Thermodynamic characterization of bilayer-nonbilayer phase transitions of phospholipid membranes

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Abstract. We determined thermodynamic properties of phase transitions between bilayer and nonbilayer for phosphatidylcholines with saturated hydrophobic chains (C18:0-PC, O-C18:0-PC) and phosphatidylethanolamines with unsaturated ones (C18:1(ε)-PE, C18:1(trans)-PE) by means of calorimetry under ambient pressure and optical measurements under high pressure. The thermodynamic quantities of the transitions between bilayer and nonbilayer were much smaller than those of the transition between bilayers (gel-liquid crystal or hydrated crystal-liquid crystal transition) for the corresponding phospholipids. Although the nonbilayer formations correspond to a dynamic transformation between lamellar structure and nonlamellar structure, we can say that the order of the lipid molecule in both structures may not appreciably change judging from the smaller thermodynamic quantities. A notable feature of the bilayer-nonbilayer transitions is the large pressure dependence of the transition temperature as compared with that of the bilayer-bilayer transitions. Comparing the enthalpy and volume changes of the bilayer-nonbilayer transitions with those of the bilayer-bilayer transitions, we concluded that the former transitions can be regarded as the volume-driven transitions for the reconstruction of molecular packing.

1. Introduction
Phospholipids usually form bilayer aggregates in aqueous solutions. However, several phospholipids form nonbilayer ones or their membranes occur a phase transition from bilayer to nonbilayer under a certain condition. For example, membranes of phosphatidylcholines (PCs) with saturated acyl chains linked to the glycerol backbone by ester bonds form the interdigitated gel (LβI) phase [1], which is the structure interpenetrating the hydrophobic chains of lipid molecules on the one side of bilayers into those on the other side of bilayers, under high pressure while PCs with saturated alkyl chains linked to the glycerol backbone by ether bonds form the LβII phase by only hydration under ambient pressure. Membranes of phosphatidylyethanolamines (PEs) with unsaturated hydrophobic chains form the inverted hexagonal (Hn) phase [2], which is the structure packing the hexagonal lattices of a cylinder formed by lipid molecules surrounding a water-cored rod, by adding oils and electrolytes. Since nonbilayer-forming phospholipids are considerably limited, the thermodynamic properties of phase transitions between bilayer and nonbilayer have not been still elucidated. Further, few reports were

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made on the formation of the H₄ phase for phospholipid membranes under high pressure [3,4] although there are some reports on the formation of the L₄I phase [5-7].

In the present study, we characterize the phase transitions between bilayer and nonbilayer for membranes of four kinds of phospholipids with hydrophobic chains of carbon number 18, distearoyl-PC (C18:0-PC), dioctadecyl-PC (O-C18:0-PC), dioleoyl-PE (C18:1(cis)-PE) and dielaidoyl-PE (C18:1(trans)-PE), in terms of the thermodynamic quantities of bilayer-nonbilayer transitions.

2. Experimental

All phospholipids were purchased from Avanti Polar Lipids Inc. (Alabaster, AL) and used as received. Water used in this study was distilled twice. Multilamellar vesicle solutions of each phospholipid were prepared by a method described elsewhere [7,8]. The concentrations of the lipid were 1 or 2 mmol kg⁻¹ for PCs and 5 or 10 mmol kg⁻¹ for PEs, respectively.

The phase transitions of phospholipid membranes under ambient pressure were observed by use of a Microcal MCS high-sensitivity differential scanning calorimeter (Northampton, MA, USA). The phase transitions under high pressure were observed by two kinds of optical measurements. One is an isothermal barotropic observation by scanning pressure at a constant temperature and the other is an isobaric thermotropic observation by scanning temperature at a constant pressure. The detailed procedures for the calorimetric and optical measurements were described elsewhere [7,8].

The enthalpies (ΔH) and entropies (ΔS) of phase transitions were determined from differential scanning calorimetry. The volumes (ΔV) of the transition were calculated by applying the Clapeyron’s equation to the phase-transition data obtained from the optical measurements with the ΔH values.

3. Results and Discussion

3.1. Phase diagrams of nonbilayer formation

Figure 1 shows the temperature and pressure phase diagrams of the C18:0-PC, O-C18:0-PC, C18:1(cis)-PE and C18:1(trans)-PE membranes, which were constructed by use of the phase-transition data obtained from the thermal and pressure experiments. The C18:0-PC membrane undergoes the pretransition from the lamellar gel (Lₚ') to the ripple gel (Pₚ') phase and subsequently the main transition from the Pₚ' phase to the liquid crystalline (Lₐ) phase with increasing temperature under ambient pressure. Applying pressure, the LₚI phase is induced in the vicinity of 70 MPa, and extended the region with pressure. On the other hand, the O-C18:0-PC membrane also exhibits the pre- and main transitions under ambient pressure. Although the main transition of both membranes is the Pₚ'/Lₐ transition, the pretransition of the O-C18:0-PC membrane is the transition from the LₚI phase to the Pₚ' phase. Since the interaction of lipid molecules in ether-linked PC membranes is weaker than that in ester-linked PC membranes [9], the ether-linked PC membranes spontaneously form the LₚI phase by only hydration at a low temperature while the ester-linked PC ones require another driving force like pressure.

We observed two-phase transitions in the C18:1(cis)-PE and C18:1(trans)-PE membranes in the

![Figure 1. Temperature-pressure phase diagrams of nonbilayer-forming phospholipid membranes: (a) C18:0-PC, (b) O-C18:0-PC, (c) C18:1(cis)-PE, (d) C18:1(trans)-PE.](image-url)
whole temperature and pressure ranges. A high-temperature transition corresponds to the transition from the $L_\alpha$ phase to the $H_{II}$ phase. A low-temperature transition is different between each membrane; the C18:1($cis$)-PE membrane undergoes the transition from the lamellar crystal ($L_\alpha$) to the $L_\alpha$ phase while the C18:1($trans$)-PE membrane does the main transition from the $L_\alpha$ phase to the $L_\alpha$ phase, respectively. This is because that the $L_\beta$ phase of the C18:1($cis$)-PE membrane is unstable at temperatures below 0 °C and cannot be observed even as the metastable phase [10]. The phase-transition temperatures of both the PE membranes increased with increasing pressure, but no pressure-induced phase was observed.

3.2. Thermodynamic properties of nonbilayer formation

The thermodynamic properties of phase-transitions for four kinds of phospholipids under ambient pressure are summarized in table 1. Here the values of the interdigitation for the C18:0-PC membrane could not be determined because the $L_\beta$ phase is observed only in the high-pressure region, and the $\Delta H$ value of the $L_\alpha$/H$_{II}$ transition for the C18:1($cis$)-PE membrane was taken from the literature [11] due to the poor reproducibility of thermal data for the membrane. The $\Delta H$, $\Delta S$ and $\Delta V$ values of the $L_\beta$/P$_\beta$ transition for the O-C18:0-PC membrane were much smaller than those of the main transition for both the PC membranes and had a close value of the pretransition for the C18:0-PC membrane. In contrast with the thermodynamic quantities, the $dT/dp$ value of the $L_\beta$/P$_\beta$ transition for the O-C18:0-PC membrane and that for the C18:0-PC membrane in the high-pressure region (see figure 1(a)) had larger values than those of any other transitions. Further it should be noted that the $\Delta V$ and $dT/dp$ values of the $L_\beta$/P$_\beta$ transition for the O-C18:0-PC membrane were about twice as large as those of the pretransition for the C18:0-PC membrane. This indicates that the $L_\beta$/P$_\beta$ transition is highly pressure-sensitive although they are both the transitions between the gel phases.

Similar behavior was found for the $L_\alpha$/H$_{II}$ transition of the C18:1($cis$)-PE and C18:1($trans$)-PE membranes. The thermodynamic-quantity values of the $L_\alpha$/H$_{II}$ transition for both the PE membranes were extremely smaller than those of the $L_\alpha$/L$_\alpha$ transition for the C18:1($cis$)-PE membrane and the main transition for the C18:1($trans$)-PE membrane while the $dT/dp$ values of the $L_\alpha$/H$_{II}$ transition became larger than those of the $L_\alpha$/L$_\alpha$ and main transitions. In the case of the C18:1($cis$)-PE membrane, it had the largest value (0.391 K MPa$^{-1}$).

| Lipid | Transition | Temperature | $dT/dp$ | $\Delta H$ | $\Delta S$ | $\Delta V$ |
|-------|------------|-------------|---------|-----------|-----------|-----------|
|       |            | (K)         | ($^\circ$C) | (K MPa$^{-1}$) | (kJ mol$^{-1}$) | (J K$^{-1}$ mol$^{-1}$) | (cm$^3$ mol$^{-1}$) |
| C18:0-PC | L$_\beta'/P_\beta'$ | 324.1 | 50.9 | 0.140 | 5.0 | 15 | 2.2 |
|         | P$_\beta'/L_\alpha$ | 328.2 | 55.6 | 0.230 | 45.2 | 137 | 31.6 |
| O-C18:0-PC | L$_\alpha'/P_\alpha'$ | 327.2 | 53.0 | 0.314 | 4.6 | 14 | 4.4 |
|          | P$_\alpha'/L_\alpha$ | 330.1 | 55.9 | 0.245 | 45.2 | 137 | 33.5 |
| C18:1($cis$)-PE | L$_\alpha'/L_\alpha$ | 267.7 | -5.5 | 0.146 | 28.0 | 105 | 15.3 |
|          | L$_\alpha'/H_{II}$ | 287.7 | 14.5 | 0.391 | 1.4$^a$ | 5 | 1.9 |
| C18:1($trans$)-PE | L$_\alpha'/L_\alpha$ | 311.2 | 38.0 | 0.209 | 33.0 | 106 | 22.2 |
|          | L$_\alpha'/H_{II}$ | 337.9 | 64.7 | 0.250 | 2.1 | 6 | 1.6 |

$^a$ From data of Shalaev et al. (Ref. [11]).

The $L_\beta$/P$_\beta'$ and $L_\alpha$/H$_{II}$ transitions correspond to packing changes in lipid membranes. They produce a dynamic transformation between lamellar structure and nonlamellar structure. However, the $L_\beta$/P$_\beta'$ transition is the transition between the gel phases, and the hydrophobic chains of the PC molecules take high-ordered conformation with all $trans$ forms in both phases. Whereas in the $L_\alpha$/H$_{II}$ transition, the hydrophobic chains of the PE molecules take low-ordered conformation including gauche forms in both phases. The order of hydrophobic chains of the lipid molecules in both structures
may not change appreciably at a phase below and above the transition. Therefore, judging from the smaller thermodynamic quantities, we can say that the difference in thermodynamic state in both phases (the $L_d\beta$ and $P_b'$ phases or the $L_d$ and $H_{II}$ phases) is small. Contrary to this, the main transition with melting of acyl chains and the $L_c/L_d$ transition with the chain melting and the hydration change in the polar head groups bring about the larger change in thermodynamic quantities. On the other hand, the $dT/dp$ values are conversely larger in the bilayer-nonbilayer transitions, which implies the transformation between bilayer and nonbilayer structures is markedly influenced by pressure.

3.3. Similarity and difference in formation of the $L_d\beta$ and $H_{II}$ phases

Comparing the results of the $L_d\beta/P_b'$ transition with those of the $L_d/H_{II}$ transition, we can confirm that the degree of hydrophobic chain order also influences the bilayer-nonbilayer transitions. The thermodynamic quantities of the $L_d\beta/P_b'$ transition had twice or three times larger value than those of the $L_d/H_{II}$ transition. This fact suggests that the transition between high-ordered chain conformations produces large thermodynamic quantities as compared with the transition between low-ordered chain conformations in the bilayer-nonbilayer transitions.

Finally, we consider the thermal and volume contributions to each transition by comparing the $\Delta H$ and $\Delta V$ values of the bilayer-nonbilayer transitions with those of the bilayer-bilayer transitions. The ratio of the $\Delta H$ and $\Delta V$ values between bilayer-nonbilayer and bilayer-bilayer transitions are listed in Table 2. The volume contributions were larger than the thermal contribution in both the $L_d\beta/P_b'$ and $L_d/H_{II}$ transitions, especially the former contribution became more than twice larger for the C18:1($cis$)-PE membrane[2]. It is further noticed from the table that the membrane of C18:1($cis$)-PE with $cis$ double bonds exhibits the greater pressure-sensibility than that of C18:1($trans$)-PE with $trans$ double bonds, indicating that the formation of the $H_{II}$ phase is markedly influenced by the difference in molecular structure of PE with unsaturated acyl chains. Taking into account that the large volume contribution of the bilayer-nonbilayer transition reflects the large $dT/dp$ values characteristic of the transition through the Clapeyron's equation, it turned out that the bilayer-nonbilayer transitions can be regarded as the transitions dominated by volume contribution, in other words, the volume-driven transitions for the reconstruction of molecular packing.

| Lipid               | Ratio of $\Delta H$ | Percentage (%) | Ratio of $\Delta V$ | Percentage (%) |
|---------------------|---------------------|----------------|---------------------|----------------|
| O-C18:0-PC          | $\Delta H(L_d\beta/P_b')/\Delta H(P_b'/L_d)$ | 10.2           | $\Delta V(L_d\beta/P_b')/\Delta V(P_b'/L_d)$ | 13.1           |
| C18:1($cis$)-PE     | $\Delta H(L_c/H_{II})/\Delta H(L_c/L_d)$    | 5.0            | $\Delta V(L_c/H_{II})/\Delta V(L_c/L_d)$    | 12.4           |
| C18:1($trans$)-PE   | $\Delta H(L_c/H_{II})/\Delta H(L_c/L_d)$    | 6.4            | $\Delta V(L_c/H_{II})/\Delta V(L_c/L_d)$    | 7.2            |

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