Effect of pressure on the bilayer phase transitions of asymmetric lipids with an unsaturated acyl chain in sn-1 position

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Abstract. The bilayer phase transitions of a series of mixed-chain phosphatidylcholines (PCs) with an unsaturated chain in the sn-1 position and saturated chain with different lengths in the sn-2 position, 1-oleoyl-2-myristoyl-PC (OMPC), 1-oleoyl-2-palmitoyl-PC (OP PC) and 1-oleoyl-2-stearoyl-PC (OSPC), were observed by differential scanning calorimetry (DSC) under ambient pressure and light transmittance measurements under high pressure. All the PC bilayer membranes exhibited not only the main transition from the lamellar gel (Lβ) phase to the liquid crystalline (Lα) phase but also the transition from the lamellar crystal (Lc) to the Lβ (or Lα) phase. The thermodynamic quantities of the transition between the Lc and Lα phases for bilayers of unsaturated mixed-chain PCs were compared with those of the corresponding mixed-chain PCs with saturated acyl chains such as 1-stearoyl-2-myristoyl-PC (SMPC), 1-stearoyl-2-palmitoyl-PC (SPPC) and 1,2-distearyloyl-PC (DSPC). It turned out that the partial molar quantities of the unsaturated mixed-chain PC bilayers in the Lc phase are larger than those of the saturated mixed-chain PCs bilayers. The increase in the partial molar quantities in the Lc phase by the introduction of a cis double bond into the sn-1 acyl chain is attributable to the loose packing of the saturated and unsaturated acyl chains in the Lc phase.

1. Introduction
Naturally occurring phospholipids usually have a saturated hydrocarbon chain in the sn-1 position while an unsaturated chain in the sn-2 position. Despite the biological importance of unsaturated mixed-chain phospholipids, only a few calorimetric studies on these lipids have been undertaken [1–5]. Since unsaturated lipids frequently undergo phase transitions at temperatures below 0 °C under ambient pressure, it is difficult to obtain the information regarding their phase behavior by using conventional techniques, but such information is readily accessible from high-pressure experiments.

In our previous study [6], we evaluated the difference in thermodynamic properties between bilayers of saturated mixed-chain phosphatidylcholines (PCs) and those of the corresponding unsaturated PCs with an unsaturated acyl chain sn-2 position to consider the effect of introduction of an unsaturated chain into the sn-2 position of PC molecules. It is now interesting to examine the introduction effect of an unsaturated chain into the reverse position, namely sn-1 position. In the

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present study, we report the bilayer phase transitions of a series of mixed-chain PCs with an oleate chain in the sn-1 position and a saturated acyl chain with different lengths in the sn-2 position. Comparing the thermodynamic quantities such as enthalpy, entropy and volume changes of the phase transitions for the unsaturated mixed-chain PC bilayers with those for the saturated mixed-chain PCs obtained previously [7–9], the effect of introduction of an unsaturated chain into the sn-1 position is discussed thermodynamically.

2. Experimental
Asymmetric mixed-chain phospholipids containing a monounsaturated acyl chain in the sn-1 position, 1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholine (OMPC), 1-oleoyl-2-palmitoyl-PC (OPPC) and 1-oleoyl-2-stearoyl-PC (OSPC), were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). These phospholipids were used without further purification. The vesicle 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 of each lipid. The lipid concentration was adjusted to 1.0, 5.0 and 10.0 mmol kg\(^{-1}\) in water or in aqueous 50 wt % ethylene glycol solution.

The phase transitions of phospholipid bilayer membranes under ambient pressure were observed by differential scanning calorimetry (DSC) using SSC5200-DSC120 calorimeter (SII Nanotechnology Co. Ltd., Chiba). The heating rate was 0.30 K min\(^{-1}\). The phase transitions under high pressure were observed by one of high-pressure light transmittance techniques, the isobaric thermotropic determination, which was described elsewhere [9].

3. Results and Discussion

3.1. Bilayer phase diagrams of unsaturated mixed-chain PCs
The transmittance vs. temperature profile for bilayers of unsaturated mixed-chain PCs, OMPC, OPPC and OSPC, at a given pressure exhibited an abrupt increase at a certain temperature (data not shown), and we adopted the temperature of this inflection point as the phase transition temperature at the pressure. On the basis of the phase-transition temperature and pressure data obtained, the temperature (T)–pressure (p) phase diagrams of the unsaturated mixed-chain PC bilayers were constructed. The resulting diagrams are shown in Figure 1(A). We have reported [6,10] that bilayers of unsaturated mixed-chain PCs containing an unsaturated acyl chain in the sn-2 position undergo only the main transition from the lamellar gel (L\(_{\beta}\)) phase to the liquid crystalline (L\(_{\alpha}\)) phase. However, this series of unsaturated mixed-chain PCs containing an unsaturated acyl chain in the sn-1 position exhibited two kinds of transitions as is seen in the figure: the lamellar crystal (L\(_c\))/L\(_\alpha\) (or L\(_c\)/L\(_\beta\)) and main (L\(_\beta\)/L\(_\alpha\))

Figure 1. Temperature-pressure phase diagrams of phospholipid bilayer membranes: (A) Unsaturated mixed-chain PC series (1: OSPC, 2: OPPC, 3: OMPC), (B) DSPC, (C) SPPC. In Figure 1(A), the “a” curves show the main transition, whereas “b” curves show the transition between the L\(_c\) and the L\(_\beta\) (or L\(_\alpha\)) phases, and open and closed symbols refer to transitions in water and aqueous 50 wt % ethylene glycol solution, respectively.
transitions were observed. The temperature of each transition increased with increasing pressure and length of the saturated acyl chain in the sn-2 position. The extrapolation of the transition temperatures observed in the high-pressure region to an ambient pressure gave a good agreement with the transition temperature determined by the DSC. The slope of the phase boundary (dT/dp) for the main transition of the unsaturated mixed-chain PC bilayers increased in proportion to the acyl-chain lengths in the sn-2 position. In Figures 1(B) and 1(C) are also shown the T–p phase diagrams for bilayers of symmetric saturated and asymmetric saturated PCs, 1,2-distearoylphosphatidylethanolamine (DSPC) and 1-earoyl-2-palmitoyl-phosphatidylethanolamine (SPPC) bilayers [8,9], for comparison. The phase diagrams of the DSPC and SPPC bilayers are more complicated than those of the unsaturated mixed-chain PC bilayers due to the appearance of the pressure-induced interdigitated gel phase in the high-pressure region. The dT/dp values of DSPC and SPPC bilayers were found to be larger than those of unsaturated mixed-chain PC bilayers.

3.2. Effect of a cis double bond in the sn-1 chain on the bilayer phase transition
The enthalpy (ΔH) and entropy (ΔS = ΔH/T) changes associated with the phase transitions were determined by the DSC measurements, and the volume changes (ΔV) for the transitions were calculated from Clapeyron’s equation,

\[
\frac{dT}{dp} = \frac{T\Delta V}{\Delta H}
\]

The thermodynamic properties for phase transitions of unsaturated mixed-chain PC bilayers are summarized together with those of saturated PC mixed-chain bilayers in Table 1. All the thermodynamic property values for the main transition of the unsaturated mixed-chain PC bilayers except for the undetermined thermodynamic quantity values of the OMPC bilayer increased with an increase of the saturated acyl-chain length in the sn-2 position. Similar behavior was found in those for the main transition of the saturated mixed-chain PC bilayers, but the values of the unsaturated mixed-chain PC bilayers were smaller than those for the saturated mixed-chain PC bilayers.

In our previous paper [6], the difference in the thermodynamic properties of the phase transition between the saturated and unsaturated mixed-chain PC bilayers was evaluated to clarify the effect of a cis double bond in the sn-2 chain on the bilayer phase transition quantitatively. We applied the similar procedure in this study to reveal the effect of a cis double bond in the sn-1 chain. The difference between the thermodynamic properties for the saturated and unsaturated mixed-chain PC bilayer are

Table 1. Thermodynamic properties for the phase transition of mixed-chain phospholipid bilayer membranes.

| Lipid | Transition | Temperature | dT/dp | ΔH  | ΔS  | ΔV  |
|-------|------------|-------------|-------|-----|-----|-----|
|       |            | (K)         | (°C)  | (K MPa⁻¹) | (kJ mol⁻¹) | (J K⁻¹ mol⁻¹) | (cm³ mol⁻¹) |
| OSPC  | Lα/Lα     | 8.7         | 281.9 | 0.201 | 26.7 | 95  | 19.1 |
|       | Lα/Lβ     | 5.2         | 278.4 | 0.101 | 1.1  | 4   | 0.4  |
| OPPC  | (Lα'/Lα)  | -7.9        | 265.3 | 0.172 | 19.2  | 73  | 12.5 |
|       | Lα/Lα'    | -3.2        | 270.0 | 0.097 | 29.4  | 109 | 10.6 |
| OMPC  | (Lα'/Lα)  | -7.9        | 254.0 | 0.150 | —    | —   | — |
|       | Lα/Lα'    | -8.0        | 264.9 | 0.062 | 38.5  | 145 | 9.0  |
| DSPC  | Pα'/Lα    | 55.6        | 328.8 | 0.230 | 45.2  | 137 | 31.6 |
|       | Lα'/Pα    | 50.9        | 324.1 | 0.140 | 5.0   | 15  | 2.2  |
|       | Lβ'/Lα    | 28.2        | 301.4 | 0.220 | 28.1  | 93  | 20.5 |
| SPPC  | Pα'/Lα    | 45.0        | 318.2 | 0.271 | 36.3  | 114 | 30.9 |
|       | Lα'/Pα    | 32.2        | 305.4 | 0.100 | 0.4   | 1   | 0.1  |
|       | Lβ'/Lα    | 22.1        | 295.3 | 0.140 | 23.0  | 78  | 10.9 |
| SMPC  | Pα'/Lα    | 33          | 306.2 | —     | 33.1  | 108 | — |
|       | Lα'/Pα    | 24          | 297.2 | —     | 25.1  | 85  | — |

*a From data of Davis et al. (Ref. [1]). b From data of Stümpel et al. (Ref. [11]).
defined by the equation,

\[ \Delta Y = Y^{(\text{sn}-1 \text{ saturated})} - Y^{(\text{sn}-1 \text{ unsaturated})} \]  

(2)

where \( Y \) refers to thermodynamic quantities related to the phase transition such as transition temperature, enthalpy, entropy and volume changes. The difference values were obtained by subtracting of the values for the unsaturated mixed-chain PC bilayers from those for the saturated mixed-chain PC bilayers. Taking into account that the main (L\( _b \)/L\( _a \)) transitions of the OPPC and OMPC bilayers are the transitions between metastable phases [10], and the thermodynamic quantity values of the OMPC bilayer are not determined experimentally, we focused our attention on the transition between the L\( _c \) and L\( _a \) phases, which is the transition between stable phases. Because the thermodynamic quantity values of the L\( _c \)/L\( _a \) transition for the saturated mixed-chain PC bilayers is not directly calculated, but indirectly estimated by use of the values for the sub- (L\( _c \)/L\( _b' \)) and pre- (P\( _b' \)/L\( _a \)) transitions. The difference in the thermodynamic quantity changes, \( \Delta (\Delta Y) \), except for that in the transition temperature is rewritten by

\[
\Delta (\Delta Y) = \left\{ \left[ Y_{\text{sat}}^{(\text{liq})} - Y_{\text{sat}}^{(\text{gel})} \right] - \left[ Y_{\text{sat}}^{(\text{gel})} - Y_{\text{sat}}^{(\text{cryst})} \right] \right\} - \left\{ \left[ Y_{\text{uns}}^{(\text{liq})} - Y_{\text{uns}}^{(\text{gel})} \right] - \left[ Y_{\text{uns}}^{(\text{gel})} - Y_{\text{uns}}^{(\text{cryst})} \right] \right\}
\]

\[
= \left\{ Y_{\text{sat}}^{(\text{liq})} - Y_{\text{uns}}^{(\text{liq})} \right\} - \left\{ Y_{\text{sat}}^{(\text{cryst})} - Y_{\text{uns}}^{(\text{cryst})} \right\}
\]

where \( Y^{(\text{liq})} \), \( Y^{(\text{gel})} \) and \( Y^{(\text{cryst})} \) are the partial molar quantities of bilayer lipid in the liquid crystalline, gel and lamellar crystal phases, respectively, and subscripts, sat and uns refer to the saturated and unsaturated mixed-chain lipids. The results are listed in Table 2. The values of \( \Delta (\Delta Y) \) were markedly large in all lipid combinations. Since the difference in the partial molar quantities between the liquid crystalline state of the saturated and unsaturated mixed-chain PC bilayers seems to be very small, the large difference values in \( \Delta (\Delta Y) \) is attributable to the large difference in the partial molar quantities between the L\( _c \) phase of the saturated and unsaturated mixed-chain PC bilayers. We can say that the partial molar quantities of the unsaturated mixed-chain PC bilayers in the L\( _c \) phase are obviously larger than those of the saturated mixed-chain PCs bilayers. The increase in the partial molar quantities in the L\( _c \) phase by the introduction of a cis double bond into the sn-1 acyl chain is understandable by the loose packing of the saturated and unsaturated acyl chains in the L\( _c \) phase. The large values of \( \Delta (\Delta S) \) also reflect the loose packing of saturated and unsaturated acyl chains. Further, the \( \Delta (\Delta V) \) values increased with an increase of the saturated acyl-chain length in the sn-2 position. This suggests that the difference in van der Waals interaction between the saturated and unsaturated mixed-chain PC bilayers is enhanced with the chain length in the sn-2 position. Regarding the \( \Delta (\Delta V) \) values, although the large value of \( \Delta (\Delta V) \) is consistent with the above consideration, the values of \( \Delta (\Delta V) \) for two lipid combinations are not so different with each other. The behavior is originated from the anomalously small volume changes of the L\( _c \)/L\( _a \) transition for the OPPC bilayer [10], which is responsible for a large partial molar volume, even in the L\( _c \) phase, due to loose chain packing by large inequivalence between the sn-1 and sn-2 acyl chains.

### Table 2. Difference in thermodynamic quantities of the L\( _c \)/L\( _a \) transition between saturated and unsaturated mixed-chain PC bilayers.

| Lipid combination | \( \Delta (\Delta H) \) kJ mol\(^{-1} \) | \( \Delta (\Delta S) \) J K\(^{-1} \) mol\(^{-1} \) | \( \Delta (\Delta V) \) cm\(^3\) mol\(^{-1} \) |
|-------------------|-----------------|-----------------|-----------------|
| SMPC – OMPC       | 19.7            | 47.6            | —               |
| SPPC – OPPC       | 30.3            | 84.3            | 31.3            |
| DSPC – OSPC       | 50.5            | 146.2           | 34.8            |

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