Biochimica et Biophysica Acta, 644 (1981) 89–95
Elsevier/North-Holland Biomedical Press

BBA 79244

SLOW CHANGE IN THE REPEAT PERIOD OF MULTILAMELLAR DIMYRISTOYL PHOSPHATIDYLCHOLINE ACCOMPANIED BY THERMAL PHASE TRANSITION

MORIO AKIYAMA

Department of Physics, Sapporo Medical College, Sapporo (Japan)

(Received August 25th, 1980)

Key words: Phase transition; Repeat period; Dimyristoyl phosphatidylcholine; (Multilayer)

The slow change of the repeat period of multilamellar structures accompanied by a thermal phase transition in dimyristoyl phosphatidylcholine containing 50% water was measured by a small-angle X-ray diffraction method employing the temperature-jump technique. The repeat period immediately after the application of a temperature jump from 25°C (fluid state, L_α) to 22.5°C (crystalline state, P'_β) was observed to be about 1 Å larger than that at the steady state (65.1 Å), and was then followed by a decrease to a steady value with a relaxation time of 70 min. The slow decrease in the repeat period seems to be due to the transformation from loosely stacked to closely stacked multilamellar structures, the looseness being caused by the formation of contracted domains in each lamella at an early stage of the phase transition from the fluid to the crystalline state.

Introduction

The kinetics of the thermal phase transition of phospholipid multilamellar structures have been investigated by means of the temperature-jump technique [1–5]. In most cases, optical measurements such as turbidity, fluorescence and light scattering have been used to discriminate between the fluid and crystalline states of the phospholipid bilayer. For example, relaxation phenomena in an aqueous dispersion of synthetic phosphatidylcholine were studied by Tsong and Kanehisa [4] using turbidity measurements, with the result of two relaxation times (1 and 0.01 s time ranges) being obtained. The methods enumerated above offer information as to the lateral arrangement of phospholipid molecules in each lamella.

The kinetics with respect to the change in the multilamellar structure at the phase transition have been scarcely studied. The only example to be found which has any point of similarity to the kinetics of the multilamellar structure is the work of Lentz et al. [5] on the kinetics of the pretransition in phosphatidylcholine multilamellar structures. According to these authors, the relaxation time for the pretransition in DPPC is 4–30 min.

Relative to the change in multilamellar structures on application of a temperature jump, the relaxation may be expected to be slower than that of the lateral arrangement of the phospholipid molecule in a bilayer, because the interlamellar distance is longer than the intermolecular distance of phospholipids.

The phase transition temperature, corresponding to the crystalline-fluid transition, of multilamellar DMPC containing more than 30% water occurs within a temperature range of 23–24°C [4–8]. In addition to the above-mentioned transition, there is a transition at 11°C, a so-called 'pretransition', which is associated with a structural transformation from a one-dimensional lamella to a two-dimensional monoclinic lattice consisting of lipid lamellar structures distorted by a periodic ripple. The hydrocarbon chains tilt

Abbreviations: DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine.

below the phase transition temperature.

The present report describes the relaxation phenomenon of the repeat period of DMPC multilamellar structures when the temperature jumps from 25 to 22.5°C. The repeat period was measured by the small-angle X-ray diffraction method.

Experimental Procedure

Synthetic DMPC was obtained from Sigma and was used without further purification. Small amounts of DMPC were mixed with an equal weight of 0.05 M phosphate buffer (pH 7.0) containing 0.1 M NaCl and $2 \cdot 10^{-5}$ M EDTA. In this water-containing region, a slight difference in the water content from the desired water content does not change the crystal parameters [9,10]. The mixture was then incubated at 30°C, which is about 6°C higher than the phase transition temperature, for at least 1 h.

Samples were sealed in a sample cell made of hard rubber, the window of which was made of polyester film. The dimensions of the sample cell were 1.5 × 1.5 × 1.5 mm.

Temperature-controlled fluid flowed around the sample cell. A temperature jump from 25 to 22.5°C or in the reverse direction was obtained by switching the flow from two individual temperature-controlled fluid baths. The temperature jump was completed in less than 2 min.

Nickel-filtered CuKα radiation from a DX-7 X-ray generator (JEOL, Japan) operating at 35 kV and 30 mA was collimated on the sample through two slits (0.1 × 10 mm) separated by 300 mm from each other for small-angle X-ray diffraction. The second slit was located near the sample. The diffracted X-ray intensity was measured by means of a proportional counter employing the usual step-scanning method. Large-angle X-ray diffraction profiles were measured by means of a standard type scanning diffractometer.

The diffraction profile must be obtained within a short time. The measurements relative to the time course of the first-order diffraction profile were taken in turn in 5–8, 10–13, 20–23, 40–43 and 100–103 min after application of the temperature jump. To improve the level of accuracy, the measurements were repeated ten times for the small-angle region and six times for the large-angle region under identical conditions with the data obtained being averaged.

Results

Small-angle X-ray diffraction

An X-ray diffraction profile obtained by the slit-collimating system does not reflect the exact diffraction profile. Therefore, a diffraction profile obtained by the slit-collimating system is unacceptable for an appraisal of precise crystallographic parameters. It may, however, be acceptable for the evaluation of changes in the crystallographic parameters under various conditions.

Fig. 1 shows a small-angle X-ray diffraction profile obtained at 25°C which corresponds to the L_α structure. The nomenclature, L_α and P_β (to be used later), is that of Luzzati [11]. The full line indicates the observed diffraction profile which was smeared by slit collimation, while the dotted line indicates the desmeared diffraction profile *. The repeat period of the multilamellar structure obtained from the desmeared first-order diffraction peak was 61.5 Å, which is close to the repeat period obtained by Tardieu and Luzzati [12]. The repeat period obtained

---

* For the deconvolution to obtain the desmeared diffraction profiles, the slit length was assumed to be infinite. The true diffraction intensity $I(\epsilon)$ is expressed by:

$$I(\epsilon) \propto -\int_0^\infty \frac{\bar{I}'(\sqrt{\epsilon^2 + s^2})}{\sqrt{\epsilon^2 + s^2}}\, ds \qquad (1)$$

$$\bar{I}'(x) = \frac{d\bar{I}(x)}{dx}$$

where $\bar{I}(x)$ is the diffraction intensity at the diffraction angle x, ϵ the diffraction angle and s the angular parameter. The practical calculation was performed with the following equation instead of Eqn. 1, dividing the angular interval from 1 to 3° by $\Delta s = 0.02°$:

$$I(\epsilon) \propto -\sum_{n=0}^{n'} [\bar{I}(\sqrt{\epsilon^2 + \{(n+1)\,\Delta s\}^2}) + -\bar{I}(\sqrt{\epsilon^2 + (n\,\Delta s)^2})]\,[\sqrt{\epsilon^2 + (n\,\Delta s^2}]^{-1} \qquad (2)$$

where n' is the largest integer which satisfies the equation:

$$\epsilon^2 + (n'+1)^2 \Delta s^2 < 3^2 \qquad (3)$$

As an exact diffraction profile is not required, the infinite slit length approximation is permitted.

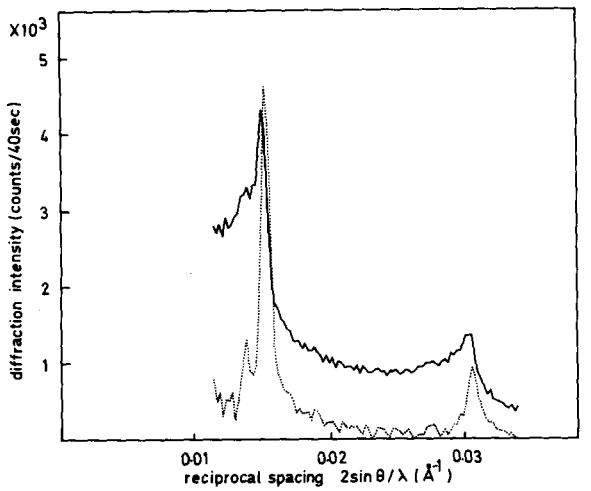

Fig. 1. Small-angle X-ray diffraction profile of DMPC containing 50% water, at 25°C. (———) Observed diffraction profile, (· · · · ·) smeared diffraction profile. The step interval was 0.02°. The measuring time at one point was 40 s.

from the observed first-order diffraction peak was about 1 Å larger than that of the desmeared one. Fig. 2 shows the same profile at 22.5°C (P'_β structure). The repeat period obtained from the desmeared first-order diffraction peak was 65.8 Å.

The time course of the diffraction profile after the

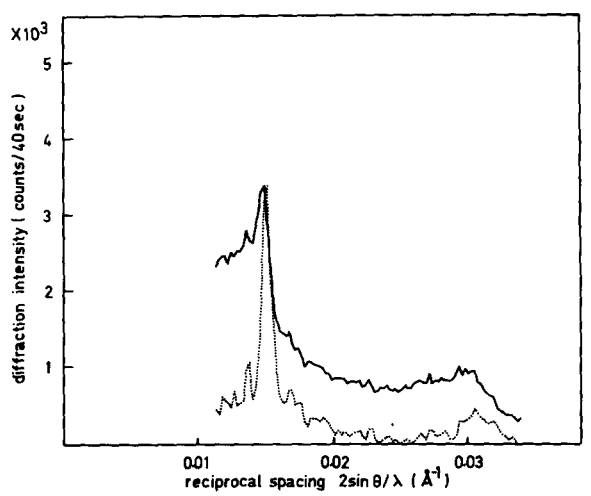

Fig. 2. Small-angle X-ray diffraction profile of DMPC containing 50% water, at 22.5°C. (———) Observed diffraction profile, (· · · · ·) smeared diffraction profile. The step interval was 0.02°. The measuring time at one point was 40 s.

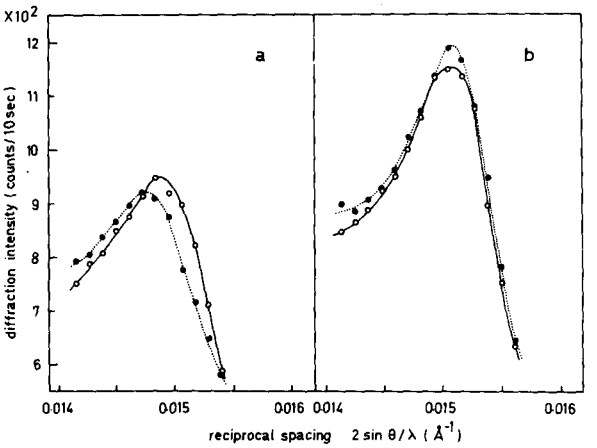

Fig. 3. The observed first-order peak profile in the small-angle region. (a) Temperature jump from 25 to 22.5°C (downward temperature jump). (b) Temperature jump from 22.5 to 25°C (upward temperature jump). (●· · · · ·●) Measured during 5–8 min after application of the temperature jump; (○———○) measured during 100–103 min after application of the temperature jump. The step interval was 0.01° and the measuring time at one point was 10 s.

application of the temperature jump was studied. Fig. 3 shows the profiles of the first-order diffraction measured during 5–8 (dotted line) and 100–103 min (full line) after application of the temperature jump from 25 to 22.5°C (downward temperature jump, a) or in the reverse direction (upward temperature jump, b). The peak position shifted toward the large reciprocal spacing side with the passage of time after application of the downward temperature jump. On the other hand, the peak position did not shift when the temperature jumped in the reverse direction. The apparent repeat period (d), which denotes the repeat period obtained from the observed first-order diffraction peak, is shown in Fig. 4 as a function of time after application of the temperature jump. This apparent repeat period may be about 1 Å larger than the repeat period obtained from the desmeared first-order diffraction peak. Immediately after application of the downward temperature jump, the apparent repeat period increased up to nearly 68 Å from 66.4 Å, which was the value at 25°C, and then decreased gradually to 67.2 Å. The time course of the diffraction intensity at various diffraction angles is shown in Fig. 5. In the case of the downward temperature jump, the diffraction intensity at the large-angle

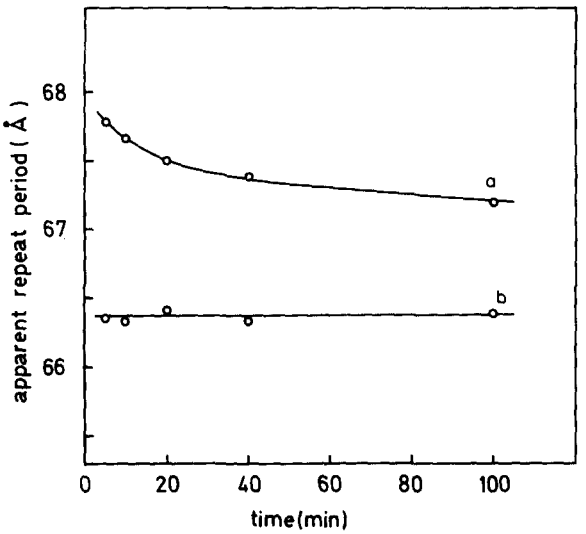

Fig. 4. Apparent repeat period obtained from the observed first-order diffraction peak after application of the temperature jump. (a) Downward temperature jump (25 to 22.5°C). (b) Upward temperature jump (22.5 to 25°C).

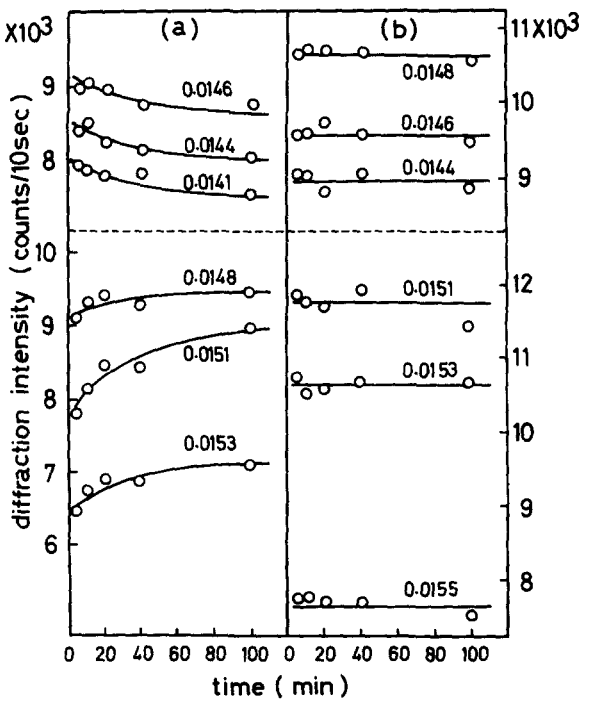

Fig. 5. The time course of the diffraction intensity at various diffraction angles after a downward temperature jump (a) and an upward temperature jump (b). The numerical quantities indicate the value of $2\sin\theta/\lambda$.

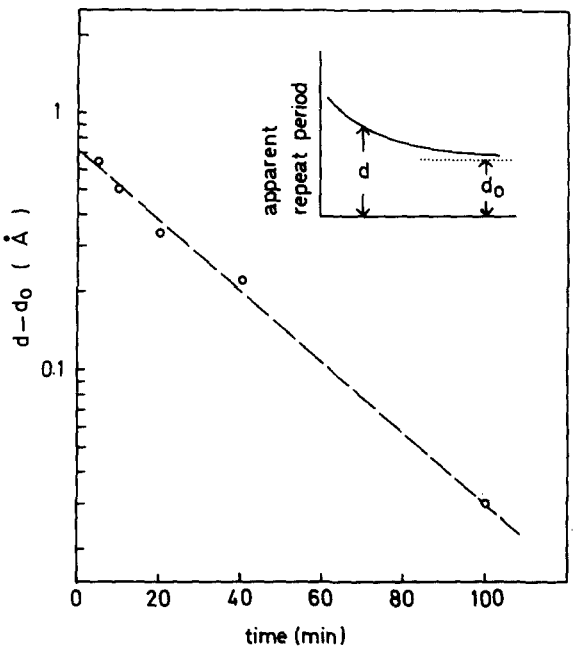

Fig. 6. Logarithmic expression of the apparent repeat period which is assumed to have the form of $d - d_0 = D\exp(-t/\tau)$. The value of d_0 is taken as 67.16 Å. $\tau \approx 70$ min, $D \approx 0.7$ Å.

side of the diffraction peak increased with the passage of time, while that at the small-angle side decreased. In the case of the upward temperature jump, on the other hand, the diffraction intensity did not show any significant change at any diffraction angles although the plots were dispersed. The relaxation time of the decrease in the apparent repeat period was about 70 min, assuming the apparent repeat period to be at the steady state (d_0) of 67.16 Å. The plots of $\log(d - d_0)$ vs. time after application of the temperature jump are shown in Fig. 6. Since there is a considerable uncertainty about the apparent repeat period at the steady state, the accuracy of the relaxation time was low.

It was confirmed by the following method that the second-order diffraction peak shows the same behavior as the first-order diffraction peak. The proportional counter was fixed at an angle corresponding to the second-order diffraction peak at 22.5°C. The X-ray intensity detected gradually increased up to a steady value after the downward temperature jump. The velocity of the increase was comparable to the relaxation time of the repeat period.

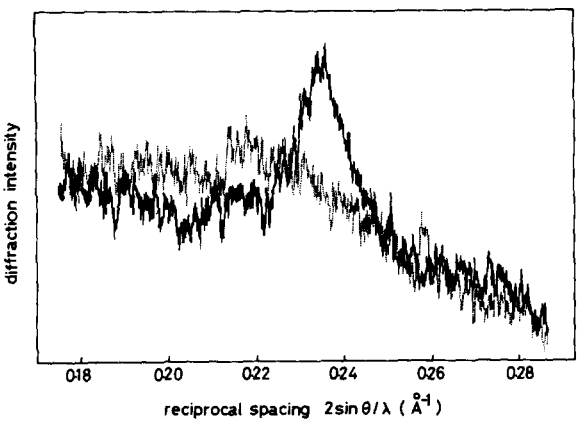

Fig. 7. Large-angle X-ray diffraction profile of DMPC containing 50% water. (———) Profile at 22.5°C. The sharp diffraction peak (approx. 4.25 Å) can be seen. (· · · · · ·) Profile at 25°C. The broad diffraction peak (approx. 4.50 Å) can be seen faintly.

Large-angle X-ray diffraction

Large-angle X-ray diffraction reflects the lateral arrangement of phospholipid molecules. Large-angle X-ray diffraction profiles were obtained by using a standard diffractometer. Fig. 7 shows the large-angle X-ray diffraction profiles obtained at 25 and 22.5°C. A sharp diffraction peak (approx. 4.25 Å) was observed at 22.5°C, and a broad one (approx. 4.50 Å) at 25°C. The large-angle spacing as a function of time after application of the temperature jump is shown in Fig. 8. Since the large-angle diffraction width at

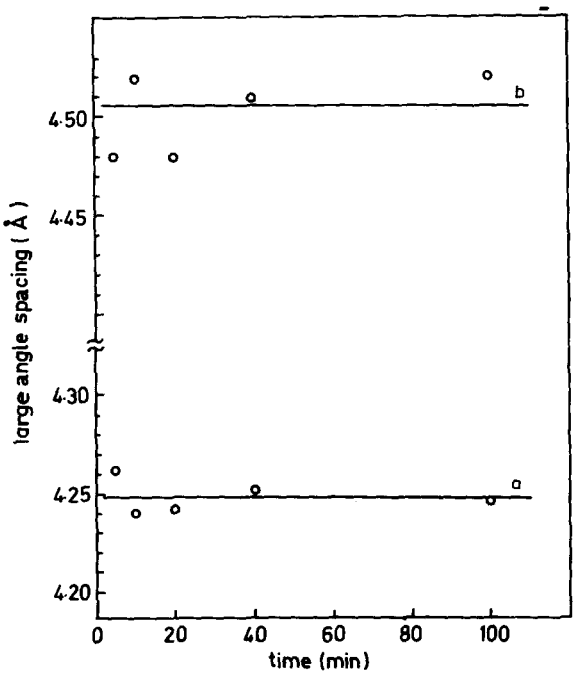

Fig. 8. Large-angle spacing of DMPC containing 50% water after application of the temperature jump. (a) Downward temperature jump (25 to 22.5°C). (b) Upward temperature jump (22.5 to 25°C).

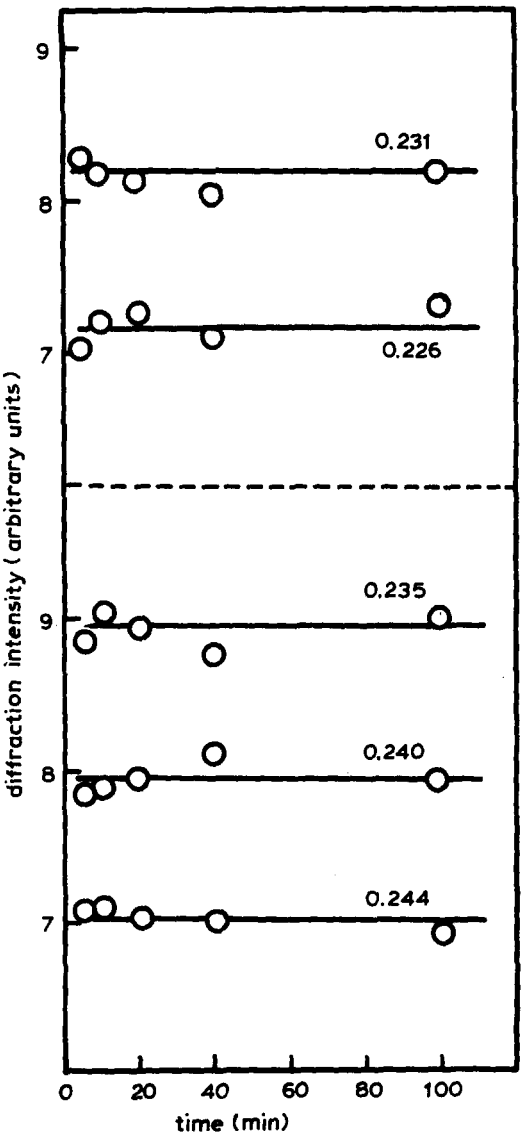

Fig. 9. The time course of the diffraction intensity at various diffraction angles after a downward temperature jump. The numerical quantities indicate the value of $2\sin\theta/\lambda$.

25°C (L_α structure) was very broad, the plots of the spacing at 25°C were dispersed. The time course of the diffraction intensity at various diffraction angles in the case of the downward temperature jump is shown in Fig. 9. The remarkable change as seen in Fig. 5a cannot be seen here. In the case of the upward temperature jump, the plots of diffraction intensity were too dispersed to illustrate. It can be concluded from Figs. 8 and 9 that the change of the lateral arrangement of phospholipid molecules is faster than the change in the stacking of lamellar structures.

Discussion

The phase transition phenomenon is essentially a three-dimensional property of matter. Since an interlamellar interaction, however, may be weaker than the lateral interaction between phospholipid molecules, it is reasonable to assume that phase transition occurs with regard to the lateral arrangement of phospholipid molecules and that, consequently, the arrangement of lamellar stacking occurs gradually. The many papers published hitherto have shown the fast relaxation (less than 10 s) of phase transition [1–4]. As shown in Fig. 8, the phase transition is too fast to detect by the X-ray diffraction technique with respect to the lateral arrangement of phospholipid molecules. As mentioned in Results, the apparent repeat period of multilamellar structures increased up to near 68 Å, at which time the temperature abruptly fell from 25 to 22.5°C. Subsequently, it decreased to 67.2 Å with a relaxation time of 70 min. For this phenomenon only the following hypothesis seems possible.

Each of the lamellar structures which make up the multilamellar structure contracts due to the decrease in the intermolecular distance at the phase transition from the fluid to the crystalline state. At the first stage of the phase transition from the fluid to the crystalline state, a large number of contracted domains must appear in each domain. These domains give rise to wrinkles on the lamellar structures. The average repeat period of a wrinkled lamellar stack (loosely stacked multilamellar structures) must be larger than that of a periodic rippled lamellar stack (closely stacked multilamellar structures, steady state P'_β). As the interlamellar interaction must be considered to be weak, the looseness of stacking remains for some time. Overcoming resistance, the loosely stacked multilamellar structure tends to form a closely stacked multilamellar structure, the resistance being due to, for example, the entrance and exit of water through a lamellar or interlamellar gliding. It cannot be supposed at which step the formation of rippling of a lamella occurs. The process of the phase transition proposed above is summarized as follows:

multilamellar structure	closely stacked	loosely stacked	loosely stacked	closely stacked
lamellar morphology	flat	wrinkled	wrinkled or rippled	rippled
lateral arrangement	fluid	fluid + crystalline	crystalline	crystalline
	temperature jump		→ fast	→ slow

When the temperature jumped upward, the relaxation of the repeat period was not observed. This may have been due to the diminution of the resistance by the fluidity of the lamella.

The relaxation time of 70 min implies a fairly weak interlamellar interaction, or a large resistance which is obstructive to the formation of stacked lamellar structures. A relaxation time of a similar order has been obtained for the DPPC pretransition [5] as mentioned in the Introduction, and for myelin swelling [14] of which there will be further mention. The pretransition is associated with a structural transformation intermediate between the stacking of a flat lamella and that of a rippled lamella. This transition does not occur in a unilamellar vesicle but does occur in a multilamellar vesicle [13]. Therefore, the pretransition is associated with a long-range structural transformation. Such a long-range transformation might be expected to be slow.

Padron et al. [14] studied the kinetics of myelin lattice swelling. According to these authors, the swelling process from subnormal or reassociated myelin to swollen myelin, or from swollen myelin to subnormal myelin, takes place in 1 h or less. Although the two above-mentioned examples are not of the same kind as that of the present work, they do hold in common a structural change associated with the

stacking of a lamella which is slower than that of the lateral arrangement of lipid molecules. An accurate diffraction profile extending over several orders was not measured, so that details of the multilamellar structure such as the thickness of the lamella, the tilt angle of the hydrocarbon chain, the repeat period of ripple, etc., could not be estimated. Only the repeat period of the multilamellar structure could be determined.

References

1 Hammes, G.G. and Tallman, D.E. (1970) J. Am. Chem. Soc. 92, 6042–6046
2 Owen, J.D., Hammes, P. and Eyring, E.M. (1970) Biochim. Biophys. Acta 219, 276–282
3 Tsong, T.Y. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 2684–2688
4 Tsong, T.Y. and Kanehisa, M.I. (1977) Biochemistry 16, 2674–2680
5 Lentz, B.R., Freire, E. and Biltonen, R.L. (1978) Biochemistry 4475–4480
6 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 3862–3866
7 Chapman, D. (1971) Symp. Faraday Soc. 5, 163–174
8 Sklar, L.A., Hudson, B.S. and Simoni, R.D. (1977) Biochemistry 16, 819–835
9 Janiak, M.J., Small, D.M. and Shipley, G.G. (1976) Biochemistry 15, 4574–4580
10 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Biol. Chem. 245, 6068–6078
11 Luzzati, V. (1968) in Biological Membranes (Chapman, D., ed.), pp. 71–124, Academic Press, London
12 Tardieu, A. and Luzzati, V. (1973) J. Mol. Biol. 75, 711–733
13 Lentz, B.R., Barenholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4521–4528
14 Padron, R., Mateu, L. and Kirschner, D.A. (1979) Biophys. J. 28, 231–241