ELSEVIER

Biochimica et Biophysica Acta 1374 (1998) 1–8

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Barotropic phase transitions of dioleoylphosphatidylcholine and stearoyl-oleoylphosphatidylcholine bilayer membranes

Shoji Kaneshina [a,]*, Hayato Ichimori [b], Takashi Hata [a], Hitoshi Matsuki [a]

[a] Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, Minamijosanjima, Tokushima 770-8506, Japan

[b] Anan National College of Technology, Minobayashi, Anan, Tokushima 774-0017, Japan

Received 12 May 1998; revised 29 June 1998; accepted 2 July 1998

Abstract

In order to understand the effect of *cis* unsaturation on the thermotropic and barotropic phase behavior of phospholipid bilayer membranes, the phase transitions of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) bilayer membranes were observed by high-pressure optical method. With respect to DOPC bilayer membrane, the so-called main transition between the liquid crystalline (L_α) and the lamellar gel (L_β) phases was observed in water at above 0°C under high pressure, in addition to the transition between the L_α and the lamellar crystalline (L_C) phases in 50% aqueous ethylene glycol. The pressure of main transition increased linearly with an increase in temperature. Extrapolation of temperature (T)-pressure (P) phase boundary to ambient pressure suggests the temperature of the main transition to be −40.3°C, which has never been found by the DSC method. On the other hand, the temperature of L_C/L_α phase transition in 50% aqueous ethylene glycol was found to be −12.0°C at ambient pressure. The main transition temperatures for DSPC, SOPC and DOPC are 55.6, 6.7 and −40.3°C, respectively, at ambient pressure. The substitution of *cis* unsaturated chain for saturated chains of DSPC brings about the depression of the main transition temperature by about 48 (±1)°C for each chain. The volume changes (ΔV) associated with the transitions were calculated from the transition enthalpy (ΔH) and the slope of T-P diagram ($\mathrm{d}T/\mathrm{d}P$) by means of the Clapeyron-Clausius equation. The value of ΔV for the main transition of SOPC bilayer membranes was reduced to half the volume change for DSPC bilayers, which means the introduction of the *cis* double bond in the acyl chain of lipids brings about the reduction of ΔV because of the disordered packing of unsaturated chains in the gel phase of lipid bilayer membranes.

Keywords: Barotropic phase transition; Bilayer membrane; *cis* unsaturation; Main transition; Phospholipid; Pressure; Vesicle

1. Introduction

The phospholipids of most biological membranes contain a high percentage of unsaturated fatty acyl chains. 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, with the result that membrane lipids containing unsaturated acyl chains tend to have lower temperatures of transition from the gel phase to the liquid crystalline phase. Since many of the unsaturated lipids studied exhibit their phase transition at temperatures below 0°C, many investi-

---

* Corresponding author. Fax: +81 (886) 55-3162.

PII: S0005-2736(98)00122-9

gators have used aqueous ethylene glycol as an antifreeze solvent and observed marked differences in the phase properties of the unsaturated lipids. The reported value of the phase transition temperature of dioleoylphosphatidylcholine (DOPC) bilayer membrane [1–7] scatters between −23.4 and −11.8°C. Lewis et al. [1] have reported by the differential scanning calorimetry (DSC) that DOPC bilayer membrane in both water and aqueous ethylene glycol undergoes the thermotropic phase transition from a condensed, subgel-like phase rather than a lamellar gel phase to the liquid crystalline phase.

On the other hand, the effect of pressure on lipid bilayer membranes is of particular interest to the studies of pressure reversal of anesthesia [8], pressure adaptation in deep sea organism [9] and high-pressure sterilization in food processing [10]. Although bilayer membranes composed of phosphatidylcholines containing two identical linear saturated fatty acyl chains have been most thoroughly studied under high pressure [11–23], there have been few studies on the phase properties of unsaturated phosphatidylcholine bilayer membranes under high pressure [24–27]. Wong and Mantsch [26] measured Raman and infrared spectra of DOPC bilayer membrane at increasing pressures up to 3700 MPa, and observed a phase transition at 28°C and 500 MPa from a disordered liquid crystalline phase to a highly ordered gel phase. In the gel phase the methylene chains of each molecule are fully extended and the two all-*trans* chain segments on the both sides of the rigid *cis* double bond form a bent structure. The bent oleoyl chains in each molecule are packed parallel to each other. To achieve this parallel interchain packing, the double bonds of the *sn*-1 and *sn*-2 chains of each molecule must be aligned at the same position with respect to the bilayer interface which is achieved by a rotation of the C-C bonds in the glycerol moiety in the head group. Their results suggest also that in the pressure-induced gel phase of DOPC the olefinic CH bonds are rotated out of the plane of the bent oleoyl chains and that the oleoyl chains of opposing bilayers bent towards opposite directions. However, the temperature-pressure phase diagram of DOPC bilayer membrane is still unknown. Since the phase transition of DOPC bilayer at ambient pressure has been observed at temperature below 0°C, information on phase behavior has been difficult to obtain by conventional techniques, but such information is readily accessible from high-pressure experiments.

The present study demonstrates the pressure effect on the phase behavior of bilayer membranes of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), and 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC), as well as 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and reveals the effect of acyl chain unsaturation on the phase behavior of lipid bilayer membranes.

2. Experimental procedures

2.1. Materials

Synthetic DOPC, 1,2-di[cis-9-octadecenoyl]-*sn*-glycero-3-phosphocholine, SOPC, 1-octadecanoyl-2-[cis-9-octadecenoyl]-*sn*-glycero-3-phosphocholine, and DSPC, 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine, were obtained from Sigma (St. Louis, MO, USA) and used without further purification. Water was distilled twice from dilute alkaline permanganate solution. The phospholipid multilamellar vesicles were prepared by suspending each phospholipid in water at 1.0×10^{-3} or 2.0×10^{-3} mol kg^{-1}, using a Branson model 185 sonifier and a cup horn at a temperature above the main transition. The phospholipid suspension was sonicated for a short time (ca. 3 min) in order to prepare the multilamellar vesicle suitable for the optical measurements of the phase transition. The average size of vesicles was determined by the light scattering method, which was found to be 320 ± 90 nm.

2.2. DSC

The phase transitions of phospholipid bilayer vesicles under ambient pressure were observed by a MicroCal MCS high-sensitivity differential scanning calorimeter (Northampton, MA, USA) or a Seiko SSC 560U calorimeter (Seiko Instruments and Electronics, Japan). The heating rate was 0.75 K min^{-1}.

2.3. Phase transitions under high pressures

The phase transitions of lipid bilayer membranes under high pressures were observed by two kinds of optical methods. One is the observation of isothermal barotropic phase transition and the other is the isobaric thermotropic phase transition. A high-pressure cell assembly with sapphire windows, which was made of SUS 630 stainless steel and supplied by Hikari High Pressure Instruments (Hiroshima, Japan), was connected to a spectrophotometer through an optical fiber. The light transmittance of the vesicle suspension was determined at a suitable interval of pressure (or temperature) by a Photal model IMUC 7000 spectrophotometer equipped with the photodiode array of 512 ch. (Otsuka Electronics, Osaka, Japan). Pressures were generated by a hand-operated KP-3B hydraulic pump (Hikari High Pressure Instruments, Hiroshima, Japan) and measured with an accuracy of ±0.2 MPa by a Heise gauge. The temperature of the high-pressure cell was controlled by circulating water from a water bath through the jacket enclosing the pressure cell. With respect to the isobaric thermotropic phase transition measurements, the abrupt change in transmittance accompanying the phase transition was followed at 560 nm. The heating rate at a given pressure was 0.67 K min^{-1}. Regarding the isothermal barotropic phase transition, vesicle suspension was compressed slowly and stepwise, i.e. the pressure was increased by ca. 5 MPa in each step in the vicinity of the phase transition, and was allowed to stand for 15 min in each step.

3. Results and discussion

3.1. Phase transitions of DOPC bilayer membrane

A typical measurement of isothermal barotropic phase transition for DOPC bilayer membrane is shown in Fig. 1. The light transmittance of vesicle suspensions in the range of 540–580 nm was measured at 0°C and various pressures (Fig. 1A). The transmittance at 560 nm was depicted in Fig. 1B as a function of pressure. The transmittance-pressure curve shows the sudden decrease of the transmittance accompanying the pressure-induced transition. The phase transition pressure was determined as a function of temperature from the transmittance-pressure profiles at various temperatures, which increased linearly with an increase in temperature. This situation is depicted on the temperature (T)-pressure (P) phase diagram shown in Fig. 2. Extrapolation of this T-P line to ambient pressure suggests the temperature of the phase transition to be −40.3°C, which has never been found in the literature.

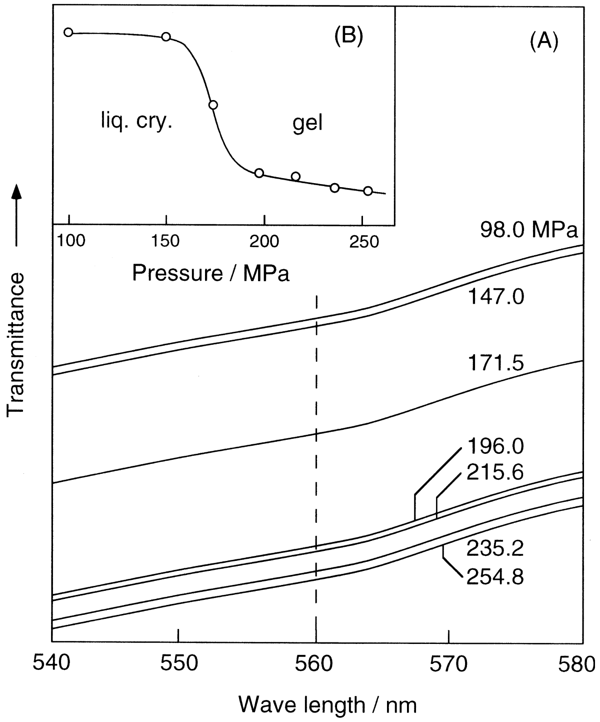

Fig. 1. Isothermal barotropic phase transition of DOPC bilayer membrane at 0°C. A: Transmittance-wave length curves at various pressures. B: A typical pressure-induced phase transition of DOPC bilayer membrane.

The phase transition of DOPC bilayer membrane at ambient pressure has been observed at temperature below 0°C by the DSC method. Many workers have used aqueous ethylene glycol as an antifreeze solvent [1–4]. The phase transition temperature has been reported to be −11.8, −14.0, −15.0 and −15.8°C in 50% aqueous ethylene glycol [1–4] and −16.5, −17.3 and −21.0°C in water [1,5,6], respectively. The transition temperatures and associated enthalpy changes are significantly different in between water and aqueous ethylene glycol. Lewis et al. [1] have described that DOPC bilayer membrane can form more stable lamellar crystalline (L_C) phases in aqueous ethylene glycol than in water. Therefore, the transition temperatures between −11.8°C and −21.0°C at ambient pressure can be regarded as the transition from the L_C-like subgel phase to the

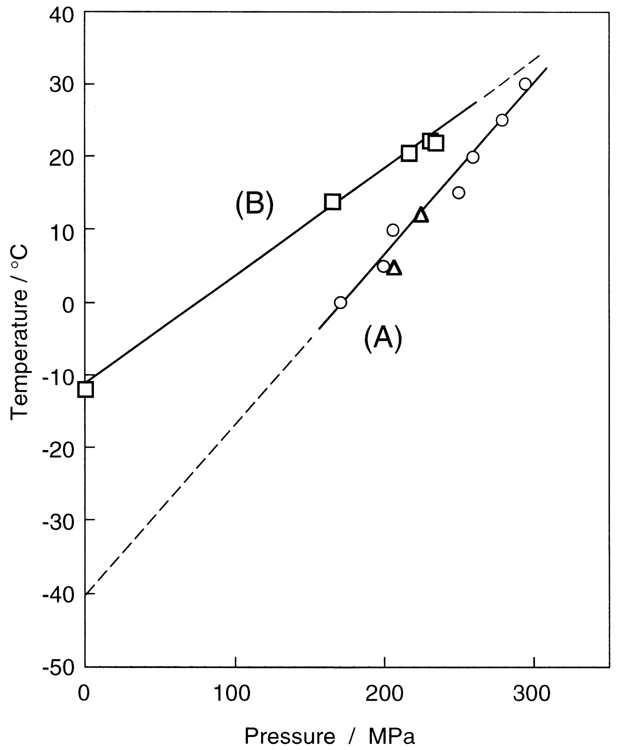

Fig. 2. Phase diagram of DOPC bilayer membranes. The concentration of DOPC was 1.0 m mol kg^{-1}. A: Main transition in water (○) and in 50% aqueous ethylene glycol (△). Linear extrapolation to ambient pressure suggests the temperature of the main transition to be −40.3°C. B: The L_C/L_α transition in 50% aqueous ethylene glycol (□).

liquid crystalline (L_α) phase, namely the L_C/L_α phase transition.

In order to observe the L_C/L_α phase transition under high pressure, the isobaric thermotropic change of transmittance in aqueous ethylene glycol suspensions was measured as follows. DOPC suspension prepared in 50% aqueous ethylene glycol was pressurized to a desired pressure at ca. 0°C and was heated slowly (0.65 K min^{-1}) after the vesicle suspension was allowed to stand for a definite period of time. The transmittance-temperature profiles under high pressures are shown in Fig. 3, which shows the existence of two kinds of transitions. When the vesicle suspension was heated at once after it was pressurized, a lower-temperature transition was observed (curve A in Fig. 3). Curve B in Fig. 3 shows clearly two step increase in transmittance, which was obtained in the case of solution standing for a relatively short time (ca. 2 h) at 0°C and ca. 220 MPa. In the case of solution standing for a relatively long time (ca. 4 h) at 0°C, only one transition was observed (curve C in Fig. 3), which is consistent with higher-temperature transition. As is seen from *T-P* diagram in Fig. 2, the higher-temperature transition in 50% aqueous ethylene glycol refers to the L_C/L_α phase transition, since extrapolation of this *T-P* line to ambient pressure suggests the temperature of transition to be −12.0°C, which is obtained by DSC measurements. This higher-temperature transition, namely L_C/L_α phase transition, could not be observed in the cooling scan or in the pressurizing scan, because the formation of the lamellar crystalline (L_C) phase is extremely slow. On the other hand, lower-temperature transition in 50% aqueous ethylene glycol (curve A in Fig. 3) was consistent with the transition of DOPC bilayer membrane in water, which can be regarded as the so-called main transition between the lamellar gel (L_β) phase and the L_α phase. This lower-temperature transition, i.e. the L_β/L_α phase transition, could be observed by not only heating but also pressurizing scans in the range of temperature above 0°C. At ambient pressure, how-

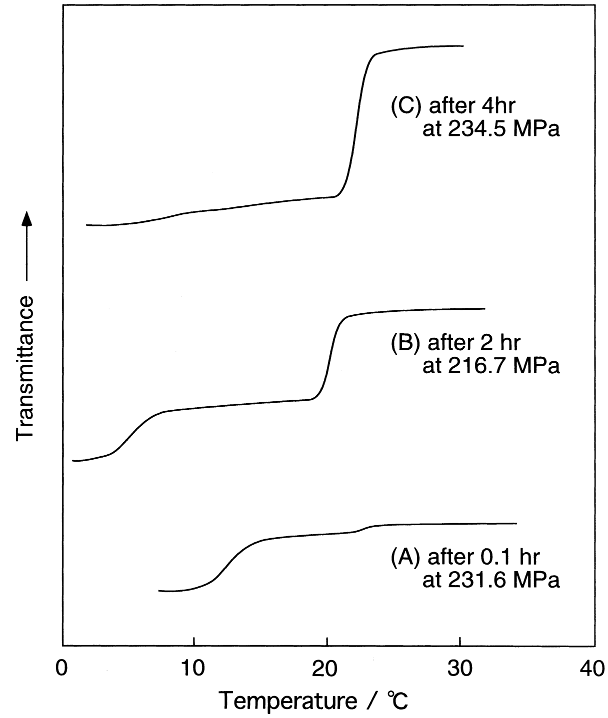

Fig. 3. Two kinds of phase transitions for DOPC bilayer membranes in 50% aqueous ethylene glycol. DOPC suspension was pressurized first to about 200 MPa at 0°C and then heated after it was allowed to stand for A: 0.1 h; B: 2 h; and C: 4 h.

ever, the transition at about −40°C has never been observed by the DSC method. From these facts it may be concluded that the L_β phase is a metastable phase in the pressure range studied. As is seen from Fig. 2, the slope of phase boundary between L_C and L_α phases is smaller than that between L_β and L_α phases. Therefore, two lines of phase boundaries intersect each other at around 340 MPa and 40°C. At higher pressure than 340 MPa the L_β phase is expected to become a stable phase. Other basis for the L_β/L_α phase transition will be described below.

Regarding the barotropic phase transition of DOPC bilayer membrane, Wong and Mantsch [26] observed a phase transition at 28°C and 500 MPa by the method of Raman and infrared spectra. They concluded that the effect of pressure on the liquid crystalline phase of DOPC bilayer membrane is to induce intrachain conformational and interchain reorientational ordering processes which trigger a structural phase transition from a structurally and dynamically disordered liquid crystalline phase to a highly ordered gel phase in which the reorientational fluctuations are completely damped and the hydrocarbon chains are highly extended with a bent configuration at the *cis* double bond. Wong and Mantsch [26] described also in their report that in the liquid crystalline phase of DOPC (at below 500 MPa) the '*gauche*' band at 1080 cm^{-1} is much stronger than the 'all-*trans*' bands; yet, the relative intensity of the *trans* bands increases with increasing pressure, which implies that external pressure induces some conformational ordering in the liquid crystalline phase. Above 500 MPa, all-*trans* C-C stretching bands become very strong whereas the *gauche* C-C stretching band can hardly be recognized in the spectra, indicating that in the pressure-induced gel phase, the conformation of the chains is highly ordered and that both CH_2 chain segments are highly extended.

In the present study, the L_C/L_α phase transition at 28°C has to be observed at around 260 MPa, which differs from the observation at 500 MPa by Wong and Mantsch [28]. As mentioned before, the phase transition from the L_α phase to the L_C phase is difficult to observe in the pressurizing scan because the transformation into the L_C phase is extremely slow. Therefore we observed the L_C/L_α phase transition by the heating scan under high pressure. The superpressing to transform into the L_C phase may be responsible for the pressure difference in the L_C/L_α phase transition. Alternatively, the observation by Wong and Mantsch may be a new pressure-induced phase transition. It is unlikely that the phase transition at 500 MPa and 28°C is the same kind of transition as the L_C/L_α phase transition at ambient pressure and −12°C. They observed that the relative intensity of the *trans* band increases with increasing pressure in the liquid crystalline phase at that below 500 MPa and that the conformation of the chains is highly ordered at high pressure above 500 MPa. Since the L_β phase is expected to appear as a stable phase at higher pressure than 340 MPa, it may be concluded that the lower-temperature transition found in the present study can be assigned as the main transition between the L_α and L_β phases, and that the higher-pressure transition observed by Wong and Mantsch may be assigned as the phase transition between the lamellar gel (or the L_C-like subgel) and highly ordered lamellar crystalline phases.

3.2. *Effect of acyl chain unsaturation on the phase behavior*

Saturated (in the *sn*-1 position) and unsaturated (in the *sn*-2 position) mixed-chain phosphatidylcholines are the major components of naturally occurring phosphatidylcholines. In order to understand the influence of *cis* double bond in the hydrocarbon chains on the thermotropic and barotropic phase behavior, the phase transitions of SOPC bilayer membrane were compared with those of DSPC and DOPC bilayer membranes.

An example of the barotropic phase transition of SOPC bilayer membrane at 30°C is shown in Fig. 4. The transmittance of SOPC vesicle suspensions in the range of 540–580 nm was measured at various pressures (Fig. 4A). Transmittance at 560 nm was depicted in Fig. 4B as a function of pressure. The transmittance-pressure curve shows the main transition from the L_α phase to the L_β phase, accompanying with a sudden decrease in transmittance. The phase transition pressures were determined at various temperatures and increased linearly with an increase in temperature. The *T-P* diagram for SOPC bilayer membrane is shown in Fig. 5 together with that for DOPC and DSPC bilayer membranes. Data for DSPC bilayer membrane were taken from our pre-

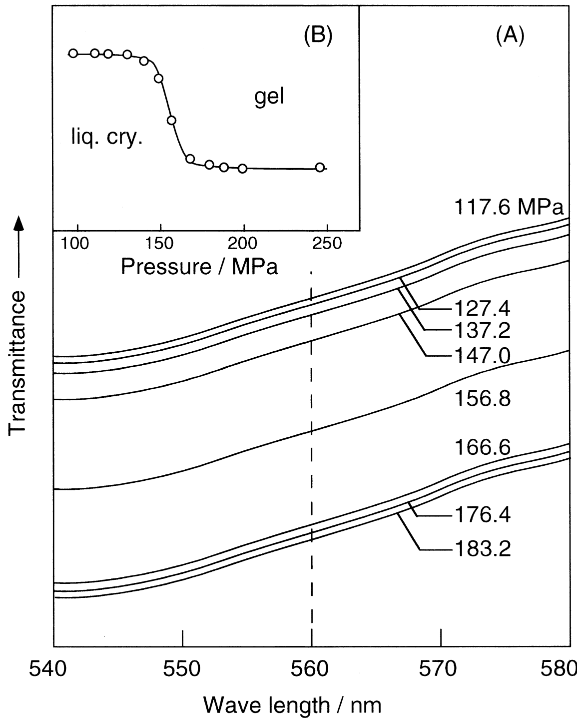

Fig. 4. Isothermal barotropic phase transition of SOPC bilayer membrane at 30°C. A: Transmittance-wave length curves at various pressures. B: A typical pressure-induced phase transition of SOPC bilayer membrane.

vious results [23]. The pretransition for both SOPC and DOPC bilayer membranes was not observed, which means the substitution of unsaturated (oleate) chain for saturated (stearate) chain of DSPC brings about the disappearance of the ripple gel phase and non-tilted bilayer structures of the gel state. The main transition temperature was 6.7°C at ambient pressure, which is in good agreement with previous data [6,28]. As is seen from Fig. 5, the main transition temperatures for DSPC, SOPC and DOPC are 55.6, 6.7 and −40.3°C, respectively, at ambient pressure. The introduction of the *cis* double bond in SOPC and DOPC thus decreases the main transition temperature by about 49 and 96°C, respectively. We may conclude that the substitution of *cis* unsaturated chain for saturated chains of DSPC brings about the depression of main transition temperature by about 48(±1)°C for each chain. With respect to SOPC bilayer membrane, there has been no study on the phase behavior under high pressures. The slope of the *T*-*P* diagram, d*T*/d*P*, for the main transition of SOPC bilayer membrane was 0.181 K MPa^{-1}, which is comparable to that of palmitoyl-oleoyl-phosphatidylcholine bilayer membrane, 0.21 K MPa^{-1} [24]. The values of d*T*/d*P* for the main transition of DSPC and DOPC bilayers were 0.230 and 0.233 K MPa^{-1}, respectively. Chong and Weber [29] have studied on fluorescence polarization measurement of 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated in DOPC vesicle. Even though there is no phase transition, they estimate the d*T*/d*P* for DOPC to be about 0.21 K MPa^{-1} from the temperature to pressure equivalence of the DPH polarization increase. This value is in good agreement with our present result.

3.3. Thermodynamic properties of the phase transitions

The values of d*T*/d*P* and phase transition temperatures for DSPC, SOPC and DOPC bilayer membranes are listed in Table 1. The enthalpy (Δ*H*) and entropy (Δ*S* = Δ*H*/*T*) changes associated with the phase transition were determined by the DSC meth-

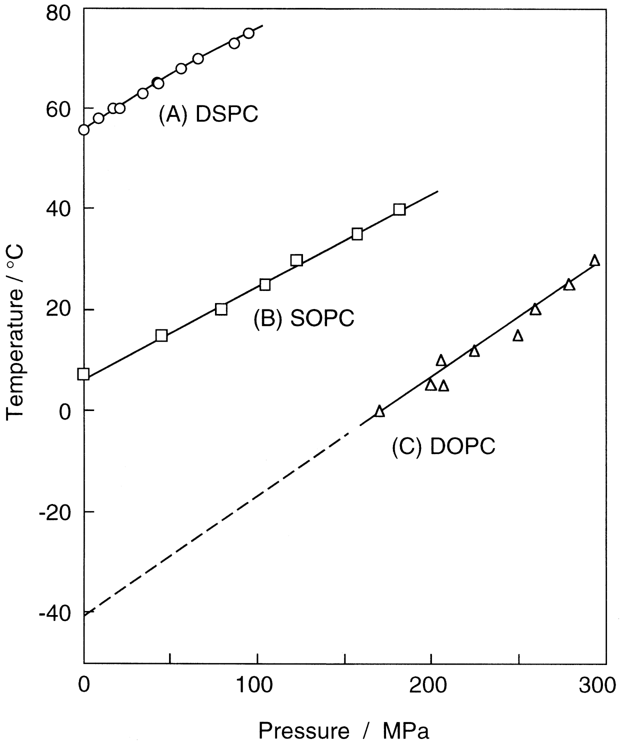

Fig. 5. Temperature-pressure phase diagrams for main transitions of bilayer membranes of phosphatidylcholine with saturated and unsaturated acyl chains. A: DSPC; B: SOPC; and C: DOPC.

Table 1
Thermodynamic properties of phase transitions for the bilayer membranes of DSPC, SOPC and DOPC

Lipid	Phase transition	Transition temperature		dT/dP	ΔH		ΔS	ΔV
		(K)	(°C)	(K MPa^{-1})	(kJ mol^{-1})	(kcal mol^{-1})	(J K^{-1} mol^{-1})	(cm^3 mol^{-1})
DSPC	$(P_{\beta}' \rightarrow L_{\alpha})$	328.8	55.6	0.230	45.2	10.8	137	31.6
SOPC	$(L_{\beta} \rightarrow L_{\alpha})$	279.9	6.7	0.181	22.6	5.4	80.7	14.6
DOPC	$(L_{\beta} \rightarrow L_{\alpha})$	232.9	−40.3	0.233				
	$(L_C \rightarrow L_{\alpha})$	261.2	−12.0	0.147	65.3	15.6	250	36.7

od. The values of ΔH for DSPC and SOPC were taken from our previous data [23] and results by Davis [6,28], respectively, which are listed in Table 1. With respective to DOPC bilayer membrane, the L_{β}/L_{α} phase transition at −40.3°C has never been observed by the DSC method because of the transition between metastable phases at ambient pressure. It is likely that the lamellar gel phase of membrane lipid containing unsaturated acyl chains is more disordered than that of the saturated lipid. Therefore, the value of ΔH for the main transition of DOPC bilayer is expected to be smaller than that for DSPC bilayer. Regarding the L_C/L_{α} phase transition of DOPC bilayer, the reported value of ΔH scatters between 65.3 [1] and 32.2 kJ mol^{-1} [5]. The higher the temperature of the transition observed, the larger the value of ΔH for the L_C/L_{α} phase transition. The temperature of the L_C/L_{α} phase transition in the present study was −12.0°C, which is consistent with the highest temperature reported [1]. The value of ΔH for the L_C/L_{α} phase transition of DOPC bilayer membrane was taken from literature [1], 65.3 kJ mol^{-1}, which is shown in Table 1.

The volume change (ΔV) associated with the transition was calculated from the Clapeyron-Clausius equation

$$\frac{dT}{dP} = \frac{\Delta V}{\Delta S}$$

and is also summarized in Table 1. The volume change associated with the main transition of DSPC bilayer membrane has been directly measured by the density method. The values of ΔV for DSPC have been reported to be 28.4 [30], 32.9 [31] and 39.0 cm^3 mol^{-1} [32], which are comparable to the present value, 31.6 cm^3 mol^{-1}, estimated from the Clapeyron-Clausius equation. The value of ΔV for SOPC bilayer was reduced to half the ΔV of DSPC. Therefore, it may be concluded that the substitution of *cis* unsaturated chain for saturated chains of DSPC brings about the reduction of ΔV because of the disordered packing of unsaturated chain in the gel phase of DOPC bilayer membrane. In the case of the L_C/L_{α} phase transition for DOPC membrane, large value of ΔV was estimated from the Clapeyron-Clausius equation, which reflects probably the transformation from the ordered L_C-like phase to the disordered liquid crystalline phase.

References

[1] R.N.A.H. Lewis, B.D. Sykes, R.N. McElhaney, Biochemistry 27 (1988) 880–887.
[2] P.W.N. Van Dijck, B. De Kruijff, L.L.M. Van Deenen, J. De Gier, R.A. Demel, Biochim. Biophys. Acta 455 (1976) 576–587.
[3] B. De Kruijff, R.A. Demel, A.J. Slotboom, L.L.M. Van Deenen, A.E. Rosenthal, Biochim. Biophys. Acta 307 (1973) 1–19.
[4] J.R. Silvius, R.N. McElhaney, Chem. Phys. Lipids 25 (1979) 125–134.
[5] P.G. Barton, F.D. Gunstone, J. Biol. Chem. 250 (1975) 4470–4476.
[6] P.J. Davis, K.M.W. Keough, Biochemistry 22 (1983) 6334–6340.
[7] J.F. Santaren, M. Rico, J. Guilleme, A. Ribera, Biochim. Biophys. Acta 687 (1982) 231–237.
[8] K.T. Wann, A.G. Macdonald, Prog. Neurobiol. 30 (1988) 271–307.
[9] A.R. Cossins, A.G. Macdonald, J. Bioenerg. Biomembr. 21 (1989) 115–135.
[10] R. Hayashi, C. Balny (Eds.), High Pressure Bioscience and Biotechnology, Elsevier, Amsterdam, 1996.
[11] J.R. Trudell, D.G. Payan, J.H. Chin, E.N. Cohen, Biochim. Biophys. Acta 373 (1974) 436–443.
[12] N.I. Liu, R.L. Kay, Biochemistry 16 (1977) 3484–3486.

[13] S. Utoh, T. Takemura, Jpn. J. Appl. Phys. 24 (1985) 356–360.

[14] P.T.T. Wong, H.H. Mantsch, Biochemistry 24 (1985) 4091–4096.

[15] N.D. Russell, P.J. Collings, J. Chem. Phys. 77 (1982) 5766–5770.

[16] J.R. Lakowicz, R.B. Thompson, Biochim. Biophys. Acta 732 (1983) 359–371.

[17] L.F. Braganza, D.L. Worcester, Biochemistry 25 (1986) 2591–2596.

[18] S.K. Prasad, R. Shashidhar, B.P. Gaber, S.C. Chandrasekhar, Chem. Phys. Lipids 43 (1987) 227–235.

[19] J. Jonas, C.L. Xie, A. Jonas, P.J. Grandinetti, D. Campbell, D.A. Driscoll, Proc. Natl. Acad. Sci. USA 85 (1988) 4115–4117.

[20] D.A. Driscoll, J. Jonas, A. Jonas, Chem. Phys. Lipids 58 (1991) 97–104.

[21] X. Peng, J. Jonas, Biochemistry 31 (1992) 6383–6390.

[22] S. Maruyama, H. Matsuki, H. Ichimori, S. Kaneshina, Chem. Phys. Lipids 82 (1996) 125–132.

[23] H. Ichimori, T. Hata, T. Yoshioka, H. Matsuki, S. Kaneshina, Chem. Phys. Lipids 89 (1997) 97–105.

[24] R. Winter, W.C. Pilgrim, Ber. Bunsenges. Phys. Chem. 93 (1989) 708–717.

[25] D.J. Siminovitch, P.T.T. Wong, H.H. Mantsch, Biochemistry 26 (1987) 3277–3287.

[26] P.T.T. Wong, H.H. Mantsch, Biophys. J. 54 (1988) 781–790.

[27] D.J. Siminovitch, P.T.T. Wong, R. Berchtold, H.H. Mantsch, Chem. Phys. Lipids 46 (1988) 79–87.

[28] P.J. Davis, B.D. Fleming, K.P. Coolbear, K.M.W. Keough, Biochemistry 20 (1981) 3633–3636.

[29] P.L.G. Chong, G. Weber, Biochemistry 22 (1983) 5544–5550.

[30] D.L. Melchior, H.J. Morowitz, Biochemistry 11 (1972) 4558–4562.

[31] K. Ohki, K. Tamura, I. Hatta, Biochim. Biophys. Acta 1028 (1990) 215–222.

[32] R. Koynova, A. Koumanov, B. Tenchov, Biochim. Biophys. Acta 1285 (1996) 101–108.