evaporation of an equimolar chloroform solution of the two compounds in a test tube and addition of a known amount of the vesicles (1 mg lipid/0.25 ml buffer) which had been prepared by the vortex suspension method. The samples were subjected to vortex suspension again and incubated at 37°C for 30 min.

Differential scanning calorimetry. The temperature and heat content of the thermotropic transition of the vesicle preparations were determined with a differential scanning calorimeter (Perkin-Elmer DSC-2) using scanning rates of 1.25—5°C/min. Samples were heated, cooled and reheated several times and the scans were completely reproducible after heating to 52°C at least five times. No transition due to the protein is observed. Some samples were also measured on a Brandts high-sensitivity Microcal DSC at a heating rate of 0.5°C/min and a sensitivity of 10 μV.

Enthalpy determinations for vesicles prepared by dialysis from 2-chloroethanol were made of both the endothermic liquid crystalline-to-gel phase transition and of the exothermic gel-to-liquid crystalline phase transition by heating and cooling the samples at a rate of 5°C/min, using a fast chart speed of 80 mm/min. Four pans were filled and measured for each sample and peak areas were determined by weighing. The pans were opened and the amount of phospholipid in the pan was determined as described earlier [8].

Freeze-fracture electron microscopy. Vesicles containing lipophilin, prepared by dialysis from 2-chloroethanol, were used for freeze-fracture electron microscopy within hours after preparation. The samples were made 30% (v/v) in glycerol, reincubated at 37°C for a few minutes before use, and droplets were mounted in gold cups. The samples were frozen from various temperatures, 42, 19 and 4°C by placing the gold cups on a heat sink in a beaker of water at the desired temperature. After leaving the samples at the desired temperature

for a few minutes, they were quickly frozen in liquid Freon 22. A Balzer BA-360 Freeze Etch Apparatus and Phillips EM-201 electron microscope were used as described earlier [4].

Results

The effect of methodological differences between earlier studies was investigated. The human myelin protein lipophilin used in studies from our laboratory [3,4,8], is fractionated and delipidated by chromatography on Sephadex LH-20 in acidified chloroform/methanol and has been shown to be a pure protein [14]. In the study of Curatolo et al. [5], the bovine white matter proteolipid is delipidated by dialysis from acidified chloroform/methanol but is not fractionated [16,18]. Vesicles containing lipophilin used in the earlier studies were prepared by methods i and ii and gave similar results [3,8]. Method iii was used earlier for the bovine proteolipid apoprotein [5].

Effect of lipophilin on the lipid phase transition

The effect of increasing amounts of lipophilin on DSC thermograms of dimyristoyl phosphatidylcholine vesicles prepared by method i is shown in Fig. 1 (heating rate 1.25°C/min). As reported earlier [3,8], no shift in the phase transition temperature, T_c, no significant broadening of the peaks, and no

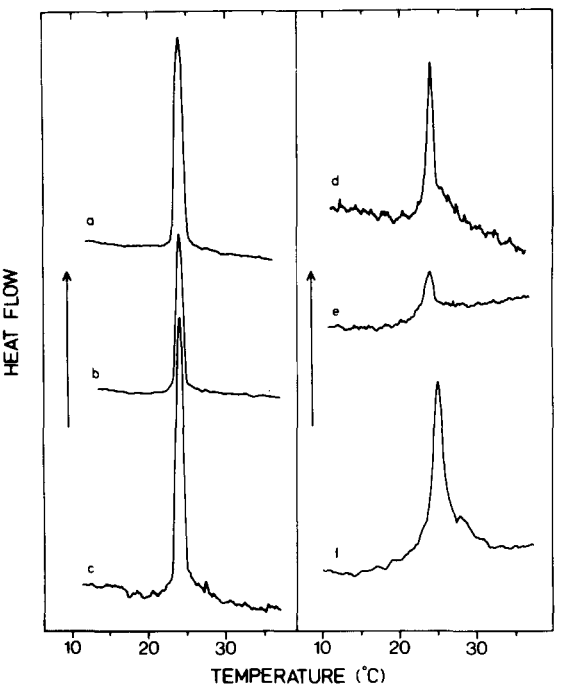


Fig. 1. DSC thermograms obtained by heating at 1.25°C/min (except f) of dimyristoyl phosphatidylcholine vesicles containing lipophilin prepared by dialysis from 2-chloroethanol. Protein concentration (wt.%) in the vesicles was: (a) 0; (b) 7.9; (c) 15.4; (d) 34.9; and (e) 56. f is the same sample as in e run at 5°C/min. The pans do not contain the same amount of lipid.

TABLE I

ENTHALPY OF MELTING AND FREEZING FOR DIMYRISTOYL PHOSPHATIDYLCHOLINE VESICLES CONTAINING VARYING CONCENTRATIONS OF LIPOPHILIN

Data are an average of four determinations at each protein concentration.

Protein concentration in vesicles (% by wt.)	Molar ratio protein:lipid	ΔH melting (kcal/mol)	ΔH freezing (kcal/mol)
0	0	8.57 ± 0.35	8.64 ± 0.37
15.7 ± 0.7	0.00517 ± 0.00023	6.11 ± 0.52	6.49 ± 0.32
30.3 ± 1.8	0.01208 ± 0.00073	4.38 ± 0.43	4.48 ± 0.21
42.8 ± 1.5	0.02078 ± 0.00072	2.82 ± 0.33	2.30 ± 0.27

higher-melting peak occurred at concentrations of lipophilin up to 35% (wt.%) (Fig. 1a—d). This was also true for samples measured on a high-sensitivity differential scanning calorimeter at a heating rate of 0.5°C/min and a sensitivity of 10 μV. At a heating rate of 5°C/min as shown in Fig. 2b, the premelt transition is observed and is retained up to 35% lipophilin content, although it decreases in intensity [8]. The main effect of lipophilin is to decrease the heat of the transition in a linear fashion with increasing concentration of protein as shown earlier [3,8] and in Table I. However, a concentration of lipophilin of 56% broadens the transition and results in a shoulder on the high-temperature side

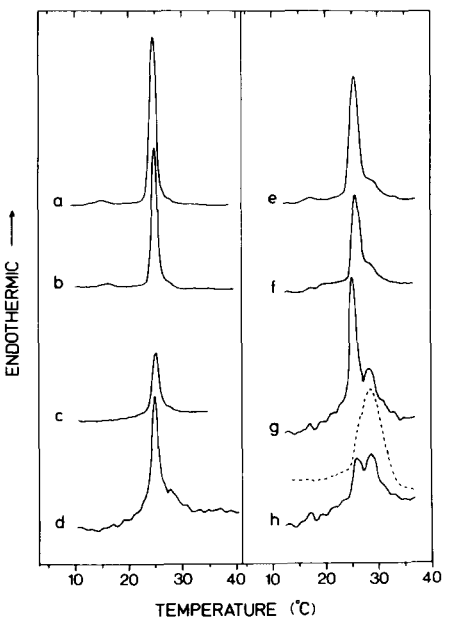


Fig. 2. DSC thermograms obtained by heating at 5°C/min of dimyristoyl phosphatidylcholine vesicles containing lipophilin prepared by dialysis from 2-chloroethanol. Protein concentration was (a and e) 0; (b and f) 22.5%; (c and g) 44%; (d and h) 56%; and the dashed line, 34.9% protein. Samples a—d were measured immediately after preparation. Samples e—h are the same samples measured after storage at room temperature for 2 days. Sample indicated by dashed line is sample d in Fig. 1 measured after storage at room temperature for 7 days. The pans do not contain the same amount of lipid.

of the main peak (Fig. 1e). This can be seen more distinctly at a heating rate of 5°C/min as shown in Fig. 1f. These results are in distinct contrast to the results reported earlier on the bovine myelin proteolipid apoprotein [5], where a higher-temperature shoulder was observed at even 3.2% protein content and only a broad peak 2°C above that of the pure lipid was observed at 20% protein content.

The DSC scans of one set of vesicles containing lipophilin changed markedly in appearance and resembled the scans previously reported for bovine proteolipid after they had been stored at room temperature for 2 days. The DSC scans of these samples measured immediately after preparation are shown in Fig. 2a—d and after storage at room temperature for 2 days in Fig. 2e—h (heating rate 5°C/min). A peak approx. 3°C higher than the lipid transition is observed after storage. This peak increases in intensity with increasing protein concentration but is apparent also in a sample of the lipid only (Fig. 2e). Remeasurement of a sample containing 34.9% lipophilin (Fig. 1d shows the appearance immediately after preparation) after leaving at room temperature for 7 days showed complete conversion to this higher-temperature peak (Fig. 2, dashed line). Application of the leftover samples to TLC revealed the presence of small amounts of lysophosphatidylcholine and free fatty acid in all samples in which a higher melting peak was seen. No lysocompounds were seen for lipid which had been stored in the freezer. This behavior did not recur with new batches of

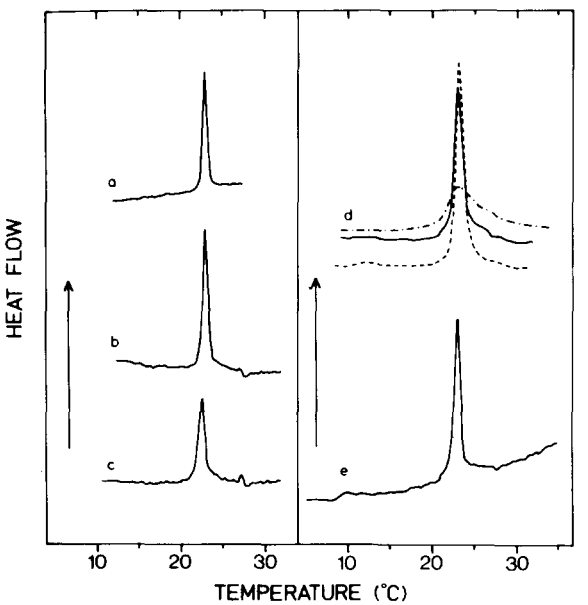


Fig. 3. DSC thermograms obtained by heating at 1.25°C/min for a, b, c and e and 5°C/min for d, of dimyristoyl phosphatidylcholine vesicles containing the bovine white matter proteolipid apoprotein. a—c contain batch II of this protein and were prepared by solvent evaporation and vortex suspension in buffer. a, dimyristoyl phosphatidylcholine only; b, with 10% protein; c, with 20% protein. d, vesicles containing 20% of different batches of the protein, batch I (— · — ·), batch II (———) and batch III (- - - - -). Vesicles were also prepared by solvent evaporation and vortex suspension in buffer. e, vesicles containing 10% of batch II of the protein prepared by evaporation directly into the pan (1 mg lipid) and hydration with distilled water, run at 1.25°C/min. The pans do not contain the same amount of lipid.

lipid obtained from the same and other sources so that the amount of decomposition could not be quantitated.

Effect of bovine white matter proteolipid, method of delipidation and method of vesicle preparation

The effect of the bovine white matter proteolipid apoprotein (batch II) on DSC thermograms of vesicles prepared by method ii is shown in Fig. 3a—c (heating rate 1.25°C/min) and is similar to that of lipophilin. Even at a concentration of 20%, no higher-temperature peak or shoulder is observed. The effect of increasing delipidation of the proteolipid on vesicles containing 20% of each batch is shown in Fig. 3d. Batch I, which still contains a high content of phospholipid, broadens the transition considerably but does not shift the transition temperature while batch II broadens the transition slightly. The transition is even sharper for batch III and the premelt transition is observed as for lipophilin delipidated in the same manner. Similar results were obtained for vesicles prepared by methods i or iii as shown in Fig. 3e for vesicles prepared by method iii containing 10% of batch II protein.

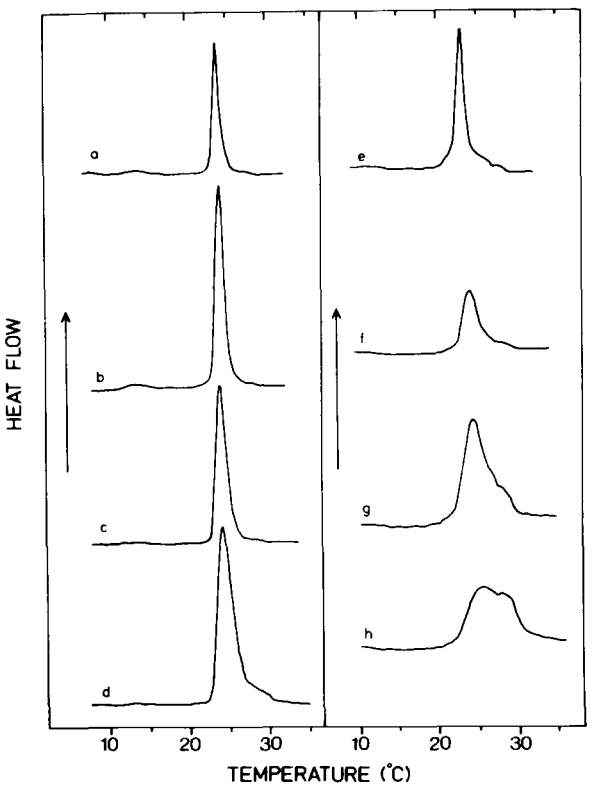


Fig. 4. DSC thermograms obtained by heating at 5°C/min of (a—d) dimyristoyl phosphatidylcholine vesicles and (e—h) dimyristoyl phosphatidylcholine containing 20% of batch II of the proteolipid apoprotein in the presence of increasing amounts of an equimolar mixture of lysodimyristoyl phosphatidylcholine and myristic acid. Vesicles were prepared by solvent evaporation and vortex suspension in buffer. Concentration (mol%) of lysodimyristoyl phosphatidylcholine and myristic acid was (a and e) 0; (b and f) 2%; (c and g) 4%; (d and h) 6%. The pans do not contain the same amount of lipid.

Effect of addition of decomposition products of dimyristoyl phosphatidylcholine

The effect of adding the decomposition products of dimyristoyl phosphatidylcholine, equimolar amounts of lysodimyristoyl phosphatidylcholine and myristic acid at a concentration of 2—6 mol% directly to vesicles with or without 20% of batch II protein is shown in Fig. 4. These compounds have little effect on pure dimyristoyl phosphatidylcholine, other than a slight broadening of the transition (Fig. 4a—c), until a concentration of 6% where a higher temperature shoulder occurs about 3°C above the dimyristoyl phosphatidylcholine transition (Fig. 4d). A much more marked effect occurs on vesicles containing 20% protein as shown in Fig. 4e—h. Even 2% lysodimyristoyl phosphatidylcholine and myristic acid broaden the transition (Fig. 4f) while 4% results in a higher-temperature shoulder (Fig. 4g). At 6%, two broad peaks are observed, 1.3 and 3.7°C above the pure lipid. The presence of two peaks both at higher temperatures than the T_c of dimyristoyl phosphatidylcholine may indicate incomplete equilibration of these compounds with the lipid. A similar effect of these compounds is observed on samples containing lipophilin.

Freeze-fracture electron microscopy on vesicles containing lipophilin

An electron micrograph of vesicles containing 17.5% lipophilin, frozen from 42°C (Fig. 5), shows that above the phase transition the intramembranous particles are randomly dispersed in the fracture plane. When the vesicles are frozen from 19°C, between the premelt and main phase transition temperatures, the characteristic lipid ridge pattern [19] is observed even in the presence of protein as shown in Fig. 6A, B and C for vesicles containing 0, 17.5 and 23.6% lipophilin, respectively. In the absence of protein it can be seen that the ridge pattern changes direction occasionally, probably due to defects in the bilayer structure. In vesicles containing 17.5% lipophilin the intramembranous particles are located at the discontinuities between the fields of the uniform ridge pattern (Fig. 6B). The ridge pattern in the particle-free areas appears similar to that in Fig. 6A but the fields are smaller in size in vesicles containing protein than in the pure lipid. Thus, the protein may locate preferentially at defects in the gel phase structure and may also induce more defects (Fig. 6B). At a higher protein concentration, 23.6% (Fig. 6C), the particle-free areas are even smaller, the ridge pattern in the particle-free areas is more distorted and is of a greater periodicity than in the pure lipid. The ridge pattern is nearly gone in the regions containing particles. Although the particles are localized in clusters at this temperature, they are not closely aggregated.

When the vesicles are frozen from 4°C, below the premelt transition, the appearance depends more strongly on the protein concentration. At a protein concentration of 13.6% the fracture surface has an irregular jumbled appearance with some areas of broad ridges and some smooth areas (Fig. 7A). There are particle-free areas and particle-rich areas, but the particles are not aggregated even in the particle-rich areas. If the vesicles are kept at 4°C for several days, however, the intramembranous particles become aggregated. At a higher protein concentration (21%), vesicles frozen from 4°C have a nearly smooth background with intramembranous particles almost randomly dispersed (Fig. 7B) as reported earlier [3].

Comparison of protein-induced decrease of enthalpy of freezing and melting of lipid

The effect of lipophilin on the enthalpy of the gel-to-liquid crystalline phase transition (melting) as well as the liquid crystalline-to-gel phase transition

Fig. 5. Freeze-fracture micrograph of vesicles of dimyristoyl phosphatidylcholine with 17.5% (wt.%) lipophilin, quenched from 42°C, above the phase transition temperature. Magnification, ×54 675.

(freezing) is shown in Table I. The data were fitted to the best straight line by linear regression and extrapolated to $\Delta H = 0$ to estimate the amount of lipid removed from the phase transition as described previously [8], giving a value of

Fig. 6. Freeze-fracture micrographs of vesicles of dimyristoyl phosphatidylcholine with (A) 0, (B) 17.5 and (C) 23.6% (wt.%) lipophilin, quenched from 19°C, between the premelt and main transition temperature. Magnification, ×54 675.

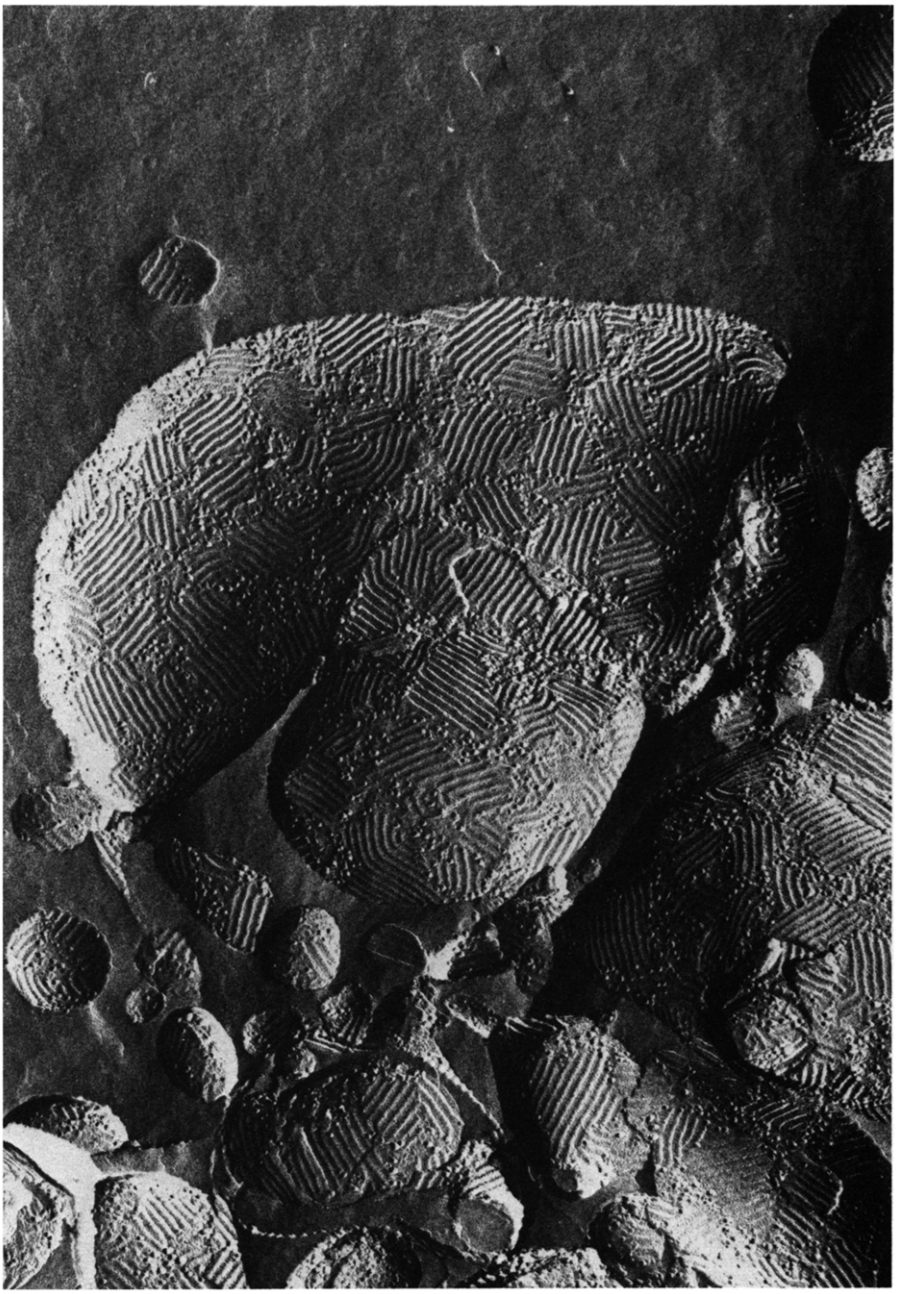


Fig. 6B.

34.5 ± 5.5 and 36.3 ± 3.5 molecules of lipid per monomer of protein for the heating and cooling scans, respectively. Although this is a higher value than reported in our earlier study, these results indicate that when the protein is dis-

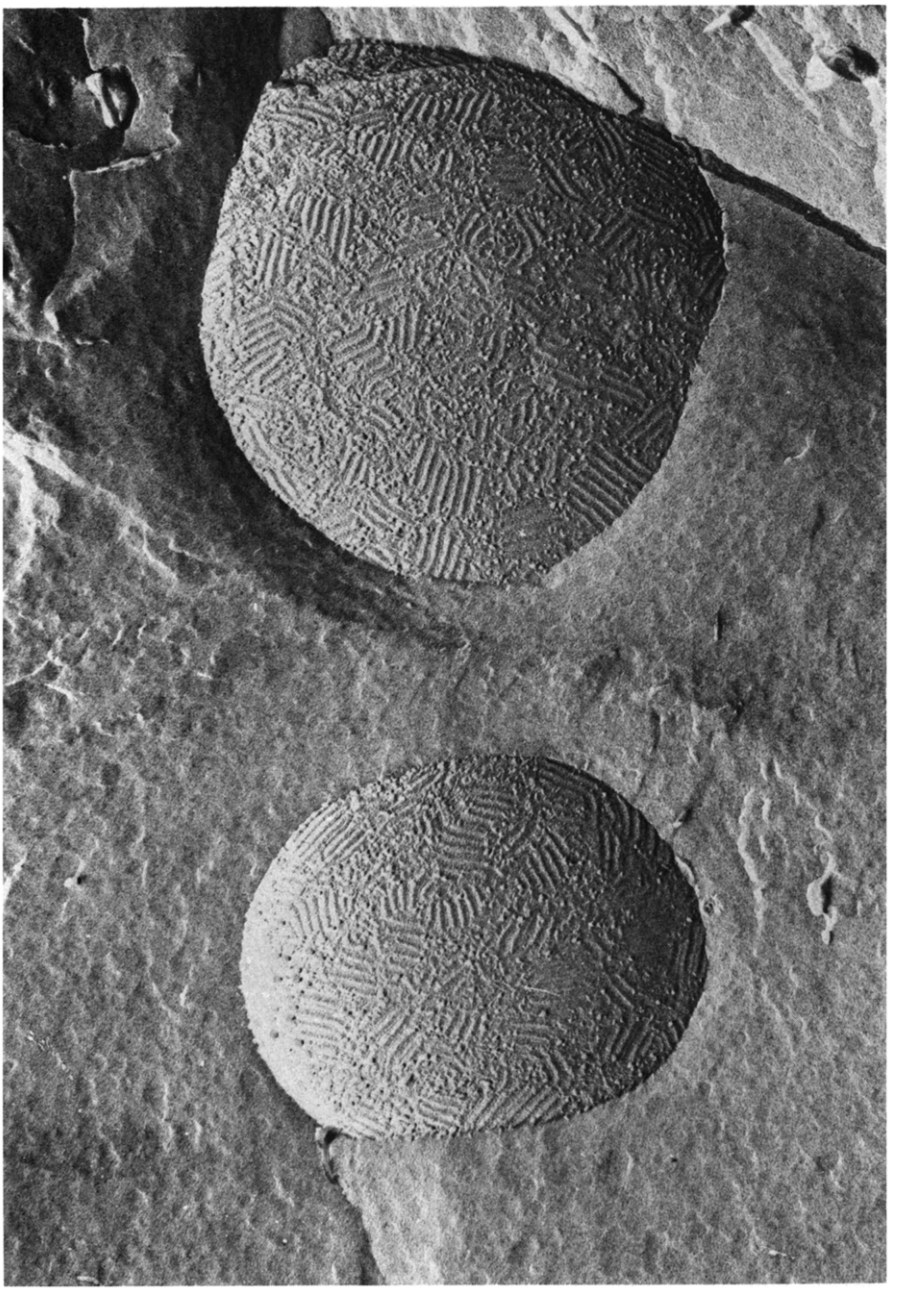
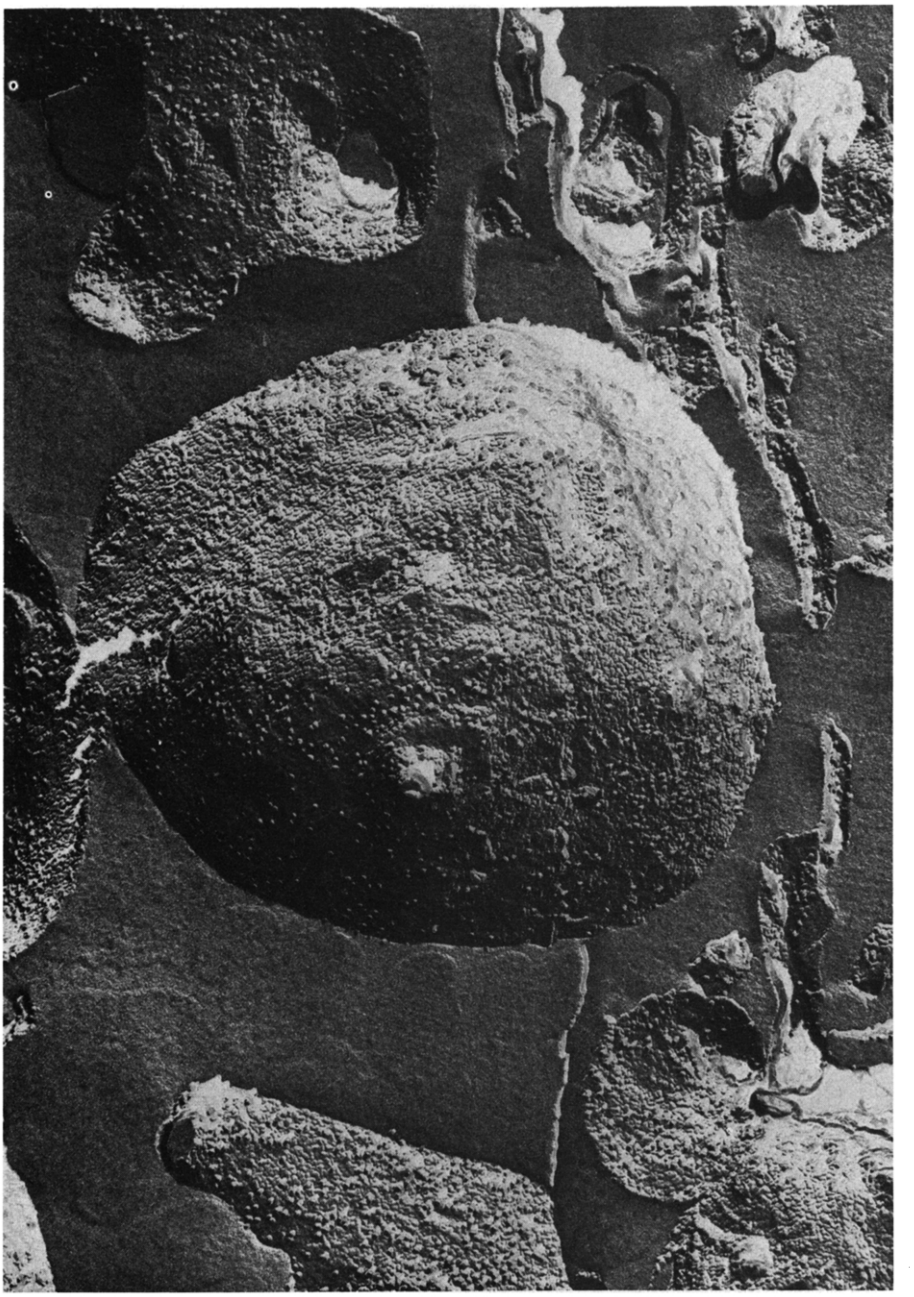


Fig. 6C.

(A)

Fig. 7. Freeze-fracture micrographs of vesicles of dimyristoyl phosphatidylcholine with (A) 13.6% and (B) 21% (wt.%) lipophilin, quenched from 4°C, below the premelt and main transition temperatures. Magnification, ×54 675.

persed in the plane of the bilayer in the liquid crystalline phase it prevents as much lipid from refreezing as it prevents from melting even though the protein is more clustered in the gel phase.



(B)

Fig. 7B.

Discussion

The results presented here indicate that there is no higher melting component in dimyristoyl phosphatidylcholine vesicles containing either the human myelin proteolipid apoprotein fraction called lipophilin or the bovine white

matter proteolipid apoprotein, contrary to the results reported by Curatolo et al. [5]. We have examined whether the reason for the discrepancy between these two studies might be the source of the proteolipid, the method of preparation and delipidation, the method of incorporation into vesicles, the aqueous solution used for hydration, the presence of residual lipid bound to the proteolipid, and the heating rate used for DSC and have ruled out all of these possibilities.

The only time we observed a higher melting component which resembled that reported by Curatolo et al. [5] was when decomposition to lysodimyristoyl phosphatidylcholine and free fatty acid had occurred in our samples after being left at room temperature for 2 days. Our subsequent samples were stable for many days even at 37°C suggesting that there may have been some impurity in one batch of dimyristoyl phosphatidylcholine which caused decomposition. Addition of as little as 6% lysodimyristoyl phosphatidylcholine and myristic acid to the vesicles caused the appearance of a higher melting peak similar to that in the samples which had decomposed. The effect of these decomposition products resembles the observations of Curatolo et al. [5] not only in the appearance of a peak 3°C higher than the T_c of dimyristoyl phosphatidylcholine but also in the increased intensity of this peak with increasing protein concentration. It has been reported elsewhere that both lysophosphatidylcholine and myristic acid increase the lipid phase transition temperature [20]. Decomposition during the course of X-ray diffraction measurements on complexes of lipophilin with phosphatidylcholine also was found to be the cause of an increase in lipid phase transition temperature [21]. However, the increased effect of these compounds with increasing concentration of protein as shown in Fig. 4 was not expected and suggests that the results in Fig. 2 are not due to increased decomposition or contamination due to the presence of the protein. This increased effect in the presence of protein may be due to exclusion of these compounds from the boundary layer of the protein, which would increase their concentration in the bulk lipid, or to greater uptake of these compounds in vesicles containing proteins.

We have found that lipophilin and the proteolipid apoprotein behave similarly in that they have little effect on the transition temperature, peak width or premelt transition until concentrations of approx. 35% by weight where the premelt transition disappears and the peak broadens somewhat but does not change in temperature. We have not measured the effect of the bovine white matter proteolipid apoprotein on the enthalpy of the transition but expect it to be similar to that of lipophilin in decreasing the enthalpy linearly with concentration, indicating that it prevents some lipid from participating in the phase transition. The amount of lipid removed from the phase transition is greater in this study than in our previous study [8] and may depend on the protein preparation or some other unknown variable. It is of the order of 20—35 molecules per monomer of protein, enough to form one layer of boundary lipid surrounding the protein. However, it should be pointed out that it may not necessarily completely surround the protein and may also occupy the interstitial space between the polypeptide chains of the protein. These results are consistent with the NMR results on these proteins, suggesting an increase in disorder (but not motion) of the boundary lipid [7] but not with the Raman spectro-

scopic data indicating an increase in *trans* character of the lipid above the phase transition temperature [10]. This increased *trans* character may occur in the bulk lipid rather than in the boundary lipid as suggested by the ESR spectra in which components due to both the bulk lipid and the boundary lipid could be detected [4].

Myelin proteolipid, like other intrinsic membrane proteins, e.g., cytochrome oxidase [1] and glycophorin [23], produces detectable effects on fatty acid chain motion and the enthalpy of the phase transition without broadening the transition endotherm significantly [3,4,8] at lipid-to-protein ratios considerably higher than that which is considered to be in the boundary layer. This is different from the behavior of gramicidin, a small polypeptide which restricts fatty acid spin label motion only at very high ratios of protein-to-lipid (mol ratio 1 : 5 in contrast to 1 : 150 for lipophilin [4]) and broadens the lipid phase transition endotherm significantly [27]. This has led to the suggestion that these effects of gramicidin and possibly membrane proteins on lipids are due to entrapment of lipid between protein aggregates [2].

It could be argued that the occurrence of a premelt transition and sharp phase transition at a concentration of 30% lipophilin (mole ratio of lipid-to-protein 84 : 1) might indicate aggregation of the protein below the phase transition, leaving most of the lipid unperturbed. However, if the protein occurs as a trimer (mol. wt. 75 000) in the bilayer, as suggested earlier [8] the lipid-to-trimer mole ratio would be 252 : 1 of which 90 are in the boundary layer and 162 are free. This is enough free lipid to give a cooperative transition as observed [24,25]. Furthermore, the location of the protein particles at sites of defects in the bilayer crystalline structure at temperatures between the premelt and main transition temperatures results in larger areas of free lipid than expected from the lipid-to-protein mole ratio. The appeance in freeze-fracture micrographs of considerable areas of ridge pattern, believed to be associated with changes in orientation of the lipids at the premelt transition [19], in vesicles containing 23.6% protein (Fig. 6B) is consistent with the occurrence of the premelt transition at this concentration.

At high concentrations the protein is located more randomly, the ridge pattern is distorted or abolished and the premelt transition decreases in size, indicating a perturbation of the crystalline gel structure allowing the protein to be accommodated. Below the premelt transition the protein is nearly randomly dispersed at concentrations above 20%. It is not frozen out of the gel phase lipid into aggregates unless it is left at low temperatures for a prolonged period of time, in contrast to reported results for glycophorin [26] and the sarcoplasmic reticulum ATPase [28]. Furthermore, lipophilin has been shown by DSC not to interact preferentially with the lowest melting lipid in a mixture of non-cocrystallizing lipids [8], in contrast to gramicidin which decreases the enthalpy of the lowest melting lipid first [27].

However, lipid entrapment due to clustering of protein particles below the phase transition could still be responsible for the decrease in enthalpy of the melting transition. Therefore, the reduction in enthalpy of the freezing transition was also measured and found to be identical to the reduction in enthalpy of the melting transition. This indicates that when the protein is dispersed in the liquid crystalline phase it interacts with a similar percentage of lipid as in

the gel phase and prevents it from freezing when the bulk lipid freezes. The protein is unlikely to aggregate rapidly enough to remove this lipid from the phase transition by entrapment.

Although membrane proteins may trap some lipid between adjacent molecules of protein at high protein concentrations, the available evidence indicates that at lower concentrations they interact with the lipid in the immediate layer surrounding them in a different way from the bulk lipid. There may also be a gradient of perturbed lipid for some distance away from the protein as predicted by theoretical studies [11,17], but there is, at present, no evidence for such a gradient in the presence of myelin proteolipid.

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References

1 Jost, P.C., Griffith, O.H., Capaldi, R.A. and Vanderkooi, G. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 4756—4763
2 Chapman, D., Gomez-Fernandez, J.C. and Goni, F.M. (1979) FEBS Lett. 98, 211—223
3 Papahadjopoulos, D., Vail, W.J. and Moscarello, M.A. (1975) J. Membrane Biol. 22, 143—164
4 Boggs, J.M., Vail, W.J. and Moscarello, M.A. (1976) Biochim. Biophys. Acta 448, 517—530
5 Curatolo, W., Sakura, J.D., Small, D.M. and Shipley, G.G. (1977) Biochemistry 16, 2313—1319
6 Moscarello, M.A. (1976) in Current Topics in Membranes and Transport (Bronner, F. and Kleinzeller, A., eds.), Vol. 8, pp. 1—28, Academic Press, New York
7 Rice, D.M., Meadows, M.D., Scheinman, A.O., Goni, F.M., Gomez, J.C., Moscarello, M.A., Chapman, D. and Oldfield, E. (1979) Biochemistry 18, 5893—5903
8 Boggs, J.M. and Moscarello, M.A. (1978) Biochemistry 17, 5734—5739
9 Boggs, J.M., Wood, D.D., Papahadjopoulos, D. and Moscarello, M.A. (1977) Biochemistry 16, 2325—2329
10 Curatolo, W., Verma, S.P., Sakura, J.D., Small, D.M., Shipley, G.G. and Wallach, D.F.H. (1978) Biochemistry 17, 1802—1807
11 Owicki, J.C., Springgate, M.W. and McConnell, H.M. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1616—1619
12 Pink, D.A. and Chapman, D. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1542—1546
13 Lowden, J.A., Moscarello, M.A. and Morecki, R. (1966) Can. J. Biochem. 44, 567—577
14 Gagnon, J., Finch, P.R., Wood, D.D. and Moscarello, M.A. (1971) Biochemistry 10, 4756—4763
15 Wood, D.D., Gagnon, J., Finch, P.R. and Moscarello, M.A. (1971) Am. Soc. Neurochem. Trans. 2, 117
16 Folch Pi, J. and Stoffyn, P.J. (1972) Ann. N.Y. Acad. Sci. 195, 86—107
17 Marcelja, S. (1976) Biochim. Biophys. Acta 455, 1—7
18 Folch Pi, J. and Lees, M.B. (1951) J. Biol. Chem 191, 807—817
19 Luna, E.J. and McConnell, H.M. (1977) Biochim. Biophys. Acta 466, 381—392
20 Papahadjopoulos, D., Hui, S., Vail, W.J. and Poste, G. (1976) Biochim. Biophys. Acta 448, 245—264
21 Rand, R.P., Papahadjopoulos, D. and Moscarello, M.A. (1976) Abstracts, Biophys. Soc., 20th Annual Meeting
22 Nussbaum, J.Z., Rouayreng, J.F., Mandel, P., Jolles, J. and Jolles, P. (1974) Biochem. Biophys. Res. Commun. 57, 1240—1247
23 Van Zoelen, E.J.J., van Dijck, P.W.M., de Kruijff, B., Verkleij, A.J. and van Deenen, L.L.M. (1978) Biochim. Biophys. Acta 514, 9—24
24 Papahadjopoulos, D., Moscarello, M.A., Eylar, E.H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317—335
25 Hinz, H.J. and Sturtevant, J.M. (1972) J. Biol. Chem. 247, 6071—6075
26 Grant, C.W.M. and McConnell, H.M. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 4653—4657
27 Chapman, D., Cornell, B.A., Eliasy, A.W. and Perry, A. (1977) J. Mol. Biol. 113, 517—538
28 Kleemann, W. and McConnell, H.M. (1976) Biochim. Biophys. Acta 419, 206—222