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High-pressure study on bilayer phase behavior of oleoylmyristoyl- and myristoyloleoyl-phosphatidylcholines

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ABSTRACT

We investigated the thermotropic and barotropic bilayer phase behavior of 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC) and 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (OMPC) by means of the differential scanning calorimetry (DSC) and high-pressure light-transmittance technique. Water could be used as a solvent for measurements at high pressures because of the elevation of the transition temperatures above 0 °C by pressurization, whereas aqueous 50 wt.% ethylene glycol solution was used mainly for those at low pressures. Only one phase transition was observed in the DSC thermogram of the MOPC bilayer membrane as an endothermic peak, and also observed at high pressures as an abrupt change of the light-transmittance. The transition was assigned as a main transition between the lamellar gel (L_β) and liquid-crystalline (L_α) phases on the basis of the values of enthalpy change (ΔH) and slope of the transition temperature with respect to pressure (dT/dP). The DSC thermogram of the OMPC bilayer membrane similarly showed a single endothermic peak but two kinds of phase transitions were observed at different temperatures in the light-transmittance profile at high pressures. The extrapolation of the lower-temperature transition in the high-pressure range to an ambient pressure coincided with the transition observed in the DSC thermogram. This transition was identified as a transition between the lamellar crystal (L_c) and L_α (or L_β) phases from the ΔH and dT/dP values. The higher-temperature transition, appearing only at high pressures, was identified as the L_β/L_α transition considering the topological resemblance of its temperature–pressure phase diagram as that of the dioleoylphosphatidylcholine bilayer membrane. The phase diagram of the OMPC bilayer membrane demonstrated that the L_β phase cannot exist at pressures below ca. 190 MPa while it can exist stably in a finite temperature range at pressures above the pressure.

1. Introduction

Biological membranes contain many kinds of phospholipids and the biological significance of the diversity of the lipid species is still a matter of great interest. Unsaturated phospholipids, which have mono- or poly-unsaturated acyl chains such as oleoyl-, linoleoyl- and arachidonoyl-chains, are known as major components of biomembranes. The bilayer phase behavior of unsaturated phospholipids has been intensely investigated. Lewis et al. [1] have demonstrated that the transition temperatures of bilayer membranes of the dioleoylphosphatidylcholine (DOPC) and the six kinds of its longer acyl-chain homologues are much lower than those of their saturated *n*-acyl counterparts. They have also revealed that the phase transition of the DOPC bilayer observed at −11.8 °C is a transition between the lamellar crystal (L_c) and liquid-crystalline (L_α) phases and not the main transition between the lamellar gel (L_β) and L_α phases. Our previous studies on the DOPC bilayer membrane [2–4] has shown that the L_β phase can exist as a stable phase at high pressures above ca. 300 MPa in aqueous 50 wt.% ethylene glycol solution and above 234 MPa in water. Further, the linear extrapolation of the high-pressure data to the atmospheric pressure has given not only the confirmation that the transition temperature of the L_c/L_α phase transition is about −12 °C at the atmospheric pressure, but also that the temperature of a *hypothetical* main transition at the atmospheric pressure is −40.3 °C. This temperature is 95 °C lower than that of the distearoylphosphatidylcholine (DSPC) bilayer membrane (55 °C).

An outstanding feature in naturally occurring unsaturated phospholipids is that they usually have one unsaturated acyl chain in the *sn*-2 position of the glycerol backbone and that almost all the double bonds have the *cis*-configuration [5]. Such phospholipids are generally classified as asymmetric unsaturated phospholipids. Despite the biological importance of why biomembranes show such configurational preference as well as the scientific interest in how the chain unsaturation and the asymmetry of the *sn*-1 and *sn*-2 acyl chains affect the membrane properties, only a few researches have been conducted [3,6–11]. Fundamental data are thought to be necessary in order to clarify the effect of the acyl-chain asymmetry on the membrane properties in a systematic way. Although the effect has been often dealt

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with for asymmetric phospholipids with two saturated acyl chains of different length in relation to the bilayer interdigitation [12], it has not yet been understood for those with a saturated and an unsaturated chain.

In this paper, we report on the thermotropic and barotropic phase behavior of the 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC) and 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (OMPC) bilayer membranes revealed by using differential scanning calorimetry (DSC) and high-pressure light-transmittance technique. The chemical structures of these phospholipids are illustrated in Fig. 1. In the case of symmetric saturated phospholipids, the *sn*-1 acyl chain is virtually longer than the *sn*-2 acyl chain by 1.5 carbon–carbon bond lengths due to the inherent difference of the conformation near the ester linkage on the glycerol backbone between the *sn*-1 and *sn*-2 acyl chains [13]. As seen from the figure, this inherent separation at the terminal methyl ends enhances the chain inequivalence for the OMPC molecule whereas it conversely reduces the inequivalence for the MOPC one. This means that MOPC and OMPC are just a pair of positional isomers but very different from each other in terms of the acyl-chain inequivalence. As far as we know, there is no report on the bilayer phase behavior of the MOPC and OMPC bilayer membranes. The lack of data on these lipids is mainly attributable to the experimental difficulty in detecting the lower phase-transition temperatures of these lipids than the freezing temperature of water. To prevent water from freezing we used aqueous 50 wt.% ethylene glycol solution as an antifreeze solvent. Ethylene glycol solution is generally used for studies on low-temperature transitions of phospholipid bilayer membranes. It is known for bilayer membranes of typical saturated PCs that the bilayer interdigitation is induced in the presence of 20–30 wt.% ethylene glycol [14]. For phospholipid bilayer membranes that are inherently difficult to transform into the interdigitated structure, such as unsaturated PC bilayers, however, its presence is believed to produce no drastic effect on the bilayer phase behavior itself though it surely tends to shift the transition temperature to slightly higher values and to bring about larger quantity of enthalpy change for the bilayer transition relating to the L_c phase [1,2].

The application of pressure produces several advantages. First, it allows us to determine the pressure dependence of the transition temperature (i.e., dT/dP). It is one of important thermodynamic properties because its value is closely related to the kind of the bilayer phase transition, such as the main transition and subtransition, as expected from the Clapeyron equation ($dT/dP = T\Delta V/\Delta H$). Second, the pressurization causes the elevation of the transition temperature, and the transition temperature below 0 °C at an ambient pressure can be shifted toward higher-temperature region above 0 °C at high pressures. This elevation allows us to use water as a solvent in the high-pressure region and to compare the results for the phospholipid bilayer systems in water and in ethylene glycol. This paper discusses the phase behavior by constructing the temperature–pressure phase diagrams and the thermodynamic properties of the phase transitions for the MOPC and OMPC bilayer membranes.

2. Experimental

2.1. Materials and sample preparation

Asymmetric unsaturated phospholipids, 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC) and 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (OMPC), were purchased from Avanti Polar Lipids Inc. (Alabaster, AL) and used without further purification. Ethylene glycol (purity>99.5%) was purchased from Kanto Chemical Co., Inc. (Tokyo). A translucent phospholipid suspension was obtained by sonicating the mixture of an appropriate amount of the phospholipid sample and water purified by double distillation or aqueous 50 wt.% ethylene glycol solution for about 10 min. The lipid concentrations were adjusted to 10.0 mmol kg^{-1} (7.26×10^{-1} wt.%) in 50 wt.% ethylene glycol solution for the DSC measurements, and to 1.0 mmol kg^{-1} (7.26×10^{-2} wt.%) in water and 2.0 mmol kg^{-1} (1.45×10^{-1} wt.%) in 50 wt.% ethylene glycol solution for the light-transmittance measurements, respectively. The dispersion in ethylene glycol solution was usually more transparent than that in water at the same lipid concentration. In order to obtain the sufficient turbidity for the light-transmittance measurements, the lipid concentration in ethylene glycol solution was adjusted to be two times higher than that in water. All the sample solutions were prepared without thermal pretreatments, such as cold storage for a long time.

2.2. DSC and light-transmittance measurements

DSC measurements were carried out using an SSC 5200-DSC 120 calorimeter (SII Nanotechnology Co. Ltd., Chiba) with an externally attached equipment for cooling sample and reference solutions below 0 °C. A 60-μL aliquot of sample solution and an identical volume of solvent (reference solution) were separately poured into DSC silver cells, and the silver cells were sealed with silver lids. The sample and reference cells were kept standing at ca. −30 °C in the furnace chamber for 10 min or more before every scan for the equilibration. The heating rate was 0.30 K min^{-1}. The enthalpy changes of the bilayer phase transitions were determined from areas of endothermic peaks in the DSC thermograms and averaged over at least 5 scans.

Light-transmittance measurements were performed using a U-3010 spectrophotometer (Hitachi High-Technologies Corp., Tokyo) equipped with a high-pressure cell assembly PCI-400 (Teramecs, Kyoto). Pressure applied to the sample solution was generated by a

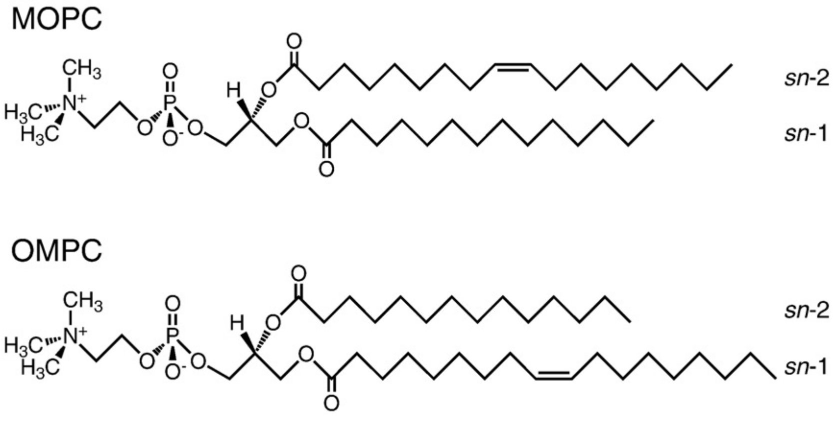

Fig. 1. Chemical structures of MOPC and OMPC molecules.

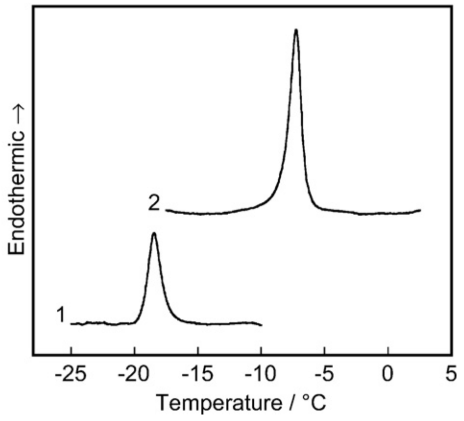

Fig. 2. DSC thermograms of (1) MOPC and (2) OMPC bilayer membranes in aqueous 50 wt.% ethylene glycol solutions.

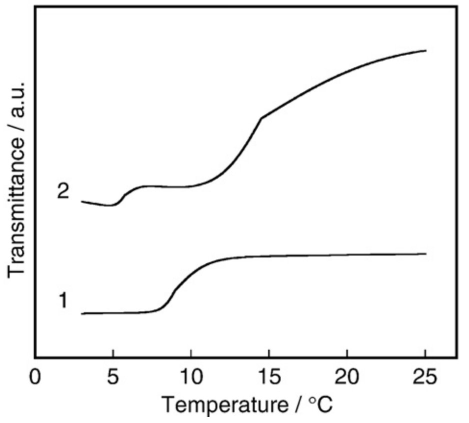

Fig. 3. Light-transmittance vs. temperature curves for (1) MOPC bilayer membrane in water at 160 MPa and (2) OMPC bilayer membrane in water at 267 MPa. The abrupt changes of the transmittance correspond to bilayer phase transitions. The light-transmittance profiles were vertically shifted to avoid overlapping each another. The scale of the transmittance is arbitrary for each profile and not unified for both profiles.

hand-operated hydraulic pump (Hikari High Pressure Instruments, Hiroshima) and monitored using a Heise model CM dial pressure gauge (Ashcroft Inc., Stratford, CT) within the accuracy of 0.2 MPa. Temperature of the sample was controlled by circulating thermostated water from an EYELA programmable circulator PCC-7000 (Tokyo Rikakikai Co. Ltd.,) through the jacket enclosing the high-pressure cell. Relative change of the intensity of the monochromatic light (wavelength: 560 nm) transmitted through the quartz cell filled up with the sample solution was measured with varying temperature at a heating rate of 0.33 K min^{-1} under high pressure. The transition temperature was determined as a temperature at the midpoint of an abrupt increase of the intensity of transmitted light in a narrow temperature range.

3. Results and discussion

3.1. Phase transitions of MOPC and OMPC bilayer membranes

Fig. 2 shows the DSC thermograms of the MOPC and OMPC bilayer membranes in aqueous 50 wt.% ethylene glycol solutions at an ambient pressure. Each of the bilayer membranes showed a single endothermic peak at −19.1 °C for the MOPC bilayer membrane or at −8.0 °C for the OMPC bilayer membrane, indicating that the bilayer membranes show only a kind of thermotropic phase transition at least in the measured temperature range from −30 °C to ca. 20 °C. However, it is evident that the areas of those peaks are different from each other: the values of the transition enthalpy (Δ*H*) were estimated to be 20.1 kJ mol^{-1} for the MOPC bilayer membrane and 38.5 kJ mol^{-1} for the OMPC bilayer membrane. These thermal data obtained by DSC are summarized in Table 1 in the form of average value ± standard deviation. The difference in the Δ*H* values suggests the possibility that both the endothermic peaks correspond to different types of thermotropic phase transitions, respectively. For 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) and 1-oleolyl-2-stearoyl-*sn*-glycero-3-phosphocholine (OSPC) bilayer membranes [11], the Δ*H* values of the main transition (i.e., L_β/L_α transition) have been determined to be 29.3 and 28.0 kJ mol^{-1}, respectively. The corresponding values for 1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (SMPC) and 1-myristoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (MSPC) have also been revealed as 21.8 and 29.0 kJ mol^{-1} [15], or 24.7 and 33.0 kJ mol^{-1} [16], respectively. These data indicates that the difference in the Δ*H* value between the MOPC and OMPC bilayer membranes is too large to explain in terms of the chain-length asymmetry originated from the positional difference of the introduction of an unsaturated chain as long as both the bilayer membranes are presumed to undergo the same kind of the phase transition. Moreover, we notice that the Δ*H* value of the OMPC bilayer membrane is remarkably larger than those of all the other bilayer membranes of the asymmetric phospholipids. The thermotropic gel-to-liquid-crystalline phase transition of phospholipid bilayers is attributable to the conformational change of hydrocarbon chains in the lipid molecules from all-*trans* conformers to conformers containing several *gauche* bonds (i.e., chain-melting). Therefore, the Δ*H* value, as a rule, becomes larger according as the hydrocarbon chains of the phospholipid molecule become longer. Taking this into account, it can be reasonably inferred from the large Δ*H* value of the OMPC bilayer membrane, larger even than that of the OSPC bilayer membrane, that the transition observed at −8.0 °C for the OMPC bilayer is not the L_β/L_α phase transition, but possibly the L_c/L_α phase transition.

In order to clarify the phase transitions of the MOPC and OMPC bilayer membranes, we observed those transitions under high pressure using light-transmittance technique. Fig. 3 shows some typical light-transmittance vs. temperature curves of the MOPC and OMPC bilayer membranes in water at high pressures. Water can be used as a solvent in the high-pressure region because the pressurization causes the elevation of the phase-transition temperature of the bilayer membranes above 0 °C. The curve 1 obtained at 160 MPa exhibited an abrupt change in transmittance at 9.0 °C, and similar

Table 1
Thermodynamic properties of bilayer phase transitions for MOPC and OMPC bilayer membranes

Lipid	Transition	Transition Temperature		d*T*/d*P* (K MPa^{-1})	Δ*H* (kJ mol^{-1})	Δ*S* (J K^{-1} mol^{-1})	Δ*V* (cm^3 mol^{-1})
		(°C)	(K)				
MOPC	L_β/L_α	−19.1 ± 0.05[a]	254.1 ± 0.05[a]	0.173	20.1 ± 2.94[a]	79	13.7
OMPC	(L_β/L_α)	−26.5	246.7	0.150	–	–	–
	L_c/L_α	−8.0 ± 0.40[b]	265.2 ± 0.40[b]	0.062	38.5 ± 1.30[b]	145	9.0

Transition temperature, Δ*H* and Δ*S* are from DSC, d*T*/d*P* is from light-transmittance, Δ*V* is from calculation using Clapeyron equation.

[a] Averaged value over 6 individual data ± standard deviation.

[b] Averaged value over 5 individual data ± standard deviation.

profiles were obtained in the pressure range from 145 to 252 MPa, signifying that the MOPC bilayer membrane undergoes one phase transition also under high pressure. On the other hand, the OMPC bilayer membrane in water showed a different profile at 267 MPa as seen in the curve 2: there are two stepwise changes in transmittance at 5.7 and 13.5 °C. This means that the OMPC bilayer membrane passed through two kinds of phase transitions with increasing temperature under high pressure, in other words, there exist three kinds of phase states in the bilayer under high pressure.

Since the OMPC bilayer membrane takes different phase behavior in the high-pressure range, we examined the phase behavior of the bilayer in the pressure region including intermediate pressures (70–170 MPa). In the pressure region, we used the aqueous 50 wt.% ethylene glycol solution instead of water as a solvent because the solvent water can completely freeze. The resulting typical profiles are shown in Fig. 4. The curve 1 obtained at 268 MPa exhibited two stepwise changes in transmittance at 7.2 and 11.7 °C even in the ethylene glycol solution. As compared to the profile of the OMPC bilayer in water at 267 MPa (curve 2 in Fig. 3), the transition temperatures for the ethylene glycol system are similar to those for the water system although the line-shapes of both the profiles are rather different from each other. The difference in line-shape is probably due to the difference of the dispersion states of the samples in water and in ethylene glycol solution. The transmittance profile obtained at 77 MPa (curve 2) had only one steep change. This finding implies that the OMPC bilayer membrane undergoes a phase transition at the pressure, and that the transition seems to be the same transition as observed in the DSC thermogram at an ambient pressure. Similar profiles were confirmed in the pressure range up to ca. 170 MPa. Thus, we can say that the phase behavior of the OMPC bilayer alters below and above the pressure.

3.2. Temperature–pressure phase diagrams of MOPC and OMPC bilayer membranes

The transition temperatures were plotted against pressure to draw the temperature (T)–pressure (P) phase diagrams for the MOPC and OMPC bilayer membranes. The phase diagrams of both the bilayer membranes are presented in Figs. 5 and 6, respectively. For the MOPC bilayer membrane, the transition temperature is elevated almost linearly with increasing pressure. The linear extrapolation of the transition temperatures at high pressures to the atmospheric pressure (i.e., 0.1 MPa) gave a value of −18.5 °C as the transition temperature at the atmospheric pressure. The temperature is almost equal to the transition temperature of −19.1 °C obtained from the DSC measurement at the atmospheric pressure. This good agreement suggests that both measurements detected the same kind of the phase transition of the MOPC bilayer membrane. Thus, the MOPC bilayer membrane undergoes only a single kind of phase transition in the whole temperature and pressure ranges in the present study. The dT/dP value was evaluated to be 0.173 K MPa^{-1}, and it is comparable to those for the L_β/L_α transitions of various asymmetric unsaturated phospholipids [3]: 0.181 K MPa^{-1} for the SOPC bilayer, 0.175 K MPa^{-1} for the OSPC bilayer, and 0.186 K MPa^{-1} for the 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayer. Therefore, it is reasonably valid to assign the phase transition of the MOPC bilayer membrane as the L_β/L_α transition. Furthermore, this phase assignment is also consistent with the ΔH value of 20.1 kJ mol^{-1} considering that the ΔH values for the L_β/L_α transition of the SOPC [11] and POPC bilayer membranes [17] are 29.3 kJ mol^{-1} and 22.6 kJ mol^{-1}, respectively. The thermodynamic properties of the bilayer phase transition are listed in Table 1, where the volume change (ΔV) was calculated by using the Clapeyron equation.

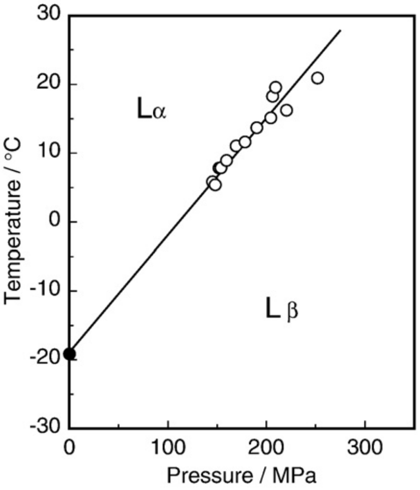

Fig. 5. Temperature–pressure phase diagram for MOPC bilayer membrane. Open and filled symbols represent data for the system in water and for the system containing 50 wt.% ethylene glycol, respectively.

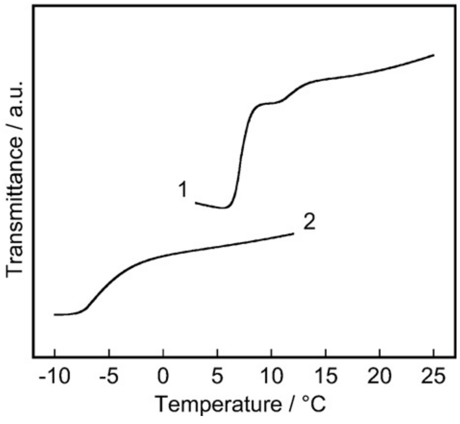

Fig. 4. Light-transmittance vs. temperature curves for OMPC bilayer membrane in aqueous 50 wt.% ethylene glycol solution at (1) 268 MPa and at (2) 77 MPa. The abrupt changes of the transmittance correspond to bilayer phase transitions. The light-transmittance profiles were vertically shifted to avoid overlapping each another. The scale of the transmittance is arbitrary for each profile and not unified for both profiles.

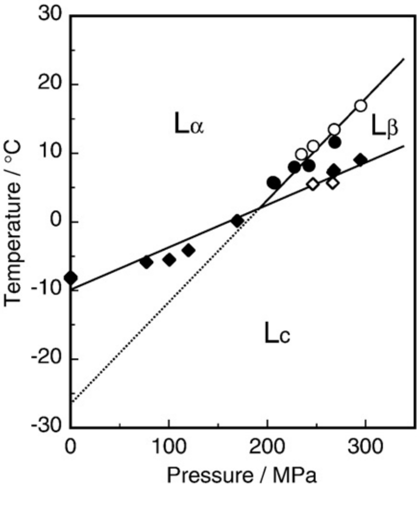

Fig. 6. Temperature–pressure phase diagram for OMPC bilayer membrane. Open and filled symbols represent data for the system in water and for the system containing 50 wt.% ethylene glycol, respectively.

The *T*–*P* phase diagram of the OMPC bilayer membrane was apparently different and rather complicated than that of the MOPC bilayer membrane as shown in Fig. 6. There were two lines of phase boundaries with different positive slopes with respect to pressure in the diagram: one had a comparatively gentle slope over the whole pressure range and the other appeared at high pressures above ca. 190 MPa with a somewhat steeper slope. The former curve had a slope of 0.062 K MPa^{-1} and gave an extrapolated temperature of −10 °C for the transition at the atmospheric pressure. The temperature is close to the transition temperature of −8.0 °C observed in the DSC thermogram. Thus, the phase transition detected by the DSC measurements can be regarded as the same transition as the lower-temperature transitions at high pressures in the light-transmittance measurements. The d*T*/d*P* value is definitely small as compared to those for the L_β/L_α phase transitions of various asymmetric unsaturated phospholipids [3]. The small value is characteristic of the L_c/L_α or L_c/L_β transitions, though it is still smaller than the typical value [3,18]. Considering the above facts with the large Δ*H* value, we judged that the transition is the transition between the L_c and L_α (or L_β) phases.

The latter curve had a slope of 0.150 K MPa^{-1} at pressures above ca. 190 MPa and intersected with the former curve at ca. 1.8 °C and ca. 190 MPa. We have similar phase diagrams for several kinds of lipid bilayer membranes such as the DOPC [2–4] and dilauroylphosphatidylcholine (DLPC) bilayer membranes [19]. Taking into account such topological resemblance of the phase diagrams, we assigned the latter transition as the L_β/L_α transition. The linear extrapolation of the transition temperatures at high pressures to the atmospheric pressure gave the hypothetical L_β/L_α transition temperature of −26.5 °C, which is about 18 °C lower than the L_c/L_α transition temperature. In the case that the difference in temperature between the L_c/L_α and L_β/L_α transitions is small, the L_β/L_α transition is often detectable as a transition between metastable phases (i.e., supercooled state) even at the atmospheric pressure [19]. This is because the complete formation of the thermodynamically stable L_c phase is a kinetically slow process accompanying dehydration around polar head groups [20]. However, the L_β/L_α transition of the OMPC bilayer membrane was not observed in the present DSC measurements, which suggests that the bilayer membrane cannot exist even as a metastable L_β phase at such lower temperatures because of the thermodynamic instability. The falling process into the L_c phase may be a relatively fast process in the OMPC bilayer membrane.

The transformation from the L_c phase to the L_α phase is considered to include mainly two processes; the hydration at the bilayer surface and the chain-melting at the bilayer core. These processes are generally accompanied by large enthalpy and volume changes in comparison with those for the L_β/L_α transition [4]. In the case of the OMPC bilayer membrane, the value of Δ*H* for the L_c/L_α transition is significantly large, on the contrary, the Δ*V* value is smaller than that for the L_β/L_α transition of the MOPC bilayer membrane by about 5 cm^3 mol^{-1} as is seen from Table 1. The large Δ*H* value is attributable to the total amount of the enthalpy changes associated with the processes of the hydration and the chain-melting, which is consistent with the normal tendency. On the other hand, the reason for the unexpectedly small volume change of the L_c/L_α transition has not yet been fully understood, but we currently count the following two possibilities as the reason: one is the melting of ice-like structure water around polar head groups with the transition from the L_c phase to the L_α phase, and the other is a large partial molar volume of the OMPC molecule in the L_c state due to the acyl-chain asymmetry and the presence of a *cis*-double bond. As for the former possibility, considering that about 10% decrease in molar volume occurs at the transition from pure ice to pure water, a simple calculation reveals that the melting of three or four water molecules per one OMPC molecule can cause the negative volume change of about 5.4–7.2 cm^3 mol^{-1}. This decrease can sufficiently compensate for the shortage of Δ*V* for the L_c/L_α transition of the OMPC bilayer membrane as compared to Δ*V* of the chain-melting transition for the MOPC bilayer. Although the possibility seems valid in quantity, it is rather theoretical or hypothetical and not probable because the calculation is based on several tacit assumptions. For example, water existing in interlamellar spaces must freeze in spite of the presence of a large amount of ethylene glycol, and moreover, the frozen water must have the same structure as ice-I, which is unique of all the known ice structures in that the decrease in molar volume occurs on melting. The latter possibility refers to the intrinsic nature of the chain asymmetry. Our previous study on the bilayer membranes including those of DSPC, DOPC, SOPC and OSPC [3] has shown that the chain asymmetry including chain unsaturation markedly decreases the volume change of the chain-melting transition. As shown in Fig. 1, the inequivalence between the *sn*-1 and *sn*-2 acyl chains is greater for OMPC than for MOPC because of the inherent separation of 1.5 carbon–carbon bond lengths along the molecular axis between the terminal methyl ends of the *sn*-1 and *sn*-2 chains. Taking this into consideration, the greater inequivalence of the acyl chains can bring about a looser molecular packing, corresponding to the large partial molar volume, even in the L_c state. Consequently, the chain-melting does not produce so large volume change as expected. This possibility is rather qualitative but probably relevant to the small Δ*V* value of the L_c/L_α transition for the OMPC bilayer membrane.

In conclusion, we clarified the thermotropic and barotropic phase behavior and thermodynamic properties of the phase transitions of the MOPC and OMPC bilayer membranes. The phase diagrams of both bilayer membranes were essentially different from each other. The MOPC bilayer membrane exhibited only the L_β/L_α phase transition in the whole temperature and pressure ranges in this study. On the other hand, the OMPC bilayer membrane underwent the L_c/L_α phase transition at pressures below ca. 190 MPa and did the L_c/L_β and L_β/L_α transitions above the pressure. This fact means that the L_β phase in the OMPC bilayer membrane cannot exist even in a metastable state below the pressure but can exist as a stable phase in a finite temperature range above the pressure. Therefore, we can say that the chain-length asymmetry caused by the positional difference of an unsaturated chain provides a significant influence on the gel-phase stability of the bilayer membranes. It should be noted that there is a possibility that the MOPC bilayer membrane could also fall into the L_c state if a thermal pretreatment such as a cold storage for a long time or repeated thermal cycle by alternative heating and cooling, what is called annealing [20], is applied to the sample solution, although we did not perform any thermal annealing on both the MOPC and OMPC samples in this study. In the near future, we will report the thermodynamic properties of the bilayer membranes for various pairs of an asymmetric phospholipid with a saturated and an unsaturated chain and its acyl-chain positional isomer to reveal the effect of the acyl-chain unsaturation and asymmetry on the bilayer phase behavior.

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