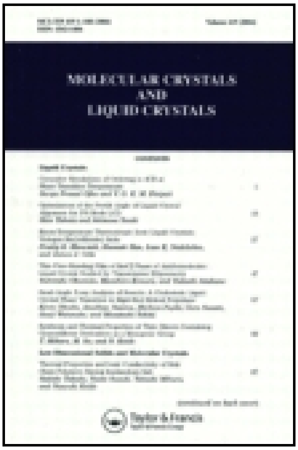

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Magneto-Orientation of Phospholipids

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Magneto-Orientation of Phospholipids†

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Magneto-orientation effects were observed for platelets of thin layered single crystals of L- and DL-dipalmitoylphosphatidylcholine (DPPC), and for single crystals of polyethylene (PE) suspended in xylene. For both L- and DL-DPPC crystals, a biaxial magneto-orientation was observed, in which both directions of hydrocarbon chain and phosphorylcholine group were directed perpendicular to the applied field direction. For PE, the hydrocarbon chain direction was oriented perpendicular to the field.

From the orientational behaviour under the magnetic field, the anisotropy of diamagnetic susceptibility, the difference between susceptibilities parallel and perpendicular to the hydrocarbon chain direction, is evaluated as -9×10^{-8} and -1×10^{-8} emu/cm^3 for L-DPPC and PE, respectively.

The origin of the diamagnetic anisotropy of DPPC is briefly discussed in terms of the molecular and crystal structures.

I INTRODUCTION

The magnetic properties of, and the magnetic field effects on, biological systems have recently attracted much interest because of the increasing number of developing technologies that utilize rather high magnetic fields. It seems, however, that studies on the magnetic properties of materials of biological interests, which may be extracted from biological systems or in some cases synthesized chemically, are very few, and the observed magnetic field effects on biological systems are discussed without detailed knowledge on the magnetic properties of constituent materials.

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In the magneto-orientation effects of biological membrane systems such as chloroplasts[1–3] or retinal rod outer segments[3–5] in aqueous suspensions, the origin of the magnetic anisotropy that makes them orient in a homogeneous magnetic field are considered to be the summed diamagnetic susceptibility anisotropy of ordered proteins or part of proteins that would exist in the biological membranes.[6–11] The observed orientation of biological membranes is such that the membrane normal is directed to the direction of the applied magnetic field. Phospholipids, the major component of biological membranes, therefore, are oriented parallel to the field. If the diamagnetic anisotropy of phospholipids with two long hydrocarbon chains is assumed to be similar to that reported for a long hydrocarbon-chain fatty acid such as stearic acid,[12] the membrane normal should orient perpendicular to the field, instead of parallel. Thus the observed magneto-orientation effects of biological membranes are in contradiction to what is expected from the assumed diamagnetic anisotropy of phospholipids, and usually explained that they result from the summed anisotropy of proteins in a membrane which is partially cancelled by that of phospholipids. Therefore, to give further convincing proofs of the role of phospholipids and proteins in the magneto-orientation of membranes, it is necessary to study the diamagnetic anisotropy of phospholipids.

In this paper, we will report on a study of the magneto-orientation effect of thin single micro-crystals of phospholipids, i.e. L-α-dipalmitoylphosphatidylcholine (L-DPPC) and DL-α-dipalmitoylphosphatidylcholine (DL-DPPC). Also will be reported a study on polyethylene (PE) single crystals, which are composed only of hydrocarbon chains and are useful in discussing the contribution of polar head parts of phospholipids to the magnetic anisotropy. For L-DPPC and PE, we have evaluated the diamagnetic susceptibility anisotropies from the magneto-orientation behaviours. Experimental results on L-DPPC and PE have been already submitted elsewhere in some detail.[13]

II MATERIALS

The sample of L-DPPC used in the present experiments was obtained from Fluka Ltd., and DL-DPPC from Sigma Chem. Co. They were used as received. The as-supplied samples were fine powders, and from the long spacings obtained by X-ray powder diagrams it was known that they contained one water molecule per DPPC molecule at most, probably by far the less.

Single crystals of DPPC's were obtained from xylene suspensions of DPPC's. The method of preparation of crystals together with electron and X-ray diffraction studies of structural features of the present crystals of L- and DL-DPPC have been already reported elsewhere.[14,15] We, therefore, will refer to a few points on structures of these crystals needed to explain the present experiments.

In Figures 1 (a) and (b), typical views of crystalline platelets of L- and DL-DPPC, respectively, are shown. These photographs were taken by use of a polarizing microscope between crossed polarizers. The shape of crystals

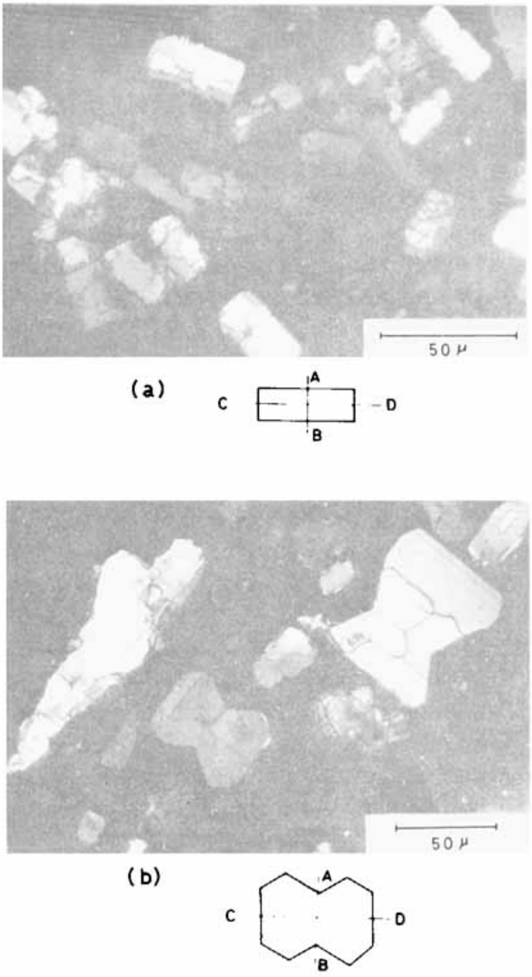

FIGURE 1 Typical views of thin single micro-crystals of L-DPPC (a), and DL-DPPC (b), grown from xylene suspension. These photographs were taken by use of a polarizing microscope just after deposition on glass slide. The polarizers are oriented horizontally and vertically. In addition to photographs, a schematic drawing of the typical shape of each crystal is shown, in which the direction of the optic axis (AB) and the direction of crystal oriented to the magnetic field (CD) are also shown.

of L-DPPC is a thin rectangular platelet, and they have rather uniform dimensions with one another. The average dimensions of crystals used in the present experiments were 36 and 18 μm in length of the longer and the shorter sides, respectively, and about 0.1 μm in the thickness. The shape of crystals of DL-DPPC, on the other hand, is complicated, and the crystals vary in size from the order of 100 μm for a larger one to the order of 10 μm or less for a smaller one. From diffraction studies, DL-DPPC crystal is shown to have nearly hexagonal packing, and the characteristic shape of it is a hexagon, as can be seen in Figure 1 (b), though a little deformed and paired.

The crystals of L- and DL-DPPC give beautiful electron diffraction spots suggesting that they are single crystalline platelets.[14,15] In the platelet, DPPC molecules form a bilayer, which is stacked on and on giving a stacked bilayer structure to the platelet. The plane of bilayer is parallel to that of the platelet. Hydrocarbon chains of DPPC are oriented nearly perpendicular to the plane of bilayer, hence that of the platelet.[14,15] In the plane of platelet, an optical anisotropy was observed by a polarizing microscope (see Figures 1 (a) and (b)). As hydrocarbon chains are nearly perpendicular to the plane of the platelet, and phosphorylcholine groups of polar head parts are on bilayer planes,[16–18] the in-plane optical anisotropy should mainly be a consequence of the oriented polar head parts. From optical observations by a polarizing microscope, therefore, it is considered that phosphorylcholine groups of polar head parts are oriented parallel to the shorter side of the platelet for L-DPPC, and to the longest side of the hexagonal platelet for DL-DPPC, hence giving optic axes to the respective directions (see Figure 1). The crystals of both DPPC's are known from the long spacing by X-ray diffraction to be in dihydrated forms.

Single crystals of PE used in the present study, were grown from xylene solution at 80°C. They are thin rhombic platelet-like crystals with the average dimensions of 16, 11, and 0.1 μm for the longer diagonal, the shorter one, and the thickness, respectively. They consist of multi-layered crystalline lamellae and hydrocarbon chains are also packed perpendicular to the plane of platelet.[19] In the plane of platelet, the PE crystal is optically isotropic.

III EXPERIMENTAL RESULTS

1 Microscopic observations of magneto-orientation

Using a polarizing microscope, we observed the effect of the application of the magnetic field on crystals of both DPPC's and PE suspended in xylene at about 22°C. We used an electro-magnet with pole pieces of 30 mm in diameters and 6 mm in the gap width between them.

On application of the magnetic field of about 5 kG, L-DPPC, DL-DPPC, and PE crystals in xylene were observed to be oriented in such a way that their thickness direction, i.e. hydrocarbon chain directions, were put perpendicular to the field direction. This orientation of hydrocarbon chains is consistent with the reported anisotropy of diamagnetic susceptibilities for long hydrocarbon chain compounds.[12] The distinctive feature in the magneto-orientation of L- and DL-DPPC crystals was that they showed a biaxial orientation in the magnetic field. For L-DPPC, the shorter side of a crystalline platelet, and for DL-DPPC, the longest side of hexagonal crystalline platelet, were oriented perpendicular to the field direction, whereas for PE crystals no evidence of biaxial orientation could be observed. Therefore, in addition to hydrocarbon chains, phosphorylcholine groups of DPPC's are also oriented perpendicular to the field direction.

We also made preliminary experiments of the effect of temperature on the magneto-orientation of L-DPPC crystals. From electron diffraction study, it is known that the in-plane symmetry of L-DPPC crystals transforms, by a temperature rise, from a low temperature form into a high temperature form with a higher symmetry.[14,15] On the other hand, from microscopic observations it was observed that above 30°C the in-plane optical anisotropy of xylene suspended L-DPPC nearly vanished. The observation of magneto-orientation of L-DPPC crystals above 30°C showed that there was a tendency to obscure the biaxial orientation observed in lower temperatures. Further experiments on the orientational behaviour in higher temperatures are under way.

2 Transmitted light intensity measurements through suspensions under the magnetic field

To evaluate the magnitude of the magnetic anisotropy of phospholipid from the magneto-orientation behaviour of the crystals, we measured the intensity of light transmitted through the suspensions under the crossed polarizers. Details of experimental results will be found elsewhere.[13] In Figure 2, the experimental set-up is shown schematically, where the intensity of polarized light through the sample suspension and the analyzer, i.e. the second polarizer crossed to the first one at the right angle, is measured. The magnetic field was applied by the same electro-magnet used in the microscopic observations, and it required about 150 msec to attain 90% of the final field strength at 9 kG. The light through the suspension was led to a photo-electric cell through a microscope whose eyepiece was replaced by the cell. The intensity of light detected by the cell was recorded in a digital memory with the resolution of 12 bits in magnitude and 20 msec in time. Before each run of

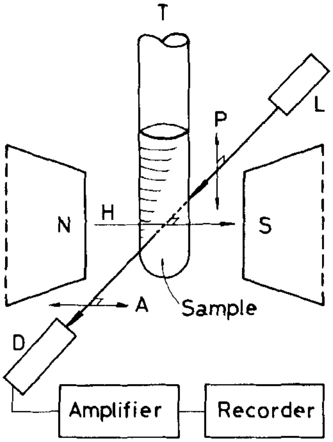

FIGURE 2 Schematic diagram of the experimental set-up. T, the glass tube with sample suspension; L, the light source of a tungsten lamp; D, the photo-electric cell; P and A, the crossed polarizers, P for the polarizer and A for the analyzer. As the recorder, a digital memory of 12 bits in resolution was used.

measurements the suspension was stirred up thoroughly by shaking the glass container. Measurements were made at about 22°C.

Examples of the magnetic field induced change in the transmitted light intensity are shown in Figure 3 for L-DPPC and PE. In this Figure, the polarizer was put so as the polarizing direction of the incident beam to be parallel to the field direction. The possibility of the intensity change in transmitted light by Cotton–Mouton effect of xylene was also examined and it was shown to be negligible compared with the change caused by the magneto-orientation of suspended crystals.

The transmitted light intensity just after stirring the suspension of L-DPPC was due to randomly oriented crystals some of which should be in the diagonal position between crossed polarizers. On application of the magnetic field, the orientational motion of crystals started and such orientation as has been stated in the preceding section was accomplished within ten seconds or so. As the optic axes, one in the direction of hydrocarbon chains and the other in that of phosphorylcholine groups, were oriented perpendicular to the field, the crystals were in the extinction position hence giving a reduced transmitted light intensity as compared with the initial intensity level. When the field was removed, the oriented crystals would turn again into disordered state and the transmitted light intensity went toward the initial intensity level before the application of the field. For PE, the behaviour of transmitted light intensity is explained, as for the case of L-DPPC, by the orientation of crystal having the optic axis parallel to the hydrocarbon chain direction.

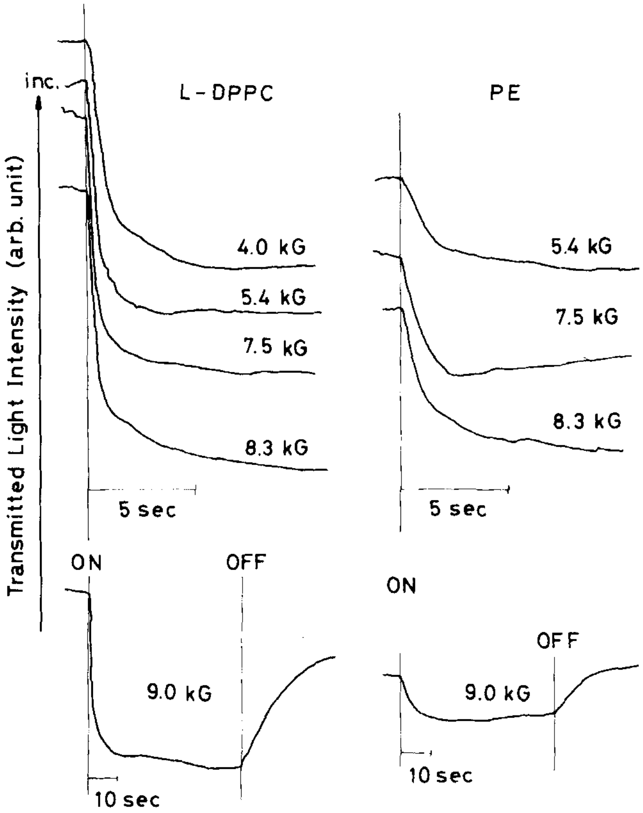

FIGURE 3 Examples of the magnetic field induced change in the transmitted light intensity through suspensions of both L-DPPC and PE crystals between crossed polarizers are shown as a function of time after the application of the magnetic field. The field strength is shown beside each curve. For $H = 9.0$ kG, the whole behaviour of the transmitted light intensity, when the field has been turned on and off, is shown. The polarizing direction of the incident beam are parallel to the magnetic field.

For DL-DPPC suspension, we could observe the similar behaviour of the transmitted light intensity as for L-DPPC. As the crystals were irregular in size, however, any further analysis was not made on DL-DPPC.

The magnitude of the magnetic field induced change in the transmitted light intensity, was nearly linear to the squared field strength, and for L-DPPC the effect of the field seemed nearly to saturate around 7 kG. As densities of both L-DPPC and PE crystals are higher than xylene, a slow sedimentation process took place during the intensity measurements. Thus an intensity change resulting from a slow change in number of crystals in the observation volume by the sedimentation process, was inevitably superimposed on the intensity change by the magneto-orientation effect of crystals. This effect manifested itself as a lack in reproducibility of the transient behaviour of intensity change after the field was turned off. The transient change in light intensity after the field was turned on, however, showed a good reproducibility at least within the first five seconds or so, and showed a simple exponential decay with time as shown in Figure 4, giving an orientational relaxation time as a function of the field strength.

We, therefore, utilized the relaxation time, τ, of the magnetic field induced orientational motion for the evaluation of the anisotropy of diamagnetic susceptibility, $\Delta\chi$, which is the difference of the principal volume susceptibilities parallel to the hydrocarbon chain direction, $\chi_{\parallel}$, and perpendicular to it, $\chi_{\perp}$. In a linear approximation, τ is given as[3]

$$\tau = \zeta/\Delta\chi \cdot V \cdot H^2,$$

where ζ is the rotatory frictional coefficient, V the volume of a crystalline platelet, and H the field strength. Figure 5 shows the plot of τ^{-1} against H^2. As expected from the above expression for τ, τ^{-1} varies almost linearly with H^2 in the lower field region. In making a rough estimation of $\Delta\chi$ from the slope of τ^{-1} vs. H^2 plot, we evaluated ζ after Perrin[20] assuming the thin crystalline platelet as an oblate ellipsoid of revolution and using the average dimensions of it, which are also used to evaluate V.

The volume susceptibility anisotropies thus obtained were, -9×10^{-8} and -1×10^{-8} emu/cm^3 for L-DPPC and PE, respectively.

IV DISCUSSION

The sample crystals, obviously without permanent magnetic moment, might also be acted on in a magnetic field by torques resulted from an anisometric shape of the crystals. This "form" anisotropy or the effect of the demagnetizing field, however, should be negligible because of the small value of susceptibility of the present diamagnetic materials.

The smaller value of $\Delta\chi$ of PE seems to suggest the contribution from polar head part in $\Delta\chi$ of L-DPPC, as the only difference in molecular structures is the depletion of polar head part in PE and as the structure of hydrocarbon chains in both crystals is nearly the same with the orthorhombic symmetry. If we take the thickness of a crystalline lamella of PE as 130 Å as reported for crystals grown from xylene solution at 80°C,[19] and the part of a PE chain just contained in the lamella as one molecule with the fiber period along the chain of 2.5 Å,[19] we have the molar susceptibility anisotropy of this tentative hydrocarbon chain molecule with about hundred CH_2 units as about -15×10^{-6} emu/mol, by use of the volume anisotropy, -1×10^{-8}

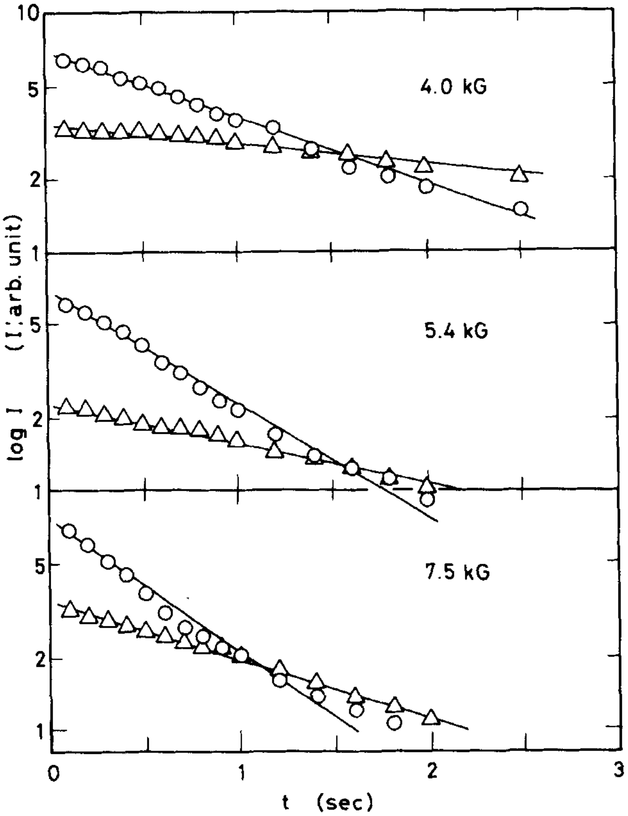

FIGURE 4 The plot of logarithm of the transmitted light intensity against the time after the application of magnetic field. Circles are for L-DPPC and triangles for PE. The field strength is shown beside each set of data.

emu/cm^3, and assuming the density as $\rho \simeq 1$ g/cm^3. As well known, the solution grown PE crystals have amorphous region between lamellae due to the chain folding.[19] Therefore, the value of molar anisotropy of PE should be considered as the lower bound of the anisotropy of crystalline hydrocarbon chain with about hundred CH_2 units.

The volume anisotropy of L-DPPC, -9×10^{-8} emu/cm^3, gives -68×10^{-6} emu/mol for the molar anisotropy. As the value of molar anisotropy of PE given above must be taken as only the lower bound, we can not say much about the contribution of hydrocarbon chains to the observed molar anisotropy of L-DPPC from the present result on PE. Instead, if we take the value of molar anisotropy of crystalline stearic acid, -26×10^{-6} emu/mol reported by Lonsdale,[12] into consideration, the most of the anisotropy of

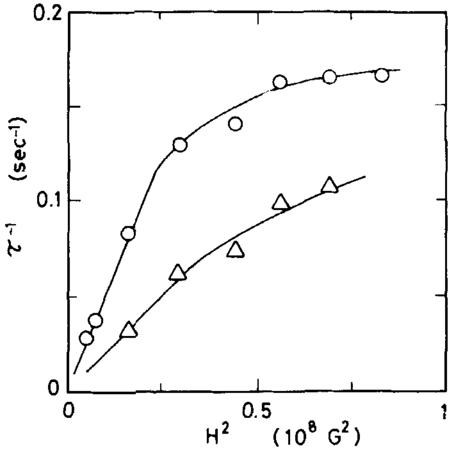

FIGURE 5 The plot of inverse relaxation time, τ^{-1}, against squared field strength, H^2. Circles are for L-DPPC and triangles for PE.

L-DPPC with two hydrocarbon chains can be explained. The rest of the anisotropy should arise from the polar head part.

We also measured the mean diamagnetic susceptibility, $\bar{\chi} = (1/3)(\chi_{\parallel} + 2\chi_{\perp})$, using a vibrating sample magnetometer (PAR Model 155). The value of $\bar{\chi}$ was $-(600 \pm 30) \times 10^{-6}$ and $-(560 \pm 30) \times 10^{-6}$ emu/mol for L- and DL-DPPC, respectively. As $\bar{\chi}$ must be determined by the molecular structure, we take the mean value of the two, -580×10^{-6} emu/mol, as $\bar{\chi}$ of DPPC. The lower bound of $\bar{\chi}$ estimated from Pascal's law, which states that the susceptibility of a diamagnetic molecule is given as a sum of that of constituent atoms and that specific to some type of chemical bondings, was about -540×10^{-6} emu/mol for DPPC, and was consistent with the measured values. Therefore, the measured anisotropy is about 10% of $\bar{\chi}$. The magnitude of anisotropy is reasonable compared to the reported data for several organic molecules.[12]

The principal susceptibilities evaluated by putting $\bar{\chi} \simeq -580 \times 10^{-6}$ and $\Delta\chi \simeq -70 \times 10^{-6}$ emu/mol were $\chi_{\parallel} \simeq -630 \times 10^{-6}$ and $\chi_{\perp} \simeq -560 \times 10^{-6}$ emu/mol. The latter susceptibility, $\chi_{\perp}$, should be the mean value of susceptibilities in the plane of crystalline platelet.

The origin of in-plane anisotropy of both DPPC's is not clear at present stage of our investigation, because the crystal structures around polar head parts have not been fully clarified. The possible candidates may be the phosphorylcholine group and two ester bonds which connect hydrocarbon chains to the polar head. The planar ester bond is reported to have rather large magnetic anisotropy, -4.4×10^{-6} emu/mol,[12,21] so as to align the plane parallel to the field, whereas for a tetrahedral group such as phosphate and choline groups the reported anisotropy is nearly as order of magnitude smaller.[22] We, therefore, might attribute the origin of this anisotropy to ester groups. In fact, if the crystal structure of DL-dilauroylphosphatidylethanolamine reported by Hitchcock *et al.*[23] is assumed to be acceptable at least for local structure of the polar head part of DPPC, the two ester planes perpendicular to each other are in the right orientation to explain the observed biaxial orientation of crystalline platelet of DPPC.

In the lyotropic liquid crystalline Lα phase, Boroske *et al.* have reported $\Delta\chi$ of egg lecithin as -0.28×10^{-8} emu/cm^3.[24] In their measurements, they used cylindrical vesicles about 20 μm in length, which had rather large ends compared with the central cylindrical part. The smaller $\Delta\chi$ of egg lecithin may be due to the different degree of ordering of hydrocarbon chains resulted from being in the Lα phase and the inhomogeneity of chains, and also probably due to the disordered polar head parts and the effect of ends of vesicles.

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