TILTED HYDROCARBON CHAINS OF DIPALMITOYL LECITHIN BECOME PERPENDICULAR TO THE BILAYER BEFORE MELTING

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ABSTRACT Differential scanning calorimetry studies of dipalmitoyl lecithin show two reversible transitions as the temperature is changed between 20 and 50°C. A pretransition endotherm occurs at 35°C prior to the main chain melting endotherm which occurs at 42°C. X-ray diffraction studies show that below 33°C the chains of the lecithin are fully extended, packed in a hexagonal crystalline lattice but tilted with respect to the plane of the bilayer. Between 35 and 42°C the chains are similarly packed but oriented perpendicular to the bilayer plane. Above 44°C the chains are "melted" or disordered. Monolayer studies of dipalmitoyl lecithin using continuous recording of pressure with molecular area reveal the existence of two solid condensed phases corresponding to these tilted and verticle chain structures. The tilted to perpendicular transition would account for the pretransition endotherm of the lipid; the crystalline to melted change corresponds to the larger transition observed at 42°C.

INTRODUCTION

Early studies of pure lecithin-water systems using a range of physical techniques including X-ray, nuclear magnetic resonance (NMR) and calorimetry showed the occurrence of a large endothermic phase transition associated with the melting of the lipid chains (for dipalmitoyl lecithin at 42°C). This transition temperature varies with the chain length and also the degree of unsaturation with the chain (Chapman et al., 1967). A small endotherm (a pretransition) was also observed to occur prior to this main transition at 35°C with dipalmitoyl lecithin. Techniques such as NMR spectroscopy showed that there was an increase in molecular mobility of the polar group at the temperature of this pretransition (Veksli et al., 1969; Chapman and Chen, 1972; Darke et al., 1972). The occurrence of the pretransition endotherm has been shown to be affected by the presence of small amounts of other molecules included in the bilayer, e.g., cholesterol (Ladbrooke et al., 1968), drug molecules (Cater et al., 1974), and also by the effects of various salts present in the aqueous channels separating the bilayers (Chapman et al., 1975).

Tardieu et al. (1973) have carried out a very comprehensive extension of earlier structural studies on lecithin-water systems. In excess water the lipids form lamellar phases in which bimolecular layers of lipid are separated to a limited extent by layers of water

of uniform thickness. The conformation of the hydrocarbon chains in these bilayers can be one of three kinds. Following the nomenclature of Tardieu et al. (1973), in the $L\beta^1$ and $L\beta$ conformations the chains are stiff, fully extended and organized with rotational disorder in a two-dimensional hexagonal lattice. In the $L\beta$ phase the chains are oriented perpendicular to the plane of the bilayer; in the $L\beta^1$ phase the chains are oriented at an angle to the plane of the bilayer, an angle that can vary with the concentration of the lipid. In the $L\alpha$ conformation the hydrocarbon chains are melted and in liquid-like disorder with the average orientation perpendicular to the plane of the bilayer. It is the melting of the chains that represents the large endothermic transition observed by differential thermal analysis at 42°C for dipalmitoyl lecithin. Tardieu et al. (1973) report that no single lecithin-water system including dipalmitoyl lecithin exhibits all three conformations. Recently Gottlieb and Eanes (1974) have reported a structural investigation of the thermal transitions from the $L\beta^1$ to the $L\alpha$ phase of dipalmitoyl lecithin. They conclude that the transition takes place over a rather large temperature range, within which the two phases coexist in varying proportions, and, not being isothermal, anomalously disobeys the phase rule.

Numerous studies of lecithin monolayers on water have been reported (Phillips and Chapman, 1968; and Munden and Swarbrick, 1973). Only one solid condensed monolayer is described in earlier reports. However by continuously recording the pressure versus molecular area, as reported here, it is possible to detect phase transitions between solid condensed phases with high sensitivity.

By using this sensitive monolayer technique and by a systematic X-ray diffraction study in the region of the transitions we have attempted to determine the structural cause of the premelt transition at 35°C.

MATERIALS AND METHODS

Dipalmitoyl lecithin was used that gave only one spot by thin-layer chromatography and that contained greater than 95% palmitic residues by gas-liquid chromatography. X-ray samples were prepared by mixing the dipalmitoyl lecithin to 50% dry weight with water then sealing it between mica windows. X-ray diffraction measurements of the repeat distance d and of the high angle region were obtained by conventional means (for example see Rand and Luzzati, 1968) using a Guinier camera operating in vacuum and using a bent quartz crystal monochromator to isolate the $CuK\alpha_1$ line. The X-ray sample was mounted in a brass block in the vacuum and its temperature was controlled to $\pm$ 0.1°C with thermal electric elements and was monitored continuously with a thermocouple. The sample was not removed during a temperature run and changes in the repeat distance of the lamellar phase could be measured to $\pm$ 0.1 Å. X-ray diagrams were also taken on a camera that records the X-ray reflections as the temperature of the sample changed, in this case at the rate of 6°C/h, and yields a diffraction vs. temperature diagram (DPT diagram).

For the monolayer studies, a continuously recording balance of the Wilhelmy type was used. A few isotherms run on a horizontal balance of the Langmuir type showed the same characteristic features except for a somewhat higher collapse pressure. The dipalmitoyl lecithin sample was dissolved in ethanol-hexane 9 : 1 and spread by a micrometer syringe. The compression was achieved by moving the barriers symmetrically in relation to the Wilhelmy plate at a velocity of about 4 $Å^2$/molecule per minute. Isotherms were run at every 3°C in the range 4–42°C. Double distilled water was used as substrate.

The calorimetric studies were carried out using a Perkin-Elmer DSC 2 calorimeter (Perkin-Elmer Corp., Norwalk, Conn.). Heating rates of 10°C/min were used.

RESULTS

Differential Scanning Calorimetry (DSC)

The DSC curve for a pure dipalmitoyl lecithin-water system (excess water) is shown in Fig. 1. This shows the "pretransition" endotherm (1.6 cal/mol) at 35°C and the main endotherm (8.7 cal/mol) at 42°C. This is similar to previous results obtained with lecithin-water systems (Chapman et al., 1967, and Hinz and Sturtevant, 1972).

X-Ray Diffraction

Fig. 2 illustrates the changes that take place in the lamellar phase of dipalmitoyl lecithin in excess water as the temperature is raised or lowered through the transition region. The low angle reflections, giving the repeat distance of the lamellar phase, show the existence of three distinct regions representing three different lamellar phases: (*1*) up to 34°C a lamellar repeat of 65 Å, (*2*) from 36°C to 41°C a lamellar repeat of approximately 70 Å, and (*3*) from 44°C and higher a lamellar repeat of approximately 68 Å. Over the narrow temperature ranges between these regions the two phases on either side coexist. The transitions are reversible.

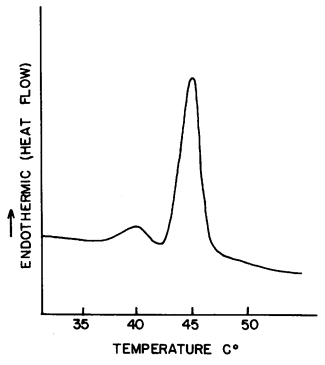

FIGURE 1

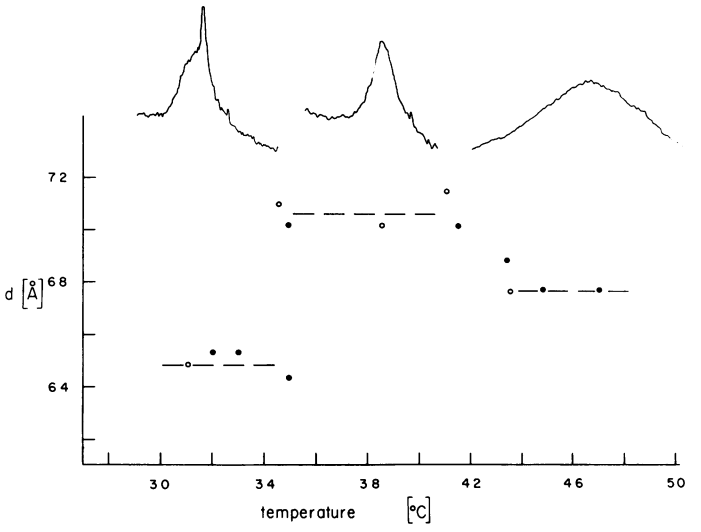

FIGURE 2

FIGURE 1 Differential scanning calorimetry curve for a pure DPL-water system (excess water). A small "pretransition" endotherm at 35°C and the main endotherm at 42°C are shown.

FIGURE 2 A plot of repeat distance *d* of the lamellar phase formed by DPL in excess water as a function of temperature. Open and closed circles represent different samples. Three distinct phases, d = 65 Å, d = 70 Å, and d = 68 Å, exist with two phases coexisting at the transition temperatures at 35°C and 44°C. Upper curves give the densitometer profile of the high angle diffraction in the 4.2 Å region characterizing the conformation and packing of the hydrocarbon chains in the temperature regions indicated.

Changes in the profile of the high-angle diffraction in the 4.2 Å region, characteristic of the conformation of the hydrocarbon chains, are shown in the microdensitometer traces in Fig. 1 as well. Below 33°C a sharp 4.2 Å line with a diffuse scattering on its higher-angle edge is indicative of the $L\beta^1$ phase with the chains crystalline but tilted with respect to the bilayer plane (Tardieu et al., 1973). At 40°C, a line at 4.2 Å indicates crystalline chains perpendicular to the plane of the bilayer; the $L\beta$ phase. The hydrocarbon chains are packed hexagonally. At 46°C a diffuse band at 4.5 Å indicates melted hydrocarbon chains of the $L\alpha$ phase. The DPT diagram in Fig. 3 more clearly illustrates the same two transitions. Table I indicates the dimensions of the lamellar repeat and the high-angle region for the three phases and shows the transitions occur at 35°C and 42°C. The diffraction lines of the $L\beta$ phase are significantly broader than those of the $L\beta^1$ of $L\alpha$ phase. This suggests that there is more long-range disorder in this phase and the diffuseness of the lines and differences in water contents of samples prepared may account for the difference in the lamellar repeat of this phase taken on the two cameras.

Therefore these X-ray diffraction experiments confirm the calorimetric data and provide a structural basis for the two transitions.

We have not attempted to extend the studies into the region of the phase diagram

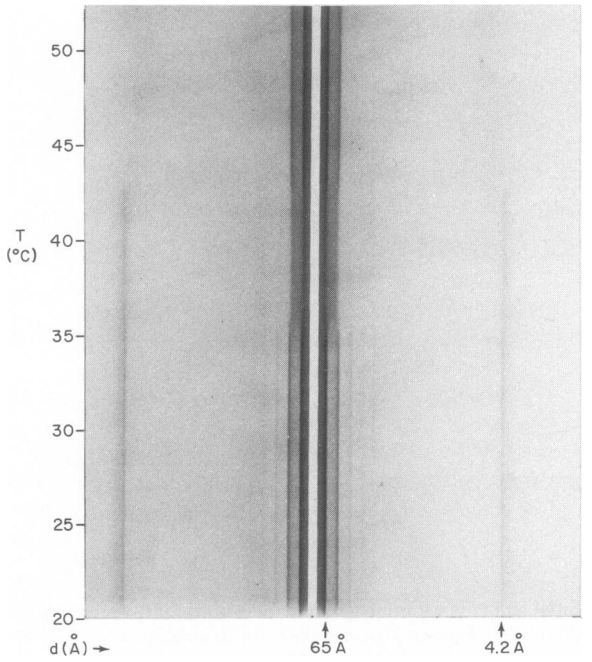

FIGURE 3 Diffraction pattern vs. temperature, continuously recorded, of DPL in excess water (50:50). The data are comparable to those of Fig. 2 showing the phase transitions at 34°C and 42°C. Both the change in *d* spacing at low angle and the change in high-angle profile in the 4.2 Å region are evident. Temperature program: 6°C/h.

TABLE I

X-RAY DATA OF DIPALMITOYL LECITHIN/WATER (50/50) TAKEN FROM FIG. 3

Temperature	Long spacings	Short spacings
°C	Å	
22 - 35	64.2 ± 0.2	4.25 + diffuse band
35 - 42.5	77.0 ± 0.8	4.23 *
42.5 - 50	67.0 ± 0.3	None 4.5 - 4.6 diffuse band

* The occurence of a single line is consistent with the hexagonal chain packing, and the spacing 4.23 Å corresponds to a cross section of about 21 $Å^2$ per hydrocarbon chain. This is slightly higher than in n-paraffins where the chains possess rotational characteristics according to X-ray data (K. Larsson, Nature 213; 383, 1967).

where the lipid is not fully hydrated or swelled. Preliminary indications are that the thermal transitions become more complicated than when water is in excess. Therefore we cannot obtain the thicknesses of the bilayers and of the interbilayer space from our results.

Monolayer Studies

A typical isotherm is shown in Fig. 4 and is taken at a temperature where all three phases are evident. These phases are not as clearly defined as they are at higher or lower temperatures where only one or two of the three phases form, but this isotherm illustrates the existence of three phases and supports the X-ray evidence. A liquid phase is first formed which, starting at about 62 $Å^2$/molecule (arrow *a*) is transformed into a solid condensed phase. This phase shows high compressibility; it exists between about 52 and 48 $Å^2$/molecule (between arrows *b* and *c*). At further compression a second solid-condensed phase with smaller compressibility is formed. This higher pressure phase exists between 35 and 41 dyn/cm. The molecular areas of the three phases indicate that they are closely related in structure to the three phases Lα, Lβ, and Lβ^1. The liquid condensed phase shows, at its highest pressure, a cross-section area per hydrocarbon chain of 31 $Å^2$ which is in good agreement with the values of lamellar liquid-crystalline phases. The lower pressure solid condensed phase must have tilted chains as the cross-section area per chain is so high, whereas the value of the area for the high-pressure phase of about 20.5 $Å^2$ (per hydrocarbon chain) Phillips and Chap-

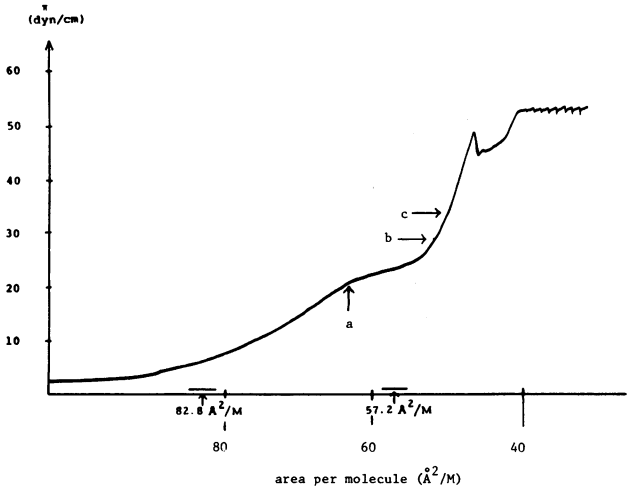

FIGURE 4 Pressure-area isotherm of DPL at 29.8°C. The photographically recorded curve is shown. Calibration points at 82.8 and 57.2 $Å^2$/molecule are shown. Three phases are evident: (*1*) a liquid phase up to about 62 $Å^2$/molecule (arrow *a*), (*2*) a solid condensed phase from about 52 to 48 $Å^2$/molecule (between arrows *b* and *c*), (*3*) a higher pressure solid condensed phase from 35 to 41 dyn/cm.

man, 1968, report a molecular limiting area of 44 $Å^2$ on 0.1 N sodium chloride substrate for this lecithin) corresponds to vertical chains.

DISCUSSION AND CONCLUSIONS

These X-ray diffraction studies of dipalmitoyl lecithin in excess water show the structural changes, indicated schematically in Fig. 5, that occur for the two thermal transitions observed by differential scanning calorimetry. The nature of the structural change at 42°C, a melting of the hydrocarbon chains, has been clear but the nature of the "pretransition" at 35°C has not. These results show that at 35°C a transition takes place from a phase where the chains are crystalline and tilted to the bilayer plane to one where the chains remain crystalline but become perpendicular to the plane. The monolayer studies also show two condensed phases corresponding to these tilted and vertical chain structures.

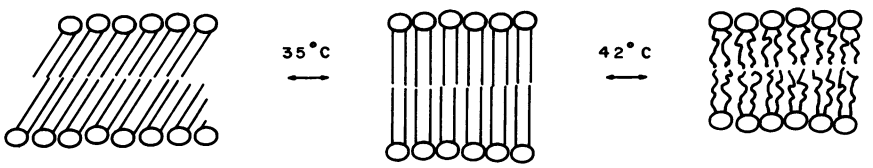

FIGURE 5 Schematic diagram of cross section of the DPL bilayer showing the structural changes of DPL in excess water observed at 35°C and 42°C. Circles represent the DPL head groups, straight lines fully extended hydrocarbon chains, and wiggly lines melted hydrocarbon chains.

The tilted to vertical chain transition will result in a decrease in the surface area of lecithin molecules at the bilayer surface. This might have been expected to cause a restriction in mobility of the polar group. The previous NMR spectroscopic evidence on the other hand suggests that the polar groups increase the mobility at this temperature. We envisage a change in the polar group conformation occurring at the pretransition temperature correlated with the change of chain tilt. It is well known that a different polar group can affect the temperature of the main transition for a given chain length (see Chapman et al., 1974). This has been related to the polar group packing affecting the lipid chain packing. The tilted chain structure also appears sensitive to the polar group packing.

The observation of two transitions, and three phases, over the temperature range 20 to 50°C is interesting from two points of view. First it removes the anomaly reported by Gottlieb and Eanes (1974) concerning the nature of the phase transition from crystalline to melted chains. Second the three phases and the temperatures of transition coincide with three quite different regimes of kinetics of the hydrolytic enzyme phospholipase A_2 as it interacts with, and hydrolyzes, dipalmitoyl lecithin.[1] The rate of hydrolysis is highest for the phase where the chains are perpendicular to the bilayer, medium for when they are tilted to the plane and lowest for melted chains. Since, when the chains are perpendicular to the bilayer the lipids occupy a minimum of area on the lipid-water interface, the three quite different kinetics have been accounted for not on the basis of the ease of access of the enzyme to the specific bonds in the substrate but rather by the rate at which the enzyme can desorb from the lipid interface and move on to a new substrate molecule.

The "pretransition" at 35°C and presumably the tilted chain structure, is sensitive to the presence of other "foreign" molecules. Early studies by Ladbrooke et al. (1968) show that when 7.5 mole percent cholesterol is included in the bilayer of dipalmitoyl lecithin the pretransition endotherm is removed and at the same point the lipid chains transform from the tilted to the vertical condition. Drug molecules (Cater et al., 1974) or the inclusion of related lipids in small amounts (approximately 10 mole percent) such as phosphatidylethanolamines (Chapman et al., 1974) remove the pretransition and presumably are affecting the chain organization. Related studies with various salt solutions show effects on this endotherm as well (Chapman et al., 1975). This latter indicates further that interactions of the polar groups affect the tilted chain structure.

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