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Barotropic phase transitions and pressure-induced interdigitation on bilayer membranes of phospholipids with varying acyl chain lengths

Hayato Ichimori [1], Takashi Hata, Hitoshi Matsuki, Shoji Kaneshina *

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

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Abstract

The bilayer phase diagrams of a series of 1,2-diacylphosphatidylcholines containing linear saturated acyl chain (C=13, 14, 15, 16, 17 and 18) were constructed by two kinds of high-pressure optical methods. One is the observation of isothermal barotropic phase transition and the other is the isobaric thermotropic phase transition. The temperature of the main transition from the ripple gel (P_{β}') phase to the liquid crystal (L_{α}) phase for each lipid was elevated by pressure. The slope of the temperature-pressure diagram, dT/dP, was in the range of 0.21–0.23 K MPa^{-1} depending on the acyl chain length. The temperature of the pretransition from the lamellar gel (L_{β}') phase to the P_{β}' phase for each lipid was also elevated by pressure. The slope of phase boundary, dT/dP, for the pretransition was in the range of 0.12–0.14 K MPa^{-1}. Both temperatures of the main and pretransition under ambient pressure increased with an increase in acyl chain length. The chain length dependences of the pretransition and main transition temperatures describe smooth curves with no evidence of odd/even discontinuities. Pressure-induced interdigitated gel (L_{β}I) phase was observed beyond 300 MPa for 14:0-PC, 175 MPa for 15:0-PC, 100 MPa for 16:0-PC, 80 MPa for 17:0-PC and 70 MPa for 18:0-PC, respectively. The minimum pressure for the interdigitation of lipid bilayer membranes decreased with an increase in acyl chain length in a manner of non-linear relation. The slopes of phase boundary between L_{β}' and L_{β}I phases transformed from the negative slope to the positive slope as the pressure increases.

Keywords: Bilayer membrane; Interdigitation; Phase transition; Phospholipid; Pressure; Vesicle

1. Introduction

Pressure studies on lipid bilayer membranes have been initiated at first in the interest of a more complete understanding of pressure-anesthetic antagonism [1], and extended to the adaptation of marine organisms to extreme depths [2] and the sterilization by high pressure in food processing [3]. Bilayer membranes composed of phosphatidylcholines containing two identical linear saturated fatty acyl chains have been most thoroughly studied. Such studies have tended to concentrate on a few members of the homologous series, with the result that the thermotropic phase behavior of some of these phosphatidylcholines (especially dipalmitoylphosphatidylcholine, 16:0-PC) is relatively well understood. The succeeding high-pressure studies on the 16:0-PC bilayer membranes have been performed with various physical techniques including ESR [4], dilatometry [5,6],

* Corresponding author. Fax: +81 (886) 553162.

[1] Present address: Anan College of Technology, Minobayashi, Anan, Tokushima 774-0017, Japan.

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calorimetry [7,8], X-ray diffraction [9], dynamic light scattering [10], Raman spectroscopy [11,12], adiabatic compression [13], fluorescence [14,15], FT-IR [16], neutron diffraction [17,18], light transmittance [19–21] and NMR [22–24]. These measurements have revealed phase behavior of 16:0-PC bilayer membranes. In addition to liquid crystal (L_α), ripple gel (P_β') and lamellar gel (L_β') phases, a new pressure-induced gel phase, i.e., the interdigitated gel phase ($L_\beta I$) has been observed by the methods of small-angle neutron diffraction [17,18], light transmittance [19–21] and NMR [24]. A triple point among L_β', P_β' and $L_\beta I$ phases has been observed on the temperature (T)-pressure (P) phase diagram of 16:0-PC bilayer membrane [17–21]. Regarding the slope of phase boundary between L_β' and $L_\beta I$ phases, there still remains disagreement on which is the positive or the negative slope.

Our previous study [25] has demonstrated the pressure effect on the bilayer phase transition (especially the main transition from the P_β' phase to the L_α phase) of a series of diacylphosphatidylcholines containing linear saturated acyl chains of even- and odd-number carbons. It is apparent from a survey of the literature that there have been relatively few studies on the phase properties of diacylphosphatidylcholines whose hydrocarbon chain contains an odd number of carbon atoms [26–28].

In the present study, we focus our attention on the interdigitation and the pretransition from the L_β' phase to the P_β' phase in addition to the main transition of bilayer membranes, and reveal phase behavior of a series of diacylphosphatidylcholines containing linear saturated acyl chains of even- and odd-number carbons. We also discuss the effect of acyl chain lengths on the pressure-induced interdigitation of lipid bilayer membranes and on the thermodynamic properties of the lipid phase transition.

2. Experimental procedures

2.1. Materials

All of the phospholipids were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Abbreviations of the diacylphosphatidylcholines (1,2-diacyl-*sn*-glycero-3-phosphocholine) are as follows: 13:0-PC, ditridecanoylphosphatidylcholine; 14:0-PC, ditetradecanoylphosphatidylcholine; 15:0-PC, dipentadecanoylphosphatidylcholine; 16:0-PC, dihexadecanoylphosphatidylcholine; 17:0-PC, diheptadecanoylphosphatidylcholine; 18:0-PC, dioctadecanoylphosphatidylcholine.

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. The phospholipid suspension was sonicated at a temperature several degrees above the main phase transition for a short time (approx. 3 min) in order to prepare the multilamellar vesicle suitable for the optical measurements of the phase transition. The average size of vesicles was found to be 200–300 nm, which was determined by the light scattering method.

2.2. Differential scanning calorimeter

The phase transitions of phospholipid multilamellar vesicles under ambient pressure were observed by a MicroCal MCS high-sensitivity differential scanning calorimeter (DSC) (Northampton, MA, USA). The heating rate was 0.75 K min^{-1}. The enthalpy changes of phase transitions were determined as an average value for several DSC measurements.

2.3. Phase transition measurements under high pressures

Phase transitions 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 a photodiode array of 512 ch. (Otsuka Electronics, Osaka, Japan). Pressures were generated by a hand-operated KP-3B hydraulic pump (Hikari High Pressure Instruments) and meas-

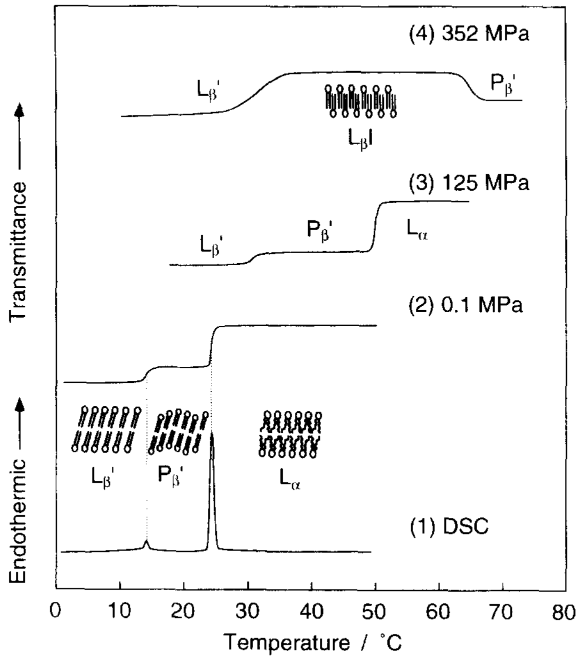

Fig. 1. Isobaric thermotropic phase transitions of 14:0-PC bilayer membrane. Main transition and pretransition by the methods of (1) DSC and (2) light transmittance at 0.1 MPa and (3) at 125 MPa. (4) Pressure-induced interdigitation by the optical method at 352 MPa.

ured within 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 approx. 5 MPa in each step in the vicinity of the phase transition, and allowed to stand for 15 min in each step.

3. Results and discussion

3.1. Thermotropic and barotropic phase transitions

An example of the thermotropic phase transition measurements for 14:0-PC bilayer membranes is shown in Fig. 1. The DSC thermogram of heating scan at ambient pressure showed two kinds of endothermic transitions (curve 1 in Fig. 1). Higher-temperature transition can be assigned as the main transition from the P_{β}' phase to the L_{α} phase. On the other hand, lower-temperature transition can be assigned as the pretransition from the L_{β}' phase to the P_{β}' phase. Light transmittance also changed clearly at two transition temperatures (curve 2 in Fig. 1). Two transition temperatures by both methods were in good agreement with each other. The main transition and pretransition temperatures of 14:0-PC bilayer membranes were 23.9°C and 13.9°C, respectively, which is in good agreement with previously published data [26]. Both temperatures of the main transition and pretransition increased with an increase in pressure (curve 3 in Fig. 1). The difference in temperature between two transitions became wide as the pressure increased. At pressures higher than 300 MPa, we observed a new pressure-induced phase transition (curve 4 in Fig. 1). It has been observed for 16:0-PC [21] and dihexadecylphosphatidylcholine (DHPC) [21,29] bilayer membranes that the transition from the L_{β}' phase to the $L_{\beta}I$ phase is accom-

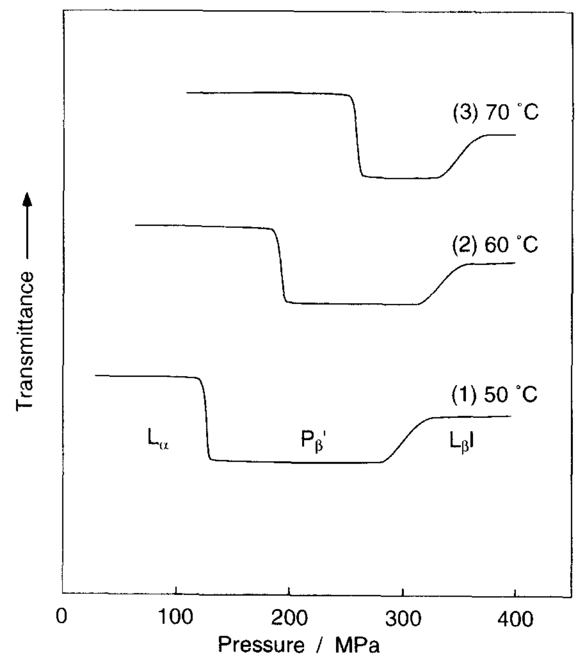

Fig. 2. Isothermal barotropic phase transitions of 14:0-PC bilayer membrane. Main transition and pressure-induced interdigitation by the optical method under various temperatures: (1) 50°C; (2) 60°C; (3) 70°C.

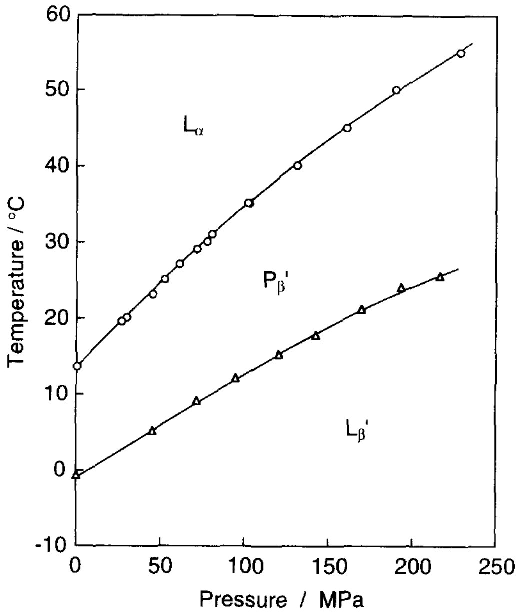

Fig. 3. Phase diagram of 13:0-PC bilayer membranes. The concentration of 13:0-PC was 1.0 mmol kg^{-1}. Phase transitions: ○, $P_{\beta}' \rightarrow L_{\alpha}$; △, $L_{\beta}' \rightarrow P_{\beta}'$.

panied by an increase in turbidity. More direct evidence for interdigitation has been observed from the spacing measurements by the neutron diffraction [17,18] or X-ray diffraction [30] method. The phase transitions by the methods of light transmittance and X-ray diffraction were in good agreement with each other. The present new phase can be assigned as the $L_{\beta}I$ phase from analogy to the previous observation for 16:0-PC [21] and DHPC [21,29].

Fig. 2 shows the isothermal barotropic phase transition of 14:0-PC bilayer membrane under various temperatures. With regard to the main transition from the L_{α} phase to the P_{β}' phase, the transmittance of vesicle suspensions decreases abruptly with increasing pressure. On the other hand, interdigitation from the P_{β}' phase to the $L_{\beta}I$ phase is accompanied by an increase in transmittance with increasing pressure. The middle point of the change in transmittance was taken as the transition pressure. The difference in pressure between two transitions becomes narrow as the temperature increases.

3.2. *Phase diagram of lipid bilayer membranes*

The temperature (*T*)-pressure (*P*) phase diagram of 13:0-PC bilayer membranes is shown in Fig. 3. The temperatures of the main transition and pretransition increase with increasing pressure. The *T-P* curves for the main transition and pretransition are slightly convex upward. The slopes of the phase boundary at ambient pressure, d*T*/d*P*, were 0.210 K MPa^{-1} for the main transition and 0.14 K MPa^{-1} for the pretransition and are listed in Table 1. The main transition of 13:0-PC bilayer membranes under high pressure has been reported only by our laboratory [25]; however, the effect of pressure on the pretransition temperature has not been reported. Extrapolation of the *T-P* line to ambient pressure suggests the temperature of the pretransition to be −1.0°C. The previous value of the pretransition temperature is −0.8°C [26], which is in good agreement with the present value. We could not observe the $L_{\beta}I$ phase of 13:0-PC bilayer membrane up to a pressure range of 400 MPa.

The *T-P* phase diagram of 14:0-PC bilayer membranes is shown in Fig. 4. The temperatures of the main transition and pretransition increase with increasing pressure. The *T-P* curves for the main transition and pretransition are slightly convex upward in a similar manner as for 13:0-PC. The values of d*T*/d*P* were 0.212 K MPa^{-1} for the main transition and 0.13 K MPa^{-1} for the pretransition, respectively.

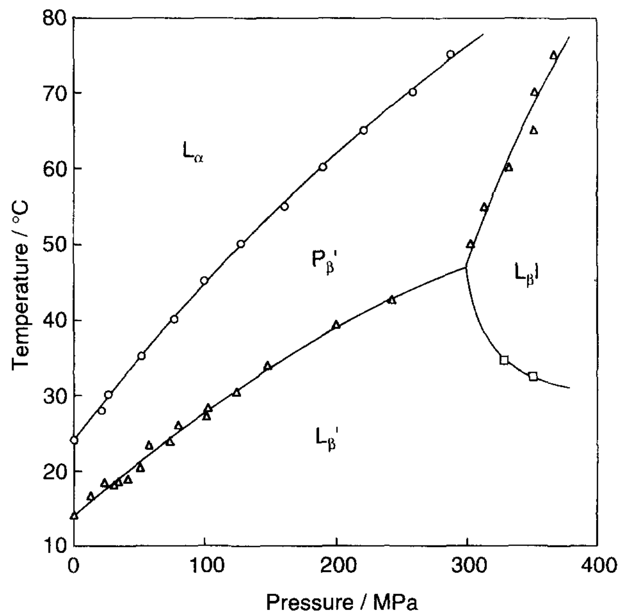

Fig. 4. Phase diagram of 14:0-PC bilayer membranes. The concentration of 14:0-PC was 2.0 mmol kg^{-1}. Phase transitions: ○, $P_{\beta}' \rightarrow L_{\alpha}$; △, $L_{\beta}' \rightarrow P_{\beta}'$ or $L_{\beta}I \rightarrow P_{\beta}'$; □, $L_{\beta}' \rightarrow L_{\beta}I$.

The main transition of 14:0-PC bilayer membranes under high pressure has been reported by several authors [12,14,15,17–19,31,32]. The previous values of d*T*/d*P* for the main transition lie between 0.20 and 0.24 K MPa^{-1} and the majority of the values are 0.22 K MPa^{-1}, which is in good agreement with the present result. Regarding the effect of pressure on the pretransition temperature, a few researchers have reported the values of d*T*/d*P* to be 0.16 [31], 0.18 [32] and 0.12 K MPa^{-1} [19], which are not consistent with each other. A detailed pressure study has been reported by Prasad and coworkers [19]. Their result, 0.12 K MPa^{-1}, is comparable to the present value, 0.13 K MPa^{-1}. As is seen from Fig. 4, a pressure-induced interdigitated gel phase, in which the hydrocarbon chains from apposing monolayers become interdigitated with the chains, was observed at pressures above 300 MPa. A triple point on the phase diagram among P_{β}', L_{β}' and $L_{\beta}I$ phases was found at 300 MPa and 47°C. The triple point would be defined as a critical interdigitation point (T_i^c, P_i^c). The value of P_i^c can be regarded as the minimum pressure for the interdigitation of lipid bilayer membranes. The phase boundary between L_{β}' and $L_{\beta}I$ phases had a negative slope. The slope of phase boundary is expressed thermodynamically by the Clapeyron-Clausius equation, and is dependent upon the sign of the volume and entropy (or enthalpy) changes for the phase transition. The negative

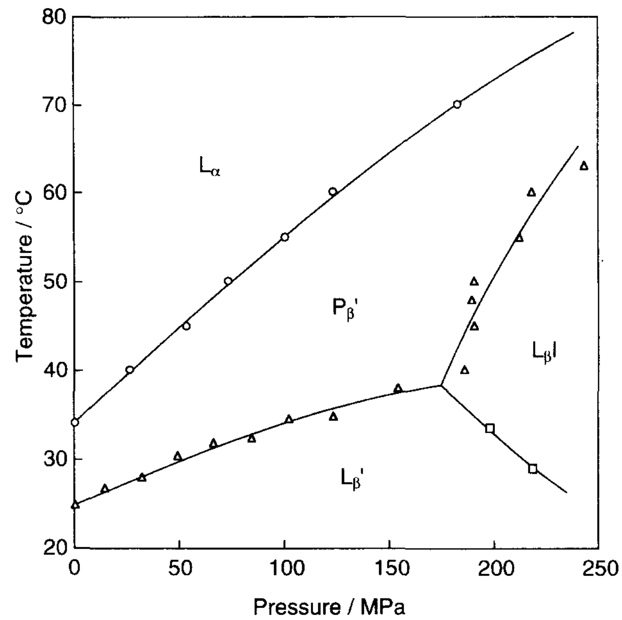

Fig. 5. Phase diagram of 15:0-PC bilayer membranes. The concentration of 15:0-PC was 1.0 mmol kg^{-1}. Phase transitions: ○, $P_{\beta}' \rightarrow L_{\alpha}$; △, $L_{\beta}' \rightarrow P_{\beta}'$ or $L_{\beta}I \rightarrow P_{\beta}'$; □, $L_{\beta}' \rightarrow L_{\beta}I$.

slope is probably attributable to the negative volume change of transition from the L_{β}' phase to the $L_{\beta}I$ phase [33], which is mentioned in detail later.

The phase diagram of 15:0-PC bilayer membranes is similar to that for 14:0-PC. The value of d*T*/d*P* was 0.215 K MPa^{-1} for the main transition and 0.12 K MPa^{-1} for the pretransition, respectively. As is

Table 1
Thermodynamic properties of phase transitions for the bilayer membranes of diacylphosphatidylcholines

Lipid	Transition temp.		d*T*/d*P* (K MPa^{-1})	Δ*H* (kJ mol^{-1})	Δ*S* (J K^{-1} mol^{-1})	Δ*V* (cm^3 mol^{-1})
	K	°C				
Main transition						
13:0-PC	286.8	13.6	0.210	16.0	56	11.7
14:0-PC	297.1	23.9	0.212	24.7	83	17.6
15:0-PC	307.0	33.8	0.215	30.3	99	21.2
16:0-PC	315.2	42.0	0.220	36.4	115	25.4
17:0-PC	322.1	48.6	0.224	41.4	129	28.8
18:0-PC	328.8	55.6	0.230	45.2	137	31.6
Pretransition						
13:0-PC	272.4	−0.8[a]	0.14	2.1[a]	8	1.1
14:0-PC	287.1	13.9	0.13	4.0	14	1.8
15:0-PC	297.8	24.6	0.12	4.2	14	1.7
16:0-PC	307.5	34.3	0.13	4.6	15	1.9
17:0-PC	316.1	42.9	0.13	4.6	15	1.9
18:0-PC	324.1	50.9	0.14	5.0	15	2.2

[a]From data of Lewis et al. [26].

seen from Fig. 5, the $L_\beta I$ phase was observed at high pressure beyond 175 MPa. The critical interdigitation point was found at 175 MPa and 38°C.

Fig. 6 shows the *T-P* phase diagram for 16:0-PC bilayer membranes, which has been reported in our previous paper [21]. The values of d*T*/d*P* for the main transition and pretransition were 0.220 and 0.13 K MPa^{-1}, respectively. The effect of pressure on bilayer membranes has been reported by several authors [5,12–21,34]. The previous values of d*T*/d*P* for the main transition lie between 0.208 and 0.249 K MPa^{-1} and the majority of the values are 0.23 K MPa^{-1}, which is in good agreement with the present result. Regarding the value of d*T*/d*P* for the pretransition, a few researchers have reported the values of d*T*/d*P* to be 0.162 [12] and 0.12 K MPa^{-1} [19], which are comparable to the present value, 0.13 K MPa^{-1}. We observed the critical interdigitation point at 100 MPa and 45°C. Braganza and Worcester [17] and Winter and Pilgrim [18] reported the observation of the $L_\beta I$ phase in D_2O by the method of neutron diffraction at high pressure beyond 150 MPa and 160 MPa, respectively, which seems to be a somewhat higher pressure than the present result. Whereas Prasad et al. [19] and Driscoll et al. [24] found the critical interdigitation point at 93 MPa, 43°C by the method of light transmittance and at 100 MPa, 39°C by NMR, respectively. Their values are in good agreement with the present result, although the slope of phase boundary between L_β' and $L_\beta I$ phases is different from the present result. The slope of phase boundary between L_β' and $L_\beta I$ phases is negative for all the phase diagrams of 14:0-PC, 15:0-PC and 16:0-PC bilayer membranes. As mentioned before, the slope of phase boundary is expressed thermodynamically by the Clapeyron-Clausius equation. Ohki and coworkers [33] have revealed the negative volume change accompanied by the transition from the L_β' phase to the $L_\beta I$ phase for 16:0-PC and 18:0-PC bilayer membranes. It is known by the DSC method that the phase transition from the L_β' phase to the $L_\beta I$ phase is accompanied by the endothermic change ($\Delta H > 0$) [35]. Consequently, the slope of phase boundary between L_β' and $L_\beta I$ phases in these lipid bilayer membranes should be negative.

Fig. 7 shows the *T-P* phase diagram of 17:0-PC bilayer membranes. We observed the critical interdigitation point at 80 MPa and 51°C. Furthermore, another triple point among L_α, P_β' and $L_\beta I$ phases was discovered. Since the slope of the phase boundary between P_β' and $L_\beta I$ phases is larger than that for the main transition, two phase boundaries inter-

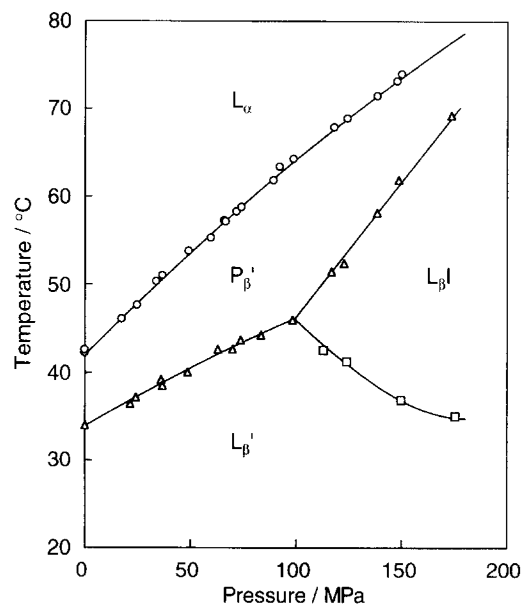

Fig. 6. Phase diagram of 16:0-PC bilayer membranes. The concentration of 16:0-PC was 2.0 mmol kg^{-1}. Phase transitions: ○, $P_\beta' \rightarrow L_\alpha$; △, $L_\beta' \rightarrow P_\beta'$ or $L_\beta I \rightarrow P_\beta'$; □, $L_\beta' \rightarrow L_\beta I$.

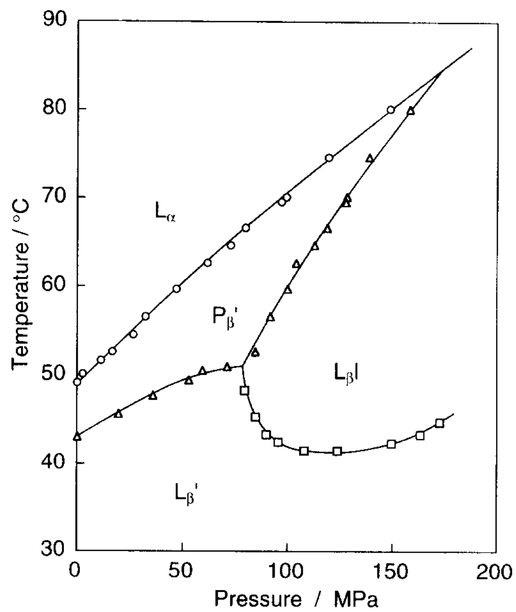

Fig. 7. Phase diagram of 17:0-PC bilayer membranes. The concentration of 17:0-PC was 1.0 mmol kg^{-1}. Phase transitions: ○, $P_\beta' \rightarrow L_\alpha$; △, $L_\beta' \rightarrow P_\beta'$ or $L_\beta I \rightarrow P_\beta'$; □, $L_\beta' \rightarrow L_\beta I$.

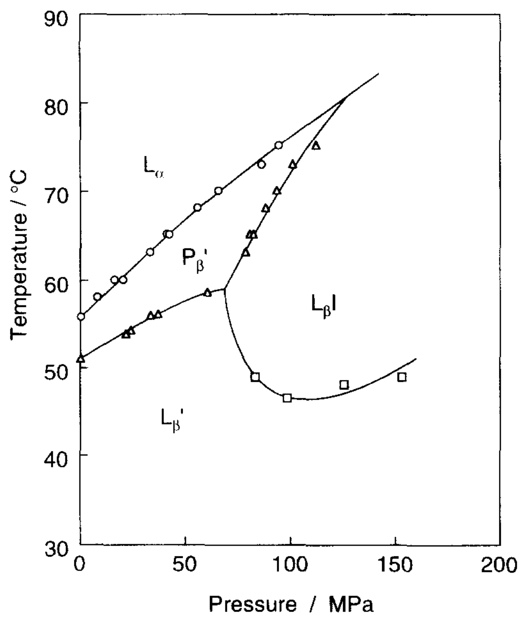

Fig. 8. Phase diagram of 18:0-PC bilayer membranes. The concentration of 18:0-PC was 2.0 mmol kg^{-1}. Phase transitions: ○, $P_\beta' \rightarrow L_\alpha$; △, $L_\beta' \rightarrow P_\beta'$ or $L_\beta I \rightarrow P_\beta'$; □, $L_\beta' \rightarrow L_\beta I$.

sect each other at 170 MPa and 84°C. The phase boundary between L_β' and $L_\beta I$ phases was transformed from the negative slope to the positive slope by pressures above 125 MPa. The negative slope is attributable to the negative volume change of transition from the L_β' phase to the $L_\beta I$ phase as mentioned before. It is expected that the two phases have different compressibilities and the L_β' phase has the larger compressibility judging from the molecular packing in both phases. Therefore, the volume change from the L_β' phase to the $L_\beta I$ phase may be transformed from a negative to a positive value as the pressure increases. The pressure reversal of the phase boundary slope may be accounted for by the reversal of the volume change on the phase transition.

The *T-P* phase diagram of 18:0-PC bilayer membranes is shown in Fig. 8. The values of dT/dP for the main transition and pretransition were 0.230 and 0.14 K MPa^{-1}, respectively. The reported values of dT/dP for the main transition were 0.249 [13] and 0.28 K MPa^{-1} [17]. The last value was determined by the method of neutron diffraction in D_2O, which seems to be somewhat large judging from the values for other lipid bilayer membranes. The value of dT/dP for the pretransition has never been known in the literature. The critical interdigitation point was observed at 70 MPa and 59°C. Braganza and Worcester reported the appearance of the $L_\beta I$ phase beyond 90 MPa [17]. These values are comparable to the present values. Another triple point among L_α, P_β' and $L_\beta I$ phases was observed at 125 MPa and 80°C. The phase boundary between L_β' and $L_\beta I$ phases was transformed from the negative slope to the positive slope by pressures above 110 MPa in a similar manner as for 17:0-PC bilayer membranes.

3.3. *Thermodynamic properties of phase transition*

The enthalpy (ΔH) and entropy ($\Delta S = \Delta H/T$) changes associated with main transition and pretransition were determined by the DSC and are listed in Table 1. The present data are comparable with previous results. The main transition enthalpies of the even acyl chain lipid have been reported by many researchers. The reported value of ΔH for 14:0-PC scattered between 22.7 kJ mol^{-1} [36] and 26.4 kJ mol^{-1} [37]. With respect to 16:0-PC, most of the reported values lie between 34.7 kJ mol^{-1} [38] and 37.2 kJ mol^{-1} [39], and the majority reported 36.4 kJ mol^{-1} [36,40,41]

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

$$dT/dP = \Delta V/\Delta S \quad (1)$$

and is also summarized in Table 1. The values of ΔV increased with an increase in acyl chain length. Direct measurements of the volume change associated with the phase transition have been reported by the methods of dilatometry [5,42,43] and density [33,44,45]. The reported values of ΔV were 19.1 cm^3 mol^{-1} [42], 24.2 cm^3 mol^{-1} [5], 24.3 cm^3 mol^{-1} [33], 27.2 cm^3 mol^{-1} [43], 27.9 cm^3 mol^{-1} [44] and 30.2 cm^3 mol^{-1} [45] for 16:0-PC, which are comparable with the present value, 25.4 cm^3 mol^{-1}, estimated from the Clapeyron-Clausius equation. With respect to 18:0-PC, several authors reported the values of ΔV to be 28.4 cm^3 mol^{-1} [42], 32.9 cm^3 mol^{-1} [33] and 38.0 cm^3 mol^{-1} [45] which are comparable with the present result, 31.6 cm^3 mol^{-1}.

The temperature and ΔH of pretransition were in good agreement with previously published data

[21,26,45–47]. Thermodynamic properties (i.e., the transition temperature, enthalpy, entropy and volume) for the main transition of lipid bilayer membranes increased with an increase in acyl chain length. Regarding pretransition, it seems that the transition temperature increases with increasing acyl chain length, but dT/dP, transition enthalpy, entropy and volume are almost constant except for 13:0-PC bilayer membranes. These phospholipid bilayer membranes undergo thermal transition due to changes in molecular packing within the hydrocarbon and head group region. With regard to main transition, the phospholipid forms a liquid crystal phase which has a bilayer structure in which the hydrocarbon chains are conformationally disordered. In this state, the acyl chains undergo extensive *trans*/*gauche* isomerization reminiscent of a fluid hydrocarbon chain. Regarding pretransition, the lipid bilayer membranes are distorted by a periodic ripple in the plane of the lamellae. The chains in this phase are relatively ordered, often tilted with respect to the bilayer normal and packed in a regular hexagonal lattice. Therefore at pretransition there were no alterations in the structure of the acyl chains. Consequently, on the main transition the influences of thermodynamic properties by the effect of chain length are much larger.

3.4. Effect of acyl chain length on phase transition

The temperatures of pretransition and main transition are plotted as a function of acyl chain length, as shown in Fig. 9. Both temperatures of pretransition and main transition increased with an increase in acyl chain length. The difference in temperature between two transitions became narrow as pressure increases. The chain length dependence of the pre- and main transition temperatures described smooth curves with no evidence of odd/even discontinuities. Consequently, the temperature difference between main transition and pretransition, $T_m - T_p$, for odd- and even-chain phospholipids lies on a single smooth curve. Previous researchers emphasized different considerations [46,48]. Silvius et al. [48] and Dörfler and Brezesinski [46] reported that the values of T_m for odd- and even-chain phosphatidylcholines lay on a single smooth curve, but the values of T_p did not, which was clearly evident when $(T_m - T_p)$ was plotted

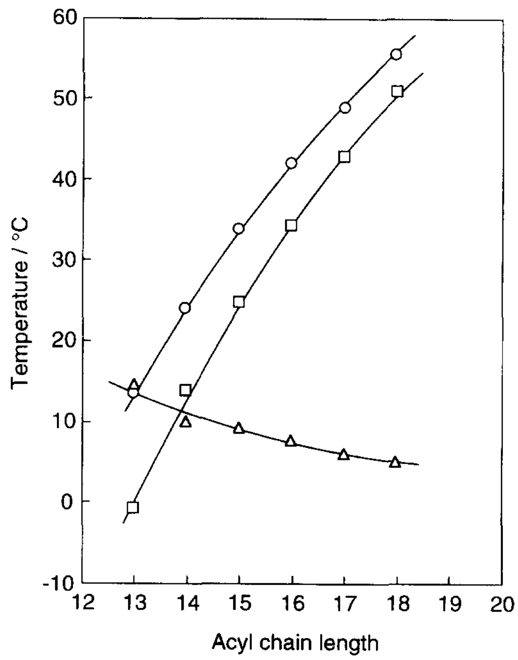

Fig. 9. Effect of acyl chain length on the phase transition temperatures of lipid bilayer membranes. ○, main transition; □, pretransition; △, temperature difference between main transition and pretransition, $T_m - T_p$.

against acyl chain length. The results of Lewis et al. [26] and the present study showed no evidence of odd/even discontinuities. The present study demonstrates that the thermotropic phase properties of lipids are not dependent on whether their acyl chains contain an odd or an even number of carbon atoms. This statement is restricted to the temperatures of the pretransition and main transition in the heating mode, because it is well known that the kinetics of P_{β}' phase formation on cooling, and the kinetics of subgel phase formation at low temperatures, do exhibit marked odd-even alterations [26], and that the structure of the subgel phases formed vary systematically with hydrocarbon chain length in a homologous series of linear, saturated diacylphosphatidylcholines [49].

The critical interdigitation points (T_i^c, P_i^c) for saturated diacylphosphatidylcholine bilayer membranes are plotted as a function of acyl chain length and shown in Fig. 10. The value of P_i^c at the critical interdigitation point can be regarded as the minimum pressure for the interdigitation of lipid bilayer membranes. The present study for a series of 14:0-, 15:0-, 16:0-, 17:0- and 18:0-PC membranes demonstrates that the value of P_i^c decreased with increasing acyl chain length in a manner of non-linear relation. It seems that the chain length dependence of P_i^c describes a hyperbolic curve. This is clearly a consequence of the larger energy gain from van der Waals interactions and the expected larger decrease in hydrocarbon volume per mole of lipid when longer chains interdigitate, so that it becomes favorable for longer chains to interdigitate at lower pressures, whereas the T_i^c vs. acyl chain length curve had a minimum at 15:0-PC bilayer membranes.

We attempted an approach by an empirical perspective in order to adequately explain the chain length dependence on the critical interdigitation point of saturated symmetric chain phosphatidylcholine bilayers. The ratio of T_i^c to P_i^c, i.e., T_i^c/P_i^c, was found to be linearly correlated to the acyl chain length as shown in Fig. 11. Although the physical meaning of the ratio T_i^c/P_i^c is still uncertain, the ratio T_i^c/P_i^c as a thermodynamic quantity would be proportional to the volume change associated with the interdigitation of bilayer membranes. The volume change on the interdigitation would be expected to be dependent upon the acyl chain length; the longer the acyl chain length of lipids, the larger the absolute change of volume on the interdigitation. The present result shown in Fig. 11 may suggest that the ratio T_i^c/P_i^c increases linearly with an increase in acyl chain length via the linear increase of the volume change on the interdigitation of lipid bilayer membranes. Fig. 11 also suggests that any diacylphosphatidylcholine with less than 13 carbons per acyl chain does not undergo pressure-induced interdigitation.

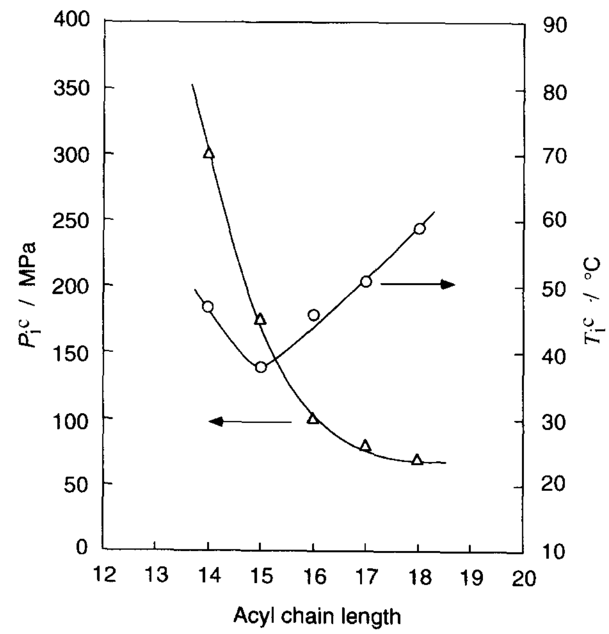

Fig. 10. Effect of acyl chain length on the critical interdigitation point (T_i^c, P_i^c) of lipid bilayer membranes. ○, T_i^c; △, P_i^c.

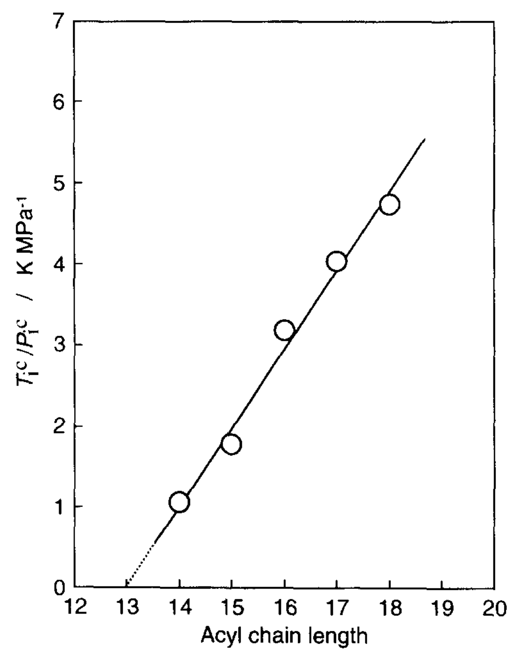

Fig. 11. Linear correlation between the ratio T_i^c/P_i^c and acyl chain length. The critical interdigitation point (T_i^c, P_i^c) refers to the triple point among P_{β}', L_{β}' and $L_{\beta}I$ phases on the phase diagram of bilayer membranes.

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