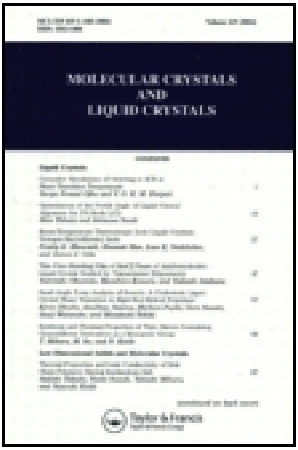

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Defects in the Ripple Structure of Phospholipids

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Defects in the Ripple Structure of Phospholipids†

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The authors propose a model for the arrangement of the phospholipid molecules in a lipid bilayer membrane, based upon both the structure of the fatty acyl chains packed in highly ordered hexagonal arrays below the main transition temperature and the ripple structures in which the ripple lines have a hexagonal symmetry. This assumption results in three kinds of the configurations, whose boundaries make the angle of 120° among them. According to this domain structure, the texture appearing on the freeze-fractured surface in the ripple phase can be explained consistently, and furthermore, the kinetics of the domain boundary motion can be understood satisfactorily.

Keywords: phospholipid, ripple structure, phase transition, freeze-fracture electronmicrograph, transient state, domain

I. INTRODUCTION

The thermotropic behavior of phospholipid membranes is characterized by several phase transitions, i.e., dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and distearoyl-

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phosphatidylcholine (DSPC) undergo a subtransition, pretransition and main transition, separating the phases L_c, $L_{\beta'}$, P_β (or $P_{\beta'}$) and L_α, respectively. Each phospholipid consists of a polar head group (phosphorylcholine) and two long flexible chains of fatty acyl groups. Both of the polar head group and the two fatty acyl chains are connected to a glycerol backbone. In the case of the phospholipids, DMPC, DPPC, DSPC, etc., they have a modulated phase with ripple structure. Then, many researchers have assumed that the interaction of the polar head groups is responsible for this modulated or so-called rippple phase. This phase takes place at temperatures just below the main transition, i.e., in the P_β (or $P_{\beta'}$) phase. The detailed structure of the ripple phase is not yet agreed. There are two proposed cases: one indicates that the average tilt angle of the fatty acyl chains is non-zero, i.e., it is $P_{\beta'}$ phase, whereas the other indicates that the tilt angle is zero, i.e., it is P_β phase.

Below the main transition temperature the hydrocarbon chains are packed in highly ordered hexagonal arrays. It should be paid attention to the fact that the two acyl chains of each phospholipid molecule make a pairing in the hexagonal arrays, and furthermore, all the hexagonal lattice points in the plane of lipid bilayer membrane are regularly filled up exhaustively by the bonds by which the neighboring hexagonal lattice points are connected. Then, the latter fact is caused from the interaction of the polar head groups. There has however been done little consideration on the arrangement of the polar head groups.

On the other hand, the ripple structure has been studied using freeze-fracture electronmicroscopy.[1,2] The ripple repeat distance is ranged between 10 and 20 nm. The pattern observed on the freeze-fractured surface in part exhibits regular parallel lines with the spacing of the ripple repeat distance. The entire region is separated by a lot of parts with the regular parallel lines. All of these lines as a result look like having a hexagonal symmetry. In the present study, this will be confirmed by the optical Fourier transform of the electronmicrograph.

It is worthwhile pointing out that the ripple structure is strongly related to the configuration of the polar head groups. From this viewpoint we shall propose a possible configuration of the polar head groups and, in accordance with this configuration, explain the texture of the ripple lines having the hexagonally symmetric structure. Furthermore, the proposed configuration will describe the kinetics of the boundary among domains composed of the parts of the regular parallel ripple lines.

II. ANALYSIS OF RIPPLE STRUCTURE

The texture of regular undulation is usually observed on the freeze-fractured surface of lipid bilayer membranes quenched from a temperature in the ripple phase. The texture associated with the ripple structure is also observed in the transient states in the formation or disappearance of the ripple structure. This texture depends on time for which phospholipid membrane is kept at a temperature of the ripple phase after heating up from the $L_{\beta'}$ phase and also at a temperature of the $L_{\beta'}$ phase after cooling down from the ripple phase. This fact is due to a slow relaxation process at the $L_{\beta'}$-to-ripple phase transition.[3,4] We carried out a preliminary observation of the texture appearing in the transient states in DPPC liposomes.

When the temperature of the liposomes is jumped from the $L_{\beta'}$ phase to the ripple phase, in the freeze-fracture electronmicrographs the texture associated with the ripple structure grows up as a function of the holding time in the ripple phase. The texture observed in this transient state exhibits a structure having the hexagonally symmetric arrangement of the ripple lines. In the final state after a sufficiently long holding time the ripple lines running one direction become dominant.

Figure 1 (a) shows a freeze-fracture electronmicrograph taken under the condition that in DPPC liposomes the starting temperature is 25°C in the $L_{\beta'}$ phase; the temperature is jumped up to 35°C in the ripple phase; at this temperature the liposomes are held for 20 min; then they are quenched. In this electronmicrograph, the structure with the ripple repeat distance of about 13 nm appears over the whole region. We could not find however the structure with a long repeat distance in our electronmicrographs as in Ref. 4. This might be due to the fact that in our experiments we took special care to make the suspension so as not to include calcium ions. The long repeat distance is observed in the presence of calcium ions and the distance returns to normal upon the removal of Ca^{2+} by EGTA.[5]

To analyze this observed texture, we made the optical Fourier transform of Figure 1 (a) as shown in Figure 1 (b). There take place six spots reflecting the hexagonal symmetry. In a rather wide region this sort of the image was observed. This fact strongly suggests that the fatty acyl chains, which construct the base structure of the texture, are packed in the ordered hexagonal arrays. It should however be pointed out that the image is not focused. This is partly due to the incomplete linearity of the regular parallel ripple lines. In fact, we can find the slight bent of them in Figure 1 (a). Ruocco and Shipley[6]

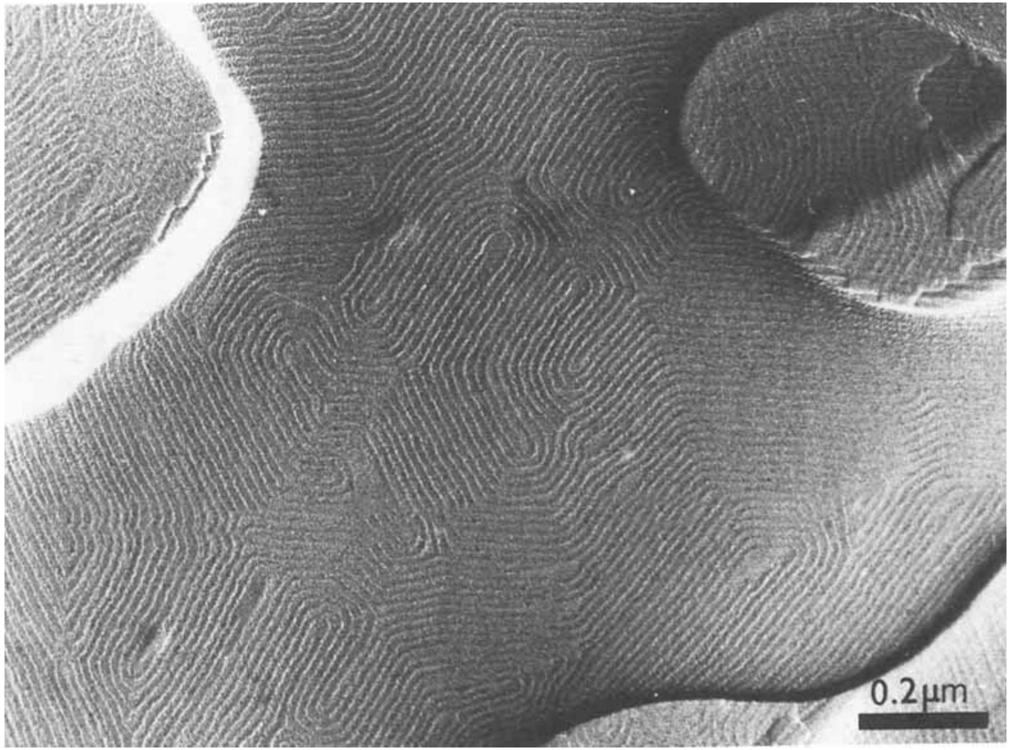

FIGURE 1 (a) A freeze-fracture electronmicrograph taken in the transient state from the L_{β}' phase to the ripple phase. The starting temperature is 25°C in the L_{β}' phase. The final temperature is 35°C in the ripple phase. The holding time at the final temperature is 20 min. (b) Image of the optical Fourier transform of (a). Six spots reflecting a hexagonal symmetry and their higher order spots can be seen.

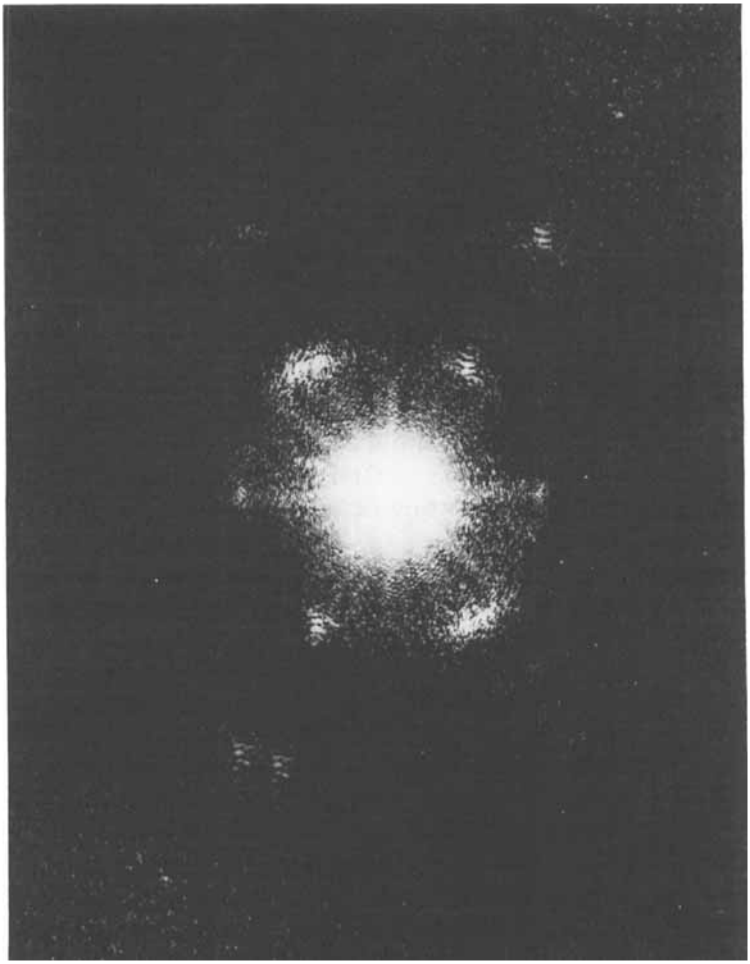

FIGURE 1(b) (*continued*)

have estimated the two-dimensional hydrocarbon chain packing density from the result of their X-ray diffraction. The corresponding area per a hydrocarbon chain is reduced with decreasing the temperature.[6] In the ripple phase it has been pointed out that the fatty acyl chains are mainly in the all-trans conformation, although some gauche conformers may be present, and packed in a regular hexagonal lattice. It is inferred however that the existence of the gauche conformers causes internal strain and then, the hexagonal lattice is distorted. As a result of it, the regular parallel ripple lines might bend slightly.

Lastly, it has been found from the observation of the freeze-fractured surfaces that the uniform region with the regular ripple lines grows, as the holding time in the ripple phase becomes long.

III. A POSSIBLE RIPPLE STRUCTURE WITH HEXAGONAL SYMMETRY

In Figure 1 (a) we can see the texture that three kinds of the domains, each of which is composed of the regular parallel ripple lines, hold with the angle of 120° among them; the chevronel structure with the ripple repeat distance takes place; their threefold structure makes trident. As a result of the structure described above, there appears the structure that elongated hexagons, composed of the tridents, fill up the surface exhaustively, and each elongated hexagon includes successive homologous smaller hexagons separated from each other by the ripple repeat distance.[4]

Attention should be paid to the fact that the pattern of the regular parallel ripple lines reflects the arrangement of the polar head groups of the phospholipid molecules. It is quite natural to consider that the regular parallel ripple pattern is brought on by the neat arrangement of the polar head groups. So far little information has been given however for this arrangement by experiments. On the other hand, a few considerations have been performed from the theoretical viewpoint. Lee[7] has pointed out from the knowledge obtained until that time that the polar head groups are folded down successively below the main transition temperature and have a similar arrangement even above that. This fact indicates that the polar head groups cooperate to make similar configuration against its anisotropic axis. Nagle[8] has discussed a phase transition in terms of an order-disorder model involving the polar head groups. In this case, a definite order of the polar head groups appears at the low temperatures. Frischleder and Peinel[9] have proposed a double cylinder model for their molecular dynamic method, in which a phospholipid molecule with the two fatty acyl chains is approximated by connected double cylinders with a certain distance. Therefore, the projection of a phospholipid molecule on the surface perpendicular to the long direction of the cylinder exhibits a dumb-bell shape. In their consideration, the bars of the dumb-bells projected on the two-dimensional map are arranged in herring-bone configuration as an energetically stable state.[9] When we take further account of the interaction energy between the polar head groups, the darning configuration of the bars might be much stable as discussed briefly by Lee.[7] In this configuration, the bars having a directional nature lie parallel to each other and line up neatly.

We propose the darning configuration of the bars of the dumb-bell as a plausible configuration. Furthermore, it is assumed that not only the uniform darning configuration but also the three kinds of domains, in which the directions of the bars lie in hexagonal symmetry as a whole, reflects the stereochemical interaction among the polar head groups. A typical configuration of the bars is shown in Figure 2. Under the above conditions, three kinds of the domains are realized, whose boundary is denoted by dotted lines, and over all the surface there is no point defect, i.e., the dumb-bells occupy all the hexagonal lattice points. For simplicity, we ignored the indication of the directional nature of the bars. Around all the intersection of the three dotted lines, the direction of the bars makes a directed closed triangle. In Figure 2, every hexagonal lattice point is connected by thin lines. These lines are parallel to the ripple ridges. This is consistent with the result of X-ray diffraction.[10] Therefore, the structure composed of these thin lines should resemble the texture of the ripple structures. The above configuration makes the distortion energy small at the domain boundary, because the strong steric hindrance among the tilted fatty acyl chains at the boundaries is avoided. From Figure 2, it has been proved that the most textures observed on the freeze-fractured surface can be interpreted in such a way.

The chevronel pattern, the trident pattern, the successive elongated hexagon pattern, etc. are frequently observed in the transient state. In the stationary state, these patterns are overcome by the regular parallel ripple lines. This fact can also be expressed in terms of the domain boundary motion, when we adopt the three kinds of the domains drawn in Figure 2. When a phospholipid molecule at the domain boundary changes its configuration from that of the base domain to that of the adjacent domains, the molecules in the two successive hexagonal lattice lines at the domain boundary change their arrangement at the same time and it propagates immediately

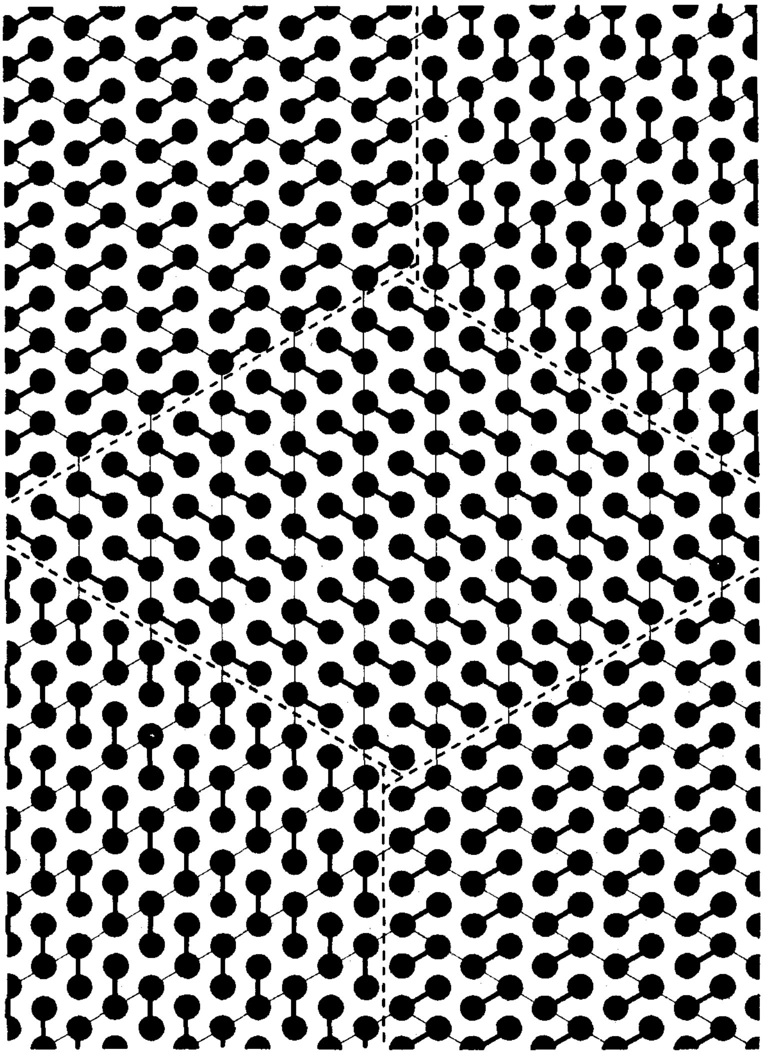

FIGURE 2 A schematic view of the darning configuration of the dumb-bells. Each dumb-bell indicates a phospholipid molecule; a closed circle denotes the average cross-section of fatty acyl chain and the bar connecting the two circles denotes both the glycerol backbone and the polar head group. Owing to the polar head group on the glycerol backbone, the bar should have a directional character, which is not shown in this illustration. There are three kinds of the darning configurations. They make domain structure. The thin lines linking the closed circles are parallel to the ripple ridges, which is expected from the result of X-ray diffraction. Therefore, the pattern formed by the thin lines resembles the texture of the ripple structures. From this illustration we can understand the relation between the configuration of the phospholipid molecules and the texture of the ripple structures.

along the boundary, i.e., the domain boundary moves perpendicularly to it by the two hexagonal lattice points as a fundamental step. As a result, the boundary of the two neighboring domains moves strictly and the regular chevronel pattern of the freeze-fractured surfaces is left under any conditions.

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