Barotropic Phase Transitions of Dilauroylphosphatidylcholine Bilayer Membrane

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Abstract
The effect of pressure on the bilayer phase behavior of dilauroylphosphatidylcholine (DLPC), containing two linear saturated acyl chains with 12 carbon atoms, was studied by the high-pressure light transmittance measurements. The DLPC bilayer under high pressure underwent three kinds of transitions in aqueous 50 wt % ethylene glycol solution, whereas only one transition was observed in water. The middle−temperature transition corresponds probably to the main transition because the middle transition is consistent with that in water. The lower−temperature transition is probably assigned to the transition from the lamellar crystalline (Lc) phase to the ripple gel (Pβ') phase. The higher−temperature transition refers to the transition from the intermediate liquid crystalline (Lx) phase to the liquid crystalline (Lα) phase judging from previous data.

Keywords: bilayer, dilauroylphosphatidylcholine, lipid, phase transition, pressure

1. Introduction
The effect of pressure on biological membranes is of particular interest to the studies of pressure adaptation of the deep sea organisms [1], high pressure sterilization in food processing [2-5], and pressure reversal of anesthesia [6]. Lipid bilayer membranes composed of phosphatidylcholines containing two identical linear saturated acyl chain have been most thoroughly studied. Especially, dipalmitoyl phosphatidylcholine (DPPC) containing two linear saturated acyl chains with 16 carbon atoms is one of the most extensively studied phospholipids, but there are few studies on the bilayer phase transition of dilauroylphosphatidylcholine (DLPC) containing acyl chains with 12 carbon atoms [7-14]. It has been known that the phase behavior of DLPC bilayer membrane is different from that of DPPC bilayer. There exists an intermediate liquid crystalline (Lx) phase between the ripple gel (Pβ') and the liquid crystalline (Lα) phase. The Lx phase would be a nontilted, partially disordered liquid crystalline phase [10]. However, there is no consistency in understanding of experimental results [7-14]. In addition, since the phase transition of DLPC bilayer membrane at ambient pressure have been observed at a temperature below 0 °C, the information on the phase behavior has been difficult to obtain by conventional techniques, but such information is readily accessible from high-pressure experiments. So far there have been few studies on the phase behavior of DLPC bilayer membrane under high pressure [13, 14].
The present study demonstrates the pressure effect on the phase behavior of DLPC bilayer membranes.

2. Materials & methods

Synthetic phospholipid DLPC (1,2-dilauroyl-sn-glycero-3-phosphocholine) was purchased from Sigma, and used without further purification. The sample solutions of multilamellar vesicles were prepared by suspending DLPC in water or in aqueous 50 wt% ethylene glycol solution at 5.0 m mol kg\(^{-1}\). Aqueous ethylene glycol solution was used as an antifreeze solvent. Samples were kept in a freezer (at \(-30^\circ\text{C}\)) for duration of about 1 ~ 2 weeks and in high pressure apparatus for 1 hr before measurement starts. The phase transitions of DLPC bilayer membrane under high pressure were observed by the method of isobaric thermotropic measurements, which were described in our previous papers [14, 15]. We observed the abrupt change in transmittance accompanying the phase transition which was followed at 560 nm. The heating rate at a given pressure was 0.3 K min\(^{-1}\).

3. Results & discussion

Since the phase transition temperature of DLPC bilayer membrane at ambient pressure has been reported to be low temperature below 0 °C [7], we used 50 wt% aqueous ethylene glycol solution as an antifreeze solvent as well as water solvent. An example of the thermotropic phase–transition measurements for DLPC bilayer membrane in 50 wt% aqueous ethylene glycol solution and in water is depicted in Fig.1.

The transmittance–temperature profiles under high pressure of 100 MPa show the existence of three kinds of transitions in 50 wt% aqueous ethylene glycol solution (curve 1), whereas there exists only one transition in water (curve 2). This transition in water was reported as the main transition from the P\(_b\)' phase to the L\(_\alpha\) phase. As is seen from Fig. 1, the middle transition–temperature in 50 wt% aqueous ethylene glycol solution was consistent with the main transition temperature in water. The middle transition–temperature in 50 wt% aqueous ethylene glycol solution at 100 MPa is consistent with the main transition in water.

![Fig. 1 Isobaric thermotropic phase transitions of DLPC bilayer observed by light transmittance method at 100 MPa. (1) Three kinds of phase transitions in 50 wt% aqueous ethylene glycol solution. (2) The main transition in water.](image-url)
aqueous ethylene glycol solution and the main transition in water are shown in Fig. 2 as a function of pressure. Two kinds of temperature–pressure lines could be superimposable each other although two lines give a much less overlap at lower pressure regions. Therefore, the middle transition–temperature shown in Fig. 1 can be regarded as the main transition from the gel to the liquid crystalline phase compared with the transition temperature in water. As is seen from Fig. 2, the main transition temperature was almost unaffected by the addition of ethylene glycol.

![Fig. 2 Effect of pressure on the main transition temperature of the DLPC bilayer in water (◇) and that in 50 wt% aqueous ethylene glycol solution (●)](image)

The temperature ($T$) – pressure ($p$) phase diagram of DLPC bilayer membrane in 50 wt% aqueous ethylene glycol solution is shown in Fig. 3. Lower–temperature transition shown in Fig. 1, which shows the most obvious change in transmittance, was plotted as a function of pressure in Fig. 3-A. Broken line in Fig. 3-A represents the main transition shown in Fig. 2. This transition was observed by keeping in a freezer (at $-30$ °C) for duration of about 1 ~ 2 weeks. Addition of ethylene glycol is known to facilitate to reach the most stable state of the lamellar crystalline ($L_c$) state, so-called subgel phase [16, 17]. Therefore, the lower–temperature transition may be regarded as the transition from the $L_c$ phase to the P$_\beta'$ phase judging from the following conditions: (1) presence of ethylene glycol added, (2) low temperature conservation for a long time, (3) under high pressure. All of these conditions seem to facilitate to reach the stable $L_c$ phase.

Higher-temperature transition shown in Fig. 1, which shows the slight change in transmittance, was plotted as a function of pressure in Fig. 3-B. Broken lines in this figure represent the transitions shown in Fig. 3-A. Extrapolation of this $T$ – $p$ line to ambient pressure suggests the temperature of the phase transition to be 4.7 °C. This transition temperature was in good agreement with the transition from the $L_x$ phase to the $L_\alpha$ phase, which has been published previously [9]. Therefore, the higher–temperature transition in 50 wt% aqueous ethylene glycol solution can be decided as the transition from the $L_x$ phase to the $L_\alpha$ phase. The $L_x$ phase disappeared at about 160 MPa. This $L_x$ phase was reported previously to be intermediate partially disordered liquid crystalline phase [9-10], but the phase state is not defined precisely.
Fig. 3 Temperature-pressure phase diagram of DLPC bilayer. (A) The phase transition from the Lc phase to the Pβ' (or Lx) phase. Broken line refers to the main (chain-melting) transition. (B) The phase transition from the Lx phase to the Lα phase. Broken lines represent two kinds of transitions shown in Fig.3-A.

As is seen from Fig. 3, two phase boundaries, namely Lc / Pβ' and Pβ' / Lx curves, crossed each other at about 40 MPa. In the region of low pressure below 40 MPa, three kinds of transitions were observed. A lower-temperature transition refers to the transition from the Pβ' phase to Lα phase, which is a transition between metastable phases. Other two transitions refer to the transition from the Lc phase to Lx phase and subsequently from the Lx phase to the Lα phase, which are transitions between stable phases. In the region of high pressure above 40 MPa, the DLPC bilayer undergoes phase transitions in turn from the Lc phase to the Pβ' phase, from the Pβ' phase to the Lx phase and finally from the Lx phase to the Lα phase. All phases of Lc, Pβ', Lx and Lα are able to exist as the stable phase at pressures above 40 MPa.

4. Conclusion

In 50 wt% aqueous ethylene glycol solution, three kinds of phase transitions of DLPC bilayer were observed under ambient pressure and high pressure. The middle-temperature transition under high pressure corresponds probably to the main transition. The lower-temperature transition may correspond to the transition from the Lc phase to the Pβ' phase. The higher-temperature transition was decided as the transition from the Lx phase to the Lα phase judging from the previous results. Two phase boundary curves (namely, Lc / Pβ' and Pβ' / Lx) crossed each other at about 40 MPa.
5. References


