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Liquid Crystalline Phases of a Diacetylenic Phosphocholine Lipid

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We have studied the liquid crystalline behavior of a polymerizable diacetylenic phosphocholine lipid 1,2-bis(10,12-tricosadiynoyl-sn-glycero-3-phosphocholine ($DC_{8,9}PC$). Optical polarized microscopy reveals several types of textures corresponding to normal as well as tilted smectic phases for the dry sample while only the oily streak texture of the L_α phase is seen for the sample with 5% water. X-ray diffraction data show that the lamellar order in the hydrated sample is more pronounced than that in the dry sample. Also, a pronounced change in the layer structure occurs for both the dry and 5% hydrated samples at the chain melting temperature. These structural changes are discussed in relation to the optical microscopic observations.

INTRODUCTION

Lipid bilayers provide relatively simple models for biological membranes. Polymerizable lipids give rise to many potential applications in which mechanically rugged membranes are desired. Polymerized membranes have attracted intense theoretical interest[1] and two dimensional polymer networks are predicted to have properties strikingly different from conventional linear polymer chains.[2] The diacetylenic phospholipids, in which a diacetylenic moiety is inserted into each of the alkyl chains, are photo-polymerizable and therefore can be good systems to test some of the theoretical predictions.

Another interesting property of these diacetylenic lipids is that they form on cooling below the chain melting temperature, hollow cylindrical structures known as 'tubules'[3] (see Figure 1). Tubules formed from 1,2-bis(10,12-tricosadiynol)-sn-3-phosphocholine[4,5] or $DC_{8,9}PC$ (see Figure 2) are approximately 0.5 μm in diameter and may range from several tens to hundreds of μm in length. The potentialities of the tubules for many technological applications were recognized[6,7] soon after their first observation. The mechanism of tubule formation is however not well understood. Even though the role of the liquid crystalline phase in tubule

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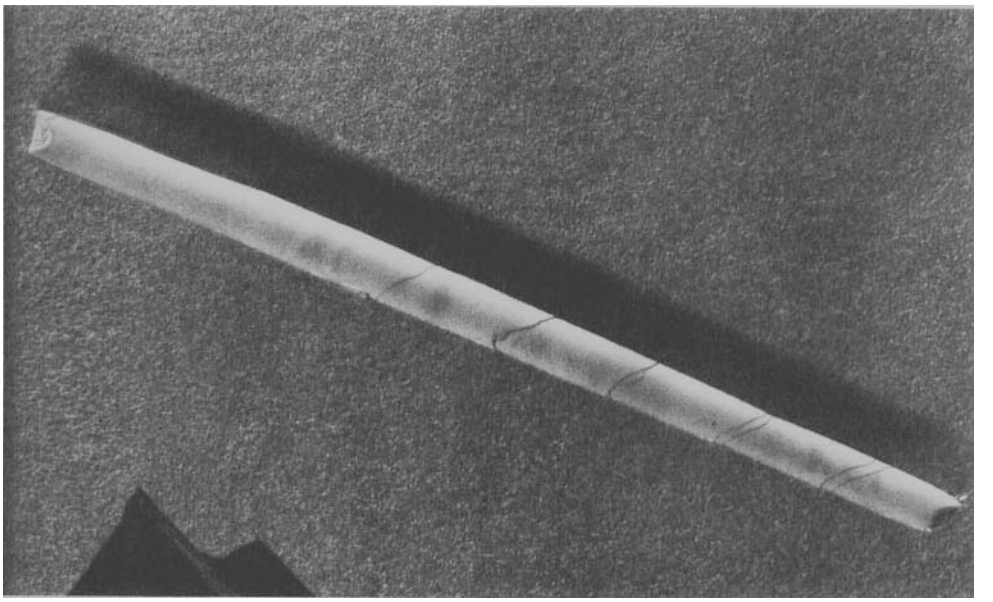

FIGURE 1 Electron micrograph of a "tubule" (hollow cylinder) formed from a diacetylenic phosphocholine lipid ($DC_{8,9}PC$) dissolved in a solvent system. The diameter of the tubule in this picture is $\approx$0.5 μm while the length of the tubule is $\approx$16 μm. The wall of the tubule is made up of helically wrapped bilayers.

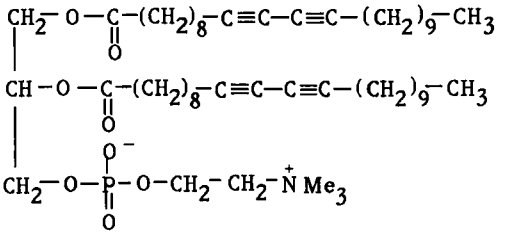

FIGURE 2 Chemical structure of $DC_{8,9}PC$ molecule.

formation has been emphasized theoretically,[8,9] there has not been any experimental study of the mesophase behavior of $DC_{8,9}PC$ so far. We have undertaken such a study as a first step in understanding the formation of tubules.

In this paper we present the results of our studies on the liquid crystalline behavior of $DC_{8,9}PC$ in the 'dry' and 5% hydration states.

EXPERIMENTAL

The lipid ($DC_{8,9}PC$) was purchased from Avanti Polar Lipids and stored at $-20°C$. Small quantities were recrystallized in the dark (to prevent photo-polymerization) three times from freshly dried acetone. The recrystallized lipid was dried overnight under vacuum and stored under dry nitrogen in paraffin sealed amber vials at

−20°C until used. The residual moisture in the lipid was measured using a Karl-Fisher Coulometer (Model 684-KF). An accurately weighed amount of $DC_{8,9}PC$ was heated inside a drying oven. The moisture which was given off by the lipid was removed by a stream of nitrogen which takes it into a titration vessel. The moisture content could then be measured to an accuracy of ±0.01%. Such an analysis showed that the residual moisture in the lipid in its driest state is about 8% by weight which makes the "dry" lipid a dihydrate, as is usually the case with any lipid. Thus the "dry" lipid is hereafter assumed to be that with about 8% residual moisture. Samples of 5% hydrated lipid (the extent of hydration is 5% by weight of water over and above the dry state) were prepared gravimetrically by adding 5 μL of triply distilled water to 100 mg of the lipid. The samples were then sealed under nitrogen and allowed to stand in the dark at room temperature for several weeks. The samples of fully hydrated lipid were also prepared in the same way but by adding an excess amount of water to the lipid.

The optical observations were made using a Nikon polarization microscope in conjunction with a Linkam heating/cooling stage. X-ray diffraction data were collected using a Rigaku diffractometer operated in the reflection geometry and in the θ-2θ mode. The Cu K_α line (λ = 1.5419) from a Rigaku RU200B rotating anode x-ray generator, operated at 50 kV and 200 mA, was used for the experiments. The temperature of the sample was controlled by a circulating water bath to an accuracy of ±0.2°C. The layer spacing was calculated using 4–6 orders of diffraction. As many as 14 orders of diffraction could be indexed for some samples.

RESULTS AND DISCUSSION

A. Optical observations

The "dry" lipid exhibits a number of smectic (Sm) mesophases. The sequence of phases on heating and the transitions between is given below:

$$X_1\ 51°C\ Sm1\ 75°C\ Sm2\ 101°C\ Sm3\ 203°C\ SmA\ 212°C\ I$$

Sm1 exhibits a sandy schlieren texture, while in the Sm2 phase, the sample appears dark under crossed polars. Figure 3 shows the transition between the Sm1 and Sm2 phases, the dark regions corresponding to the Sm2 phase. On further heating, a more well defined schlieren texture appears (Figure 4) at 110°C signaling the onset of Sm3 phase. This phase is stable till 203°C when the sample transforms to the smectic A phase characterized by a focal conic texture (Figure 5). On the basis of these optical texture observations it appears that both Sm1 and Sm3 are tilted smectic phases while Sm2 could be a uniaxial phase with a strong homeotropic orientation of the molecules. Considering that in the Sm3 phase it is possible to displace the cover slips between which the sample is sandwiched, it would also appear that the Sm3 phase is likely to be a tilted 'fluid' smectic. As far as we are aware, no other instance of a fluid tilted smectic has been reported in any lipid system so far. The structure of these tilted smectic phases on a molecular level is yet to be established.

Adding even a small quantity of water to the dry lipid alters the phase behavior

FIGURE 3 Optical texture showing the transition from the Sm1 (schlieren texture) to the Sm2 (dark regions) phase in 'dry' $DC_{8,9}PC$. (400 X, T = 75°C).

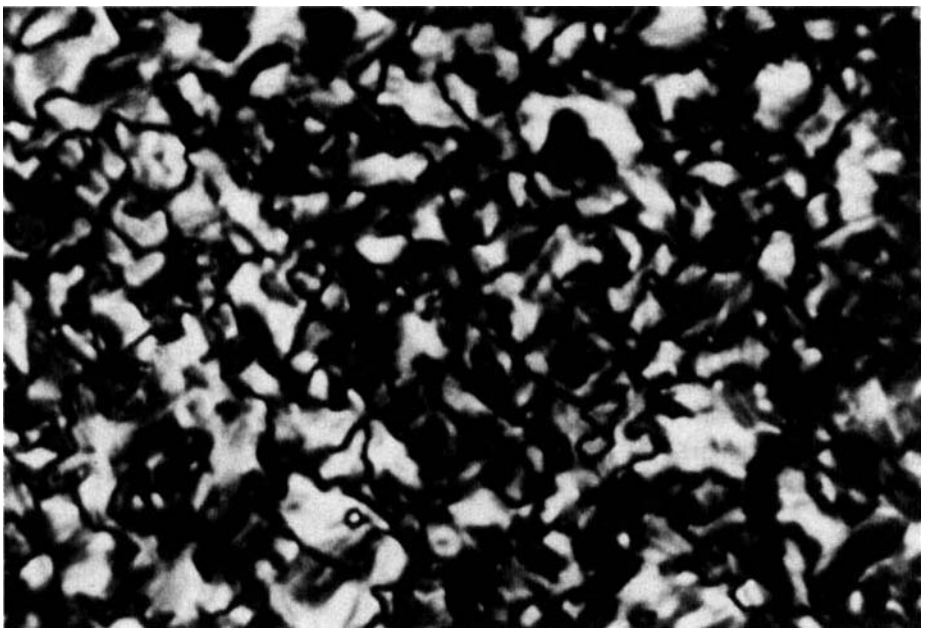

FIGURE 4 The well defined schlieren texture of the Sm3 phase of dry $DC_{8,9}PC$. (T = 120°C).

FIGURE 5 Focal conic texture of the Sm A phase for dry $DC_{8,9}PC$. (T = 205°C).

drastically. The tilted smectic phases are suppressed and only the L_α phase exhibiting the characteristic oily streaks (Figure 6) is observed. The transition from the crystal X_2 (referred to as X_2 to distinguish from the crystal phase (X_1) exhibited by the dry lipid) to the L_α phase occurs at about 50°C. This is about the same temperature at which the crystal (X_1) to the Sm1 transition occurred for the dry lipid. Thus, although the melting temperature is about the same for the dry and 5% hydrated $DC_{8,9}PC$, the high temperature phases are different in the two cases, viz., the classical L_α phase in the case of the 5% hydrated sample and the tilted smectic phase (Sm1) in the case of the dry lipid. To investigate if the smectic layer spacing reflects this difference, we have studied the variation of the layer thickness with temperature across the melting transition for the dry and 5% hydrated lipid samples. The results of these studies are presented in the following section.

X-ray Studies

(1) Dry lipid: The data on the layer spacing variation for the dry sample are shown in Figures 7 and 8. The data in Figure 7 have been taken in the heating mode while those in Figure 8 have been obtained on cooling the sample from the Sm1 phase. Although a pronounced hysteresis is seen with regards to the temperature at which the X_1-Sm1 transition occurs, the layer spacing values in the two phases are independent of the thermal history of the sample. The layer spacing in the X_1 phase which is about 60 Å drops to 45 Å in the Sm1 phase. A recent x-ray study by Caffrey et al.[10] of the bilayer packing for $DC_{8,9}PC$ in the dry state has identified this state as L_c (lamellar crystal). Our value for the lamellar repeat

FIGURE 6 Oily streaks of the L_α phase for the 5% hydrated $DC_{8,9}PC$.

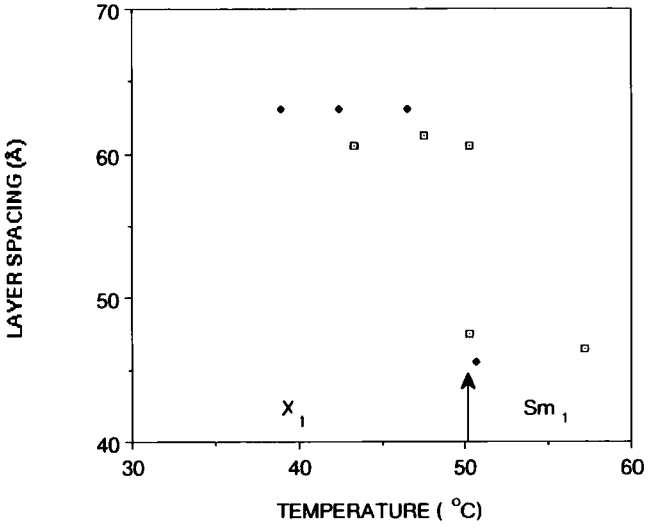

FIGURE 7 Temperature variation of the lamellar periodicity (d in Å) in the X_1 and Sm1 phases of dry $DC_{8,9}PC$. A pronounced jump in d is seen at the transition. X_1 is the lamellar crystalline phase[10] while Sm1 is a highly ordered (unidentified) tilted smectic phase. The symbols denote the data for 2 independent sets of experiments.

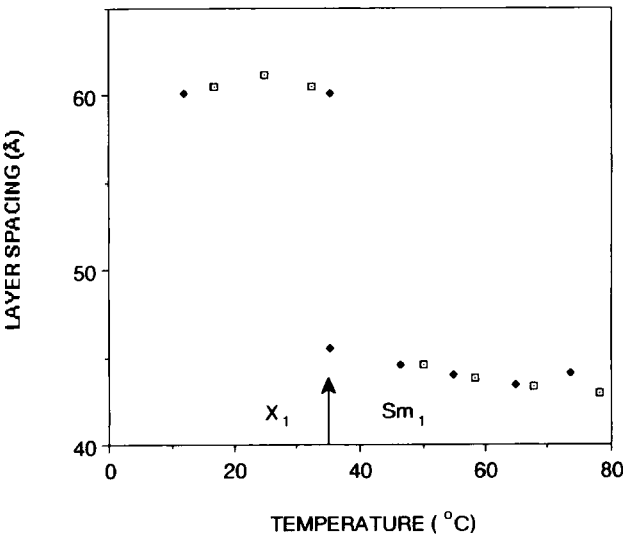

FIGURE 8 Temperature variation of the lamellar periodicity on cooling from Sm1 to X_1 phase.

distance (60 Å) in the low temperature phase for dry $DC_{8,9}PC$ is in good agreement with the value (59 Å) of Caffrey et al. in the L_c phase. Caffrey et al. have also formulated a model for the $DC_{8,9}PC$ crystalline bilayer in which 1) the methylene segments are in the fully extended all-trans configuration, 2) the long axis is tilted with respect to the layer normal by 32° and 3) the chain interdigitation is minimal, if any. The optical texture exhibited in the Sm1 phase (Figure 3) seems to indicate that the tilt of the crystalline state is maintained in the high temperature phase. The reason for such a large decrease in the lamellar periodicity on going to the Sm1 could be due to an increase in the fluidity of the chains or due to an interdigitation of the chains on melting. We wish to emphasize that we observed 8–10 orders of diffraction peaks corresponding to the lamellar order in the X_1 phase compared to only three or four orders in the Sm1 phase. This indicates that the layering is less defined in the Sm1 phase than in the X_1 phase.

(2) 5% hydrated $DC_{8,9}PC$: The layer spacing for this sample (Figure 9) also shows a decrease at the X_2 to L_α phase transition, the values of the layer spacing in the two phases being very similar to those in the X_1 and Sm1 phases of the dry lipid respectively (Figures 7 & 8). This is somewhat surprising since we know from optical textures described earlier (see Figures 3 and 6) that the high temperature phases for the dry lipid and the 5% hydrated lipid are different: Sm1 phase of the dry lipid is probably a tilted smectic (C-like ?) phase whilst L_α is clearly the high temperature phase in the case of the 5% hydrated sample. Also, the fact that we see 12 to 14 diffraction orders in the X_2 phase compared to only 4 to 6 orders in the L_α phase shows that the fluidity of the layers is enhanced in the L_α phase. Similar results were observed for excess hydration of 10%.

Thus our studies on dry and hydrated forms of the diacetylenic phospholipid $DC_{8,9}PC$ have shown that unusual polymorphic liquid crystalline phases are exhib-

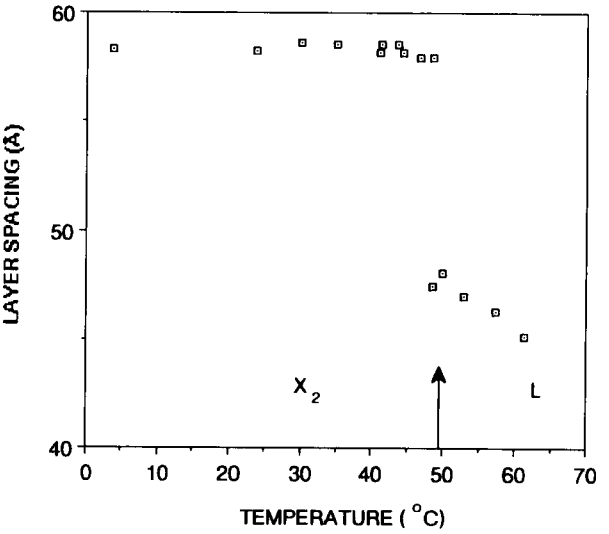

FIGURE 9 Temperature variation of d in the X_2 (solid) and L_α phases of 5% hydrated $DC_{8,9}PC$.

ited by the dry lipid, but are suppressed even for small levels of hydration. The lamellar periodicity shows a pronounced decrease at the chain melting transition regardless of the nature of the higher temperature phase. It will be important to probe the nature of the phases in the high dilution regimes (the work reported in this paper is entirely in the low dilution regime), particularly because tubule formation has been seen so far only in excess water suspensions of $DC_{8,9}PC$. Such a study assumes more relevance in view of recent theories[8,9] which indicate that the nature of the liquid crystalline phase should be playing an important role in tubule formation.

Acknowledgment

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