Raman Analysis of the Thermotropic Behavior of Lecithin–Fatty Acid Systems and of Their Interaction with Proteolipid Apoprotein†

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ABSTRACT: We have used Raman spectroscopy to study the thermal transitions of the binary mixtures containing lecithins, i.e., dimyristoyllecithin (DML), dipalmitoyllecithin (DPL), distearoyllecithin (DSL), or egg yolk lecithin (EYL), plus saturated/unsaturated fatty acids, i.e., stearic, oleic, linoleic, linolenic, and linolelaidic acids, as well as ternary systems containing also myelin proteolipid apoprotein (PLA). Our analyses have focused on the 2800–3000-cm^{-1} (CH stretching) regions of the Raman spectra and have employed plots of temperature vs. the intensity of the ~2880-cm^{-1} band relative to that of the ~2850-cm^{-1} feature, i.e., the ratio $I_{\sim 2880}/I_{\sim 2850}$, or of ln $I_{\sim 2880}/I_{\sim 2850}$ vs. $1/T$. For the binary mixtures, the Raman data are in accord with calorimetric analyses and show that lecithin–fatty acid mixtures in general undergo multiple spectroscopic transitions. Each of these transitions is most reasonably ascribed to a separate phase with distinctive proportions of lecithin and fatty acids. The data further suggest that phases rich in unsaturated fatty acids and low in lecithin undergo state changes at lower temperatures than phases enriched in lecithin. When unsaturated fatty acids are employed in binary mixtures, acids with cis double bonds are found to decrease the order ↔ disorder transition temperatures observed with a given lecithin, while the fatty acids studied that have trans unsaturations increase the transition temperatures. Among the cis double bonded fatty acids, linoleic acid interferes most with cooperative interactions between lecithin acyl chains. Distinctly different thermotropic behavior is observed when ternary mixtures of saturated lecithins–fatty acid PLA are examined. The low-temperature spectroscopic transitions of the binary mixtures shift to higher temperatures (generally by 14 °C), and the higher temperature transitions reduce in width and increase in transition temperature by a few degrees. We interpret these results to indicate that insertion of PLA into domains composed of saturated lecithin and unsaturated fatty acids causes preferential partitioning of unsaturated fatty acids into the lipid boundary gradients surrounding the PLA molecules.

Proteolipids abound in myelin (Folch & Lees, 1951) but also occur in appreciable amounts in nonneural tissue (Folch-Pi & Sakura, 1976), in mitochondria (Cattel et al., 1971; Tzagoloft & Meagher, 1972), and in sarcoplasmic reticulum (MacLennan et al., 1972). A primary characteristic is their solubility in chloroform–methanol. Proteolipid apoprotein (PLA),[1] the delipidated form of bovine white matter proteolipid (Folch & Lees, 1951), is the most abundant protein in bovine myelin membranes (Autilio et al., 1964; Gonzalez-Sastre, 1970). It is very similar to the N-2 protein (Gagnon et al., 1971) of human myelin or lipophilin (Boggs et al., 1976) and contains covalently bound fatty acids and more than 50% apolar amino acids (Gagnon et al., 1971; Folch-Pi & Stoffyn, 1972). PLA appears to account for the intramembranous particles observed by freeze–fracture electron microscopy in myelin (Pinto da Silva & Miller, 1975) and in recombinants of lipophilin with dipalmitoylphosphatidylcholine (DPL) (Papahadjopoulos et al., 1975).

Bovine PLA incorporated into bilayers of dimyristoyllecithin (DML) and egg yolk lecithin (EYL) has been shown to be surrounded by boundary gradients of lecithin and to disturb the order ↔ disorder transitions of the lecithin molecules in these gradients (Curatolo et al., 1977, 1978). Also, lipophilin, incorporated into bilayers of dipalmitoylphosphatidylglycerol, decreases the enthalpy of the lipid order ↔ disorder transition (Papahadjopoulos et al., 1975), although Boggs & Moscarello (1978) found no calorimetric evidence that variations in acyl chain length appreciably influence lipophilin–lipid interactions.

All of the above studies indicate that PLA (or lipophilin) structures the lecithin molecules in the boundary gradients into arrays that differ from those assumed by bulk lipid. Moreover, Raman spectroscopy and differential calorimetry (Curatolo et al., 1978) suggest different boundary layer effects in recombinants of PLA with EYL vs. DML. The EYL–PLA recombinants exhibit, in addition to the usual EYL order ↔ disorder transition at −5 °C, a transition at 12 °C, i.e., 17 °C higher than the normal EYL transition. This behavior of PLA–EYL recombinants was attributed to the mixture of chain lengths and saturation in EYL (Curatolo et al., 1978). We have therefore investigated the thermotropic behavior of PLA–lipid recombinants where the lipids consisted of lecithins with differing acyl chain lengths mixed with fatty acids of varied chain lengths and unsaturations. In this study we monitored transition temperatures by Raman spectroscopy as before (Curatolo et al., 1978), analyzing the CH stretching spectra of various recombinants over a wide range of temperatures.

Materials and Methods

Synthetic DLL, DML, DOL, DPL, and DSL as well as stearic, oleic, linolenic, linoleic, and linolelaidic acids were obtained from Sigma (St. Louis, MO), and egg yolk lecithin (EYL) was from Lipid Products Ltd. (Epsom, U.K.). Mul-

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[1] Abbreviations used: DLL, dilauryllecithin; DML, dimyristoyllecithin; DPL, dipalmitoyllecithin; DSL, distearoyllecithin; DOL, dioleioyllecithin; EYL, egg yolk lecithin; PLA, proteolipid apoprotein.

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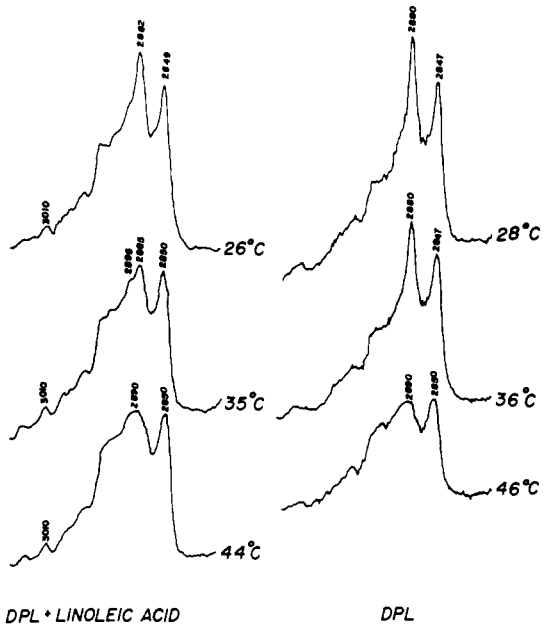

FIGURE 1: Raman spectra in the 2800–3000-cm^{-1} (C–H stretching) region of dipalmitoyllecithin ± linoleic acid at various temperatures.

tilayered liposomes were prepared as follows. Lecithin–fatty acid mixtures (molar ratio 1.0:0.6, unless stated otherwise) were dissolved in chloroform–methanol (1:1 w/w). The solvent was removed by first gassing with nitrogen and then drying under vacuum for several hours. The dried samples were then hydrated with $2H_2O$–H_2O (1:1 v/v) under nitrogen and sealed. Prior to spectroscopic measurements, hydrated sealed samples were placed for 2 h in a water bath at temperatures 5 ± 2 °C higher than those of the respective transition temperatures of the phospholipids and were periodically agitated by using a Vortex mixer.

Multilayered liposomes of lecithins combined with saturated or unsaturated fatty acids plus PLA were prepared as follows. Stock solutions of PLA in chloroform–methanol (2:1 v/v) were mixed with appropriate proportions of phospholipids and fatty acids (4:1 w/w) under a stream of nitrogen. The ratio of PLA to phospholipid was 20:1 (w/w). The solvent was removed by nitrogen gas, followed by desiccating under vacuum for 2–4 h. The samples were hydrated with ^2H_2O–H_2O (1:1 v/v) in a nitrogen atmosphere, and the tubes were sealed and kept in a water bath at 40 ± 2 °C for 1 h. Equilibration at temperatures greater than 45 °C was avoided because these produce a high background in the Raman spectrum probably due to PLA degradation.

Liposomes were transferred to Kimax capillaries for recording of Raman spectra as before [e.g., Curatolo et al., 1978], using a Ramalog 4 Raman spectrometer (Spex Industries, Metuchen, NJ) interfaced to an Interdata (Model 70) computer. The samples sealed in Kimax capillaries were irradiated by an Ar^+ laser (Spectra Physics, Model 164) tuned at 488 nm. Sample temperatures were controlled by a flow of temperature-regulated nitrogen gas, using a Harney-Miller cell. Temperature in the cell was measured continuously by using a thermistor as before [e.g., Curatolo et al. (1978)] placed close to the laser beam. Measured temperatures were 2–3 °C lower than sample temperatures, according to calibration curves with pure lipids.

Results

Lecithin and Lecithin–Fatty Acid Mixtures. Representative Raman spectra in the CH stretching region (2800–3000 cm^{-1}) of dipalmitoyllecithin–linoleic acid (molar ratio 1:0.6) at various temperatures are shown in Figure 1. The spectra of the other phosphatide–fatty acid mixtures are essentially similar to the data in Figure 1. Plots of the intensity, I, at 2880 cm^{-1} vs. that at 2850 cm^{-1}, i.e., the ratio $I_{\sim 2880}/I_{\sim 2850}$, against temperature yield inflections as phospholipids undergo their crystal ↔ liquid-crystal transition. The basis for this is as follows. The strong bands, at 2847 and 2880 cm^{-1}, characterize phospholipids in the crystalline state. The 2880-cm^{-1} band, due to asymmetric CH_2 stretching, is markedly enhanced in intensity because of Fermi resonance interactions between symmetric CH stretching fundamentals and HCH deformation overtones (Verma & Wallach, 1977; Snyder et al., 1978). In the liquid-crystalline state, the asymmetric CH_2 stretching mode is out of resonance; its intensity is therefore reduced and the position is shifted to 2890 cm^{-1}. In contrast, the 2847-cm^{-1} band, due to symmetric CH_2 stretching, changes only slightly in intensity as lipids shift between their crystalline and liquid-crystalline states.

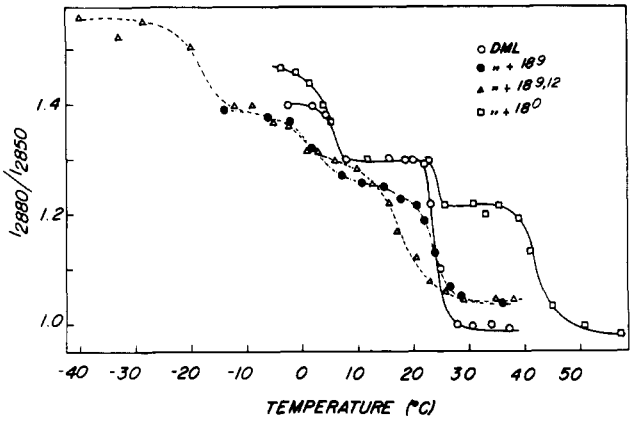

FIGURE 2: Plot of I_{2880}/I_{2850} vs. temperature for dimyristoyllecithin (DML) ● fatty acid mixtures. (○) DML; (●) DML + oleic acid (molar ratio 1:0.6); (△) DML + linoleic acid (molar ratio 1:0.6); (□) DML + stearic acid (molar ratio 1:0.6). (Error in each data point ±3–5%.)

Below, we present the results obtained with selected combinations of lecithins and saturated/unsaturated fatty acids. The plots of $I_{\sim 2880}/I_{\sim 2850}$ presented are averages of three to five readings (error ±5–7%) recorded from at least three different liposome preparations.

DML–Stearic Acid. Figure 2 represents the plots of $I_{\sim 2880}/I_{\sim 2850}$ vs. temperature for multibilayers containing DML and stearic acid (molar ratio 1:0.60). DML alone yields a small, well-defined pretransition at 12 °C and a sharp main transition at 23 °C. In the DML–stearic acid mixture, the pretransition is abolished and the main transition is broadened and skewed toward higher temperatures. Indeed, three transitions are observed in the mixture, with onset and completion temperatures at 5 and 8 °C, 23 and 25 °C, and 38 and 50 °C. The 23–25 °C transition is comparatively small, and its position suggests that it represents free DML. The 2880-cm^{-1} band, which normally either vanishes or shifts to 2888 cm^{-1} in the liquid-crystalline state, persists under 45 °C (not shown).

DML–Oleic Acid. In this case (Figure 2) the main DML transition broadens but does not shift appreciably (onset 18 °C; completion 29 °C). However, a new transition appears between −2 and 8 °C. (Oleic acid itself melts at 16 °C.)

DML–Linoleic Acid. The effect of linoleic acid (*cis*-9,-12-octadecanoic acid) on the transition behavior of DML is complicated (Figure 2). The plot suggests three poorly cooperative transitions at −28 to −15 °C, −8 to 2 °C, and 11 to

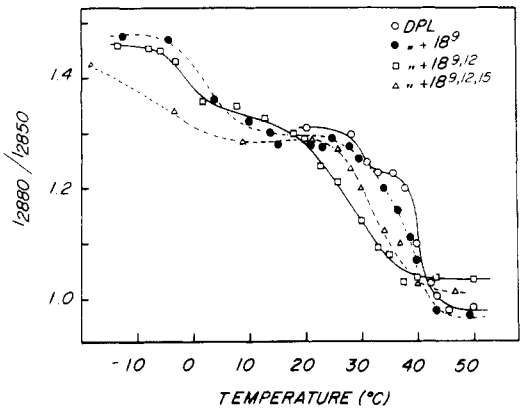

FIGURE 3: Plots of I_{2880}/I_{2850} vs. temperature for dipalmitoyllecithin (DPL) ± fatty acid mixtures. (○) DPL; (●) DPL + oleic acid (molar ratio 1:0.6); (□) DPL + linoleic acid (molar ratio 1:0.6); (△) DPL + linolenic acid (molar ratio 1:0.6). (Error in each data point ±3–5%.)

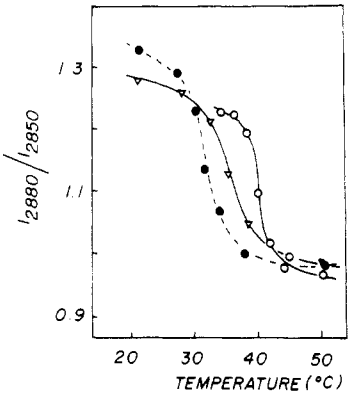

FIGURE 4: I_{2880}/I_{2850} as a function of temperature for DPL ± linoleic acid. (○) DPL; (△) DPL + linoleic acid (molar ratio 1:0.12); (●) DPL + linoleic acid (molar ratio 1:0.48). (Error in each data point ±3–5%.)

20 °C. The last appears to be the main DML transition, broadened and shifted to a lower temperature (midpoint 17 °C).

DML–Linolelaidic Acid. DML–linolelaidic acid (9-*trans*-12-*trans*-octadecanoic acid) liposomes exhibit a broadened main transition with a midpoint at 26 °C and a width of 7 °C. The effect of linolelaidic acid differs from that of linoleic acid and resembles the behavior of stearic acid.

DPL. DPL alone exhibits a sharp pretransition and a main transition at 30 and 41 °C, respectively (Figure 3). $I_{\sim 2880}/I_{\sim 2850}$ changes from ~1.0 to ~1.24 (difference 0.24) in going from the liquid-crystalline to the crystalline state and vice versa and by only ~0.08 when DPL is cycled through pretransition.

DPL–Oleic Acid. The solid circles in Figure 3 represent the thermal behavior of DPL–oleic acid. The main transition has shifted from 41 °C to a broad (13 °C wide) transition centered at ~35 °C. The pretransition is abolished. A second transition, occurring between 20 and −10 °C, is centered around 0 °C. $I_{\sim 2880}/I_{\sim 2850}$ changes from 1.0 to ~1.30 during the main transition. This difference in the ratio is approximately equal to the total difference observed during the main transition and pretransition of pure DPL. Between 20 and −10 °C, $I_{\sim 2880}/I_{\sim 2850}$ changes from ~1.30 to ~1.48, a ratio (difference of 0.18) almost double that noted during the pretransition of pure DPL. The low-temperature transition of DPL–oleic acid thus probably arises from a distinct DPL–oleic acid domain and does not represent a shifted pretransition of DPL alone.

DPL–Linoleic Acid. The rectangles in Figure 3 represent the thermal behavior of DPL–linoleic acid. Linoleic acid shifts the main transition of DPL to ~28 °C and also broadens the transition to 20 °C, an effect greater than that observed with oleic acid. The lower temperature transition resembles that found with DPL–oleic acid (0 to −10 °C). The changes in $I_{\sim 2880}/I_{\sim 2850}$ are approximately the same as observed with DPL oleic acid.

DPL–Linolenic Acid. The triangles in Figure 3 represent the behavior of DPL–linolenic acid. In spite of the presence of three double bonds in the acyl chains of this fatty acid, it has a less pronounced effect on the thermal behavior of DPL than linoleic acid. The main lipid transition is ~12 °C wide and has a midpoint near 32 °C. The low-temperature transitions are diffuse.

Figure 4 shows the effects of different DPL–linoleic acid proportions on the main transition of DPL. At a DPL–linoleic acid molar ratio of 1:0.12, the main transition shifts to ~35 °C. When the proportion of linoleic acid is increased to a molar ratio of 1:0.48, the main transition decreases further to ~31 °C.

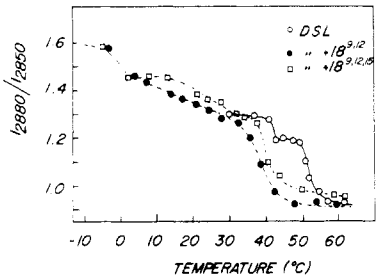

FIGURE 5: Plots of I_{2880}/I_{2850} vs. temperature for distearoyllecithin (DSL) ± fatty acids. (○) DSL; (●) DSL + linoleic acid (molar ratio 1:0.6); (□) DSL + linolenic acid (molar ratio 1:0.6). (Error in each data point ±3–5%.)

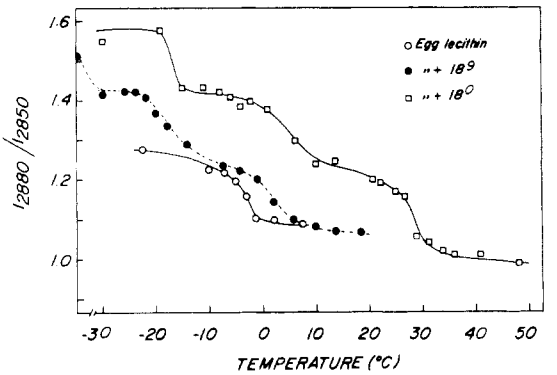

FIGURE 6: Plots of I_{2880}/I_{2850} vs. temperature for egg lecithin ± fatty acids. (○) Egg lecithin; (●) egg lecithin + oleic acid (molar ratio 1:0.5); (□) egg lecithin + stearic acid (molar ratio 1:0.5). (Error in each data point ±7%.)

DSL–Linoleic Acid. DSL alone exhibits a pretransition at 41 °C and a main transition at 52 °C (Figure 5). The maximum difference in $I_{\sim 2880}/I_{\sim 2850}$ observed as DSL is cycled through its pretransition and main transition is ~1.36. The incorporation of oleic acid (not shown), linoleic acid (Figure 5) or linolenic acid (Figure 5) tends to lower and broaden the thermal transition of DSL in a fashion similar to what was described for DML or DPL plus fatty acids. DSL–linoleic acid yields transitions at 30 to 45 °C and 0 to −5 °C. DSL–linolenic acid gives a less broad (7 °C) transition around 40 °C. The pretransition is abolished in both cases.

EYL–Stearic Acid. As shown in Figure 6, we observe three transitions in mixtures of EYL and stearic acid. The main EYL transition, normally at −5 °C, is broadened and shifted

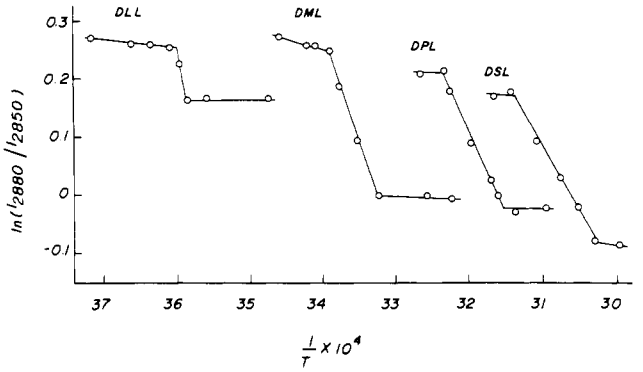

FIGURE 7: Plots of ln I_{2880}/I_{2850} vs. $1/T$ for dilauryllecithin (DLL), dimyristoyllecithin (DML), dipalmitoyllecithin (DPL), and distearoyllecithin (DSL).

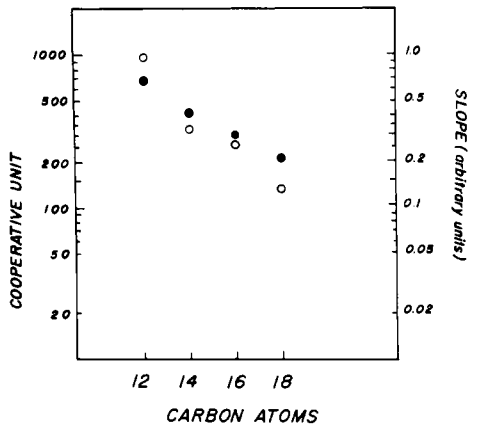

FIGURE 8: Relationship of slopes, in arbitrary units calculated from Figure 7 between onset and completion temperatures and represented by solid circles, vs. number of carbon atoms in the respective acyl chains of *lipids*. The open circles represent the corresponding sizes of cooperative units [taken from Marbrey & Sturtevant (1976) for dilauryllecithin, dimyristoyllecithin, dipalmitoyllecithin, and distearoyllecithin].

to higher temperatures (midpoint ~5 °C). Additional comparatively sharp transitions occur at ~29 and −17 °C. The $I_{\sim 2880}/I_{\sim 2850}$ values for EYL at −10 °C are equivalent to those for EYL–stearic acid at 20 °C (~1.22). However, in the transition region of EYL, $I_{\sim 2880}/I_{\sim 2850}$ rises to ~1.4 in the case of EYL–stearic acid, with final values for $I_{\sim 2880}/I_{\sim 2850}$ in the range of ~1.6 at −20 °C. For EYL alone, the maximum value observed is ~1.3.

EYL–Oleic Acid. The solid circles in Figure 6 represent the behavior of EYL–oleic acid. Two broad transitions are observed, around −19 and 0 °C.

Arrhenius Plots. Lipid transition data yield greater information if analyzed as Arrhenius plots, i.e., ln I_{2880}/I_{2850} vs. $1/T$, where T is in kelvin. This type of graphic analysis yields two breaks in the region of a phospholipid's main transition (Figure 7). The discontinuity at low temperature represents the onset of the transition and the one at high temperature its completion.

The Raman data allow one to estimate the size of a cooperative unit. For this the slopes of the lines between the onset and termination temperatures are determined and plotted against the number of carbon atoms in a single phospholipid acyl chain. This analysis (Figure 8) yields a straight line when the lipid carbons are plotted in order of increasing chain length: i.e., dilaurylphosphatidylcholine < dimyristoylphosphatidylcholine < dipalmitoylphosphatidylcholine < distearoylphosphatidylcholine. Relating these data to values from Mabrey & Sturtevant (1976), correlating the number of carbons per chain to cooperative unit size, one obtains parallel plots. This indicates that graphing ln I_{2880}/I_{2850} vs. $1/T$ can be used to estimate cooperative sizes of transition units. Cooperativity is reduced in the presence of unsaturated fatty acids and, among the fatty acids used, the most disruptive one is linoleic acid.

Table I: Summary of Thermal Transitions of Phosphatidyllecithins ± Fatty Acids ± Proteolipid Apoprotein As Revealed from the Plots of I_{2880}/I_{2850} vs. Temperature[a]

sample	thermal transitions (°C)
DML	23 ± 1
DML + oleic acid	−2 to +8 (W and B)
	18 to 29 (S and B)
DML + oleic acid + PLA	−10 to 12 (S and B)
	20 to 25 (sharp)
	25 to 38 (S and B)
DML + linoleic acid	−28 to −15 (S and B)
	−8 to +2 (W and B)
	11 to 20 (S and B)
DML + linoleic acid + PLA	5 to 15 (S and B)
	19 to 30 (S and B)
DOL	−22 ± 2
DOL + PLA	−16 to −26 (S and B)
	−9 to +5 (S and B)

[a] S = strong; W = weak; B = broad.

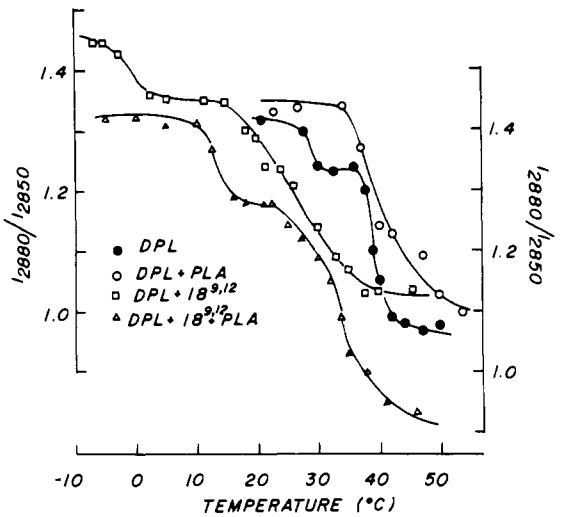

FIGURE 9: Plots of I_{2880}/I_{2850} vs. temperature for dipalmitoyllecithin (DPL) ± linoleic acid ± proteolipid apoprotein (PLA). (●) DPL; (○) DPL + PLA (20:1 w/w DPL/PLA); (□) DPL + linoleic acid (molar ratio 1:0.6); (Δ) DPL + linoleic acid + PLA (20:1 total lipid/PLA, w/w). Left-side scale for DPL, DPL + PLA, and DPL + linoleic acid; right-side scale for DPL + linoleic acid + PLA. (Error in each data point ±3–7%.)

Lipid Systems Containing PLA. The spectroscopic transitions observed with various lipid mixtures plus PLA are summarized in Table I, and we will here only treat the representative case of DPL–linoleic acid–PLA. In Figure 9 we have combined the presentation of the DPL–linoleic acid data with the results for DPL–linoleic acid–PLA. PLA produces three changes: (a) the −1 °C transition shifts to +15 °C; (b) the main spectroscopic transition of the DPL–linoleic acid–PLA system is less broad than that of DPL–linoleic acid, appearing more like the transition of pure DPL; (c) the $I_{\sim 2880}/I_{\sim 2850}$ values above the DPL transition temperature are closer to those of DPL (or DPL–PLA recombinants) than the DPL–linoleic acid system.

DOL–PLA. DOL alone undergoes a cooperative transition at −22 ± 2 °C, but DOL–PLA (20:1 w/w) recombinants show two broad spectroscopic transitions. The main transition does not shift but broadens markedly. In addition, a broad tran-

sition appears between −9 and +5 °C with a midpoint at ~1 °C (data not shown).

Discussion

Plots of $I_{\sim 2880}/I_{\sim 2850}$ vs. temperature for lecithin–fatty acid mixtures reveal multiple discontinuities. In accord with Mabrey & Sturtevant (1977), we reason that each of these spectroscopic transitions represents a phase with different proportions of fatty acids and phosphatides. In general, we obtain three transitions. One, which seems to be common to all the lecithin–fatty acid systems studied, occurs around 0 ± 5 °C. We doubt that this represents shifted pretransitions since it occurs with lecithin–fatty acid liposomes that have been sonicated, a treatment that abolishes pretransitions. Lecithin–fatty acid liposomes prepared at pH ~3.0 also show a spectroscopic transition in this temperature region, suggesting that carboxyl group ionization is not involved in the 0 ± 5 °C transition. That the 0 ± 5 °C transitions are not pretransitions is also indicated by the observation that they involve a change in $I_{\sim 2880}/I_{\sim 2850}$ about twice as much as that observed during pretransitions of pure lecithins (typically 0.2 vs. 0.08). Whatever the sources for the 0 ± 5 °C transition, it appears that the incorporation of saturated or unsaturated fatty acids into the lecithin liposomes abolishes the pretransition.

Another general trend noticed in the above systems is that the main transition shifts to higher or lower temperatures in the presence of saturated or unsaturated fatty acids, respectively. This is in agreement with Mabrey & Sturtevant (1977), who calorimetrically analyzed the thermal transitions of systems such as dilauryllecithin–lauric acid and distearoyllecithin–stearic acid. Their results led them to propose that insertion of fatty acid chains into hexagonal lecithin chain lattices eliminates the destabilizing "crowding" effect of the phosphocholine head groups. Fatty alcohols do not influence this "crowding" effect to the same extent as the fatty acid, and it is proposed that the carboxyl groups are principally responsible, allowing increased van der Waals interactions between lecithin molecules.

We have observed that cis-unsaturated fatty acids, when incorporated into lecithin liposomes, lower the transition temperatures. This may be because the double bonds in the cis form are more "bulky" and thus interfere more between the interchain interactions of phosphatides than of the trans species. Trans-unsaturated fatty acids thus behave in a fashion similar to that of saturated fatty acids.

Barton & Gunstone (1975) have shown that the position of the cis double bonds in the acyl chain influences the cooperativity of interchain interactions in a bilayer. Thus, cis double bonds positioned at 9, 10 or 10, 11 are more disruptive than at other positions (1, 8 or 12, 18) in C_{18} acyl chains. Our results, revealing a "disruptive order" of linoleic acid > linolenic acid > oleic acid (all of which have a cis double bond at position 9, 10), point to the importance of additional double bonds and chain length. The data indicate that the cooperativity of lecithin–fatty acid systems is further decreased by a second cis double bond, e.g., at the 12 position in linoleic acid. This fits well with the views of Barton & Gunstone (1975). However, linolenic acid, which has a melting point below −20 °C, has a lesser influence on the main transitions of DPL and DSL than linoleic acid, although one might expect that the double bonds at 9 and 12, if not at 15, should exert an influence similar to that seen with linoleic acid. This apparent discrepancy may relate to the effective chain lengths of the fatty acids. The "chain length equivalent" of linolenic acid is that of a 16-carbon-saturated chain (the same as that of DPL acyl chains) while that of linoleic acid corresponds to an 18-carbon-saturated chain. Nonequivalence of chain length thus appears to influence the totality of interchain interactions in the case of DPL–linoleic acid liposomes but not in the DPL–linolenic acid system. On the other hand, it seems that DSL bilayers can accommodate a shorter "chain length equivalent", with three double bonds, more readily than a chain of the same effective length with two double bonds. At present we cannot determine whether chain length or number of double bonds is more disruptive to lecithin acyl chain cooperativity.

What do the multiple spectroscopic transitions observed represent? Recently, Mabrey & Sturtevant (1976) presented calorimetric data on phospholipid–saturated fatty acid mixtures with unequal chain lengths. They demonstrated that in such systems the transition peaks shift to higher temperatures and split into transitions, each representing a separate "phase". They found that mixtures of acyl chains with unequal chain lengths do not form homogeneous phases and that, at molar ratios near unity, phase separation occurs. It appears that the peak closer to the native transition of any one of the components in the mixture is enriched in that component and vice versa. Our data support the work of Mabrey & Sturtevant (1976) and suggest that the lower temperature transitions represent "phases" rich in unsaturated fatty acids while the higher temperature ones represent phases rich in lecithin.

PLA interferes with the lateral packing of DML acyl chains in DML–PLA recombinants (Curatolo et al., 1978). PLA also abolishes the pretransition of DPL and broadens the main transition of this lecithin. Finally, the total change in $I_{\sim 2880}/I_{\sim 2850}$ during the transition of DPL–PLA is equal to the sum of the values for the pretransition and main transition.

The effects of PLA on the thermal behavior of the DPL–linoleic acid system are (a) to shift the low-temperature transition (−5 to 2 °C) to a higher temperature, 14 °C, without affecting its width, (b) to sharpen the broad main transition (15–36 °C), and (c) to produce a sharp change in $I_{\sim 2880}/I_{\sim 2850}$ at 34 °C. Following the arguments of Mabrey & Sturtevant (1976), the first modification of the DPL–linoleic acid system suggests that PLA associates preferentially with a phase represented by the transition occurring at −5 to +2 °C and presumably enriched in unsaturated fatty acid. The nature of the PLA–lipid association is such as to raise the transition temperature and suggests preferential partitioning of unsaturated fatty acid into the boundary gradient of surrounding PLA. This would reduce the proportion of such acyl chains in the DPL-rich phase, yielding the broad and shifted transition at 27 °C. The sharpening of the main transition is consistent with this suggestion. Moreover, as illustrated for the case of linoleic acid, the change in this transition depends on the fatty acid proportion.

We suggest the following: (a) PLA causes unsaturated fatty acids to partition into its boundary gradient from DPL–unsaturated fatty acid phases and (b) PLA raises the transition temperature of a phase rich in unsaturated acyl chains more than a similar phase enriched in saturated acyl chains. Our studies imply a preference of PLA for certain unsaturated fatty acyl chains but do not give unambigous information about the role of carboxyl groups in fatty acid–PLA associations.

Why might PLA "extract" unsaturated chains into its boundary gradient? The following possibilities should be considered: (a) as cis double bonds make kinks in their acyl chains, their intermixture with saturated chains would create domains more accommodating of PLA than saturated chains; (b) double bonds have unpaired electrons, giving them a lesser hydrophobicity than that of saturated bonds. Double bonds might thus provide an environment with slightly polar

"pockets" that do not exist in a single-bond environment. Conceivably, PLA bears sites that prefer such pockets and others which prefer highly apolar regions. For this reason, perhaps, the protein is more soluble in chloroethanol or chloroform–methanol than in more apolar or polar solvents. One might consider the possibility, therefore, that unsaturated domains in a membrane lipid core provide islands of lesser hydrophobicity and that these are attractive to PLA.

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Changes in the Hepatic Levels of Messenger Ribonucleic Acid for Malic Enzyme during Induction by Thyroid Hormone or Diet†

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ABSTRACT: Levels of hepatic messenger ribonucleic acid (mRNA) for malic enzyme [L-malate:NADP oxidoreductase (decarboxylating), EC 1.1.1.40] were quantitated in different dietary and hormonal states of the rat. Polysomal or total cellular poly(A)-containing RNA was translated in the rabbit reticulocyte lysate system, which had been treated to reduce endogenous mRNA activity. The relative level of incorporation of radiolabeled amino acid into malic enzyme was determined by immunoprecipitation with antibody to malic enzyme and formaldehyde-fixed *Staphylococcus aureus* (Cowens I strain) as an immunoadsorbent. The immunoprecipitated product comigrated with purified malic enzyme on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. No malic enzyme was detected when nonspecific antisera or an excess of unlabeled malic enzyme was added during immunoprecipitation. The level of malic enzyme mRNA was found to markedly increase relative to euthyroid, chow-fed rats when the animal was either fed a high carbohydrate, fat-free diet or made hyperthyroid. Animals receiving both treatments had a further increase in mRNA activity to a level which was ~0.2% of the total incorporation of [^{3}H]leucine. Levels of malic enzyme activity and the relative rate of synthesis were found to increase roughly in proportion to mRNA levels in these three states. Thus, the induction of malic enzyme by thyroid hormone or high carbohydrate, fat-free diet is due largely to an increase in the mRNA coding for this enzyme.

The activity of rat hepatic malic enzyme [L-malate:NADP oxidoreductase (decarboxylating), EC 1.1.1.40] can be modulated by both hormonal and dietary factors. The thyroidal status of the rat has profound effects on the levels of this cytosolic enzyme (Tepperman & Tepperman, 1964; Wise & Ball, 1964; Ruegamer et al., 1965; Tarentino et al., 1966). Hypothyroid rats have reduced levels of hepatic malic enzyme activity, whereas hyperthyroid rats have greatly increased levels compared to euthyroid animals. Consequently, malic enzyme activity has become a commonly employed marker of tissue effects of thyroid hormone for the rat liver (Oppenheimer et al., 1977). The level of hepatic malic enzyme activity is also known to be influenced by the diet of the animal (Tepperman & Tepperman, 1964; Wise & Ball, 1964; Tarentino et al., 1966; Fitch & Chaikoff, 1960; Pande et al., 1964). Starvation causes a dramatic decrease in enzyme activity, whereas diets

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