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Barotropic and thermotropic bilayer phase behavior of positional isomers of unsaturated mixed-chain phosphatidylcholines

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ABSTRACT

The bilayer phase transitions of six kinds of mixed-chain phosphatidylcholines (PCs) with an unsaturated acyl chain in the *sn*-1 or *sn*-2 position, 1-oleoyl-2-stearoyl- (OSPC), 1-stearoyl-2-oleoyl- (SOPC), 1-oleoyl-2-palmitoyl- (OPPC), 1-palmitoyl-2-oleoyl- (POPC), 1-oleoyl-2-myristoyl- (OMPC) and 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC), were observed by means of differential scanning calorimetry (DSC) and high-pressure light transmittance measurements. Bilayer membranes of SOPC, POPC and MOPC with an unsaturated acyl chain in the *sn*-2 position exhibited only one phase transition, which was identified as the main transition between the lamellar gel (L_β) and liquid crystalline (L_α) phases. On the other hand, the bilayer membranes of OSPC, OPPC and OMPC with an unsaturated acyl chain in the *sn*-1 position exhibited not only the main transition but also a transition from the lamellar crystal (L_c) to the L_β (or L_α) phase. The stability of their gel phases was markedly affected by pressure and chain length of the saturated acyl chain in the *sn*-2 position. Considering the effective chain lengths of unsaturated mixed-chain PCs, the difference in the effective chain length between the *sn*-1 and *sn*-2 acyl chains was proven to be closely related to the temperature difference of the main transition. That is, a mismatch of the effective chain length promotes a temperature difference of the main transition between the positional isomers. Anomalously small volume changes of the L_c/L_α transition for the OPPC and OMPC bilayers were found despite their large enthalpy changes. This behavior is attributable to the existence of a *cis* double bond and to significant inequivalence between the *sn*-1 and *sn*-2 acyl chains, which brings about a small volume change for chain melting due to loose chain packing, corresponding to a large partial molar volume, even in the L_c phase. Further, the bilayer behavior of unsaturated mixed-chain PCs containing an unsaturated acyl chain in the *sn*-1 or *sn*-2 position was well explained by the chemical-potential diagram of a lipid in each phase.

1. Introduction

Biological membranes of organisms living in various environments contain many kinds of phospholipids. Phospholipids found in most biological membranes have two different acyl chains attached to a glycerol backbone in the molecules. The acyl chain in the *sn*-1 position is typically saturated, whereas that in the *sn*-2 position is usually unsaturated and sometimes has a different carbon number compared to the *sn*-1 acyl chain. Such phospholipids are classified as asymmetric unsaturated phospholipids [1]. The biological importance of unsaturated fatty acids is believed to be related to the fact that their melting points are much lower than those of saturated fatty acids. Thus, membrane lipids containing *cis* unsaturated acyl chains tend to have lower temperatures of transition from the gel phase (L_β) to the liquid crystalline (L_α) phase. With respect to asymmetric phospholipids, the effect of the position (*sn*-1 or *sn*-2) of the unsaturated acyl chain on bilayer properties is not clear although its biological importance is very interesting. Investigations of asymmetric phospholipids under ambient pressure have been performed by conventional differential scanning calorimetry (DSC) [2–9], high-sensitivity DSC [10–13], X-ray diffraction [7,9,13], Raman and IR spectroscopy [13–17] and NMR [17,18]. Keough and Davis [2] synthesized 1-palmitoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (PMPC) and 1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (MPPC), and studied the effect of difference in acyl-chain length between the *sn*-1 and *sn*-2 positions on phospholipid bilayer membranes by DSC. Their results demonstrated that both the transition temperature and the transition enthalpy of the PMPC bilayer membrane had lower values than the corresponding values of the MPPC bilayer membrane [2]. Other reports [19–22] have also revealed for saturated mixed-chain phospholipids that a lipid containing a longer acyl chain in the *sn*-2 position has a higher transition temperature and enthalpy. However, there are few studies on unsaturated mixed-chain phospholipids. Only the transition temperatures for two pairs of positional isomers of unsaturated mixed-chain phosphatidylcholines (PCs) have been reported [3,23]. Since the phase transitions of unsaturated lipid bilayers at ambient

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pressure are sometimes observed at low temperatures below 0 °C, it is difficult to obtain information regarding the phase behavior by conventional techniques, but such information is readily accessible by high-pressure experiments. In our previous studies on 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) bilayers [24,25], a new L_{β}/L_{α} transition between stable phases was found under high pressure, as well as a transition from the lamellar crystal (L_c) phase to the Lα phase, which was observed in the entire pressure range.

In the present study, we report the effect of pressure on the bilayer phase transitions of three pairs of the positional isomers of unsaturated mixed-chain PCs, revealed by using DSC and high-pressure light transmittance techniques. The thermotropic and barotropic phase transitions of the unsaturated mixed-chain PC bilayer membranes are discussed using the temperature–pressure phase diagrams and thermodynamic quantities associated with the phase transitions.

2. Experimental procedures

2.1. Materials

Asymmetric phospholipids, 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (OSPC), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC), 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (OPPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (OMPC) and 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC), were purchased from Avanti Polar Lipids Inc. (Alabaster, AL) and/or Sigma-Aldrich Chemical Co. (St. Louis, MO). They were used without further purification. Lot numbers of phospholipids used in this study were as follows: OSPC, Avanti #181-180PC-20; SOPC, Avanti #180-181PC-47; OPPC, Avanti #181-160PC-49; POPC, Avanti #160-181PC-171; OMPC, Avanti #181-140PC-15; MOPC, Avanti #140-181PC-13 and Sigma #054H8461. Purity of the lipids was more than 99% and the positional purity of fatty acids in the lipids was more than 97%. Water was distilled twice from dilute alkaline permanganate solution. Ethylene glycol (EG) (purity>99.5%) was obtained from Kanto Chemical Co., Inc. (Tokyo). Phospholipid vesicle solutions were prepared by suspending each phospholipid in water or aqueous 50 wt.% EG solution at a definite concentration. The suspensions were sonicated for a few minutes using a Branson model 185 sonifier and a cup horn at a temperature above the main-transition temperature.

2.2. DSC measurements

DSC measurements were performed using an SSC 5200-DSC 120 calorimeter (SII Nanotechnology Co. Ltd, Chiba). Vesicle solutions prepared at concentrations from 5.0 to 10.0 mmol kg^{-1} were sealed up to the amount of 60 μl in DSC silver cells. Water or aqueous 50 wt.% EG solution was used as a reference solution. Measurements were carried out at a heating rate of 0.30 K min^{-1}. The enthalpy changes of the phase transitions were determined as average values from areas of endothermic peaks in several measurements.

2.3. Light transmittance measurements

The vesicle solutions for light-transmittance measurements were prepared at a concentration of 1.0 mmol kg^{-1} in water or at a concentration of 2.0 mmol kg^{-1} in aqueous 50 wt.% EG solution. To transform the bilayer completely into the L_c phase, the vesicle suspensions were allowed to stand in the high-pressure apparatus for 6–48 h at 5.0 °C before measurement.

Light transmittance measurements under high pressure were carried out with a Hitachi spectrophotometer, Model U-3010 (Hitachi High-Technologies Corp., Tokyo). A high-pressure cell assembly with sapphire windows (PCI-400) was supplied by Teramecs Co. (Kyoto). The pressures were generated by a hand-operated KP-3B hydraulic pump (Hikari High Pressure Instruments, Hiroshima) and monitored using a Heise gauge with an accuracy of 0.2 MPa. The temperature of the optical cell was controlled by circulating thermostated water from a water bath through a jacket enclosing the cell.

The phase transitions of the lipid bilayer membranes under ambient and high pressures were observed by an isobaric thermotropic measurement method, which was described previously [26,27]. Abrupt changes in transmittance of monochromic light (wavelength 560 nm) were observed at phase transitions. The heating rate at a given pressure was 0.33 K min^{-1}.

3. Results and discussion

3.1. Thermotropic and barotropic phase transitions for bilayers of unsaturated mixed-chain PCs

There are few reports on the phase-transition temperatures of the present unsaturated mixed-chain PC bilayers due to the difficulty in detecting such low-temperature transitions. In particular, there is no report for the MOPC and OMPC bilayers. We used not only water as a solvent but also aqueous 50 wt.% EG solution as an antifreeze solvent for DSC measurements because the phase-transition temperatures for some of the PC bilayer membranes at ambient pressure occur at low temperatures below 0 °C [28,29]. In our previous study on the DOPC bilayer membrane [24], we found that the only phase transition observed at ambient pressure was the transition from the L_c phase to the L_{α} phase. The L_{β} phase was unstable at an ambient pressure and existed as a stable phase at high pressure. In general, the metastable gel phase of bilayers is known to occur from supercooling or superpressing. To confirm the existence of metastable phases of bilayer membranes, we employed two kinds of thermal pretreatments for the OSPC and OPPC vesicle solutions in this study: (1) the vesicle solution was held at −15 °C in the heat sink of a calorimeter and the heating scan started at −15 °C, and (2) a vesicle solution which had been stored in the freezer at −30 °C was held at −30 °C in the heat sink and the scan started at −30 °C.

Typical heating DSC thermograms obtained for the vesicle dispersions of six unsaturated mixed-chain PCs are shown in Fig. 1. Curves 1–3 and 4–6 in the figure display thermograms of bilayer membranes of PCs with an unsaturated acyl chain in the *sn*-1 position (i.e., OSPC, OPPC and OMPC) and those with an unsaturated acyl chain

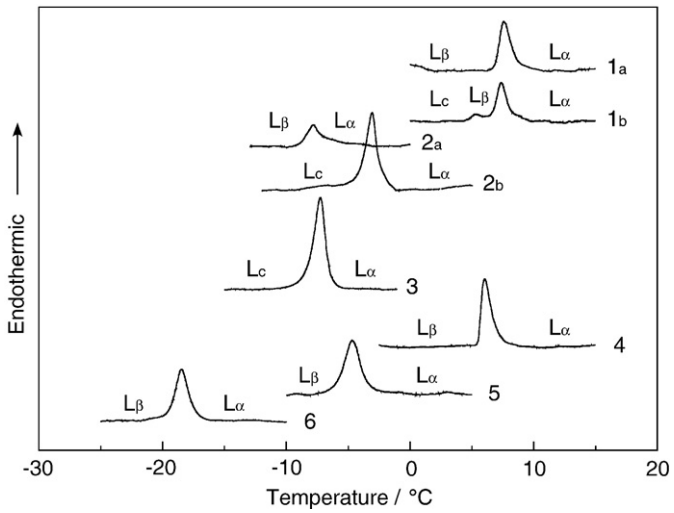

Fig. 1. DSC thermograms for bilayer membranes of mixed-chain phosphatidylcholines: (1a) and (1b) OSPC, (2a) and (2b) OPPC, (3) OMPC, (4) SOPC, (5) POPC and (6) MOPC. Thermograms (1a), (2b) and (4) were obtained for aqueous dispersions; others were measured for dispersions in 50 wt.% aqueous ethylene glycol solution. Beginning temperatures of heating scans: (1a) and (2a) −15 °C, (1b), (2b), (3), (4), (5) and (6) −30 °C.

Table 1
Temperatures and enthalpy changes obtained from DSC measurements for the bilayer phase transitions of unsaturated phospholipids

Lipid	Solvent	Transition	Transition temperature (°C)	ΔH (kcal mol^{-1})	ΔH (kJ mol^{-1})	No. of scanning
OSPC	W[a]	L_β/L_α	8.7 ± 0.09	6.4 ± 0.90	26.7 ± 3.78	7
	EG[b]	L_β/L_α	7.3 ± 0.13	6.2 ± 0.17	26.1 ± 0.69	9
	EG	L_c/L_β	5.2 ± 0.04	–	–	9
OPPC	W	L_c/L_α	−3.2 ± 0.09	7.0 ± 2.90	29.4 ± 12.14	7
	EG	(L_β/L_α)	−7.9 ± 0.05	–	–	5
OMPC	EG	(L_β/L_α)	(−26.5)	–	–	–
	EG	L_c/L_α	−8.0 ± 0.40	9.2 ± 0.31	38.5 ± 1.30	5
SOPC	W	L_β/L_α	6.7 ± 0.10	5.9 ± 0.93	24.8 ± 3.89	6
	EG	L_β/L_α	4.8 ± 0.08	5.1 ± 0.20	21.3 ± 0.84	6
POPC	EG	L_β/L_α	−4.6 ± 0.07	5.1 ± 0.35	21.3 ± 1.45	15
MOPC	EG	L_β/L_α	−19.1 ± 0.05	4.8 ± 0.70	20.1 ± 2.94	6

[a] W: water.
[b] EG: aqueous 50 wt.% ethylene glycol solution.

in the *sn*-2 position (i.e., SOPC, POPC and MOPC), respectively. Curves 1a and 1b show the DSC thermograms of the OSPC bilayer membrane in water and aqueous EG solution, respectively. The thermogram for the heating scan in water beginning at −15 °C (curve 1a) showed a single endothermic peak at 8.7 °C, which was in good agreement with the previous observation by Davis et al. [3] and assigned to the main transition from the L_β phase to the L_α phase. On the other hand, the thermogram of aqueous EG solution beginning from −30 °C (curve 1b) exhibited two kinds of endothermic peaks at 5.2 and 7.3 °C. The higher-temperature transition can be identified as the main transition, taking into account the effect of ethylene glycol, since it did not differ significantly from the transition temperature on thermogram 1a. The lower-temperature transition was observed after cold storage at −30 °C, and could be identified as the transition from the L_c phase to the L_β phase. These transition temperatures were in good agreement with previous observations [30]. A thermogram in aqueous EG solution beginning from at −15 °C (not shown) exhibited a single endothermic peak at 8.0 °C, which was actually identical with the main transition in water.

The DSC thermograms of the OPPC bilayer membrane in aqueous EG solution and water are shown as curves 2a and 2b, respectively. The thermogram for the heating scan in water beginning at −30 °C (curve 2b) exhibited a single endothermic peak at −3.2 °C. A thermogram in aqueous EG solution beginning from −30 °C (not shown) also exhibited a single endothermic peak at −3.5 °C. Since these transitions were observed newly after cold storage, the peak may be assigned to the transition from the L_c phase to the L_α phase. Phase assignment of bilayers was accomplished with the aid of the temperature–pressure phase diagram and thermodynamic quantities of the phase transition, as described below. On the other hand, the thermogram for the heating scan in aqueous EG solution beginning at −15 °C (curve 2a) showed a single endothermic peak at −7.9 °C, which was in good agreement with the previous observation by Davis et al. [3] and assigned to the main transition between the L_β and L_α phases. The temperature of the main transition for the OPPC bilayer membrane in water was also consistent with that in aqueous 50 wt.% EG solution (not shown in Fig. 1). On cooling to −15 °C, the L_β phase appeared as a metastable state because it takes some time for the OPPC bilayer to be completely transformed into the L_c phase. Regarding the peak area for the L_β/L_α phase transition of the OPPC bilayer, the reproducibility was poor owing to the metastable phase transition. Therefore, we employed the value of transition enthalpy for the main transition of the OPPC bilayer as reported by Davis et al. [3].

The DSC thermogram of the OMPC bilayer membrane (curve 3) showed a single endothermic peak at −8.0 °C, which can be regarded as a transition from the L_c phase to the L_α phase. We observed only a single endothermic peak on the DSC thermogram, although two kinds of phase transitions have been detected by an optical method under high pressure, which is discussed in detail later in the document.

The DSC thermograms of the bilayer membranes of SOPC, POPC and MOPC with an unsaturated acyl chain in the *sn*-2 position showed a single endothermic peak at 6.7, −4.6 and −19.1 °C (curves 4, 5 and 6), respectively. These can be assigned to the main transition, and the temperatures for the SOPC and POPC bilayer membranes were well consistent with the previous results [3,23,30–33]. The temperature of the main transition for the SOPC bilayer in aqueous EG solution (not shown) was 4.8 °C, which is slightly lower than that in water (6.7 °C). However, the enthalpy change for the transition was almost the same in both solvents. The values of transition temperatures and enthalpy changes (ΔH) associated with the phase transitions for the six unsaturated mixed-chain PC bilayers determined from the DSC measurements are summarized in Table 1. Solvents used for the DSC measurements and the standard deviations for the measurements are also shown.

Fig. 2 shows one of the results for the isobaric thermotropic phase-transition measurements by the optical method for the OPPC bilayer membrane. The transmittance increased abruptly at a certain temperature corresponding to the phase-transition temperature. The temperature of the inflection point on the curve increased with applied pressure. At pressures of 110 and 200 MPa, the temperatures of the main transition were found to be 12.5 and 27.7 °C (curves 1 and 2), respectively. Curve 3 exhibited a different profile from curve 2, which was obtained at the same pressure, showing a clear two-step increase in transmittance. These transition temperatures were 16.5 and 27.7 °C. The higher-temperature transition coincided with that of curve 2. The lower-temperature transition emerged only for the scan that started just after the solution was kept at 5 °C for a long time (ca. 6 h) under high pressure. Therefore, we regarded it as the transition from the L_c phase to the L_β phase.

3.2. Phase diagrams for bilayers of unsaturated mixed-chain PCs

The temperature (T)–pressure (p) phase diagrams of the POPC and OPPC bilayers are shown in Fig. 3A and B, respectively. Data for the bilayers in water and aqueous 50 wt.% EG solution are distinguished by open and closed symbols, respectively. The transition of the POPC

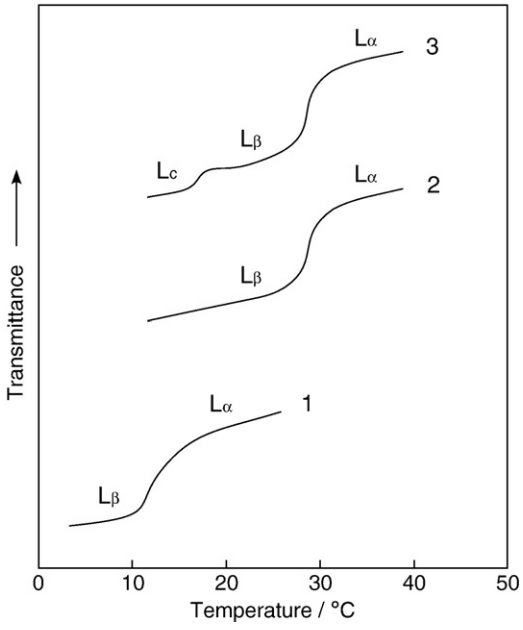

Fig. 2. Phase transitions of OPPC bilayer observed by the light-transmittance method at (1) 110 MPa, (2) 200 MPa and (3) 200 MPa. Transmittance curve 3 is for heating scan after the sample was held at 5 °C and 200 MPa for 6 h.

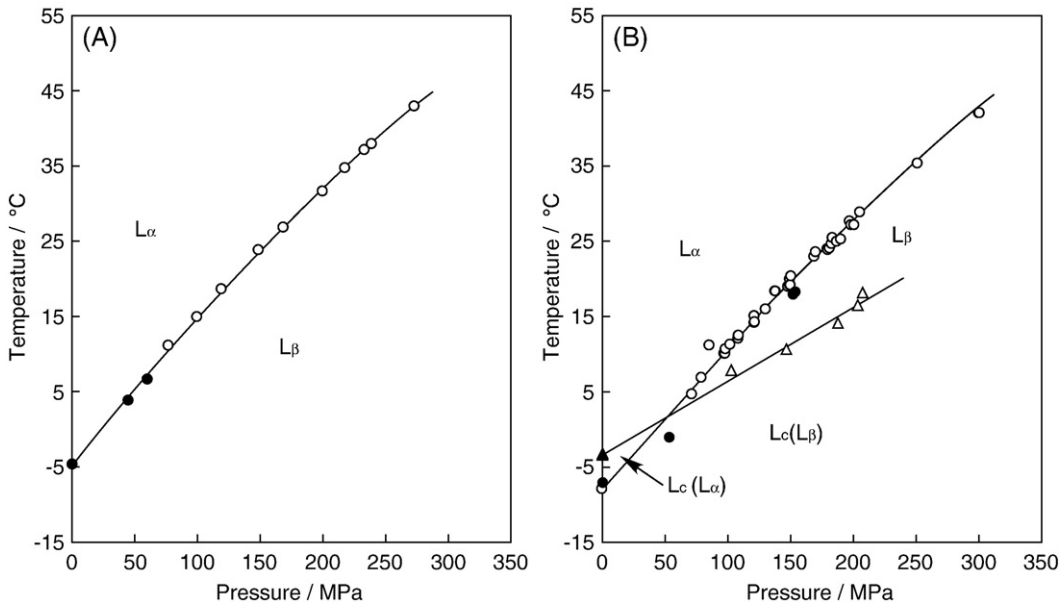

Fig. 3. Temperature–pressure phase diagrams for bilayer membranes of (A) POPC and (B) OPPC. Bilayer phases in parentheses refer to metastable phases. Open and closed symbols refer to transitions in water and aqueous 50 wt.% EG solution, respectively.

bilayer membrane was recognized as the transformation between the stable L_β and L_α phases in the entire pressure range. The temperature of the transition was −4.6 °C at ambient pressure and increased almost linearly with increasing pressure. The slope of the phase boundary (dT/dp) was 0.192 K MPa^{-1}. The barotropic phase behavior of the POPC bilayer was similar to that of the SOPC bilayer [30]. For the OPPC bilayer, the transition (L_c/L_α or L_c/L_β) temperature, as well as the main-transition temperature, increased with increasing pressure. The values of dT/dp for the two transitions were 0.172 and 0.097 K MPa^{-1}, respectively. The two curves of the phase boundaries intersected each other at about 50 MPa on the phase diagram. At high pressures (above 50 MPa), L_c/L_β transition was observed instead of L_c/L_α transition. At temperatures lower than the L_β/L_α transition temperature, the L_β phase appears as a stable phase at pressures above 50 MPa. Peculiar pressure-induced phases such as the interdigitated gel phase, which has often been observed for bilayer membranes of saturated PCs [17,27,34], were undetected for the bilayer membranes of mixed-chain PCs with an unsaturated acyl chain in the *sn*-1 or *sn*-2 position.

The *T*–*p* phase diagrams for the bilayer membranes of SOPC, POPC and MOPC, containing an unsaturated acyl chain in the *sn*-2 position, are compared in Fig. 4. As seen from the figure, only the main transition was observed. The phase transition-temperatures for the SOPC, POPC and MOPC bilayer membranes at ambient pressure were 6.7, −4.6 and −19.1 °C, respectively. The temperature of the main transition increased with an increase in pressure and length of the saturated acyl chain in the *sn*-1 position. The values of dT/dp for these three kinds of lipid bilayers were found to be 0.173, 0.192 and 0.216 K MPa^{-1} depending on the length of the acyl chain in the *sn*-1 position. Generally, polymorphism among gel phases is known in bilayers of PCs with two saturated acyl chains. Since the choline head group of a PC molecule is bulky, the acyl chains are tilted about 30° away from the bilayer normal to maintain a stable distance between the acyl chains of adjacent PC molecules in the gel state [35]. However, the acyl chains of unsaturated phospholipids are unlikely to tilt because of the introduction of a double bond in the acyl chain. The present result shows that the bilayer membranes of the PCs containing an unsaturated acyl chain in the *sn*-2 position show no polymorphism of gel phases.

The *T*–*p* phase diagrams for the bilayer membranes of OSPC, OPPC and OMPC, containing an unsaturated acyl chain in the *sn*-1 position, are compared in Fig. 5. This series of unsaturated PCs exhibited more complicated phase behavior than the series containing an unsaturated acyl chain in the *sn*-2 position. In that, two kinds of transitions, namely, the L_c/L_α (or L_c/L_β) and L_β/L_α transitions were observed. The temperature of each transition increased with increasing pressure and with increasing length of the saturated acyl chain in the *sn*-2 position. For the OMPC bilayer, the L_c/L_α phase boundary line (3b in Fig. 5) gives an extrapolated temperature of −10.0 °C for the transition at ambient pressure, which is close to the transition temperature of −8.0 °C observed by DSC. The values of dT/dp for the main transition were in the range of 0.150–0.201 K MPa^{-1} and those for the L_c/L_α or L_c/L_β transition were in the range of 0.062–0.101 K MPa^{-1} depending on the length of the acyl chain in the *sn*-2 position. The main-transition temperature at ambient pressure was lower than the L_c/L_α

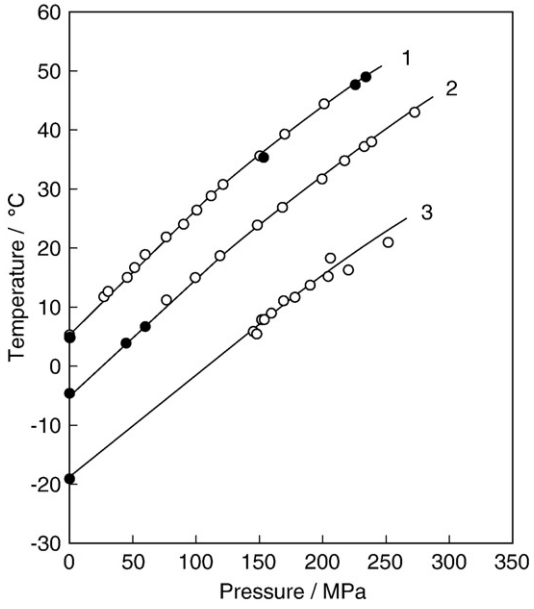

Fig. 4. Temperature–pressure phase diagrams for bilayer membranes of phospholipids with an unsaturated acyl chain in the *sn*-2 position: (1) SOPC, (2) POPC, and (3) MOPC. Open and closed symbols refer to transitions in water and aqueous 50 wt.% EG solution, respectively.

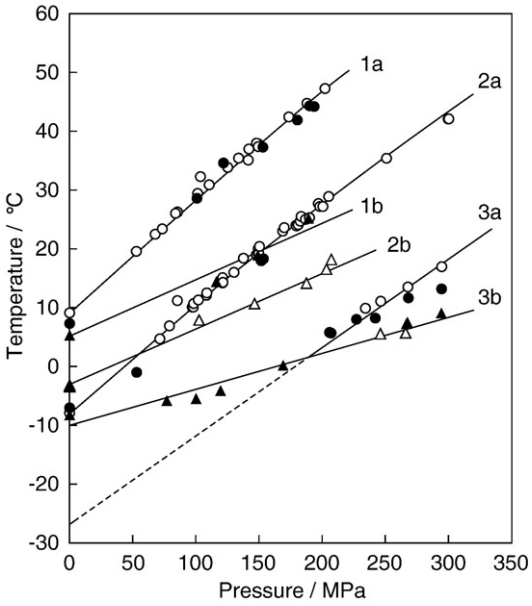

Fig. 5. Temperature–pressure phase diagrams for bilayer membranes of phospholipids with an unsaturated acyl chain in the *sn*-1 position: (1a, 1b) OSPC, (2a, 2b) OPPC, and (3a, 3b) OMPC. The "a" curves show the main transition, whereas "b" curves show the transition between the L_c and the L_β(or L_α) phases. Open and closed symbols refer to transitions in water and aqueous 50 wt.% EG solution, respectively.

transition temperature for the OPPC and OMPC bilayer membranes, whereas that of the OSPC bilayer was higher than the L_c/L_β transition temperature. Because of the larger slope of the phase boundary for the main transition, the two boundary curves of the L_c/L_α and L_β/L_α transitions for the OPPC and OMPC bilayers crossed each other at a certain pressure. As the length of the acyl chain in the *sn*-2 position is shortened, the crossing pressure at the intersection shifted upward. The OMPC molecule has a great mismatch of effective chain length between the *sn*-1 and *sn*-2 acyl chains, which is mentioned later. As Fig. 5 illustrates, the *T*–*p* phase diagrams for these three kinds of lipid bilayers exhibit a systematic change. No additional phases resulting from the chain-length mismatch were observed on the phase diagram of the OMPC bilayer membrane in the range of pressure up to 300 MPa.

3.3. Effect of ethylene glycol on the bilayer phase transition

It is well known for bilayer membranes of saturated PCs such as diacyl PC homologues that EG has a biphasic effect on transition temperatures of PC bilayers, slightly reducing the temperature of the main transition between the ripple gel and liquid crystalline phases at low concentrations of EG, but increasing the main-transition temperature and extinguishing the pretransition at high concentrations of EG (above 20–50 wt.%) [36]. This is explained by the induction of an interdigitated gel phase at high EG concentrations. However, unsaturated PC bilayer membranes have never been observed to transform into an interdigitated structure. The fact that most unsaturated phospholipids exhibit phase transitions at temperatures below the freezing point of water has encouraged many researchers to include EG in the aqueous phase to prevent the freezing of the bulk solvent phase. Lewis et al. [37] have observed the bilayer phase transition of DOPC and unsaturated longer chain homologues by DSC and ^{31}P NMR spectroscopy. The DSC thermograms of this series of unsaturated PCs in aqueous 50 wt.% EG solution were compared with those in water. Their thermograms were qualitatively similar to those observed in water, but the transition temperatures and associated enthalpy changes were significantly different. The transition temperature of the DOPC bilayer in water (−17.3 °C) was elevated to −11.8 °C by the presence of EG. The value of ΔH for the DOPC bilayer in aqueous EG solution (15.6 kcal mol^{-1}) was considerably higher than that measured in water (7.8 kcal mol^{-1}). They correlated this increase in ΔH with the formation of a more ordered Lc phase and not with the L_β phase using ^{31}P NMR spectroscopy. Our previous studies on the DOPC bilayer membrane under high pressure [24,25] showed the existence of the main transition as well as the L_c/L_α transition as observed by Lewis et al. [37]. The main (L_β/L_α) transition of the DOPC bilayer could be observed under high pressure, and the transition temperature was not affected by the presence of EG, unlike the temperature of the L_c/L_α transition [24,25]. Davis et al. [3] suggested that EG did not substantially change the estimate of the main transition temperature (−7.9 °C) for the OPPC bilayer membrane on heating scan of DSC. However, they did not find another phase transition, namely the L_c/L_α transition.

In the present study, although the transition temperature of the SOPC bilayer in water (6.7 °C) was slightly higher than that observed in aqueous EG solution (4.8 °C), the values of ΔH in both solvents were in good agreement, and the *T*–*p* curve for the main transition in water was compatible with that in aqueous EG solution, as shown in Fig. 4. A similar situation was also seen for the main transition of the OSPC bilayer in water and aqueous EG solution, which is shown in Table 1 and Fig. 5. Therefore, the effect of EG on the main-transition temperature is negligible, in accordance with our previous observation [24,25,38]. The L_c/L_β transition of the OSPC bilayer could be observed only in the aqueous EG solution. Thus, the addition of EG facilitates the occurrence of the L_c phase. The L_c/L_β transitions of the OMPC bilayer in both solvents were observed at high pressures above 190 MPa and gave identical dT/dp values, although at low pressures below 190 MPa, the L_c/L_α transition was observed only in aqueous EG solution owing to low temperatures below 0 °C.

3.4. Effect of chain asymmetry on the main-transition temperature

So far, it has been reported for bilayers of mixed-chain PCs with two saturated acyl chains that the two acyl chains do not contribute evenly to the thermodynamic properties of the main transition, and the length of the *sn*-2 acyl chain of the positional isomers primarily governs the bilayer properties [1–5]. If this rule is applicable to the present results, we expect the OPPC and OMPC bilayer membranes to have higher transition temperatures than the POPC and MOPC bilayer membranes, respectively. However, in fact, the opposite results were obtained. Now, we consider the effective chain lengths of unsaturated mixed-chain PCs. Generally, the length of the acyl chain in the *sn*-2 position is known to be virtually shortened by 1.5 carbon–carbon bond lengths relative to that of the acyl chain in the *sn*-1 position in the gel state of the bilayer membrane for saturated mixed-chain PCs [39]. In addition, the acyl-chain length is effectively shortened by the introduction of a *cis* double bond into the acyl chain of phospholipids [3]. Assuming that the introduction of a *cis* double bond into the acyl chain causes the effective chain length to be decreased by 0.2 carbon–carbon length than the corresponding saturated acyl-chain lengths [3], we calculated the difference in the effective chain length between the *sn*-1 and *sn*-2 chains (ΔC), that is, the intramolecular difference in the chain length. This is given by

$$\Delta C = C1 - C2 \tag{1}$$

where $C1$ and $C2$ are the effective lengths of the acyl chains expressed in units of carbon–carbon bond length in the *sn*-1 and *sn*-2 positions, respectively. The difference in ΔC between a pair of positional isomers is defined by

$$\Delta\Delta C = \Delta C(sn\text{-}1 \text{ unsaturated}) - \Delta C(sn\text{-}2 \text{ unsaturated}), \tag{2}$$

where ΔC (*sn*-1 unsaturated) and ΔC (*sn*-2 unsaturated) refer to the intramolecular differences in the effective chain length for lipids with

Table 2
Chain parameters of mixed-chain phospholipids and temperature difference in the main transition of bilayers between positional isomers

Lipid	OSPC		SOPC		OPPC		POPC		OMPC		MOPC	
	C1	*C2*	*C1*	*C2*	*C1*	*C2*	*C1*	*C2*	*C1*	*C2*	*C1*	*C2*
Acyl chain length	17.8	16.5	18.0	16.3	17.8	14.5	16.0	16.3	17.8	12.5	14.0	16.3
ΔC	1.3		1.7		3.3		−0.3		5.3		−2.3	
$\Delta\Delta C$	−0.4				3.6				7.6			
ΔT_m	2.0				−3.3				−7.4			

an unsaturated acyl chain in the *sn*-1 and *sn*-2 positions, respectively. The difference in the main-transition temperature between a pair of positional isomers is written by

$$\Delta T_m = T_m(sn\text{-}1 \text{ unsaturated}) - T_m(sn\text{-}2 \text{ unsaturated}). \quad (3)$$

The calculated results are summarized in Table 2, and a schematic figure of the effective chain length of a lipid molecule is illustrated in Fig. 6. We notice that the value of $\Delta\Delta C$ is closely related to the value of ΔT_m; namely, the ΔT_m values correlate well to those of the mismatch of effective chain length in the lipid molecules. The present results definitely indicate that the greater the mismatch of effective chain length, the larger the difference in the main-transition temperature between each pair of positional isomers of mixed-chain PCs.

3.5. Thermodynamic properties for bilayers of unsaturated mixed-chain PCs

The thermodynamic quantities, namely, ΔH, entropy ($\Delta S = \Delta H / T$) and the volume changes (ΔV) of the respective phase transitions, were obtained from the thermal data and by the application of the Clapeyron equation [40]

$$dT/dp = \Delta V/\Delta S = T\Delta V/\Delta H \quad (4)$$

to the dT/dp values obtained from Figs. 4 and 5. The thermodynamic properties of the phase transitions for the asymmetric unsaturated PC bilayers are summarized in Table 3. The ΔH values of the main transitions for the SOPC and POPC bilayers were 24.8 and 21.3 kJ mol^{-1}, respectively, which were comparable with the values reported previously [3,23,31,41]. In a series of bilayers of SOPC, POPC and MOPC, which have an unsaturated acyl chain in the *sn*-2 position, all the values of the thermodynamic quantities associated with the main transition increased with an increase in length of the saturated acyl chain in the *sn*-1 position. This feature is attributable to the chain-melting transition. However, the ΔH, ΔS and ΔV values were significantly lower than those for asymmetric and symmetric PCs with saturated acyl chains [22,40]. This reduction in the thermodynamic quantities of phase transitions may be attributable to the large difference in the partial molar quantities of lipids between the gel states of the saturated and unsaturated mixed-chain PCs. In other words, in the bilayer gel state the partial molar quantities of unsaturated PCs would be obviously larger than those of saturated PCs. The reduction in ΔH for the main transition of the unsaturated PC bilayers is mainly attributable to the decrease of chain–chain van der Waals interaction energy in the bilayer gel phase owing to the introduction of a double bond. Seelig and Waespe-Sarcevic [42] have found that the double bond of POPC in the L_α phase is oriented with its axis almost parallel to the bilayer normal, and it would seem that this is a likely orientation in the gel phase. Such an orientation can be achieved by introducing a 30° twist in the carbon–carbon bond adjacent to the *cis* double bond plus a *gauche* configuration in the bond between the α and β carbons. Davis et al. [3] have described how a minimum perturbation of gel-phase hydrocarbon chain packing in the unsaturated mixed-chain PCs would be achieved by the following conformation of the oleate chain. Introduction of a twist plus a *gauche* rotamer on both sides of the double bond results in a chain with all parts on the same axis, with the exception of the double bond. The double bond is displaced laterally, but its axis is nearly parallel to that of both the chains. Therefore, we can say that the introduction of a *cis* double bond into the *sn*-2 acyl chain brings about a loose packing of acyl chains in the bilayer gel phase.

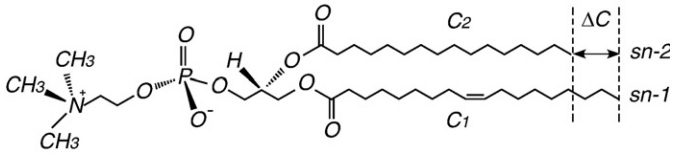

Fig. 6. Schematic figure of effective chain lengths of the *sn*-1 and *sn*-2 chains of a lipid molecule in the gel state of a lipid bilayer membrane.

On the other hand, in a series of the OSPC, OPPC and OMPC bilayers, the ΔH value of the main transition for the OMPC bilayer unfortunately remains undetermined probably because the $L_{\beta'}$ phase is unstable at ambient pressure. The ΔH value of the $L_c/L_{\beta'}$ transition for the OSPC bilayer also remains undetermined because of the poor reproducibility of its DSC thermogram in the presence of ethylene glycol. The magnitude of the ΔH values for the main transitions of the OSPC and OPPC bilayers was comparable with those of the corresponding positional isomers and the dependence on the *sn*-2 acyl-chain length seems to be typical. However, the ΔH values for the L_c/L_α transitions of the OPPC and OMPC bilayers were 29.4 and 38.5 kJ mol^{-1}, respectively. The bilayer of OMPC, which has the shortest acyl chain in the *sn*-2 position, exhibited an anomalously large enthalpy change. The ΔV value of the L_c/L_α transition for the OMPC bilayer was anomalously small despite the large ΔH value. In the case of the OPPC and OMPC bilayers, although the ΔH values for their L_c/L_α transition are sufficiently large, the ΔV value for the L_c/L_α transition of the OPPC bilayer is smaller than that for the main transition, and that of the OMPC bilayer is smaller than that for the $L_{\beta'}/L_\alpha$ transition of the MOPC bilayer by 4.7 cm^3 mol^{-1}, as shown in Table 3. Transformation from the L_c phase to the L_α phase is considered to include two main processes: hydration at the bilayer surface and chain melting at the bilayer core. These processes are generally accompanied by larger

Table 3
Thermodynamic properties for phase transitions of unsaturated phospholipid bilayer membranes

Lipid	Transition	Transition temperature		dT/dp (K MPa^{-1})	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})	ΔV (cm^3 mol^{-1})
		(°C)	(K)				
OSPC	$L_{\beta'}/L_\alpha$	8.7	281.9	0.201	26.7	95	19.1
	$L_c/L_{\beta'}$	5.2	278.4	0.101	–	–	–
OPPC	($L_{\beta'}/L_\alpha$)	−7.9	265.3	0.172	19.2[a]	73	12.5
	L_c/L_α	−3.2	270.0	0.097	29.4	109	10.6
OMPC	($L_{\beta'}/L_\alpha$)	−26.5	245.0	0.150	–	–	–
	L_c/L_α	−8.0	264.9	0.062	38.5	145	9.0
SOPC	$L_{\beta'}/L_\alpha$	6.7	279.9	0.201	24.8	89	19.2
POPC	$L_{\beta'}/L_\alpha$	−4.6	268.6	0.192	21.3	79	15.2
MOPC	$L_{\beta'}/L_\alpha$	−19.1	254.1	0.173	20.1	79	13.7

[a] The result from Davis et al. [3].

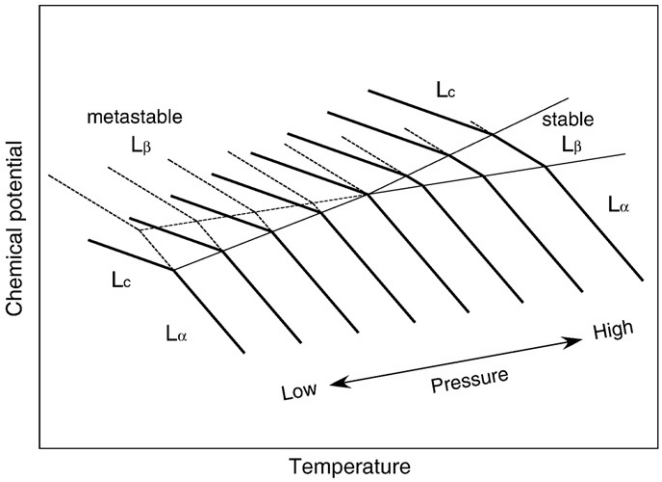

Fig. 7. Schematic diagram for a chemical potential–temperature profile for the L_α, L_β and L_c phases. Solid and dotted lines refer to stable and metastable states, respectively. The slopes reflect the partial molar entropies of lipids in each state. Break points on the chemical potential curves correspond to phase transition points. Isobaric curves are drawn at regular pressure intervals.

enthalpy and volume changes than those for the main transition. The large ΔH value of both bilayers is attributable to the total amount of enthalpy change associated with the processes of hydration and chain melting, which is consistent with the usual tendency. On the other hand, the reason for the unexpectedly small volume change of the L_c/L_α transition for the OPPC and OMPC bilayers is not yet been fully understood, but we currently consider the following two possibilities: the melting of ice-like structured water around polar head groups associated with the transition from the L_c to the L_α phase, the large partial molar volumes of the OPPC and OMPC molecules in the L_c state due to the acyl-chain asymmetry and the presence of a *cis* double bond. Regarding the former possibility, since the ice-melting brings about ca. 10% decrease in molar volume, a simple calculation reveals that the ice-melting of three or four water molecules per one lipid molecule can cause a negative volume change of about 5.4–7.2 $cm^3\ mol^{-1}$. Although the possibility seems valid in quantity, it is rather hypothetical, and it is unlikely that water existing in interlamellar spaces freezes despite the presence of a large amount of ethylene glycol. The latter possibility arises from the chain asymmetry of lipid molecules. As shown in Table 2, the inequivalence between the *sn*-1 and *sn*-2 acyl chains is remarkable for OMPC and OPPC. This inequivalence can bring about a looser molecular packing, corresponding to a large partial molar volume even in the L_c phase. Consequently, chain melting does not bring about as large a volume change as expected. This possibility is rather qualitative but probably relevant to the small ΔV value of the L_c/L_α transition for the OMPC and OPPC bilayer membranes.

Finally, let us consider the stability of the bilayer phases. Partial molar volume and entropy are defined thermodynamically as the differential of chemical potential with respect to pressure and temperature, respectively. On the basis of the thermodynamic quantities for the phase transitions of lipid bilayer membranes, we can draw a chemical potential (μ)–temperature (T) profile for the three (L_c, L_β and L_α) states of a lipid bilayer as shown in Fig. 7. In this figure, the isobaric μ–T curves are shown at regular pressure intervals. The slopes reflect the molar entropies of a lipid in the states of L_c, L_β and L_α, which increase in that order. Regarding bilayers in the low temperature and pressure region, the chemical potential of the lipid in the L_β state is larger than that in the L_c state, which means that the L_β state is metastable. The transition between the stable L_c and L_α phases is thus observed, while the transition between the L_β and L_α phases can be observed as a transition between metastable phases at a lower temperature than that of the L_c/L_α transition. As the acyl-chain length in the *sn*-2 position shortens, the temperatures of both the L_c/L_α and L_β/L_α transitions decrease, corresponding to the left-hand shift in Fig. 7. The temperature difference between the L_c/L_α and L_β/L_α transitions gradually increases; ultimately, the L_β/L_α transition cannot be observed because the L_β phase is unstable. This can be applied to the case of the OMPC bilayer membrane. On the other hand, in the region of higher temperature and pressure, we can observe two kinds of transitions between the stable phases, that is, the L_c/L_β and L_β/L_α transitions. The L_β/L_α transition is observed at a higher temperature (or a lower pressure) than the L_c/L_β transition as in the case of the OSPC bilayer membrane. In a series of bilayers for MOPC, POPC and SOPC containing an unsaturated acyl chain in the *sn*-2 position, only the main transition from the L_β to the L_α phase was observed. The present thermal treatment prior to the measurements is perhaps insufficient for the observation of the L_c phase because the transformation into the L_c phase is particularly slow in a series of the MOPC, POPC and SOPC bilayer membranes.

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