$L_{\beta'} \rightarrow L_{c'}$ phase transition in phosphatidylcholine lipid bilayers: A disorder-order transition in two dimensions

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The structure of the $L_{c'}$ phase exhibited by hydrated dipalmitoylphosphatidylcholine (DPPC) was recently determined by Raghunathan and Katsaras [Phys. Rev. Lett. **74**, 4456 (1995)] from x-ray diffraction studies on oriented multibilayers. Here, we reanalyze the powder diffraction data reported in the literature on a number of hydrated lipids possessing the phosphatidylcholine headgroup. As in DPPC, the $L_{c'}$ phase in all of these systems is found to be characterized by two-dimensional ordering of the lipid molecules on a superlattice of the hydrocarbon chain lattice. We also discuss the influence of headgroup interactions on the structure of this phase. [S1063-651X(96)00310-8]

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I. INTRODUCTION

Lipid molecules, when hydrated, exhibit a number of lamellar phases which are characterized by the absence of interlayer correlations in their molecular arrangement [1–3]. In the high temperature L_{α} phase the hydrocarbon chains of the molecules are in a ''melted'' state and, hence, the ordering of the molecules within each layer is liquidlike. On the other hand, in the lower temperature $L_{\beta'}$ and $L_{c'}$ phases the chains are practically fully stretched and are ordered within the plane of the bilayer. Most of the lipids studied to date consist of two hydrophobic hydrocarbon chains, a glycerol backbone and a hydrophilic headgroup. Although the hydrocarbon chains tend to be ordered on a two-dimensional (2D) lattice, the headgroups are not. X-ray diffraction studies indicate that the structure of the $L_{\beta'}$ phase is consistent with such a picture [Fig. 1(a)]. Thus all the reflections due to the in-plane ordering can be attributed to the hydrocarbon chains whereas the electron-rich headgroups give rise to a diffuse background [4].

The $L_{c'}$ phase was first observed calorimetrically in multilamellar suspensions of DPPC by Chen, Sturtevant, and Gaffney [5] after the sample was kept at 0 °C for 3.5 days. From x-ray diffraction studies on oriented multibilayers, we have recently shown that the $L_{\beta'} \rightarrow L_{c'}$ phase transition in dipalmitoyl phosphatidylcholine (DPPC) results in the lipid molecules being ordered in the plane of the bilayer [3]. Interestingly, this ordering process takes place without destroying the chain lattice, and the simultaneous existence of the two lattices requires the molecular lattice to be a superlattice of the chain lattice [Fig. 1(b)]. This transition can thus be looked upon as a disorder-order transition on a two-dimensional lattice and is likely to be driven by the interactions between the headgroups. Hence, lipids, with the same headgroup, can be expected to have similar structure in the $L_{c'}$ phase. We have, therefore, reanalyzed x-ray diffraction data on lipids having the phosphatidylcholine (PC) headgroup, but differing in the length of their hydrocarbon chains and their position in the glycerol backbone, reported by Stümpel, Eibl, and Nicksch [6]. The $L_{c'}$ phase of most of these systems is found to be similar to that exhibited by DPPC.

II. THE MODEL

X-ray diffraction studies reported in Ref. [6] were carried out on powder (unoriented) samples. One of the systems studied by these authors was DPPC, for which we have recently collected diffraction data from both powder and oriented samples [3]. A diffraction pattern obtained from a powder sample of DPPC in the $L_{c'}$ phase is shown in Fig. 2.

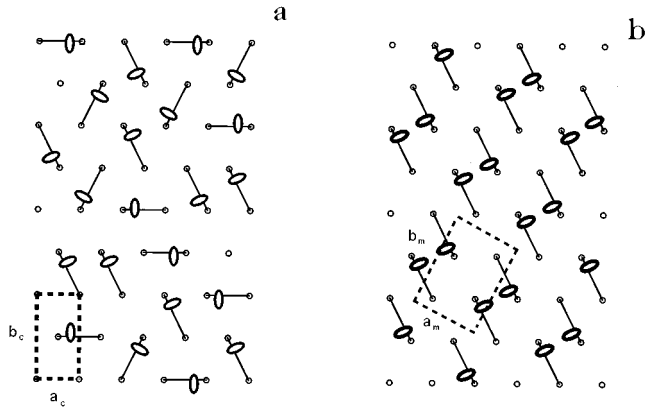

FIG. 1. (a) Schematic representation of the in-plane molecular ordering in the $L_{\beta'}$ phase of hydrated lipids. The small open circles represent the hydrocarbon chains while the larger symbols represent the phosphorylcholine headgroups. The solid black lines represent glycerol backbones, which connect two nearest neighbor hydrocarbon chains with one PC headgroup, forming one lipid molecule. Note that even though the chains are ordered on a lattice, the molecules themselves are not. (b) The in-plane structure of the $L_{c'}$ phase of DPPC deduced from x-ray studies on oriented bilayers. The molecules are now ordered on a superlattice of the chain lattice. The figure shows one out of a possible six molecular arrangements within the superlattice (please see Ref. [3] for the remaining molecular arrangements). The hydrocarbon chain lattice and molecular lattice dimensions are given by a_c, b_c and a_m, b_m, respectively.

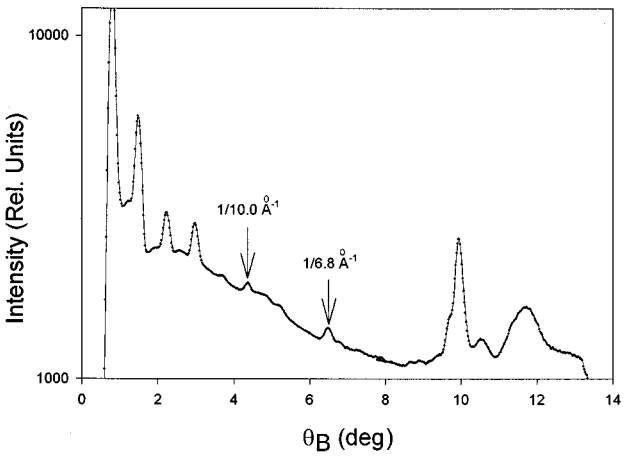

FIG. 2. Diffraction pattern of a DPPC powder sample in the $L_{c'}$ phase at 7 °C and under excess water. θ_B is the Bragg angle. The two weak reflections at 1/10.0 $Å^{-1}$ and 1/6.8 $Å^{-1}$ are a result of the headgroups forming a 2D lattice.

The four peaks in the small angle region ($\theta_B \leqslant 3°$) are due to the lamellar ordering of the bilayers. The two weak reflections at about 1/10 $Å^{-1}$ (4.4°) and 1/6.8 $Å^{-1}$ (6.5°) arise from the molecular superlattice while the reflections at larger angles ($\theta_B \geqslant 10°$) are from the hydrocarbon chain lattice. From the data presented in Ref. [6], we see that the spacings of the nonlamellar reflections observed in the $L_{c'}$ phase for most of the systems are comparable to those found in DPPC, indicating that the structure of this phase in these systems is similar to that in DPPC. In order to confirm this, we have fitted the model given in Fig. 1(b) to the powder data and obtained a very good agreement between the observed and calculated spacings of the different reflections.

Of the 13 PC lipids studied by Stümpel, Eibl, and Nicksch [6], 1*M*-2*M*-*PC* and 1*P*-2*S*-*PC* do not show the $L_{c'}$ phase, while 1*M*-2*P*-*PC* and 1*S*-2*P*-*PC* show reflections with spacings different from those seen in the other nine systems. Moreover, the data for two of these (1*S*-2*M*-*PC* and 1*P*-3*P*-*PC*) do not exhibit a sufficient number of reflections for the proper determination of the structure. Hence, we shall only consider the seven remaining systems. As discussed in Ref. [3], it is not possible to directly calculate the lattice parameters from powder data as information about molecular tilt cannot be easily retrieved. However, since we obtained diffraction data from both oriented and unoriented DPPC multibilayers, we know the specific relationship between the peaks in the powder pattern and the reflections in the oriented pattern. As the powder patterns of all the lipids considered here are similar, it is reasonable to expect the same relationship in all of them. Therefore we make the following assumptions, which can be justified on the basis of the DPPC data, while analyzing the data from the remaining seven systems. (a) Of the three reflections seen in the 1/4 $Å^{-1}$ region ($\theta_B \geqslant 10°$), the central one at $\theta_B = 10.5°$ is due to the secondary maximum in the form factor of the hydrocarbon chains [2,3]. (b) The molecules are tilted towards nearest neighbor. This is consistent with the data presented by Stümpel, Eibl, and Nicksch [6] in which one of the hydrocarbon chain reflections is ''sharp'' in all of the diffraction patterns presented [7]. (c) The unit cell of the chain lattice is rectangular. This assumption is not strictly correct since the unit cell of the chain lattice in DPPC is not rectangular, as indicated by the three distinct chain reflections in the oriented pattern [3]. However, as the obliquity of the unit cell is very small ($\gamma = 94°$), the (1 1) and ($1\ \bar{1}$) reflections merge to give a very broad peak at about 1/3.9 $Å^{-1}$ ($\theta_B \approx 11.5°$) in the powder pattern. Therefore, it is not possible to estimate the obliquity of the unit cell from the powder data and we are forced to take it to be rectangular.

In order to determine the unit cell parameters we start by indexing the sharp chain reflection at about 1/4.4 $Å^{-1}$ as (0 2) on the chain lattice and the reflection at about 1/10 $Å^{-1}$ as (0 1) on the molecular lattice. These give the parameters a_c and a_m, respectively. As the chain lattice is assumed to be rectangular, so is the molecular lattice shown in Fig. 1(b). The other parameters can then be calculated using the relations

$$b_c^2 = a_m^2 - a_c^2, \quad b_m^2 = (a_c^2 + 9b_c^2)/4.$$

The spacings of (1 1) reflections are given by

$$d_i = a_i b_i / \sqrt{(a_i^2 + b_i^2)}, \quad i = c \text{ or } m.$$

The reflection from the (1 1) molecular planes in the powder pattern can be attributed to the headgroups, which are relatively short entities, the polar region of the bilayer being only about 5 Å in thickness. Thus this reflection can be treated as due to point scatterers lying in the plane of the bilayer and, hence, the measured spacing of this reflection must be equal to d_m. In contrast, the spacing of the (1 1) planes of the chain lattice will not be equal to d_c due to the tilt of the elongated hydrocarbon chains with respect to the bilayer normal. The tilt angle θ is calculated from the expressions [8]

$$\tan\theta = \frac{\tan\phi}{\sin\psi}, \quad \phi = \cos^{-1}\left(\frac{d_{11}}{d_c}\right)$$

where d_{11} is the measured spacing of the (1 1) reflection in the powder pattern, and ψ is the angle between the (1 1) planes and the direction of the molecular tilt. As the tilt is towards nearest neighbor, i.e., along b_c, $\sin\psi = a_m/a_c$. In Table I we compare the values of the lattice parameters and the tilt angle of DPPC obtained from our oriented data and the powder data of Stümpel, Eibl, and Nicksch [6]. The values of these parameters for the other systems studied by these authors are shown in Table II. The agreement between the calculated and measured spacings is very good, confirming the model shown in Fig. 1(b) for the structure of the $L_{c'}$ phase.

III. DISCUSSION AND SUMMARY

In their 1983 paper, Stümpel, Eibl, and Nicksch [6] attempted to index all the observed nonlamellar reflections on a two-dimensional lattice in the plane of the bilayer. However, they did not get a satisfactory agreement between the calculated and measured spacings as the hydrocarbon chain reflections were not corrected for tilt and as the reflection due to the secondary maximum of the chain form factor was treated on an equal footing with the others. Consequently,

TABLE I. List of observed spacings in the $L_{c'}$ phase of DPPC and those calculated from the superlattice shown in Fig. 1(b).

Type of sample	Oriented Ref. [3]		Unoriented Ref. [6]	
Chain lattice				
a (Å)	8.80		8.65	
b (Å)	5.25		5.23	
θ (°)	34.5		34	
Chain reflections	Obs. (Å)	Cal. (Å)	Obs. (Å)	Cal. (Å)
02	4.4	4.4	4.40	4.33
11	3.9	3.9	3.88	3.88
$1\bar{1}$	3.8	3.8	3.88[a]	3.88
Molecular lattice				
a (Å)	10.15		10.15	
b (Å)	9.09		9.06	
Superlattice reflections	Obs. (Å)	Cal. (Å)	Obs. (Å)	Cal. (Å)
01	10.0	10.0	10.00	10.15
11, $1\bar{1}$	6.8	6.8	6.78	6.76

[a]It is not possible to separate the (1 1) and (1 $\bar{1}$) reflections in the powder sample.

they were not able to establish the in-plane structure of the bilayers.

The only system other than DPPC, where the structure of the $L_{c'}$ phase has been elucidated is dipalmitoylphosphatidylglycerol (DPPG) [9]. In contrast to DPPC, where only a few weak additional reflections appear below the $L_{\beta'} \rightarrow L_{c'}$ transition, a large number of strong reflections appear in the case of DPPG. Interestingly, the chain lattice present in the $L_{\beta'}$ phase is absent in the $L_{c'}$ phase of DPPG. Thus the $L_{\beta'} \rightarrow L_{c'}$ transition in this system involves a drastic change in the in-plane arrangement of the molecules. Powder diffraction patterns obtained from another lipid, namely, dilauroylphosphatidylethanolamine (DLPE), also show a large number of reflections in the L_c phase, probably indicating a similar structure [10]. Further, in DLPE this phase is highly stable and melts directly into the L_α phase on heating; the L_β phase is formed only on cooling. The difference in the structure of the $L_{c'}$ phases in these systems may be related to the differences in the headgroup interactions. The PG headgroup is charged and hence the interactions can be expected to be strong. Further, though both the PE and PC headgroups are zwitterionic, there is evidence from crystallographic data [11] that the former interact much more strongly with each other than the latter. The crystal structure of PE lipids show that these headgroups form a very compact lattice that is stabilized by strong electrostatic interaction and by hydrogen bonds between the ammonium nitrogen and the phosphate oxygens. In contrast, the fully methylated ammonium groups in PCs do not allow the formation of similar hydrogen bonds. Instead, water molecules of hydration are incorporated into the headgroup lattice linking phosphate groups into ribbons and shielding groups of similar charge [11]. As a result, the headgroup lattice in this case is much less rigid than in the PEs. It is, therefore, likely that the rather weak headgroup interactions in the PC's are responsible for the $L_{\beta'} \rightarrow L_{c'}$ transition in these systems, which involves only a subtle reorientation of the molecules within the bilayer.

In this article we have shown that the $L_{c'}$ phase exhibited by a number of hydrated lipids with the phosphatidylcholine headgroup has a similar structure. This differs from the structure of this phase in hydrated DPPG. While both these systems are made up of a stack of two dimensionally ordered bilayers, they differ in the type of in-plane arrangement of the molecules. In the PC's the ordering of the hydrocarbon

TABLE II. The spacings observed in the powder diffraction data presented in Ref. [6] and those calculated from the model shown in Fig. 1(b). The letters *M*, *P*, and *S* in the names of the lipids stand for saturated acyl chains containing 14, 16, and 18 carbon atoms, respectively. The numbers 1, 2, and 3 denote the position of the chains in the glycerol backbone.

Lipid	1*S*-2*S*-*PC*		1*M*-2*S*-*PC*		1*P*-2*M*-*PC*		1*M*-3*M*-*PC*		1*S*-3*M*-*PC*		1*S*-3*P*-*PC*	
Chain lattice												
a (Å)	8.70		8.70		8.70		8.40		8.56		8.40	
b (Å)	5.32		5.13		5.13		5.14		5.07		5.24	
θ (°)	35		33		33		25		34		37	
Chain reflections	Obs. (Å)	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.
02	4.41	4.35	4.34	4.35	4.40	4.35	4.25	4.20	4.33	4.28	4.26	4.20
11, $1\bar{1}$	3.88	3.88	3.86	3.86	3.84	3.84	4.07	4.07	3.76	3.76	3.76	3.76
Molecular lattice												
a (Å)	10.20		10.10		10.10		9.85		9.95		9.90	
b (Å)	9.09		8.84		8.84		8.79		8.73		8.91	
Superlattice reflections	Obs. (Å)	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.
01	10.10	10.20	9.98	10.10	9.96	10.10	9.73	9.85	9.82	9.95	9.82	9.90
11, $1\bar{1}$	6.80	6.78	6.75	6.65	6.74	6.65	6.69	6.56	6.69	6.56	6.64	6.62

chains present in the $L_{\beta'}$ phase is retained in the $L_{c'}$ phase, resulting in the formation of a molecular superlattice. On the other hand, in DPPG, the chain lattice is destroyed at the $L_{\beta'} \rightarrow L_{c'}$ phase transition. Thus we may conclude that there are two types of in-plane ordering possible in the $L_{c'}$ phase of hydrated lipids. This difference might be attributed to the differences in the interactions between the headgroups.

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