Nature of the Thermal Pretransition of Synthetic Phospholipids: Dimyristoyl- and Dipalmitoyllecithin†

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ABSTRACT: The hydrated synthetic lecithins, dimyristoyl- and dipalmitoyllecithins, undergo two thermal transitions, a broad low enthalpy "pretransition" prior to the sharp first-order "chain-melting" transition. Both phospholipids exhibit the same temperature-dependent structural changes associated with the thermal pretransition. At low temperatures, below the pretransition, a one-dimensional lamellar lattice is observed. The hydrocarbon chains are fully extended and tilted with respect to the plane of the lipid bilayer. The hydrocarbon chain packing displays a temperature dependence and the angle of tilt of the hydrocarbon chains decreases with increasing temperature, reaching a minimum value of 30° at the pretransition temperature of both lecithins. The pretransition is associated with a structural transformation from a one-dimensional lamellar to a two-dimensional monoclinic lattice consisting of lipid lamellae distorted by a periodic ripple. The hydrocarbon chains remain tilted in the temperature range intermediate between the pretransition and chain-melting transition. The cell parameters of this two-dimensional lattice exhibit a compositional dependence. The *a* parameter (proportional to the lamellar repeat distance) increases with increasing water content, while the *b* parameter (a measure of the ripple periodicity) decreases with increasing water content. At the chain-melting transition, the hydrocarbon chains of the phospholipid melt and assume a liquid-like conformation and the lattice reverts to one-dimensional lamellar. These structural changes observed for dimyristoyl- and dipalmitoyllecithins may be a common feature of all synthetic lecithins exhibiting a thermal pretransition. The appearance of the pretransition and accompanying two-dimensional lattice may arise from specific interactions between the choline moiety of the polar head group and the structured water matrix surrounding it.

A common feature of all membrane phospholipids is the existence of a temperature-dependent reversible transition in which the hydrocarbon chains of the phospholipid undergo a transformation from an ordered crystalline-like state to a more disordered fluid-like state. This order-disorder transition has been exhaustively examined in phospholipids by a wide variety of techniques (Chapman, 1965,1968; Luzzati, 1968), and has been determined to be the "melting" temperature of the hydrocarbon chains. This chain-melting transition is characterized by differential scanning calorimetry (DSC)[1] as a sharp first-order transition, resulting in a large endotherm on heating which is dependent on the nature and homogeneity of the hydrocarbon chains and polar head groups (Chapman, 1968). The structures associated with this transition, as revealed by x-ray diffraction, are also dependent on the nature of the phospholipid. In the case of lecithin, for example, a structure consisting of stacked lamellae containing one-dimensional order in which the hydrocarbon chains assume a liquid paraffin-like arrangement is observed above this transition (Luzzati, 1968).

Until recently, little information was available concerning the structures formed by phospholipid systems at temperatures below the chain-melting transition. Several structures of varying lattice order and chain conformation have now been described (Tardieu, 1973; Ranck et al., 1974). However, these structures have not been correlated with other thermal transitions that may exist in phospholipid systems, such as those observed in synthetic lecithins (Chapman, 1968). In dimyristoyllecithin (DML) and dipalmitoyllecithin (DPL), an additional broader transition of lower enthalpy occurs at a temperature below the chain-melting transition (see Figure 1), but it is unclear what structural changes are associated with it. This pretransition[2] has been investigated, but the interpretations of these studies concerning the structural and conformational changes associated with this transition are varied. Early proton and deuterium nuclear magnetic resonance (NMR) reports indicated that the polar head group may undergo conformational rearrangement at the pretransition (Salsbury et al., 1971; Oldfield et al., 1971; Sheetz and Chan, 1972). More recent deuterium and phosphorus NMR studies concluded that no conformational changes occur in the head group at the pretransition (Gally et al., 1975). Recent fluorescence studies have indicated the pretransition has a pronounced effect on the arrangement of the alkyl chains (Galla and Sackman, 1974; Jacobson and Papahadjopolous, 1975). These studies indicate a delicate balance is maintained between the hydrophobic and hydrophilic regions of the phospholipid and subtle modifications in structure may lead to the pretransition.

We have systematically investigated the synthetic lecithins, dimyristoyl- and dipalmitoyllecithins, as a function of temperature and water content by x-ray diffraction and DSC techniques in an effort to characterize the structures associated with the thermal pretransition.

Materials and Methods

DML was synthesized in this laboratory from glycerophosphorylcholine isolated from egg yolks (Chadha, 1970)

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[1] Abbreviations used are: DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; DML, dimyristoyllecithin; DPL, dipalmitoyllecithin; DLL, dilauroyllecithin; PE, phosphatidylethanolamine; TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

[2] The term "pretransition" used here may not be consistent with the classically associated thermotropic liquid crystalline behavior (see Barrall and Johnson, 1974; de Gennes, 1974). However, since this term has been widely used to describe this transition, we chose not to confuse matters.

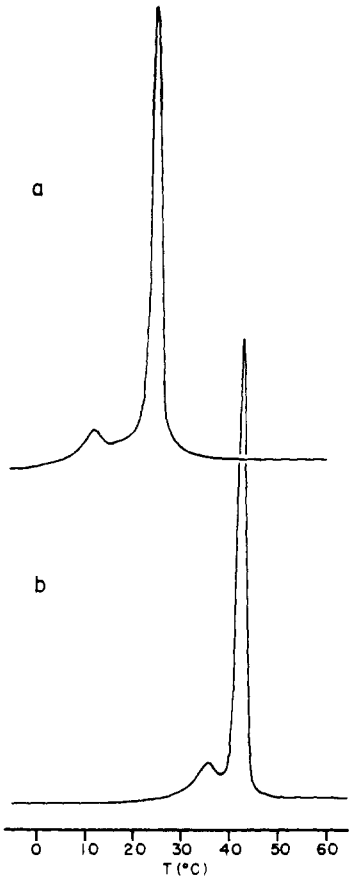

FIGURE 1: DSC traces of mixtures of DML (a) and DPL (b) containing 50% water. For DML, the pretransition occurs at 11 °C, $\Delta H \simeq 1$ kcal/mol and the chain-melting transition at 23 °C, $\Delta H \simeq 6$ kcal/mol. For DPL, the pretransition occurs at 34.5 °C, $\Delta H \simeq 2$ kcal/mol and the chain-melting transition occurs at 42.0 °C, $\Delta H \simeq 9$ kcal/mol.

and myristic acid (Nu Chek Prep, Austin, Minn.) by the method of Cubero Robles and Van Den Berg (1965). DML was isolated by silicic acid chromatography and determined to be only phosphatidylcholine by thin-layer chromatography (TLC) and comprised of 99.9% $C_{14:0}$ acyl chains by gas–liquid chromatography (GLC). DPL, obtained from Serdary Laboratories (Quebec, Canada), was further purified by silicic acid chromatography and was determined to be only phosphatidylcholine by TLC and contained 99.8% $C_{16:0}$ acyl chains by GLC. Triply distilled water was used in the study.

A known weight of phosphatidylcholine dissolved in solvent (benzene or chloroform–methanol) was added to a glass tube with a narrow constriction in the center. The solvent was removed by drying in vacuo and the appropriate amount of water was added. The tube was then sealed under nitrogen and centrifuged through the constriction repeatedly for 4–6 h at a temperature above the chain-melting transition. All samples examined by x-ray diffraction were also studied by DSC and TLC. The water content was determined gravimetrically. Samples taken for calorimetry (5–10 mg) were hermetically sealed in aluminum pans and placed in a Perkin Elmer (DSC-2) differential scanning calorimeter. Samples were studied at variable heating rates between 0.31 and 10°C/min. Each sample was heated and cooled repeatedly between −10 and 60 °C.

Nickel filtered Cu Kα x radiation from an Elliott GX-6 rotating anode generator was collimated by double-mirror optics (Franks, 1958). Samples were contained in thin-walled capillary tubes (internal diameter 0.7 mm) and x-ray diffraction patterns were recorded between −10 and 60 °C utilizing a variable temperature specimen holder. Microdensitometry of x-ray photographs was carried out on a Joyce Loebl Model III-CS microdensitometer.

Results

DSC thermograms for DML and DPL containing 50 wt % water are shown in Figure 1. Both lipids exhibit similar thermal behavior undergoing a broad, low enthalpy pretransition prior to the sharp high enthalpy chain-melting transition. The pretransition temperature is 11.0 °C for DML and 34.5 °C for DPL. The chain-melting transition temperature is 23 °C for DML and 43 °C for DPL. These values are similar to those previously reported (Chapman et al., 1967; Hinz and Sturtevant, 1972). The effect of hydration on these transitions will be discussed later (see Discussion).

X-ray diffraction studies were performed at temperatures below the pretransition, at temperatures intermediate between the two transitions and above the chain-melting transition. Three characteristic x-ray diffraction patterns can be associated with the two thermal transitions for both DML and DPL. To analyze these diffraction patterns, we consider the low- and high-angle diffraction data separately. The low-angle diffraction (equivalent to a Bragg spacing >10 Å) arises from the characteristic lattice repeat, i.e., the long-range ordering of phospholipids, whereas the high-angle region (Bragg spacing <10 Å) is characteristic of the hydrocarbon chain packing, i.e., the short-range ordering.

The three characteristic low-angle diffraction patterns for DML are shown in Figure 2a,c,e and for DPL in Figure 2b,d,f. At temperatures below the pretransition (Figure 2a,b), several sharp low-angle reflections in the ratio of 1:0.5:0.33 . . . are observed. In the high-angle region (discussed below) a sharp reflection at 4.2 Å followed by a more diffuse reflection is observed. In the temperature range between the pretransition and the chain-melting transition (Figure 2c,d), several strong sharp reflections, together with many additional sharp reflections, are observed. At high angle, a broad reflection centered at 4.2 Å is observed. At temperatures above the chain-melting transition (Figure 2e,f), several low-angle reflections, together with a broad diffuse band at 4.6 Å, are observed. This well-characterized structure consists of stacked lamellae with the hydrocarbon chains of the phospholipid in a "liquid paraffin-like" conformation and has been previously observed in many hydrated phospholipid systems (Luzzati, 1968; Shipley, 1973).

The indexed reflections obtained from such diffraction patterns over a range of temperatures are plotted in Figure 3. For both lipids, only $h0$ reflections arising from a one-dimensional lattice are observed below the pretransition and again above the chain-melting transition. The appearance of additional reflections is clearly associated with the pretransition. All reflections observed in this intermediate temperature range have been indexed according to a two-dimensional monoclinic lattice. Two-dimensional lattices of various types have been previously observed for hydrated soaps (Skoulios et al., 1961) and lecithins containing varying amounts of water (Luzzati et al., 1968).

This two-dimensional lattice has been observed for hydrated DML at 19 °C and hydrated dilauroyllecithin (DLL)[3] at −7

[3] For dilauroyllecithin, the chain-melting transition occurs at ~0 °C (Chapman et al., 1967). Since the transition temperature for DLL occurs near the ice-melting temperature, these transitions are difficult to observe and, thus, a pretransition temperature has not been reported.

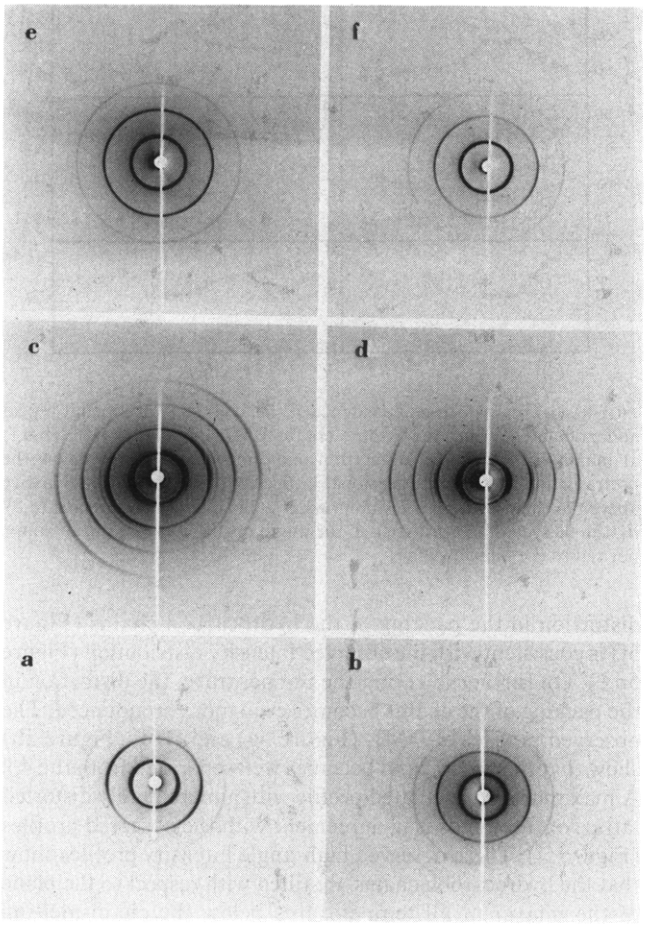

FIGURE 2: Three characteristic low-angle diffraction patterns from DML(a,c,e) and DPL (b,d,f) below the thermal pretransition (a,b), intermediate between the two transitions (c,d), and above the chain-melting transition (e,f). Shown are mixtures of DML containing 23% water at 5 °C (a), 20 °C (c), 37 °C (e), and DPL containing 24% water at 20 °C (b), 39 °C (d), 50 °C (f).

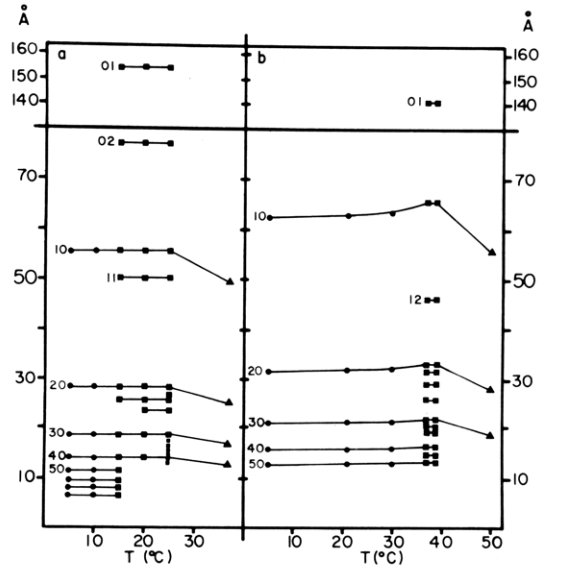

FIGURE 3: Typically observed temperature dependence of the indexed Bragg reflections for DML and DPL below the pretransition (●), intermediate between the two transitions (■), and above the chain melting transition (▲). (a) DML containing 21.2% water; (b) DPL containing 27.8% water.

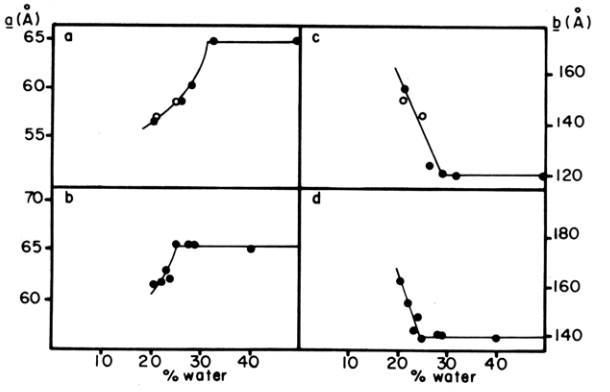

FIGURE 4: Compositional dependence of the calculated cell parameters of the two-dimensional lattice for DML at 20 °C (a,c) and DPL at 39 °C (b,d). The *a* cell parameter (a,b) is proportional to the interlamellar repeat and the *b* cell parameter (c,d) describes the length of the periodic ripple. (○) Values obtained by Tardieu (1973) for DML.

°C by Tardieu (1973). We note that this temperature for DML is intermediate between the two transitions. The structure of this two-dimensional lattice has been interpreted to consist of stacked lamellae (the *a* direction in the unit cell traversing the lamellae) distorted by a periodic ripple in the plane of the bilayer (*b* measuring the period of the ripple) (Tardieu et al., 1973).

This two-dimensional structure exhibits a compositional dependence for both DML and DPL; their diffraction pattern and resulting unit cell parameters vary as a function of water content present. A prominent feature in the diffraction pattern is the compositional dependence of the 11 and 12 reflections. At the lower compositional limit, at which the two-dimensional lattice is observed, the 11 reflection is observed while the 12 reflection is absent. At the upper compositional limit, approaching maximum hydration, the 12 reflection is present and the 11 reflection is absent. These intensity fluctuations may result from differences in the ripple amplitude as a function of composition. We are presently analyzing this feature using model electron density distributions of the rippled structure.

In conjunction with these intensity distribution fluctuations, the calculated cell parameters for DML (Figure 4a,c) and DPL (Figure 4b,d) exhibit a compositional dependence. The *a* cell parameter, being proportional to the interlamellar repeat, increases with increasing water concentration reaching a maximum value at maximum hydration. For DML (Figure 4a), the maximum value of *a* is 64.9 Å at 30% water; for DPL (Figure 4b), the *a* cell parameter reaches a maximum value of 65.6 Å at 25% water. The *b* cell parameter describes the period or wave length of the ripple parallel to the plane of the lipid lamellae and decreases with increasing water concentration. For DML (Figure 4c), *b* decreases from 156 Å at 21% water to a minimum value of 120 Å at 29% water. DPL behaves similarly (Figure 4d), decreasing from 162 Å at 21% water to a minimum value of 140 Å at 25% water.

Thus, the low-angle x-ray diffraction information indicates that a structural transformation from a one-dimensional lamellar lattice, L, to a two dimensional monoclinic lattice, P, occurs at the thermal pretransition.[4] All mixtures of DML examined containing between 20 and 50% water display this

[4] The nomenclature used is that of Luzzati (1968), see also Tardieu et al. (1973): L denotes a one-dimensional lamellar lattice; P denotes a two-dimensional monoclinic lattice; α denotes a hydrocarbon chain conformation in a liquid paraffin-like arrangement; β', crystalline-like chains tilted with respect to the bilayer plane; β, crystalline-like chains perpendicular to the bilayer plane.

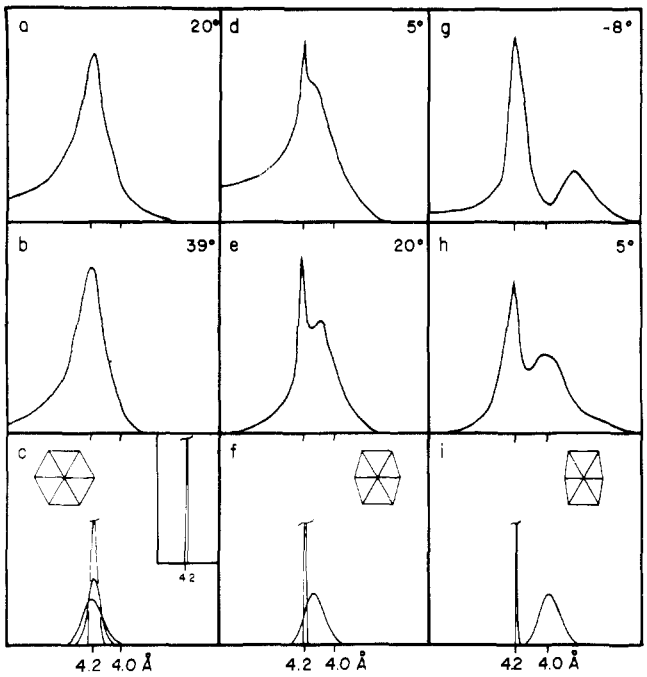

FIGURE 5: Characteristically observed high-angle diffraction patterns for DML at 20 °C (a), 5 °C (d), −8 °C (g), and DPL at 39 °C (b), 20 °C (e), 5 °C (h), together with calculated profiles from long rods (c,f,i) described by Tardieu et al. (1973) and a schematic representation of the packing of rods. (c) Profiles for hexagonally packed 36 Å rods 4.85 Å apart assuming various angles of tilt, insert–untilted; (f) each rod surrounded by four rods at 4.82 Å and by two rods at 4.75 Å tilted by 30°; (i) each rod surrounded by four rods at 4.78 Å and by two rods at 4.55 Å tilted by 30°.

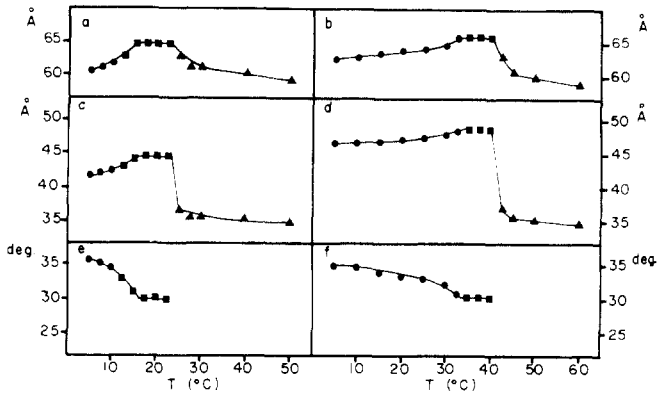

FIGURE 6: Temperature dependence of the average interlamellar repeat and calculated structural parameters for DML (a,c,e) and DPL (b,d,f) at maximum hydration. Data from one-dimensional lattice below the pretransition (●), two-dimensional lattice (■), one-dimensional lattice above the chain-melting transition (▲). d_{10}, the interlamellar repeat (a,b); d_l, the bilayer thickness (c,d); θ, the angle of tilt (e,f) measured from a normal to the bilayer plane.

two-dimensional structure between 11 and 23 °C. The two-dimensional lattice is observed for DPL over the same hydration values between 34.5 and 42 °C. At the chain melting transition, these phospholipids revert to a one-dimensional lattice. From the high-angle diffraction pattern, a broad diffuse band at 4.6 Å, the hydrocarbon chains are determined to be in a liquid paraffin-like arrangement (Luzzati, 1968). This structure is designated L_α.[4]

The conformation of the hydrocarbon chains has been derived from the high-angle intensity profiles below the chain-melting transition. Microdensitometer traces of the high-angle intensity profiles characteristically observed for DML are shown in Figure 5a,d,g and for DPL in Figure 5b,e,h. At temperatures intermediate between the two transitions (the P lattice), the observed high-angle profiles for DML and DPL show a broad maximum at 4.2 Å, as shown in Figure 5a,b. In Figure 5c are calculated intensity profiles for rods 36 Å in length with a central gap of 4 Å assuming various angles of tilt. These calculated profiles are taken from the data of Tardieu et al. (1973). This is analogous to the hydrocarbon chain of the phospholipid being rigid and fully extended, but varying in their orientation with respect to the plane of the bilayer. With a perpendicular orientation, a narrow intensity profile centered at 4.2 Å is calculated (see insert to Figure 5c). As the rods are progressively tilted, so the profile becomes progressively broadened. The observed intensity profiles are consistent with those calculated for tilted rods.

At temperatures below the pretransition (the L lattice), intensity profiles shown in Figure 5d,e,g,h is observed. For both DML at 5 °C (Figure 5d) and for DPL at 20 °C (Figure 5e), a narrow maximum at 4.2 Å, together with a broader band at higher angles, is observed. A calculated profile of a quasi-hexagonal array of tilted rods, which may be considered as a distortion in the packing of the hydrocarbon chains (Figure 5f) is consistent with the observed intensity distribution (Figure 5d,e). On further lowering the temperature, the distortion in the packing of the chains becomes even more pronounced. The observed profiles of DML (Figure 5g) and DPL (Figure 5h) show that the broad band becomes well separated from the 4.2 Å maximum. A calculated profile with a more highly distorted lattice of tilted rods is in agreement with the observed profiles (Figure 5i). These observed high-angle intensity profiles show that the hydrocarbon chains are tilted with respect to the plane of the bilayer at all temperatures below the chain-melting transition and suggest that the hydrocarbon chains become more regularly packed with increasing temperature.

In addition to the temperature effects described above, it is possible to calculate the temperature dependence of the average orientation of the hydrocarbon chains with respect to the plane of the bilayer. Knowing the interlamellar repeat distance, d_{10}, and the maximum hydration value as a function of temperature, the bilayer thickness, d_l, and angle of tilt, θ, can be calculated (see Figure 7). Maximum hydration was determined from swelling experiments, and for DML was found to be 29% water at 10 and 20 °C, and 40% water at 37 °C (Janiak et al., 1976). For DPL, maximum hydration was determined to be 25% water at 20 and 39 °C (Janiak, unpublished observations) and 40% at 50 °C (Chapman et al., 1967). As shown in Figure 6, d_{10} increases similarly for both lipids reaching a maximum value of 64.8 Å for DML (Figure 6a) and 66.0 Å for DPL (Figure 6b), at temperatures intermediate between the pretransition and the chain-melting transition. Above the chain melting transition, d_{10} decreases with increasing temperature. Similar temperature-dependent behavior is observed for the bilayer thickness d_l. Again, at temperatures intermediate between the two transitions, d_l reaches a maximum of 44.5 Å for DML (Figure 6c) and 48.8 Å for DPL (Figure 6d).

From the bilayer thickness, the angle of tilt, θ, can be calculated (Figure 6e,f). By comparing the bilayer thickness to that calculated for the phospholipid with hydrocarbon chains fully extended and normal to the bilayer plane, θ is determined. This relationship is $\cos\theta = d_l/d_l'$, where $d_l' = 51.6$ Å for DML and 56.6 Å for DPL. θ can also be derived from the cosine relationship of the calculated surface area of the phospholipid to that of a fatty acid chain in a fully extended conformation. For both lipids, an increase in temperature results in a decrease in θ to a minimum value of 30°, suggesting the difference of

the bilayer thickness is attributable solely to differences in chain length. If this is the case, then the product of twice the difference in chain length of DPL and DML, i.e., $2\Delta(C_{16:0}-C_{14:0}) = 5$ Å, and the angle of tilt, $\cos\theta$, should be equal to the difference in the bilayer thickness, Δd_l. The relationship is $2\Delta(C_{16:0}-C_{14:0})\cos\theta = \Delta d_l$, and the value of $2\Delta(C_{16:0}-C_{14:0})$ is derived assuming 2.5 Å/pair of methylene groups when the hydrocarbon chains are fully extended and normal to the bilayer plane. This would result in a bilayer thickness of 51.6 Å for DML and 56.6 Å for DPL. Selecting values of θ from Figure 6e,f, the following values of Δd_l are calculated: for $\theta = 30, 33.3, 35.0°$, Δd_l (calculated) = 4.33, 4.17, 4.09 Å, and Δd_l (observed) = 4.3, 4.2, 4.1 Å, respectively. This suggests that for a given angle of tilt, the difference in bilayer thickness between DML and DPL is due only to the difference in chain length.

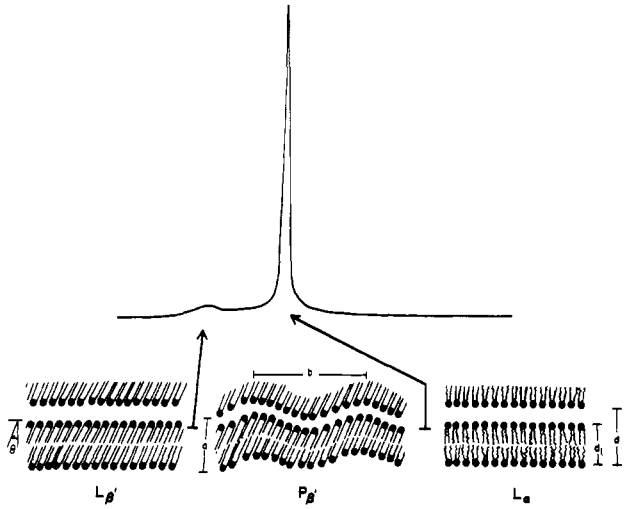

FIGURE 7: Structural models associated with thermal transitions in synthetic lecithins. d is the interlamellar repeat, d_l the bilayer thickness, and θ the angle of tilt. a and b are the cell parameters for the two-dimensional lattice.

Discussion

Our results are consistent with the following conclusions: at low temperatures both DML and DPL form a one-dimensional lamellar structure. The hydrocarbon chains are fully extended and tilted with respect to the plane of the bilayer (Figure 7, structure $L_{\beta'}$), but packed in a distorted quasi-hexagonal lattice. Increasing the temperature results in a more regular packing of the hydrocarbon chains. In addition, the angle of tilt of the hydrocarbon chains decreases with increasing temperature, reaching a minimum value of 30° at the pretransition temperature of both lecithins. This is in contrast to the calculated angle of tilt as a function of water content. Both phospholipids exhibit an increase in tilt angle with increasing hydration at a given temperature. For example, for DML at 10 °C, θ increases from 22° at 10% water to a maximum value of 34° at 30% water (Janiak et al., 1976). This behavior is similar to that of DPL at 20 °C reported by Tardieu et al. (1973) where θ increases from 26° at 11% water to 33° at 25% water.

It should be noted here that the determination of the angle of tilt involves a number of variables such as water content, partial specific volume ($\bar{\nu}_l$), lamellar thickness, etc., whose measured accuracy determines the accuracy of the calculation. The parameter least accurately defined is $\bar{\nu}_l$. Variation of $\bar{\nu}_l$ would result in some changes in the bilayer thickness and surface area, but to a much lesser degree in θ, since it is determined from a cosine relation. We have assumed $\bar{\nu}_l$ to be invariant at temperatures below the chain-melting transition in calculating θ, since little, if any, data on $\bar{\nu}_l$ is available in this temperature range. If $\bar{\nu}_l$ does vary, it would probably increase with increasing temperature. This would result in the same variation in θ, as shown in Figure 6e,f with only its absolute values being larger. Thus, it would appear that the values reported here approximate the lower limit of tilt angle.

The pretransition is associated with a structural transformation from a one- to two-dimensional monoclinic lattice consisting of lipid lamellae distorted by a periodic undulation or ripple. This two-dimensional structure exhibits a compositional dependence in its unit cell parameters, both the a and b cell parameters varying as a function of water content. The a parameter increases with increasing water concentration proportional to the amount of water intercalated between the lipid bilayers in the swelling lecithin. At the limit of swelling (maximum hydration), the a parameter reaches its maximum value. Associated with an increase in the a parameter is a decrease in the b parameter, measuring the period of the ripple. The period of the ripple decreases with increasing water concentration, reaching a minimum value at the swelling limit of the lecithin. The hydrocarbon chains are tilted with respect to the plane of the bilayer (structure $P_{\beta'}$) and are packed in a hexagonal array. In the temperature range intermediate between the pretransition and chain-melting transition, the angle of tilt is invariant.

At the chain melting transition, the hydrocarbon chains of the phospholipid melt assuming a liquid-like conformation and the lattice reverts to one-dimensional lamellar (structure L_α) as previously described (Luzzati, 1968). The interpretation of fully extended hydrocarbon chains does not rule out the possibility of local disordering in the terminal regions of the chains. However, recent studies on spin-labeled DML indicate a significant angle of tilt exists along the entire length of the lipid chain (Birrell and Griffith, 1976). In addition, a recent Raman spectroscopy study of DPL by Yellin and Bulkin (1976) is consistent with fully extended hydrocarbon chains at low temperatures and that the thermal pretransition is not a result of significant gauche conformer production.

In a recent study on the temperature-dependent behavior of DPL (Rand et al., 1975), it was concluded that the pretransition is associated with a conformational change in the hydrocarbon chains from tilted to perpendicular with respect to the plane of the bilayer ($L_{\beta'}$ to L_β). These results are not consistent with our conclusions and we are unable to account for these differences.

Having observed the same structural changes at the pretransition for both DML and DPL, and noting that a rippled structure has also been observed for DLL (Tardieu et al., 1973), we suggest that the structural transformation of $L_{\beta'}$ to $P_{\beta'}$ is common to all synthetic lecithins exhibiting a thermal pretransition. Furthermore, rippled structures have been reported for a variety of synthetic lecithins by freeze fracture electron microscopy when quenched from a temperature below the chain-melting transition (Gulik-Krzywicki, 1975, and references therein). In addition, Ververgaert et al. (1973) have observed rippled structures for dilauroyl-, dimyristoyl-, dipalmitoyl-, and distearoyllecithin. Although several ripple periodicities are observed for each lecithin, one value predominates and this value is in good agreement with the x-ray diffraction results. However, it should be borne in mind that these rippled structures are observed by freeze fracture when

samples are quenched from temperatures below or above the pretransition.[5]

Having defined the structures associated with the pretransition, it is of interest to understand those intermolecular forces responsible for this structural transformation. We have shown that the pretransition exhibits a compositional dependence and is first observed by DSC at approximately 20% water. At water concentrations of less than 20%, the pretransition is not observed and the structure $L_{\beta'}$ is preserved (Janiak et al., 1976). A water concentration of 20% corresponds to the bound water or hydration shell of lecithin, approximately 11 molecules of water/phospholipid molecule (Hauser, 1976). Mixtures of DPL and ethylene glycol–water (1:1, v:v) do not exhibit a thermal pretransition (Klopfenstein et al., 1974) and similar observations have been made for mixtures of DML and ethylene glycol–water (Janiak, unpublished observations). Thus, the appropriate hydration shell or the formation of a structured water matrix surrounding the phospholipid molecule, appears to be a requirement for the appearance of the pretransition.

Other DSC studies indicate that the choline group of the phospholipid is essential for the presence of the pretransition. Although the chain-melting transition enthalpies of DPL and dipalmitoylphosphatidylethanolamine (PE) are very similar, no pretransition is observed for phosphatidylethanolamine (Ladbrooke and Chapman, 1969). The N-methylated dipalmitoyl-PE (*N*-mono- and *N,N*-dimethyl-), which differ from DPL only in the degree of *N*-methyl substitution, also do not exhibit a pretransition (Vaughan and Keough, 1974). These results, together with the above observations, suggest that a specific interaction between a structured water matrix and the choline group at the pretransition may be responsible for the observed structural transformation of $L_{\beta'}$ to $P_{\beta'}$. However, the complexity of these interactions may prevent detailed explanations of these observations regarding the thermal pretransition until higher resolution crystallographic analyses of these structures are available.

Acknowledgments

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[5] A systematic freeze fracture study of DML and DPL by E. Luna and H. M. McConnell (private communication) has shown the presence of the regular banded appearance indicative of rippled structures only when the lecithins are quenched from a temperature between their pretransition and chain melting transition. No regular banded appearance is observed when DML and DPL are quenched from temperatures above the chain melting transition or below the pretransition.