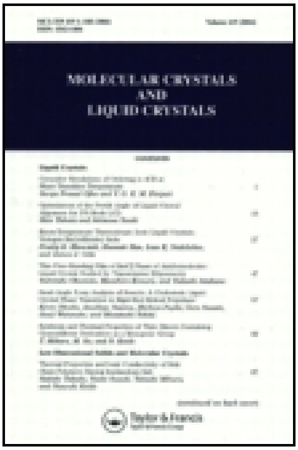

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Phase Transitions in Single Crystals of L-DPPC and DL-DPPC†

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By use of thin layered single micro-crystals obtained from xylene suspensions, the polymorphism of L- and DL-dipalmitoylphosphatidylcholines (DPPC) was studied by electron and X-ray diffractions, microscopic observations, and thermal analyses, with particular attention to the structural transformation in the bilayer plane associated with a thermotropic phase transition, which occurred at about 60°C for dihydrated crystals.

At this phase transition, the disappearance of the optical anisotropy in the bilayer plane of the low temperature phase and large changes in crystal dimensions were observed microscopically, and by diffraction studies corresponding changes in unit cell parameters and intensity distributions were observed.

The effect of water on thermotropic phase transition behaviours of DPPC's were also examined.

The combination of the present experimental methods and the use of monodomain single crystals facilitate the analysis of molecular structures and packings in bilayer plane, which will be discussed briefly.

INTRODUCTION

Lecithin is one of the major components of biological membranes and knowledge of its structure is vitally important for understanding the biological function of the membranes. This molecule consists of three parts; the two hydrocarbon chains, the phosphorylcholine group having (+) and (−) charges, and the glyceryl group connecting the former two parts. The last two parts are designated as "polar head part". There are the D- and L-type molecules which arise from the asymmetric carbon atom in the glyceryl group. The structure elements of these lipids crystals are symmetric bilayers

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in which the hydrophobic hydrocarbon chains assemble to constitute the interior and hydrophilic polar head parts are on the surface.

In low temperatures or at low water concentration, the multi-layered structure is generally obtained. Lyotropic and thermotropic phase transitions occur with varying water concentrations and temperatures, and so far many mesophases have been reported on lecithins.[1-3] Most of these phase transitions and mesophases have been investigated by crystalline powders of multi-domain mesophases and discussed mainly on the basis of the various informations in the directions along bilayer thickness. On the other hand, the informations of the structure in bilayer plane in various mesophases are very little because of difficulty in obtaining so large a crystal with a single domain as to be able to determine the structure in bilayer plane.

As we have succeeded to obtain thin layered single crystals of synthetic L- and DL-type lecithins having monodomain of sufficient area to be able to investigate the structure in bilayer plane,[4] we have made microscopic and X-ray and electron diffraction studies on both crystals to make a comparison with each other. These crystals facilitate a variety of structural and physical studies on lecithins, not possible with polycrystalline or powder-like samples.

In this paper, phase transition behaviours of both L- and DL-type thin layered crystals and powders are examined by use of a polarizing microscope, thermal analyses, and electron and X-ray diffraction methods. The results will be reported with particular attention to the conformational changes of lecithin molecules and side-by-side packing of them in layer plane at a thermotropic phase transition. This phase transition occurs at about 60°C in dihydrated crystals, above which the optical anisotropy in the low temperature phase disappears and crystal and unit cell dimensions change anisotropically.

Both electron and X-ray diffraction patterns obtained from these thin layered crystals by incident beams normal to the layer plane were "fine-net-patterns" of in-plane structures which were just the reciprocal lattice net works of them. By examining both of the variations in spacings and the whole diffraction patterns, many informations were obtained on structural and conformational changes before and after the phase transition in layer plane.

MATERIALS

L-α-dipalmitoylphosphatidylcholine (L-DPPC) was obtained from Fluka Ltd., and DL-α-dipalmitoylphosphatidylcholine (DL-DPPC) from Sigma Co. Using these DPPC's, the thin layered single crystals were grown from xylene suspensions as reported before.[4] The shape of layered crystal was changed by the crystallization conditions such as water content, concentration of DPPC in xylene suspension, and growth temperature. The crystal of L-DPPC was a thin rectangular platelet with multi-layered structure of about 0.1 μm in thickness and the longer side of about 100 μm in length for a larger one. That of DL-DPPC was a thin hexagonal platelet with multi-layered structure of about 0.1 μm in thickness and the longer side of about 300 μm in length for a larger crystal. (See Figure 1.)

The crystalline samples for diffraction studies were prepared from the suspension by evaporating xylene on a collodion substrate based on a 150 mesh electron microscope grid for electron diffraction measurements, and on a mylar film of 45 μm thick for the X-ray diffraction measurements.

EXPERIMENTAL RESULTS

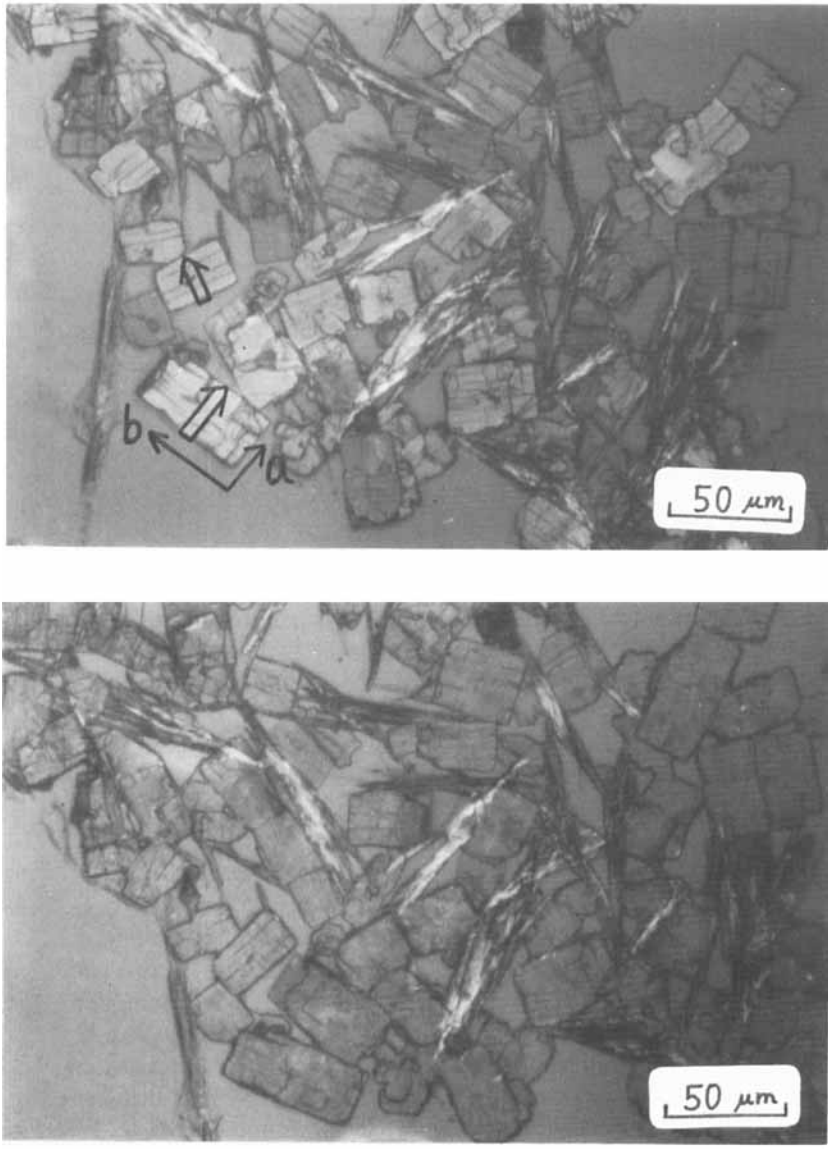
i) Diffraction studies

The electron diffraction experiments were carried out at 200 KV in a JEM–200CX electron microscope. By use of the selected area diffraction mode in which the effective selected area was limited to approximately 1 μm in diameter, electron diffraction patterns were recorded on high-sensitivity photographic films.

During the irradiation of electron beam on DPPC, a thermotropic phase transition occurred on account of the temperature rise by the irradiation.[4] The diffraction pattern of the low temperature phase obtained, within a first few seconds of irradiation, by incident beam normal to the plane of crystalline platelet of L-DPPC (Figure 2, a), shows a quadrilateral unit with spacings $d_{100} = 9.9$ Å, $d_{010} = 8.8$ Å, and that of DL-DPPC (Figure 2, c) shows $d_{100} = 17.2$ Å and $d_{010} = 8.9$ Å. The unit cell dimension along *a*-axis of DL-DPPC was nearly twice as large as that of L-DPPC. Here, *a* and *b* axes are conveniently indexed as orthorhombic system in high temperature, in which the packing of chains is in accord with that of polyethylene.[4]

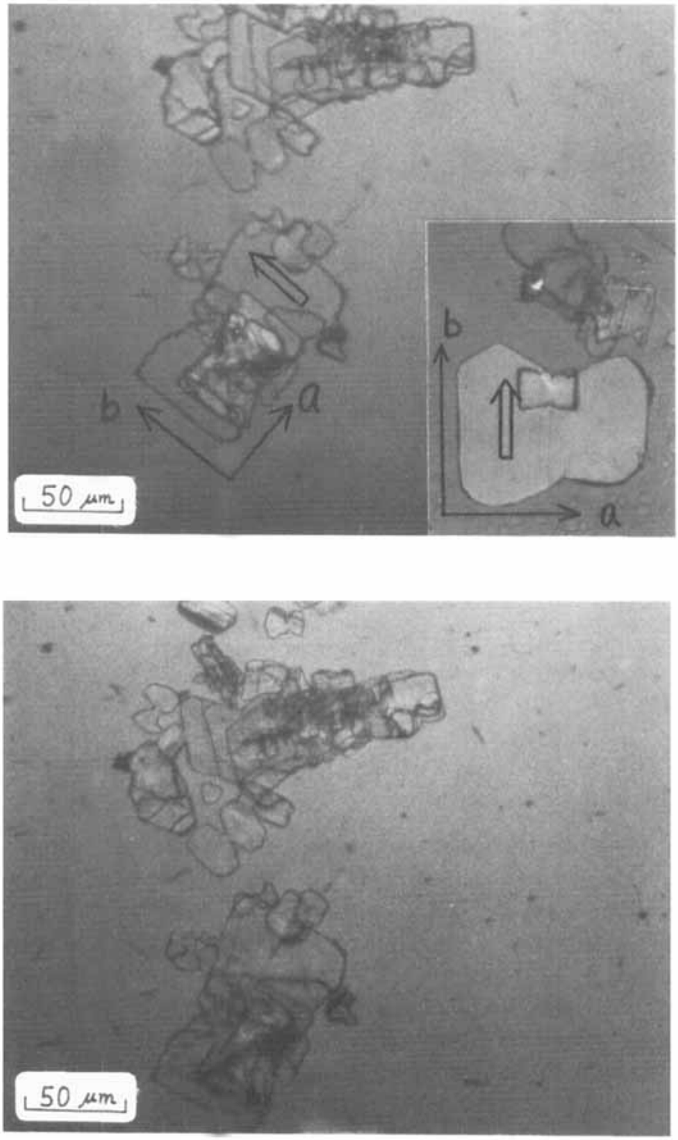
After prolonged irradiation many of the reflections disappeared and both electron diffraction patterns of L- and DL-DPPC were transformed into those of the high temperature phase basically composed of six reflection spots having about 4.1 Å spacing for L-DPPC and about 4.2 Å for DL-DPPC (Figures 2 b and d).

This thermotropic transformation behaviour was also examined by X-ray diffraction method for both L- and DL-DPPC. It was found that, in accordance with the observed phase transition in electron diffraction methods caused by temperature rise under prolonged irradiation, the thermotropic



(a)

FIGURE 1 Photographs of thin layered single micro-crystals of lecithins grown in xylene suspension taken by a polarizing microscope with a sensitive color plate. The crystal was prepared by evaporating the solvent from the xylene suspension on a glass slide. The arrows show the optical axes in the layer planes. *a* and *b* show directions of crystallographic *a*- and *b*-axis of the lattice of each crystal determined from X-ray diffraction method. (a) L-DPPC; below 60°C (Top) and above 60°C (Bottom). (b) DL-DPPC; below 60°C (Top) and above 60°C (Bottom). Inlet shows a typical shape of DL-DPPC crystalline platelet.



(b)

phase transition was also observed to occur between 60°C and 90°C by X-ray diffraction method. At the same time, it was found that a contraction of about 16% along *a*-axis and an expansion of about 7% along *b*-axis occurred in L-DPPC and a slight contraction along *a*-axis and an expansion of about 8% along *b*-axis in DL-DPPC.

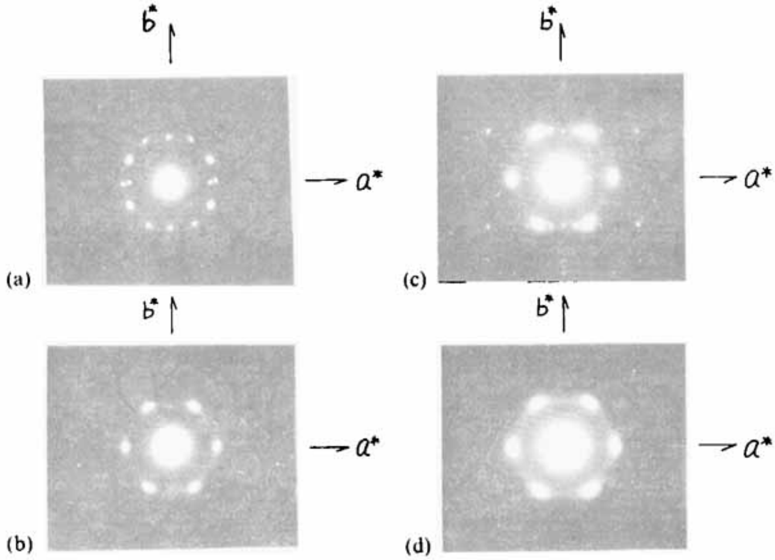


FIGURE 2 Electron diffraction pattern from a thin layered single crystal of lecithin. Several reflections are from high temperature form which appeared due to the temperature rise during the exposure to the electron beam. (a) L-DPPC at low temperature. $a^* = 0.101\,Å^{-1}$ and $b^* = 0.114\,Å^{-1}$. (b) L-DPPC at high temperature. Six intense spots correspond to $d^* = 0.244\,Å^{-1}$. (c) DL-DPPC at low temperature. $a^* = 0.058\,Å^{-1}$ and $b^* = 0.112\,Å^{-1}$. (d) DL-DPPC at high temperature. Six intense spots correspond to $d^* = 0.238\,Å^{-1}$.

ii) Microscopic observations

We have observed the phase transition behaviours of crystals under a polarizing microscope. The observations were made with crystals exposed to the air. The X-ray diffraction pattern from the aggregate of the same crystals also exposed to the air showed similar spacings in *hk*0 reflections as those reported by Pearson *et al.* on dihydrated DMPC.[5] We may, therefore, think that the crystals under this condition are in dihydrated state.

From microscopic observations at room temperature, it was known that the thin layered single crystals of both L- and DL-DPPC had a strong optical anisotropy in the bilayer plane. For L-DPPC, the optic axis lies parallel to one of the two sides of the rectangular crystalline platelet, which for the present crystal is the shorter side and corresponds to the *a*-axis determined by the diffraction study (Figures 1a and 2a). For DL-DPPC, the optic axis lies parallel to the pair of longer side of the hexagonal crystalline platelet, which corresponds to the *b*-axis determined by the diffraction study (Figures 1b and 2c).

When the temperature was increased from room temperature at the rate of 2°C/min., both L- and DL-DPPC lost their in-plane optical anisotropies in the lower temperature at about 60°C, and were transformed into states optically isotropic in layer plane (Figures 1, 2b, and 2d). At the same time, the dimension of crystalline platelet was observed to contract slightly along *a*-axis and to expand about 10% along *b*-axis for L-DPPC. These changes in dimensions of L-DPPC crystal correspond qualitatively to respective changes in unit cell dimensions obtained by the electron diffraction study. The changes in dimensions of DL-DPPC crystal at the phase transition, on the other hand, were an expansion of about 6% along *a*-axis and little change along *b*-axis. They are in contradiction with the respective changes in unit cell dimensions by the electron diffraction study.

iii) Thermal depolarization analysis of phase transition behaviours

The measurement of the transmittance of polarized light through optically anisotropic crystals as a function of an external variable such as the temperature, is a simple way of observing polymorphism of a crystal. Using a polarizing microscope, we measured the transmitted light intensity through DPPC crystals between crossed polarizers as a function of temperature (thermal depolarization analysis). The sample crystals deposited on a glass slide were inserted into a micro furnace (Mettler FP–52). The transmitted light was detected by a photo-electric cell and the intensity of it was recorded on a recording chart.

Measurements were made for both L- and DL-DPPC on their layered crystals and powders. The temperature was increased at the rate of 2°C/min. from room temperature to slightly above the melting temperature. In Figure 3, the results are summarized. In measurements on thin layered single micro-crystals, a few sample crystals were deposited randomly on a glass slide with their bilayer planes parallel to the surface of the slide. As sample crystals were exposed to the air, they were in dihydrated state as mentioned in the preceding section. The measured intensity change, therefore, should reflect the temperature change of the in-plane optical property. From Figure 3, crystals of L- and DL-DPPC are known to show similar phase transition behaviours in the measured temperature region. Both types of DPPC showed an abrupt decrease of the transmitted light intensity at about 60°C indicating the appearance of a phase without in-plane optical anisotropy. This phase lasted to about 170°C, where after a complicated change in the transmitted light intensity an optically anisotropic phase appeared. In this phase between about 170°C and melting temperature, well-developed large spherulites were observed microscopically.

For comparison with the above results, the measurements were made also on as-received powder like samples of L- and DL-DPPC, which were

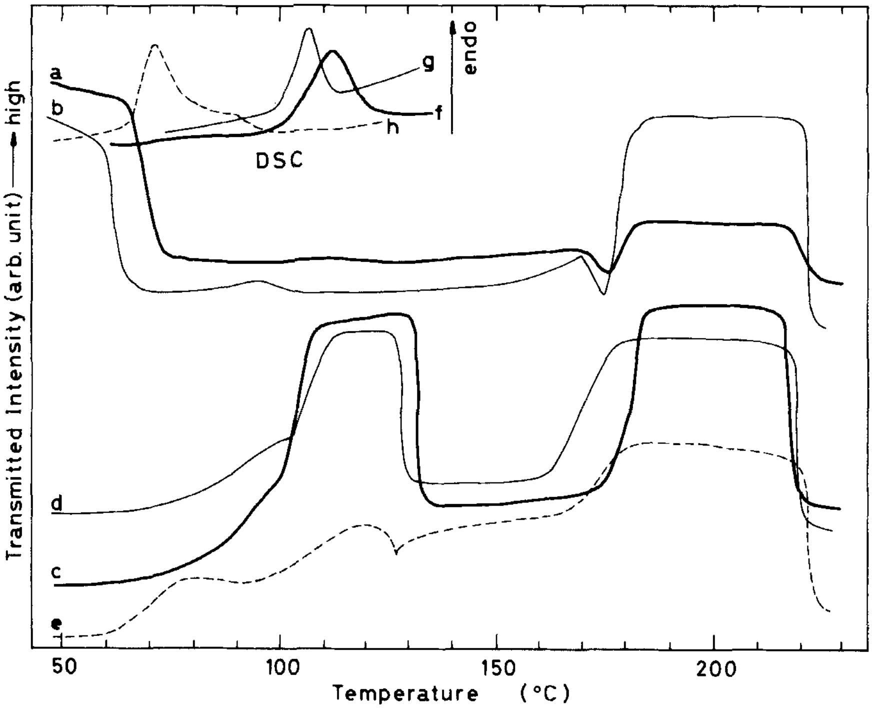


FIGURE 3 Transmitted light intensity (curves (a), (b), (c), (d), and (e)) and DSC recording (curves (f), (g), and (h)) against temperature, which was increased at the rate of 2°C/min. (a), for thin layered dihydrated crystals of L-DPPC; (b), for thin layered dihydrated crystals of DL-DPPC; (c) and (f), for monohydrated powder specimen of L-DPPC; (d) and (g), for monohydrated powder specimen of DL-DPPC; (e) and (h), for a little more hydrated powder specimen of DL-DPPC than that of (d).

sealed in a thin cell by two parallel plates of mica after keeping them sufficiently long in vacuum. The sample thus sealed showed many sharp reflections in wide angle region, and the long spacings of about 52 Å and about 54 Å for L- and DL-DPPC, respectively, in X-ray diffraction pattern. It seemed to be monohydrate as estimated from observed unit cell parameters.

As is clear from Figure 3, both types of DPPC show three thermotropic phase transitions below the melting transition, indicating four phases, including that around room temperature, below the isotropic melt. The temperature range of existence of each phase slightly differs with the type of DPPC. It was observed microscopically that the first phase looked powder-like from room temperature to about 100°C, where the phase transition to the second mesophase occurred. The second mesophase looked as aggregated small crystallites transformed from the first phase, existed up to about 130°C, where the phase transition to the third mesophase occurred. The third phase was almost isotropic optically. At about 170°C this phase was transformed to well-developed large spherulites. These spherulites were transformed to the isotropic melt.

In Figure 3 (e) is also shown the results on DL-DPPC containing water a little more than in the dihydrated state. Many X-ray diffraction rings in the crystalline state disappeared in this sample. As a result of the presence of water molecules more than the dihydrated state, the phase transition behaviour of this sample was considerably altered and the transition temperatures were distinctly shifted to lower temperatures.

The difference in phase transition behaviour between the single crystalline sample and the powder-like one of respective type of DPPC may be resulted not only from the difference in water content but also from the orientation of crystallites in the observation area. In the measurement of single crystals, we are observing the changes in the in-plane optical anisotropy determined mainly by the structure and side-by-side packing of molecules in the layer plane. A drastic change in the intensity of transmitted light, therefore, may not be observed till a large structural transformation in three dimensions occurs if the phase transition does not affect seriously on the in-plane structure. Whereas for the powder-like sample, as the orientation of crystallites is random, the observed result is the orientationally averaged one. In such case, the intensity change may be mainly influenced by a structural change in the direction of the largest refractive index, i.e. the direction parallel to the hydrocarbon chains or the thickness direction of the layer. Thus a phase transition mainly associated with a structural change of hydrocarbon chain parts may be well detected by the powder sample. A change which affects the in-plane optical property, on the other hand, may be well observed by the single crystalline sample, as we have succeeded to observe for DPPC's.

In addition to the results of transmitted light intensity measurements, those of a differential scanning calorimetry (DSC) were also shown in Figure 3 (f, g, h). An endothermic peak was observed at the first thermotropic phase transition of each powder sample of DPPC.

DISCUSSION

Among multiple thermotropic phase transitions of either L-DPPC or DL-DPPC crystal in extremely small water content region, the first one that occurs when the temperature is increasing from room temperature, is characterized microscopically by the disappearance of the optical anisotropy and the large change in size of crystal at the phase transition. For L-DPPC in dihydrated state, it was observed at about 60°C that the optical anisotropy in the bilayer plane with the optic axis parallel to *a*-axis disappeared and that the crystal size along *a*- and *b*-axis contracted slightly and expanded by about 10%, respectively. Also was observed for DL-DPPC in dihydrated state the disappearance of the optical anisotropy with the optic axis parallel to the *b*-axis, an expansion of crystal size of about 6% along *a*-axis, and little change along *b*-axis at about 60°C. If the optical anisotropy in the low temperature phase is mainly resulted from a tilt of hydrocarbon chains, the change in dimensions of a crystal by the disappearance of the optical anisotropy might be more reasonable for a larger change in the direction parallel to the optic axis to occur. The actual change in size observed for each of L- and DL-DPPC crystals, however, was larger in the direction perpendicular to the optic axis, i.e. about 10% and 6% expansion along *b*- and *a*-axis for L- and DL-DPPC, respectively. Therefore, the origin of optical anisotropy in the low temperature phase can, at least partly, be attributed to the polar head group on the bilayer plane, and if so the disappearance of the anisotropy at the phase transition is mainly attributable to a rearrangement of the polar head group in the bilayer plane. Accordingly, we think that the very thermotropic phase transition discussed here is associated with a large change in the conformation and the packing of the polar head group and with the accompanying change in the degree of freedom of rotational motion and the packing of the hydrocarbon chain.

The enthalpy of this phase transition evaluated from the DSC measurement shown in Figure 3, is about 5 kcal/mol. If we assume that nearly the half in weight of a lecithin molecule is occupied by two hydrocarbon chains and that the whole of the transition enthalpy is roughly assigned to a change in state of hydrocarbon chains, the measured value of transition enthalpy gives an approximate value of 14 cal/g for the transition enthalpy of hydrocarbon chain part. This value, a rough estimation of transition enthalpy required for the change in state of hydrocarbon chains at the present phase transition, is considerably smaller than the typical enthalpy of melting of hydrocarbon chain compounds such as paraffins and in comparable order of magnitude with that observed at the rotational phase transition of paraffinic chains to the hexagonal packing, i.e. ~ 50 cal/g or more for the former transition whereas ~ 10 cal/g for the latter transition for paraffins having about 20 carbon atoms.[6] Thus, we might think that on the phase transition of DPPC the hydrocarbon chains would not make such a large change in packing as might do in their melting transition. Anyway, hydrocarbon chains would be a little disordered at the phase transition, which, in nature, might be like that at the rotational phase transition of paraffins or like that at the melting transition occurring partially. Therefore, the change in optical property again should be mainly a consequence of a disordering of the polar head parts, as the structural change of hydrocarbon chains should not be so drastic.

In the present study, we have made measurements on both L- and DL-DPPC. By diffraction measurements in crystalline state of DPPC's with very small amount of water content, it was shown that the diffraction pattern for each type of DPPC was different with each other. The lattice constant in *a*-axis of DL-DPPC is twice as large as that of L-DPPC, and in *ab*-projection each of L-type and D-type molecule has different packing. The thermotropic phase transition behaviours of these crystals, however, were very similar with each other except for slight differences in the transition temperatures. The difference in the water content of crystal, on the other hand, affected considerably the thermotropic phase transition behaviour in low water content region even of the DPPC of the same type. A rather large shift in phase transition temperatures was observed together with the change in over all phase transition behaviour. These observations suggest that to the phase transition behaviour the contribution from hydrogen bondings by water molecules get into polar head parts may be important, as discussed by Pearson *et al.* in the paper on the structure analysis of a phospholipid crystal.[5]

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References

1. D. Chapman, R. M. Williams, and B. D. Ladbrooke, *Chem. Phys. Lipids*, **1**, 445 (1967).
2. J. Charvolin and A. Tardieu, *Solid State Physics*, Suppl. 14, (Academic Press, London and New York, 1978), pp. 209–257.
3. A. Tardieu, V. Luzzati, and F. C. Reman, *J. Mol. Biol.*, **75**, 711 (1973).
4. I. Sakurai, S. Sakurai, T. Sakurai, T. Seto, A. Ikegami, and S. Iwayanagi, *Chem. Phys. Lipids*, **26**, 41 (1980).
5. R. H. Pearson and I. Pascher, *Nature*, **281**, 499 (1979).
6. M. G. Broadhurst, *J. Res. NBS*, **66A**, 241 (1962).