Structure of the $L_{c'}$ Phase in a Hydrated Lipid Multilamellar System

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In this Letter we establish the structure of the $L_{c'}$ phase exhibited by the lipid dipalmitoylphosphatidylcholine (DPPC) under hydration. On the basis of x-ray diffraction data on oriented multibilayers, we show that the structure of this phase is characterized by two lattices: a molecular superlattice and a hydrocarbon chain sublattice. Diffraction data available in the literature suggest the presence of similar molecular superlattices in the $L_{c'}$ phase of various other lipid-water systems.

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Lipids are of fundamental importance as they are the main constituents of biological membranes. Being amphiphilic in nature, they self-assemble into a variety of phases when hydrated [1]. The vast majority of lipid molecules consist of two hydrophobic hydrocarbon chains and a hydrophilic headgroup. Dipalmitoylphosphatidylcholine (DPPC), one of the most extensively investigated lipids, belongs to this class. The lamellar phases of lipid-water systems are of current interest due to their two-dimensional (2D) nature and other novel features [2–5]. Many fully hydrated saturated phosphatidylcholines, such as DPPC, exist in four distinct lamellar phases, and on decreasing the temperature they occur in the following sequence: L_α, $P_{\beta'}$, $L_{\beta'}$, and $L_{c'}$. The structures of the L_α, $P_{\beta'}$, and $L_{\beta'}$ phases are at present well understood [1–3]. The L_α phase has no long-range order within the layers, and the hydrocarbon chains are in a "melted" state. On the other hand, both the periodically modulated lamellar $P_{\beta'}$ and the gel $L_{\beta'}$ phases are characterized by a 2D ordering of their hydrocarbon chains in the bilayers with no interbilayer correlations. Furthermore, it has been shown that the $L_{\beta'}$ phase in dimyristoylphosphatidylcholine (DMPC), in fact, consists of three phases with differing directions of the molecular tilt with respect to the 2D bond direction [3]. The in-plane arrangement of the chains is nearly hexagonal in the $L_{\beta'}$ phase, and, as each lipid molecule under consideration consists of a headgroup and two hydrocarbon chains, such a packing does not imply a regular packing of the lipid molecules themselves. Indeed, analysis of the diffuse x-ray scattering from this phase indicates a large degree of positional disorder of the headgroups [6].

The $L_{c'}$ phase was first observed by Chen *et al.* [7] in fully hydrated DPPC bilayers after a few days of incubation at $\approx 0\,^\circ$C. Since then, the $L_{c'}$ phase has been observed in other lipid-water systems [8–10]. However, only the structure of this phase in hydrated dipalmitoylphosphatidylglycerol (DPPG) has been established so far [10]. In this system, the hydrocarbon chain lattice present in the $L_{\beta'}$ phase is found to be replaced by a molecular lattice in the $L_{c'}$ phase. The lattices in the two monolayers making up each bilayer are correlated, though there are no interbilayer correlations. While the structure of the $L_{c'}$ phase of DPPC is in some respects similar to that of DPPG, we find that there is an important difference between these two systems, namely, the presence of a molecular superlattice in the DPPC. Thus at the $L_{\beta'} \rightarrow L_{c'}$ phase transition, in the DPPC-water system, the molecular lattice is formed without destroying the hydrocarbon chain lattice. The simultaneous existence of the two lattices requires the two to be commensurate, and we find the unit cell of the molecular lattice to have twice the area of that of the chain lattice. The $L_{\beta'} \rightarrow L_{c'}$ phase transition, therefore, involves the reorientation of the lipid molecules within the bilayers and can be expected to be a very slow process, consistent with the experimentally observed slow kinetics of this transition in DPPC [11–13]. X-ray diffraction data on various other lipid-water systems also indicate the presence of such molecular superlattices in the $L_{c'}$ phase [14].

Most of the detailed x-ray diffraction studies on the $L_{c'}$ phase of DPPC have been done using powder samples [9,11,15,16]. These studies show that the formation of this phase, from the $L_{\beta'}$ phase, is heralded by the appearance of a few relatively weak reflections. As is well known, the powder patterns are difficult to interpret due to the existence of a molecular tilt in DPPC multibilayers [1]. They are further complicated by the presence of "satellite" reflections arising from the secondary maxima of the form factor of the hydrocarbon chains [3]. In order to overcome these limitations, we have carried out studies on oriented DPPC multibilayers under hydration. The oriented sample was prepared on a curved glass substrate using a concentrated solution of DPPC and methanol. After evaporation of the methanol and subsequent hydration of the bilayers, the sample was kept at $4\,^\circ$C for about 5 d in order to form the $L_{c'}$ phase. Further experimental details will be published elsewhere [17]. A diffraction pattern obtained in the $L_{c'}$ phase at $7\,^\circ$C and 60% relative humidity is shown in Fig. 1. The set of reflections along c^* are due to the lamellar structure and correspond to a periodicity

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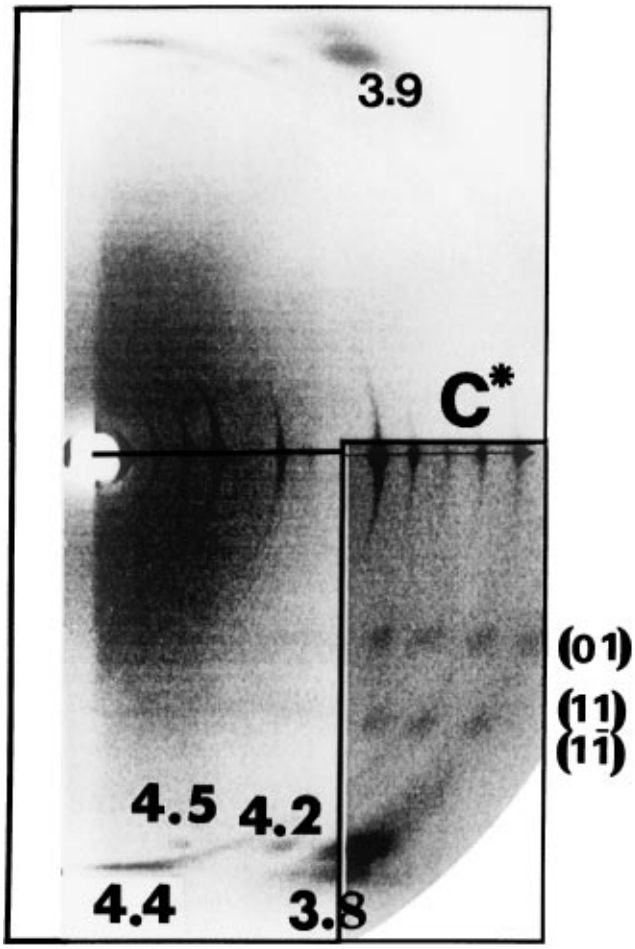

FIG. 1. The diffraction pattern obtained from oriented DPPC bilayers in the $L_{c'}$ phase at (7 ± 1) °C and at a relative humidity of (60 ± 5)%. The reflections along c^* correspond to the lamellar periodicity, while the other reflections are due to in-plane structure. The numbers against the hydrocarbon chain reflections correspond to repeat spacings and are given in angströms. The weak 1/10 (01) and 1/6.8 Å^{-1} [(11), (1$\bar{1}$)] reflections, due to the molecular superlattice, are in the form of long bars parallel to c^*. The contrast has been enhanced in a portion of the pattern in order to clearly show the modulation in their intensities.

of 56.4 Å, which is slightly smaller than the values reported from fully hydrated powder samples [9,11,15–17]. In addition, five reflections arising from the 2D packing of the hydrocarbon chains can be seen in each quarter of the photographic plate. These reflections can be unambiguously assigned to the chain lattice as they are not significantly altered across the $L_{\beta'} \rightarrow L_{c'}$ phase transition. The three main reflections from the chain lattice are distinct due to the obliquity of the unit cell. These correspond to repeat spacings of 4.4, 3.9, and 3.8 Å. The reflections as 1/4.5 and 1/4.2 Å^{-1} are collinear with the 1/3.9 Å^{-1} reflection and are due to the secondary maxima in the form factor of the hydrocarbon chains, as was shown by Smith *et al.* [3] in connection with the structure of the $L_{\beta'}$ phase. The use of an oriented sample in the present study makes it possible to unambiguously understand the origin of these "satellite" reflections. The parameters of the hydrocarbon chain lattice calculated from the diffraction data are $a = 5.3$ Å, $b = 8.8$ Å, and $\gamma = 94°$.

Of all the reflections reported in the $L_{c'}$ phase of DPPC, there are only four that cannot be attributed to the hydrocarbon chain lattice. These weak reflections correspond approximately to repeat spacings (d) of 10.0, 9.3, 6.8, and 4.9 Å in the powder patterns [9,11,15,16]. The stronger of these, namely, the reflections at 1/10 (01) and 1/6.8 Å^{-1} [(11), (1$\bar{1}$)] are visible in the diffraction pattern of the oriented sample shown in Fig. 1. They appear in the form of long bars parallel to the c^* axis and whose projections on the orthogonal a^* axis correspond to lattice spacings of 10.0 and 6.8 Å. The intensity along these reflections is modulated with a periodicity of about 40 Å. It is possible that the other two weak reflections are not seen in the diffraction pattern as they are masked by the rather strong background due to the substrate used for making the oriented sample. On the other hand, all of the four reflections were observed when we used a powder sample [17].

It is well established that only the hydrocarbon chains of the lipid molecules are ordered in the $L_{\beta'}$ phase, and all the observed nonlamellar reflections can be accounted for in terms of the chain lattice [3,6]. The chains in the two monolayers in each bilayer are in registry, and hence the width of the chain reflections in a direction parallel to c^* corresponds to twice the length of an individual chain. Evidence for the lack of molecular ordering in this phase also comes from the analysis of the diffuse scattering, which indicates a high degree of positional disorder of the headgroups [6]. The fact that the chain reflections are not significantly altered across the $L_{\beta'} \rightarrow L_{c'}$ phase transition indicates the continued existence of the 2D chain lattice in the latter phase. If we assume that in addition to the chains the entire lipid molecules are ordered within each bilayer, then the rest of each molecule, in particular the headgroup, would also scatter coherently, giving rise to additional reflections. The simultaneous existence of the molecular and chain lattices requires the two to be commensurate. Therefore, if this conjecture is correct, it should be possible to index all the additional reflections observed in the $L_{c'}$ phase on a molecular lattice that is based on the chain lattice. As we show below, this is indeed the case.

Since each DPPC molecule consists of two hydrocarbon chains linked by a glycerol backbone carrying the headgroup, it is possible to construct many molecular superlattices from a given chain sublattice. Each of these leads to a distinct set of reflections. Interestingly, there is a unique superlattice, shown in Fig. 2, that is consistent with the observed reflections. The parameters of this superlattice can be expressed in terms of those of the chain

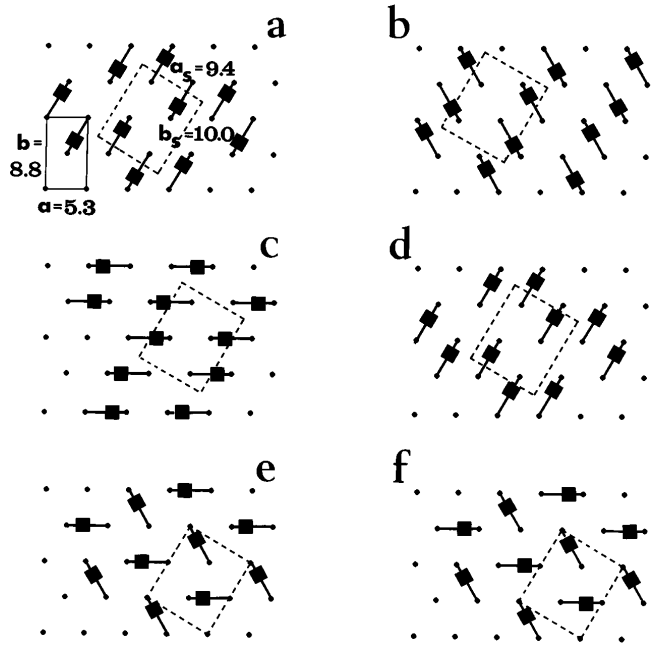

FIG. 2. The possible in-plane molecular arrangements in the $L_{c'}$ phase of hydrated DPPC. The chains are denoted by (●)'s and the headgroups by (■)'s. By connecting two nearest neighbor hydrocarbon chains with one headgroup, we obtain one DPPC molecule. The molecular superlattice is shown in dashed lines and the hydrocarbon chain sublattice in solid lines. Molecular arrangements (a)–(c) belong to the plane group $p2$, while the rest belong to $p1$. Unit cell parameters are given in Å.

lattice by the following relations:

$$a_S^2 = \frac{1}{4}(9a^2 + b^2) - \frac{3}{2}ab\cos\gamma\,,$$

$$b_S^2 = a^2 + b^2 + 2ab\cos\gamma\,,$$

and

$$\cos\gamma_S = \frac{3a^2 - b^2 + 2ab\cos\gamma}{2a_S b_S}$$

Using the values of a, b, and γ determined from the chain reflections, the superlattice unit cell parameters are found to be $a_S = 9.4$ Å, $b_s = 10.0$ Å, and $\gamma_S = 90°$. The unit cell of this lattice has twice the area of that of the chain sublattice and contains two DPPC molecules. Table I lists lattice spacings from all the observed nonlamellar reflections that are not due to the chain sublattice, as well as those calculated from this superlattice. The agreement between the two sets is excellent, confirming the existence of the molecular superlattice.

The fact that the reflections at 1/10 and 1/6.8 Å^{-1} appear as long bars parallel to the c^* axis is consistent with their origin in the ordering of relatively short entities, like the headgroups, in the plane of the bilayer. The period of modulation of the intensity along these bars is approximately 40 Å and corresponds to the distance between the headgroups across the thickness of the bilayer [18]. This modulation can therefore be understood as arising from

TABLE I. The observed and calculated repeat spacings (d) from the molecular superlattice shown in Fig. 2.

(hk)	d calc (Å)	d obs (Å) Present study	d obs (Å) Ref. 15
01	10.0	10.0 ± 0.1	10.0
10	9.4	9.4 ± 0.1[a]	9.30
11	6.8	6.8 ± 0.1	6.81
$1\bar{1}$	6.8	6.8 ± 0.1	6.81
02	5.0	5.0 ± 0.1[a]	4.9

[a]Repeat spacings obtained from powder data.

the positional correlation between the molecular lattices in the two monolayers constituting each bilayer, as was also observed in the $L_{c'}$ phase of DPPG [10].

Headgroup lattices have been previously observed in lamellar lipid systems, but only at extremely low hydrations [1]. In these systems, the headgroups of neighboring bilayers interact and give rise to interdigitated structures [19]. However, the possibility of headgroup ordering in hydrated bilayers has not been suspected until now. 1D electron density profiles show that DPPC bilayers, under excess water conditions, in the $L_{c'}$ phase are separated by a layer of water that is about 8 Å thick [20] and whose thickness can be altered under various hydration regimes [18]. This is consistent with the absence of interbilayer correlations of the in-plane structure. Moreover, the spacings of the additional nonlamellar reflections seen in this phase are found not to change as the thickness of the water layer is reduced to about 3 Å [20]. This observation confirms the fact that these reflections are due to in-plane ordering of the DPPC molecules.

In general, the determination of the lattice parameters does not by itself lead to an understanding of the arrangement of the molecules in the unit cell. However, in the present case, the existence of two commensurate lattices restricts the number of possible molecular arrangements in the bilayer to six. These are shown in Fig. 2. Packings in Figs. 2(a)–2(c) belong to the plane group $p2$, while the rest belong to $p1$. While there are no systematic absences arising from these plane group symmetries, one of these, namely the molecular arrangement in Fig. 2(d), causes extinctions due to the specific relationship between the two lipid molecules within the unit cell [21]. In Fig. 2(d) the molecules are arranged along rows parallel to the b axis of the molecular superlattice with the molecules in adjacent rows being shifted by $a/2$ along the a axis and by $b/4$ along the b axis. This leads to the equivalent positions (x, y) and $(a/2 + x, b/4 + y)$ and results in the extinction condition $h + k/2 = 2n + 1$, where n is an integer. Since the (10) reflection has been observed in many studies, it follows that the molecular arrangement in the $L_{c'}$ phase of DPPC is given by one of the other five shown in Fig. 2.

In conclusion, we have established the existence of a molecular superlattice in the $L_{c'}$ phase of DPPC on the

basis of x-ray diffraction studies using oriented multibilayers as well as other diffraction data reported in the literature. The presence of the two commensurate lattices makes it possible to list the possible molecular arrangements in the plane of the bilayer. X-ray diffraction data on this phase of some other lipids indicate the formation of similar superlattices in these systems as well.

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