Biochimica et Biophysica Acta, 687 (1982) 337–339
Elsevier Biomedical Press

BBA Report

BBA 70034

KINETICS OF PRETRANSITION IN MULTILAMELLAR DIMYRISTOYLPHOSPHATIDYLCHOLINE VESICLE

X-RAY DIFFRACTION STUDY

M. AKIYAMA [a], Y. TERAYAMA [a] and N. MATSUSHIMA [b]

[a] *Department of Physics, Sapporo Medical College, Sapporo and* [b] *Department of General Arts and Science, Hokkaido Institute of Technology, Sapporo (Japan)*

(Received December 8th, 1981)

Key words: Phase transition kinetics; X-ray diffraction; Dimyristoylphosphatidylcholine

The time course of transformation between P'_β phase and L'_β phase in a multilamellar dimyristoylphosphatidylcholine vesicle containing 50% water was followed by small-angle X-ray diffraction. The transformation of $P'_\beta \rightarrow L'_\beta$ was 90% complete at 7 min after the temperature jump, although imperfections remained for a long time. Transformation in the opposite direction was fast as compared with $P'_\beta \rightarrow L'_\beta$.

The thermal pretransition of a multilamellar phospholipid vesicle is associated with a long-range transformation. In this transition, the hysteresis phenomenon has been observed [1–3]. Therefore, it is supposed that the transformation in the pretransition may be far slower than that in the main transition.

The kinetics of pretransition was studied by Lentz et al. [4] using dipalmitoylphosphatidylcholine (DPPC). The characteristic time ($t_{1/2}$) was measured by two methods. In the first, involving observation of the fluorimetric response at different temperature scanning rates, the characteristic time obtained was 8–30 min. In the second, involving temperature-jump monitoring of the fluorimetric response, a characteristic time of 4–8 min was recorded. It is possible to follow the time course of the transformation by the X-ray diffraction method, when the relaxation time is of a few minutes time range. This paper reports the transformation of multilamellar structure after application of the temperature jump. The time course was followed by the small angle X-ray diffraction method.

Small amounts of dimyristoylphosphatidylcholine (DMPC) were mixed with an equal weight of 0.05 M phosphate buffer (pH 7.0)/0.1 M NaCl/ $2 \cdot 10^{-5}$ M EDTA, after which mixture was incubated at 30°C for 1 h. Samples were sealed in a sample cell made of hard rubber, the window of which made of polyester film. Temperature-controlled fluid flowed around the sample cell. A temperature jump from 10°C (L'_β phase) to 15°C (P'_β phase) or one in the opposite direction was carried out by switching the flow from two individually temperature-controlled fluid baths. These temperature jump were each completed in 1 min.

CuKα radiation obtained from a RIGAU RU200 rotating anode generator was collimated by mirror-monochromator optics. X-ray diffraction profiles were detected with a MOS image sensor (one-dimensional photodiode array) [5]. This detecting system was assembled by UNION GIKEN Co. Japan. Since the X-ray diffraction profile must be detected within 2 min, measurements were repeated 14 times under identical conditions in order to improve the level of accuracy, with the data obtained being accumulated.

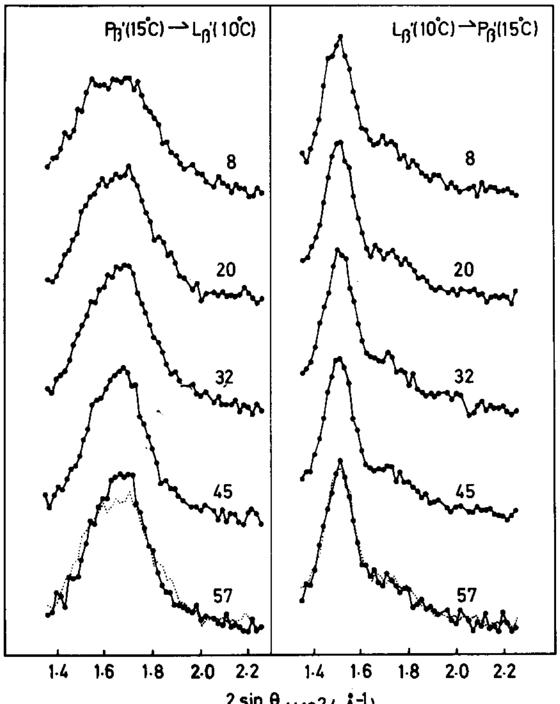

Fig. 1. First-order reflection profiles of DMPC multilamellar containing 50% water. The numerals indicate the time in minutes after temperature jump. The repeat period at 10°C was 60 Å and that at 15°C was 66 Å. The dashed line overlapping on profile 57 is the same as profile 20.

The first-order reflection profiles at various times are shown in Fig. 1. The numerals (t) written in this figure indicate the time (in minutes) after application of the temperature jump. The profiles

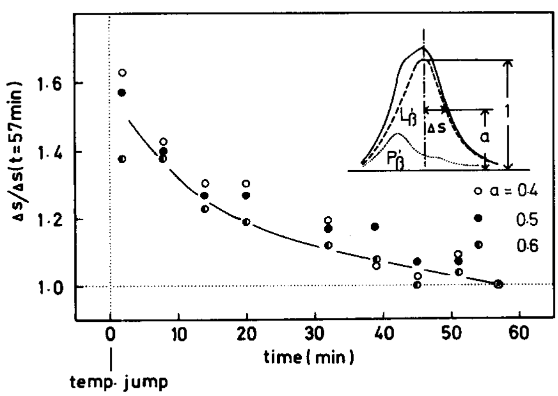

Fig. 2. Time course of the width of reflection arising from the L'_β component. The vertical axis is graduated by the ratio of the width (Δs) to that at $t=57$ ($\Delta s(t=57$ min)).

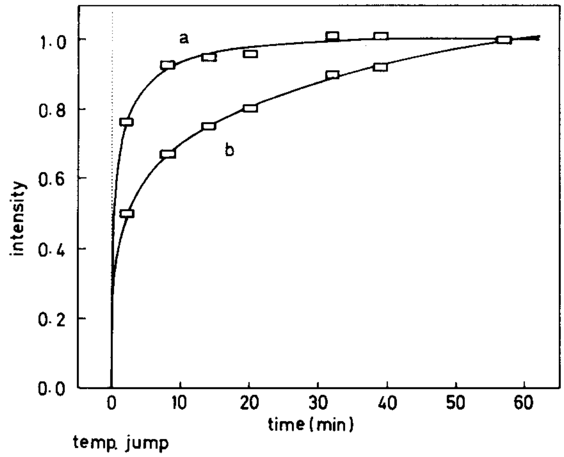

Fig. 3. Time courses of the first-order reflection intensity: (a) integrated intensity; (b) maximum intensity.

were obtained spending 2 min from $t-1$ to $t+1$ min. The multilamellar repeat period at $t=57$ was 66 Å at 15°C and 60 Å at 10°C. In the transformation $P'_\beta \rightarrow L'_\beta$, the change in the diffraction profile progressed slowly. The reflection arising from the P'_β phase ($1.51 \cdot 10^{-2}$ Å^{-1}) still remained in the profile at $t=20$. The width of the reflection on right-hand side decreased with the passage of time. For comparison, the profile at $t=20$ is overlapped on the profile at $t=57$ by a dashed line. The profile at any time can be divided in two components; one being the P'_β component ($1.51 \cdot 10^{-2}$ Å^{-1} region) and the other being the L'_β component ($1.67 \cdot 10^{-2}$ Å^{-1}). The ratio of width at $t=2$ to the width at $t=57$ was about 1.5 and it decayed with a relaxation time of 30 min as shown in Fig. 2. The time courses of the maximum intensity and the integrated intensity of the L'_β component are shown in Fig. 3, normalizing those by the maximum intensity or integrated intensity at $t=57$. It can be seen that the transformation $P'_\beta \rightarrow L'_\beta$ was 90% complete at 7 min after the temperature jump. The increase in the maximum intensity was slower than that of the integrated intensity. This was owing to the slow decrease in the reflection width. The width of reflection refers the randomness of the multilamellar structure or the smallness of domain size. Therefore, imperfection of the multilamellar structure remains for a long time. The P'_β component had 90% disappeared at 17 min after the temperature jump. On the other hand, the transformation $L'_\beta \rightarrow P'_\beta$ was completed in less than 5 min with the exception of a slight increase in maximum intensity. The slight increase in maximum intensity is perhaps due to the rearrangement at the domain boundary.

According to Lentz et al. [4], the characteristic time of the transformation $P'_\beta \rightarrow L'_\beta$ is longer than that of the opposite transformation. Our results agree with these results. Such a phenomenon may be due to the difference in the fluidity of both phases. The transient increase of the multilamellar repeat period which was observed in the main transition $L'_\alpha \rightarrow P'_\beta$ [6] was not observed in the pretransition.

References

1 Sklar, L.A., Hudson, B.S. and Simoni, R.D. (1977) Biochemistry 16, 819–835
2 Träuble, H. (1971) Naturwissenschaften 58, 277–284
3 Tsong, T.Y. and Kanehisa, M.I. (1977) Biochemistry 16, 2674–2679
4 Lentz, B.R., Freire, E. and Biltonen, R.L. (1978) Biochemistry 17, 4475–4480
5 Akiyama, M. and Terayama, Y. (1978) J. Pre-Med. Sapporo Med. Coll. 19, 59–64
6 Akiyama, M. (1981) Biochim. Biophys. Acta 644, 89–95