Molecular Chirality and the “Ripple” Phase of Phosphatidylcholine Multibilayers

J. Katsaras[1,2] and V. A. Raghunathan[1]
[1]Centre de Recherche Paul Pascal-CNRS, Avenue A. Schweitzer, F-33600 Pessac, France
[2]Atomic Energy of Canada Limited Research, Chalk River Laboratories, Chalk River, Ontario, Canada K0J 1P0

(Received 13 July 1994)

We present small- and wide-angle x-ray diffraction data of chiral and racemic dimyristoylphosphatidylcholine bilayers in the “ripple” $P_{\beta'}$ phase. In both cases, this phase is found to be characterized by asymmetric ripples. This result differs from that of Lubensky and MacKintosh [Phys. Rev. Lett. **71**, 1565 (1993)], whose model predicts symmetric ripples in achiral systems.

PACS numbers: 61.30.Eb, 64.70.–p, 87.15.By, 87.15.He

Lipid molecules are of great scientific interest for various and often very different reasons. They are the major component of biological cell membranes and, since the proposal of the “fluid mosaic model” of the latter [1], much effort has been expended in understanding their structure and function. Moreover, lipids are also lyotropic liquid crystals which exhibit a variety of interesting structures (e.g., lamellar, hexagonal, cubic, etc.) and serve as prototype models for certain 1D and 2D phase transitions [2–4].

In a landmark study, Tardieu, Luzzati, and Reman [4] described a variety of novel phases formed by lipid-water systems. One of these phases which has recently attracted much attention is the periodically modulated lamellar $P_{\beta'}$ phase [4–7] observed in many synthetic lecithin-water systems between the $L_{\beta'}$ and L_α phases and associated by Janiak, Small, and Shipley with a structural transformation from a 1D lamellar to a 2D monoclinic lattice [7]. The high temperature L_α phase has the same symmetry as a liquid crystalline smectic-*A* phase while the low temperature $L_{\beta'}$ phase is characterized by bond orientational order [8].

In a recent Letter, Lubensky and MacKintosh [5] describe many symmetric and asymmetric $P_{\beta'}$ phases using a continuum Landau theory. They show that three distinct ripple phases may be found in achiral systems, all of which are symmetric. On the other hand, in the case of chiral bilayers some of these ripple phases become asymmetric. Since ripple asymmetry is associated with unit cell obliquity [9], it can be detected directly by x-ray diffraction on oriented bilayers. The phenomenological model of Lubensky and MacKintosh [5] is in agreement with some x-ray data in the literature, which show a chiral dimyristoylphosphatidylcholine (DMPC) system having an oblique unit cell with $\gamma = 97.5°$ [2] and a racemic [10] dipalmitoylphosphatidylcholine (DPPC) system whose ripple structure forms an orthorhombic unit cell ($\gamma = 90° \pm 2°$) [6]. However, freeze-fracture images show asymmetric ripples in the $P_{\beta'}$ phase of both chiral and racemic DPPC [11], in disagreement with the above model.

Although many x-ray diffraction data concerning the $P_{\beta'}$ phase have been reported, it is not always clear as to whether or not a chiral system was used (e.g., [4,12,13]). In light of this situation, we have studied the influence of molecular chirality on the ripple symmetry by using x-ray diffraction on oriented bilayers of chiral and racemic DMPC-water systems, under identical conditions. We find that both these systems are characterized by oblique unit cells ($\gamma = 99° \pm 2°$) indicative of asymmetric ripples. In addition, both samples experience a significant reduction in the hydrocarbon chain tilt angle with respect to the layer normal, during the $L_{\beta'} \rightarrow P_{\beta'}$ phase transition.

The chiral enantiomer, *l*-DMPC, and the racemic mixture, *dl*-DMPC (purity >98%), were obtained from Fluka, and their optical activity was checked using a Perkin-Elmer 241 polarimeter. The lipids were oriented on a curved glass surface, 150 μm thick, using a concentrated solution of DMPC in methanol which was pipetted onto the glass surface. After evaporation of the methanol a clear film of the lipid was left adhering to the glass plate. The remainder of the methanol was evaporated by placing the samples under a vacuum for 24 h, after which time they were hydrated in a 100% relative humidity (RH) environment for a few days. This procedure produces a stack of about 2000 highly oriented (<5° mosaic spread) bilayers and allows the *simultaneous observation of both the in-plane and lamellar structures*, with an incident x-ray beam tangential to the curved substrate. It should be noted here that in the $P_{\beta'}$ and $L_{\beta'}$ phases, which are characterized by the ordering of the hydrocarbon chains in the bilayer, the diffracting sample is well aligned only in the direction normal to the layers. Within each layer it consists of domains of differing orientations. Consequently, the reciprocal lattice structure has cylindrical symmetry about the direction of the layer normal. As we are using a two-dimensional detector, this makes it possible to get the whole diffraction pattern with just a single setting of the sample and the detector.

The experiments were carried out with an 18 kW Rigaku Rotaflex RU300 rotating anode generator and a 2D Marresearch imaging plate detector having a plate diameter of 180 mm and pixel size of 150 μm × 150 μm. Monochromation of the Cu radiation was achieved using a flat graphite crystal having a mosaic spread of $0.4° \pm 0.1°$ $\mathrm{FWHM}_{(002)}$ and the sample-to-film

0031-9007/95/74(11)/2022(4)$06.00

distance was 186 ± 1 mm. The spot size, as defined by three sets of vertical and horizontal slits, was approximately 0.5 mm × 0.5 mm. The sample holder (volume ≈ 300 cm^3) was designed to monitor and control both the RH and the temperature.

Figure 1 shows schematics of the rippled bilayers in the $P_{\beta'}$ phase and of the corresponding diffraction pattern. As mentioned earlier, the diffracting sample consists of many domains in the plane of the bilayer. The reciprocal space structure is, therefore, cylindrically symmetric and is obtained by rotating Fig. 1(b) about the q_z axis. In Fig. 2 we present 2D diffraction patterns of *l*-DMPC [Figs. 2(a) and 2(b)] and *dl*-DMPC [Figs. 2(c) and 2(d)] multilayers at 25 °C and 98% RH. Figures 2(b) and 2(d) are identical, except for different contrast, to Figs. 2(a) and 2(c), respectively. From these patterns (Fig. 2) we find the lamellar periodicity d to be 56.5 Å for *l*-DMPC and 57.8 Å for *dl*-DMPC. Both these samples have a ripple wavelength λ of about 145 Å. Interestingly, these diffraction patterns of the two DMPC systems under identical experimental conditions are practically indistinguishable. In particular, both these systems are characterized by asymmetric ripples, indicating that molecular chirality is not essential to produce ripple asymmetry. The observation of asymmetric ripples in the $P_{\beta'}$ phase of DMPC is consistent with earlier x-ray diffraction [2,4,9], freeze-fracture [11], and scanning tunneling microscopy (STM) [14] data.

The occurrence of asymmetric ripples in *dl*-DMPC can be accounted for by the Lubensky-MacKintosh model if the racemic mixture were to phase separate into domains of d and l enantiomers within each bilayer. Though such spontaneous chiral resolution has been observed in a monolayer made up of rigid amphiphilic molecules [15], it is by no means common. As described below, we find the repeat spacing and in-plane structure of $L_{\beta'}$ phase *l*-DMPC multibilayers to be slightly different from those of *dl*-DMPC. The structures of phospholipid monolayers are also known to be influenced by the chirality of the system [16]. In addition, differential scanning calorimetry and freeze-fracture electron microscopy using a variety of DPPC enantiomer mixtures, including a racemate,

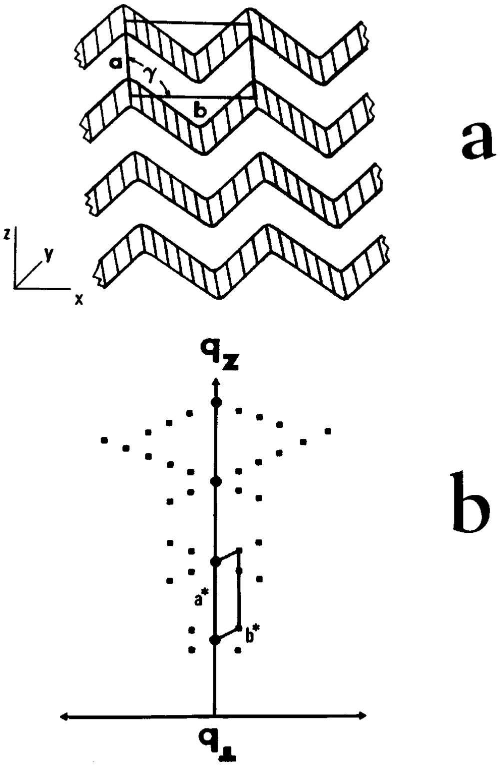

FIG. 1. Schematics of the (a) rippled bilayers in the $P_{\beta'}$ phase and (b) corresponding small-angle diffraction pattern. The main Bragg reflections are denoted by ● while the satellites due to the ripple are given by ■. The characteristic X pattern arises from the combination of the obliquity of the unit cell and the cylindrical symmetry of the diffracting sample about the z axis.

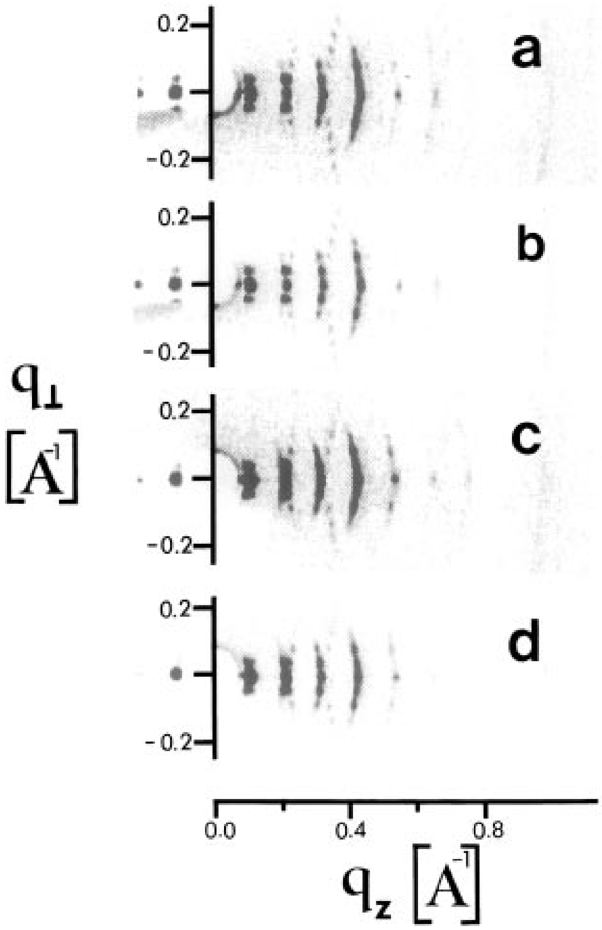

FIG. 2. (a) 2D diffraction pattern of *l*-DMPC bilayers in the $P_{\beta'}$ phase at 25 °C and 98% RH. (b) The same pattern at a lower contrast to resolve the intense reflections. $d = 56.5 \pm 0.3$ Å, $\lambda = 144 \pm 5$ Å, and $\gamma = 99° \pm 2°$. (c) Diffraction pattern of *dl*-DMPC bilayers in the $P_{\beta'}$ phase under identical conditions. (d) The same pattern at a lower contrast. $d = 57.8 \pm 0.3$ Å, $\lambda = 147 \pm 5$ Å, and $\gamma = 99° \pm 2°$.

demonstrated ideal solid and liquid mixing of the *d* and *l* enantiomers [11]. These observations indicate the stability of racemic mixtures of phospholipids against spontaneous phase separation. Hence we may conclude that the formation of asymmetric ripples in the $P_{\beta'}$ phase cannot be attributed to the chirality of the bilayers.

It should be pointed out that, although chirality seems to have little effect on the wavelength and the symmetry of the ripples, it is well known that as a function of increasing water content both λ and the angle γ of the oblique unit cell decrease in DMPC bilayers [4,12]. As the temperature was decreased across the $P_{\beta'}$ phase, we observed an approximately 15 Å increase in λ and practically no change in the obliquity of the unit cell. As such, it seems that other factors play a greater role in determining the characteristics of the ripple rather than the chirality of the system.

Figure 3 shows wide-angle x-ray diffraction patterns of *l*-DMPC bilayers in the L_{β_I} phase [Fig. 3(a)] and *dl*-DMPC multilayers in the L_{β_L} [Fig. 3(b)] and $P_{\beta'}$ phases [Fig. 3(d)] [17]. A coexistence of the $P_{\beta'}$-$L_{\beta'}$ phases in *dl*-DMPC bilayers is presented in Fig. 3(c). These reflections (Fig. 3) arise from the ordering of the hydrocarbon chains of the lipid molecules within the bilayers. Although both these systems form similar $P_{\beta'}$ phases, it seems that their in-plane structures in the $L_{\beta'}$ phase are different. Lowering the temperature results in a $P_{\beta'} \rightarrow L_{\beta_I}$ transition in *l*-DMPC bilayers, while the ripple-to-gel transition in *dl*-DMPC bilayers results in the L_{β_L} phase. Although much work needs to be done concerning this point, it may be mentioned here that chirality has been shown to influence the structure of phospholipid monolayers on water [16]. The hydrocarbon chain tilt angle θ decrease from about 30° in the $L_{\beta'}$ phase to about 12° in the $P_{\beta'}$ phase [Fig. 3(d)] for both samples. In the $P_{\beta'}$ phase we could not clearly determine the tilt direction with respect to the lattice [Fig. 3(d)], unlike the case when the bilayers were in one of the $L_{\beta'}$ phases [Figs. 3(a) and 3(b)]. It is interesting to note that the wide-angle reflection of the $P_{\beta'}$ phase [Fig. 3(d)] is in the form of a diffuse arc which is probably indicative of slightly more disordered hydrocarbon chains compared to the $L_{\beta'}$ phase. This would be in agreement with Raman spectroscopic data which indicate that the $L_{\beta'} \rightarrow P_{\beta'}$ transition in DPPC multilayers is accompanied by an increase in the *gauche* rotamer population of the hydrocarbon chains [18].

A recent x-ray diffraction study [6] reported that the $L_{\beta'} \rightarrow P_{\beta'}$ phase transition in oriented DPPC multibilayers is characterized by a change in hydrocarbon chain tilt direction from nearest neighbor to next-nearest neighbor. Although we observe a difference of about 18° in the tilt angle between the $L_{\beta'}$ and $P_{\beta'}$ phases, Hentschel and Rustichelli [6] observe a difference of only 2° between the two phases. One possible explanation for this discrepancy is that their $P_{\beta'}$ phase, as pointed out by the authors themselves, is not pure but coexists with the $L_{\beta'}$ phase. As such the wide-angle reflections are a combination of those from the two phases. Figure 3(c) is a wide-angle diffraction pattern of *dl*-DMPC bilayers in the $P_{\beta'}$-$L_{\beta'}$ coexistence region, obtained from the present study. This diffraction pattern seems to be very similar to that shown in Ref. [6].

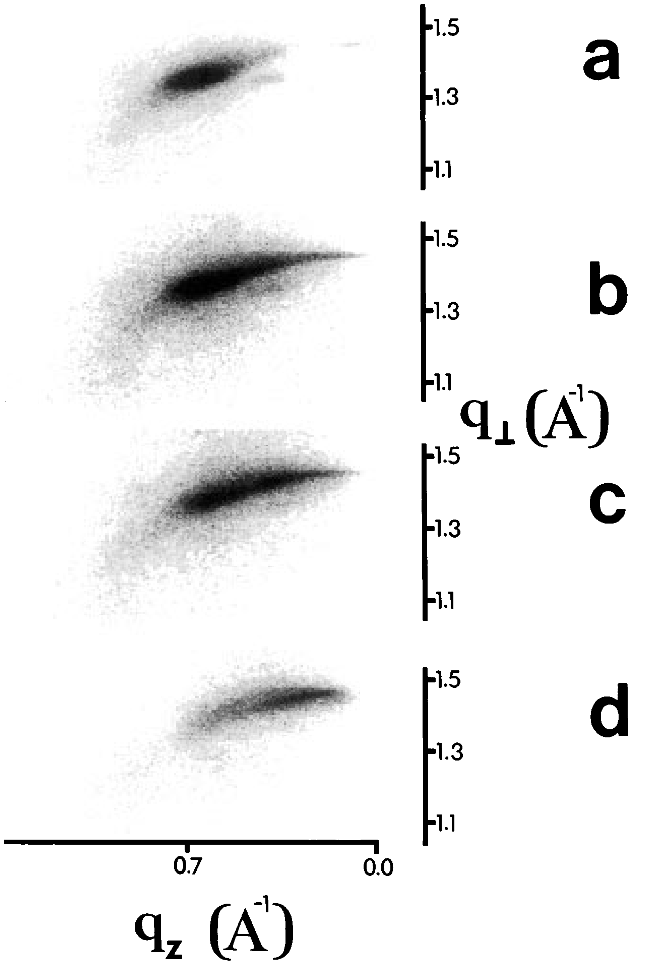

FIG. 3. Wide-angle x-ray diffraction patterns of (a) *l*-DMPC bilayers in the L_{β_I} phase having a θ of 30° ± 1°, (b) *dl*-DMPC bilayers in the L_{β_L} phase having a θ of 27° ± 2°, (c) *dl*-DMPC bilayers in the $P_{\beta'}$-$L_{\beta'}$ two-phase region, and (d) *dl*-DMPC multilayers in the $P_{\beta'}$ phase having a θ of 12° ± 3°. It should be noted that equatorial reflections (i.e., those at $q_z = 0$) are attenuated due to the diffraction geometry. The wide-angle pattern of *l*-DMPC bilayers in the $P_{\beta'}$ phase (not shown) is similar to that of *dl*-DMPC bilayers.

In conclusion, we have presented high-resolution x-ray diffraction data from oriented multibilayers of chiral and racemic DMPC-water systems under identical experimental conditions. The data clearly show the occurrence of asymmetric ripples in the $P_{\beta'}$ phase of a racemic system and is thus in disagreement with the recent model of Lubensky and MacKintosh [5]. Further, the hydrocarbon chain tilt angle is found to be drastically reduced at the $L_{\beta'} \rightarrow P_{\beta'}$ transition.

J. K. is grateful to the Centre National de la Recherche Scientifique and the National Research Council of Canada for financial support. V. A. R. acknowledges support from the Indo-French Center for the promotion of advanced research.

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