# Barotropic Phase Transitions of 1-Palmitoyl-2-stearoylphosphatidylcholine Bilayer Membrane

Hitoshi Matsuki,* Masaki Goto, Masataka Kusube, Nobutake Tamai, and Shoji Kaneshina
*Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506*

(Received November 29, 2004; CL-041438)

The barotropic phase transitions of an asymmetric phosphatidylcholine, 1-palmitoyl-2-stearoylphosphatidylcholine (PSPC), bilayer membrane were observed by the methods of high-pressure light transmittance and differential scanning calorimetry. The temperature–pressure phase diagram and thermodynamic quantities of the phase transitions were compared with those of three kinds of symmetric phosphatidylcholines. The different transition properties of PSPC bilayer from those of symmetric phosphatidylcholines may be attributable to the enhanced cohesive interaction between acyl chains in the gel state of the bilayer membrane.

Earlier studies on model membranes of phospholipids have been concentrated on phosphatidylcholines (PCs) containing saturated fatty acids with identical chain lengths in the *sn*-1 and *sn*-2 positions of the glycerol backbone (symmetric PCs). Especially, dipalmitoylphosphatidylcholine (DPPC) bilayer membranes have been the most thoroughly examined by several physicochemical techniques in pressure studies. We recently constructed the temperature ($T$)–pressure ($p$) phase diagrams for homologs of symmetric PCs and revealed that the phase behavior is appreciably influenced by a chain-length of the PC.[1] On the other hand, biological membranes have the heterogeneity concerning compositions in fatty acyl chain of phospholipids. PCs containing saturated fatty acids with different chain lengths in the *sn*-1 and *sn*-2 positions (asymmetric PCs) are also found in large numbers in the membranes, but there have been no reports of the pressure effect on such asymmetric lipids although some investigations under ambient pressure were performed.[2–12] In the present study, the phase transitions of one of asymmetric PCs, 1-palmitoyl-2-stearoylphosphatidylcholine (PSPC) bilayer membrane were observed under ambient and high pressures. The barotropic phase behavior of the asymmetric PC bilayer membrane was discussed by comparing the $T$–$p$ phase diagram for bilayer membrane of PSPC with those of three kinds of symmetric PCs with similar acyl chain length to PSPC, DPPC, diheptadecanoylphosphatidylcholine (DHPC), and distearoylphosphatidylcholine (DSPC).

PSPC, 1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphocholine, was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL) and used as received. Water was distilled twice from a dilute alkaline permanganate solution. The multilamellar vesicle solutions of PSPC at a concentration of 1.0 mmol $kg^{-1}$ were prepared by suspending PSPC in water using a vortex mixer. Then, the suspensions were sonicated for a few minutes by using a sonifier (Branson Model S-450D) at a temperature several degrees above the main-transition temperature. The phase transitions of PSPC bilayer membranes under high pressure were observed by two kinds of optical methods, the determination techniques by isobaric thermotropic and isothermal barotropic measurements, which were developed in our laboratory.[13] The phase transitions under ambient pressure were measured by a method of DSC using two calorimeters (Seiko SSC 560U and Microcal MCS) with heating rate of 0.75 K $min^{-1}$.

From the results of DSC measurements, the PSPC bilayer membrane exhibited three kinds of endothermic peaks at a first heating scan and a subsequent heating scan after cooling (second heating scan) provided two kinds of the peaks. This thermal behavior was similar to that of the DPPC bilayer membrane.[14] Two peaks observed at temperatures of 38.1 and 48.6 °C coincide with each other in both scans. They correspond to the pretransition from the lamellar gel ($L_{\beta'}$) phase to the ripple gel ($P_{\beta'}$) phase and the main transition from the $P_{\beta'}$ phase to the liquid crystalline ($L_\alpha$) phase, respectively. Unlike symmetric PCs as DPPC, the pretransition peak was remarkably small. The peak at the lowest temperature at 34.3 °C was large and only observed in the first scan after cold storage of sample solution at −4 °C for more than a week. It grew with an increase in the period of cold storage. We identified the peak as the subtransition from the lamellar crystal ($L_c$) phase to the $L_{\beta'}$ phase judging from the thermal behavior. Although it is reported in the asymmetric PC bilayers that the pretransition can be observed as a metastable transition because of the $L_{\beta'}$ phase to be unstable,[5,8] we observed the pretransition as a stable transition in the PSPC bilayer membranes.

Barotropic phase transitions of the PSPC bilayer membrane were determined by the methods described in the experimental section. Figure 1 demonstrates the resulting $T$–$p$ phase diagram of the PSPC bilayers together with those of the symmetric PC bilayers, where the pressure dependence of subtransition is only shown for the PSPS and DPPC bilayers. Three transition temperatures of the PSPC bilayer increased linearly by applying pressure and the interdigitated gel ($L_\beta I$) phase appeared between the $L_c$ (or $L_{\beta'}$) and $P_{\beta'}$ phases in the high pressure region. The thermodynamic quantities (enthalpy ($\Delta H$), entropy ($\Delta S$), and volume ($\Delta V$) changes) of respective phase transitions were calculated from the DSC data and the application of Clapeyron–Clausius[13] equation to the phase boundaries ($dT/dp$) in Figure 1. In Table 1, are compared the thermodynamic properties of phase transition for the PSPC bilayer with the corresponding ones for the symmetric PC bilayers. Here the quantities of subtransition for the PSPC bilayer were estimated from the $\Delta H$ value in the literature[4] since the value was not accurately determined due to the dependence of peak area on the period of cold storage. The temperatures and thermodynamic quantities of main transition for the bilayer membrane of PSPC were not comparable to those of DPPC or DSPC, but that of DHPC which has the same total carbon numbers in the two acyl chains as PSPC. In the gel state of bilayer membranes for symmetric PCs, the acyl chain length at the *sn*-2 position of the glycerol backbone is shortened by 1.5 carbons in the chain than that at

Copyright © 2005 The Chemical Society of Japan

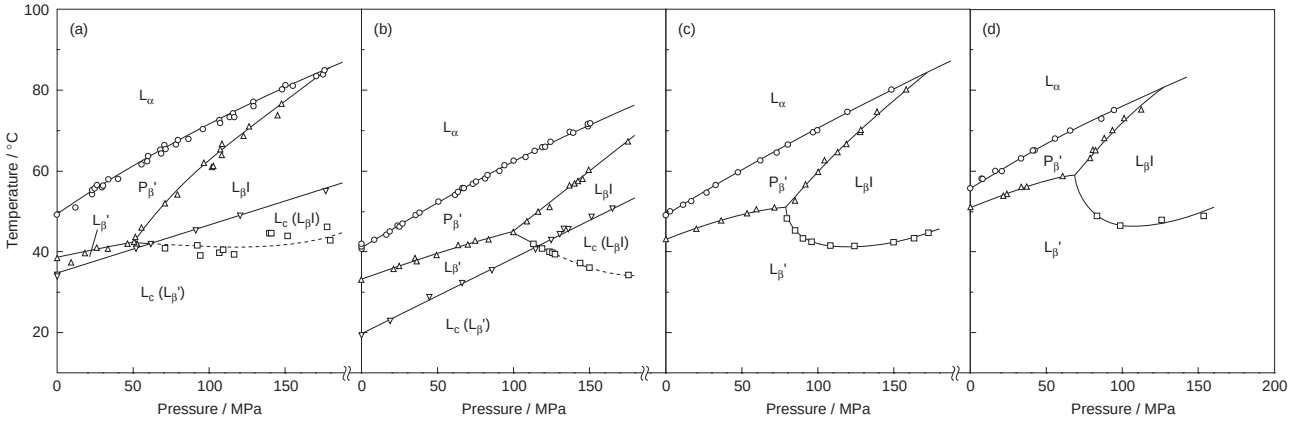


**Figure 1.** Temperature–pressure phase diagrams for bilayer membranes of asymmetric and symmetric PCs: (a) PSPC, (b) DPPC, (c) DHPC, (d) DSPC. Bilayer phase in parenthesis indicates the metastable phase.

**Table 1.** Thermodynamic properties of phase transitions for bilayer membranes of asymmetric and symmetric phosphatidylcholines at 0.1 MPa

| Lipid | Trans. | Temp. /°C | $dT/dp$ /K MPa$^{-1}$ | $\Delta H$ /kJ mol$^{-1}$ | $\Delta S$ /J K$^{-1}$ mol$^{-1}$ | $\Delta V$ /cm$^3$ mol$^{-1}$ |
|---|---|---|---|---|---|---|
| PSPC | $L_c/L_{\beta'}$ | 34.3 | 0.12 | 18.8[a] | 61 | 7.0 |
| | $L_{\beta'}/P_{\beta'}$ | 38.1 | 0.08 | 0.9 | 3 | 0.2 |
| | $P_{\beta'}/L_\alpha$ | 48.6 | 0.23 | 39.8 | 124 | 28.6 |
| DPPC | $L_c/L_{\beta'}$ | 19.6 | 0.20 | 25.9 | 89 | 17.2 |
| | $L_{\beta'}/P_{\beta'}$ | 34.3 | 0.13 | 4.6 | 15 | 1.9 |
| | $P_{\beta'}/L_\alpha$ | 42.0 | 0.22 | 36.4 | 116 | 25.4 |
| DHPC | $L_{\beta'}/P_{\beta'}$ | 42.9 | 0.13 | 5.0 | 16 | 2.0 |
| | $P_{\beta'}/L_\alpha$ | 49.1 | 0.22 | 41.4 | 129 | 28.8 |
| DSPC | $L_{\beta'}/P_{\beta'}$ | 50.9 | 0.14 | 5.0 | 15 | 2.2 |
| | $P_{\beta'}/L_\alpha$ | 55.6 | 0.23 | 45.2 | 137 | 31.6 |

[a]Stümpel et al. (Ref. 4)

the *sn*-1 position.[10] The thermodynamic properties increase with an enhancement of cohesive interaction between PC molecules in the bilayer, namely, an increase of acyl chain length of the PC molecules as seen in Table 1. In the case of PSPC bilayer membrane, the difference in chain length between the *sn*-1 and *sn*-2 chains is reduced by 0.5 carbons and the terminal methyl ends of both chains approach closely. Therefore, we may say that the thermodynamic quantities required to melt the acyl chains of PSPC bilayer increases in comparison with that of DPPC and becomes comparable to the DHPC bilayer. For the pretransition of the PSPC bilayer, the thermodynamic quantities were much smaller than those for symmetric PC bilayers. This suggests that the thermodynamic states of the $P_{\beta'}$ and $L_{\beta'}$ phases in the PSPC bilayer are close to each other.

Although the barotropic phase behavior of four PCs qualitatively resemble each other, noticeable difference is seen in the minimum pressure inducing bilayer interdigitation, which can be regarded as the critical interdigitation pressure (CIP). We noticed that the CIP value of symmetric PC bilayer decreased with an increase of acyl chain lengths of PC, that is, DPPC (100 MPa), DHPC (80 MPa), and DSPC (70 MPa).[1] This order indicates that the ease of pressure-induced interdigitation of PC bilayers is proportional to the cohesive force between acyl chains of the PC molecules. However, the value of PSPC bilayer (50 MPa) became the smallest among phospholipids in this study. Because the terminal methyl ends of *sn*-1 and *sn*-2 chains of PSPC can approach the nearest to each other among four PCs in the gel state as mentioned before, it seems probable that the enhanced cohesive interaction between acyl chains greatly reduced the CIP value to low pressure region. Present study reveals that the difference in chain length between the *sn*-1 and *sn*-2 chains of PC provides a significant influence on the barotropic phase transitions of the bilayer membrane, especially on the bilayer interdigitation.

## References

1 H. Ichimori, T. Hata, H. Matsuki, and S. Kaneshina, *Biochim. Biophys. Acta*, **1414**, 165 (1998).
2 S. C. Chen and J. M. Sturtevant, *Biochemistry*, **20**, 713 (1981).
3 J. T. Mason, C.-H. Huang, and R. L. Biltonen, *Biochemistry*, **20**, 6086 (1981).
4 T. Stümpel, H. Eibl, and A. Nicksch, *Biochim. Biophys. Acta*, **727**, 246 (1983).
5 E. N. Serrallach, G. H. de Haas, and G. G. Shipley, *Biochemistry*, **23**, 713 (1984).
6 T. J. McIntosh, S. A. Simon, J. C. Ellington, and N. A. Porter, *Biochemistry*, **23**, 4038 (1984).
7 S. W. Hui, J. T. Mason, and C.-H. Huang, *Biochemistry*, **23**, 5570 (1984).
8 J. Mattai, P. K. Sripada, and G. G. Shipley, *Biochemistry*, **26**, 3287 (1987).
9 H.-n. Lin, Z.-Q. Wang, and C.-H. Huang, *Biochim. Biophys. Acta*, **1067**, 17 (1991).
10 T. Bultman, H.-N. Lin, Z.-Q. Wang, and C.-H. Huang, *Biochemistry*, **30**, 7194 (1991).
11 R. N. A. H. Lewis, R. N. McElhaney, M. A. Monck, and P. R. Cullis, *Biophys. J.*, **67**, 197 (1994).
12 S. Tristram-Nagle, Y. Isaacson, Y. Lyatskaya, Y. Liu, K. Brummond, J. Katsaras, and J. F. Nagle, *Chem. Phys. Lipids*, **100**, 101 (1999).
13 H. Ichimori, T. Hata, T. Yoshioka, H. Matsuki, and S. Kaneshina, *Chem. Phys. Lipids*, **89**, 97 (1997).
14 M. Kodama, H. Hashigami, and S. Seki, *Biochim. Biophys. Acta*, **814**, 300 (1985).

Published on the web (Advance View) January 26, 2005; DOI 10.1246/cl.2005.270