

論文目録

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学位論文題目	BAROTROPIC AND THERMOTROPIC PHASE TRANSITIONS OF PHOSPHOLIPID BILAYER MEMBRANES			
論文の目次				
Chapter 1. General Introduction				
Chapter 2. Thermotropic and barotropic phase transition on bilayer membranes of phospholipids with varying acyl chain-lengths				
Chapter 3. Barotropic phase transitions and pressure-induced interdigitation on bilayer membranes of phospholipids with varying acyl chain-lengths				
Chapter 4. Barotropic phase transitions of dioleoylphosphatidylcholine and stearyl-oleoylphosphatidylcholine bilayer membranes				
Chapter 5. Effect of unsaturated acyl chains on the thermotropic and barotropic phase transitions of phospholipid bilayer membranes				
Chapter 6. Summary				
参考論文				
主論文				
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論文内容要旨

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学位論文題目	BAROTROPIC AND THERMOTROPIC PHASE TRANSITIONS OF PHOSPHOLIPID BILAYER MEMBRANES			
内容要旨	<p>リン脂質二分子膜は細胞モデルとして幅広く研究されてきた。しかし圧力効果の研究は炭素数 16 および 14 の飽和アシル鎖を有するリン脂質 DPPC、DMPC に集中しており、アシル鎖の鎖長依存や不飽和アシル鎖を有するリン脂質二分子膜の高圧力下における研究は極めて少ない。生物の高圧力環境適応や微生物の高圧力殺菌を理解するうえで、高圧力下における二分子膜の相挙動に対してアシル鎖がどのような効果をもつのか十分に理解されていない。本研究では、まず飽和アシル鎖をもつリン脂質二分子膜（炭素数 12-18）のゲル-液晶相転移（主転移）を観測し、高圧力下における相挙動を明らかにした。鎖長の増加に伴い、主転移温度は上昇した。相転移温度の圧力依存性 dT/dP は $0.200-0.230 \text{ K MPa}^{-1}$ で鎖長の増加に伴い僅かに大きくなった。相転移熱量、体積変化は鎖長の増加に伴い大きくなったが、鎖長の奇数、偶数による差は見られなかった。次に、飽和アシル鎖を有するリン脂質のゲル相間の転移について明らかにした。ラメラゲル相からリップルゲル相への転移（前転移）は鎖長 13 以上のリン脂質で観測された。主転移と同様に鎖長の増加に伴い、前転移温度は上昇したが、dT/dP は $0.12-0.14 \text{ K MPa}^{-1}$ で鎖長の影響はみられなかった。高圧力下でのみ観測される圧力誘起の Interdigitated ゲル相はアシル鎖長 14 の DMPC 二分子膜では 300 MPa 以上で観測された。アシル鎖長が長くなると Interdigitation を起こす圧力は低下した。</p> <p>生体膜を構成するリン脂質は、ほとんどの生物で、不飽和脂肪酸を高いパーセンテージで含有している。不飽和脂肪酸の融点は飽和脂肪酸に比べてはるかに低温であることから、不飽和脂肪酸から成るリン脂質膜のゲル-液晶相転移温度は低く、このことが生物において重要な機能をはたしていると考えられる。不飽和結合を持つリン脂質としてオレイン酸をアシル鎖に持つ DOPC の温度-圧力相図を決定した。従来常圧下で -12°C に主転移があるとされたが、さらに低温側の -40°C に新しい相転移を発見した。検討した結果、-12°C の相転移はラメラ結晶相から液晶相への転移であり、-40°C にゲル-液晶の主転移があることを明らかにした。ステアリン酸 (<i>sn</i>-1) とオレイン酸 (<i>sn</i>-2) をアシル鎖に持つ SOPC 二分子膜の相図から、1 個のシス二重結合を導入すると主転移温度を 48°C 下げることが明らかになった。ステアリン酸 (<i>sn</i>-2) とオレイン酸 (<i>sn</i>-1) が入れ替わった OSPC やトランス型二重結合のエライジン酸を持つ DEPC の主転移および結晶相から液晶相への転移の圧力効果を検討した。SOPC、OSPC、DEPC は、2 つの転移ともほぼ同じ転移温度、圧力依存性を示した。このことからシス型二重結合 1 個はトランス型二重結合 2 個と同じ効果を持つことが明らかになった。また 1 位にステアリン酸、2 位に不飽和アシル鎖を持つリン脂質、SOPC（オレイン酸 18:1）、SAPC（アラキドン酸 20:4）、SDPC（ドコサヘキサエン酸 22:6）の常圧における主転移温度は、6.7°C、-13°C、-7.2°C であり、不飽和度の増加にともない、一様に下がることはない。また圧力依存性 dT/dP は、DSPC 0.230、SOPC 0.181、SAPC 0.134、SDPC 0.165 K MPa^{-1} であり、不飽和度の増加により一様に小さくなるわけではない。この現象は脂質分子の頭部（ホスファチジルコリン）がかさ高いため、ドコサヘキサエン酸が二分子膜中でラセン状になりパッキングを密にしていることが原因と考えられる。不飽和リン脂質二分子膜でのゲル-液晶相転移はこれまでほとんど調べられていないが、相転移の熱力学量 (ΔH、ΔS、ΔV、dT/dP) から秩序性の低いゲル相を形成することが明らかになった。</p>			

BAROTROPIC AND THERMOTROPIC PHASE
TRANSITIONS OF PHOSPHOLIPID BILAYER
MEMBRANES

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November 1999

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MEMBRANES**

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CONTENTS

CHAPTER 1.	GENERAL INTRODUCTION.....	1
CHAPTER 2.	THERMOTROPIC AND BAROTROPIC PHASE TRANSITION ON BILAYER MEMBRANES OF PHOSPHOLIPIDS WITH VARYING ACYL CHAIN-LENGTHS.....	3
	1. Introduction	
	2. Experimental procedures	
	2.1 <i>Materials</i>	
	2.2. <i>Differential scanning calorimetry</i>	
	2.3. <i>Phase transition measurements under high pressures</i>	
	3. Results and discussion	
	3.1. <i>Effect of pressure on the phase transition temperature</i>	
	3.2. <i>Thermodynamic properties of the phase transition</i>	
CHAPTER 3.	BAROTROPIC PHASE TRANSITIONS AND PRESSURE-INDUCED INTERDIGITATION ON BILAYER MEMBRANES OF PHOSPHOLIPIDS WITH VARYING ACYL CHAIN-LENGTHS.....	19
	1. Introduction	
	2. Experimental procedures	
	2. 1. <i>Materials</i>	
	2. 2. <i>Phase transition measurements</i>	

- 3. Results and discussion
 - 3.1. *Thermotropic and barotropic phase transitions*
 - 3.2. *Phase diagram of lipid bilayer membranes*
 - 3.3. *Thermodynamic properties of the phase transition*
 - 3.4. *Effect of acyl chain length on the phase transition*

CHAPTER 4. BAROTROPIC PHASE TRANSITIONS OF
DIOLEOYLPHOSPHATIDYLCHOLINE AND
STEAROYL-OLEOYLPHOSPHATIDYLCHOLINE
BILAYER MEMBRANES.....43

- 1. Introduction
- 2. Experimental procedures
 - 2.1. *Materials*
 - 2.2. *Phase transition measurements*
- 3. Results and discussion
 - 3.1. *Phase transitions of DOPC bilayer membrane*
 - 3.2. *Effect of acyl chain unsaturation on the phase behavior*
 - 3.3. *Thermodynamic properties of the phase transitions*

CHAPTER 5. EFFECT OF UNSATURATED ACYL CHAINS ON THE
THERMOTROPIC AND BAROTROPIC PHASE TRANSITIONS
OF PHOSPHOLIPID BILAYER MEMBRANES.....59

- 1. Introduction
- 2. Experimental procedures
 - 2.1. *Materials*

- 2.2. *Phase transition measurements*
- 3. Results and discussion
 - 3.1. *Influence of monounsaturated acyl chain containing
eighteen carbons on the phase transition of PC bilayer
membranes*
 - 3.2. *Influence of polyunsaturated acyl chain in sn-2 position
on the phase transition of PC bilayer membranes*
 - 3.3. *Thermodynamic properties of the phase transitions*

CHAPTER 6. SUMMARY.....85

ACKNOWLEDGMENTS.....89

REFERENCES.....91

REFERENCE PAPERS.....97

CHAPTER 1.

GENERAL INTRODUCTION

As recognized at 1925 by Gorter and Grendel, a bimolecular layer, or bilayer, can satisfy the thermodynamic requirements of amphipathic molecules in an aqueous environment. A bilayer exists as a sheet in which the hydrophobic regions of the phospholipids are protected from the aqueous environment, while the hydrophilic regions are immersed in water. Only the ends or edges of the bilayer sheet are exposed to an unfavorable environment, but even these exposed edges can be eliminated by folding the sheet back upon itself to form an enclosed vesicle with no edges. The closed bilayer provides one of the essential properties of membranes. It is impermeable to most water-soluble molecules, since they would be insoluble in the hydrophobic core of the bilayer.

Membranes are complex structures composed of lipids, carbohydrates, and proteins. The basic structure of all membranes is the lipid bilayer as known by the "fluid mosaic model" proposed by Singer and Nicolson at 1972. This bilayer is formed by two sheets of phospholipids in which the hydrophilic polar head groups are directed away from each other and are exposed to the aqueous environment on the outer and inner surfaces of the membrane. The hydrophobic nonpolar tails of these molecules are oriented toward each other, in the direction of the center of the membrane. Proteins can be integral component of the membrane and span the bilayer, or they can be attached, by electrostatic charge, to the outer or inner surface of the membrane.

Recent concepts of membrane structure emphasize the functional significance of molecular mobility within the phospholipid bilayer. Under physiological conditions, the bilayer is generally thought to be in a disordered or fluid condition, the degree of molecular mobility and structural order of the hydrocarbon chains having a direct effect upon the

dynamic structure, segmental mobility, and catalytic activity of at least some membrane-bound enzymes. It is also generally accepted, if only implicitly, that the fluidity of the bilayer is precisely regulated. However, the most direct method of investigating the regulation of membrane order is to disturb the system and to observe corrective responses. Temperature and pressure are the most powerful disturbing influences, and studying the adjustment of membranes to these modalities reveals, in a particularly dramatic way, the capacity of cells, in general, to regulate adaptively the structure and functional properties of their constituent membranes. Perhaps the most consistently observed adaptive responses of microorganisms, plants and animals to temperature variations involves changes to the lipid composition and dynamic structure of their cellular membranes. Invariably, low temperature adaptation is associated with the incorporation of greater proportions of unsaturated fatty acids in membrane lipids and this causes an increase in the disorder of the bilayer that overcomes the direct ordering influences of low temperature. Furthermore, by the pressure adaptation deep-sea organisms contain more unsaturated fatty acid. The low temperature and high pressure have similar effect for the composition of biomembrane. The fluidity of biomembrane was decided by the degree of unsaturation in biomembrane lipids.

Phospholipid membranes undergo thermotropic phase transition of which temperature depends strongly on the nature of the hydrophobic chain. Temperature-pressure phase diagrams of lipid bilayer membranes give us many informations on membrane properties. In this paper, in order to understand the effect of acyl chain length and unsaturation on the thermotropic and barotropic phase behavior of phospholipid bilayer membranes, we performed the high-pressure light transmission measurements on the phase transitions of phospholipid bilayer membranes under various pressures. In addition to the high-pressure experiments, the phase transitions of lipid bilayer membranes under ambient pressure were observed by high-sensitivity differential scanning calorimeter.

CHAPTER 2.

**THERMOTROPIC AND BAROTROPIC PHASE TRANSITION
ON BILAYER MEMBRANES OF PHOSPHOLIPIDS
WITH VARYING ACYL CHAIN-LENGTHS**

1. Introduction

The effect of pressure on lipid bilayer membranes is of particular interest to the studies of pressure-anesthetic antagonism [1], pressure adaptation in deep-sea organisms [2], and high-pressure sterilization in food processing [3]. Lipid 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 isothermal barotropic phase behavior of phospholipid bilayer membranes has been well described by Wong et al. [4]. It is also apparent from a survey of the literature that there have been relatively few studies on the phase properties of diacylphosphatidylcholines whose hydrocarbon chains contain an odd number of carbon atoms [5, 6] and that there is no study on the effect of pressure. Pressure studies on the 16:0-PC bilayer membranes have been reported by various physical techniques including ESR [7], dilatometry [8, 9], calorimetry [10, 11], X-ray diffraction [12], dynamic light scattering [13], Raman spectroscopy [14, 15], adiabatic compression [16], fluorescence [17, 18], FT-IR [19], neutron diffraction [20, 21], light transmittance [22, 23] and NMR [24-27]. These measurements have revealed phase behavior of 16:0-PC bilayer membranes. In addition to liquid crystal, ripple gel and lamellar gel phases, a new pressure-induced gel phase, i.e., the interdigitated gel

phase has been observed under high pressure [20-23, 26].

In the present study, we focus our attention on the main transition from the ripple gel to the liquid crystal phase of bilayer membranes, and reveal the pressure effect on the bilayer phase-transition of a series of diacylphosphatidylcholines containing linear saturated acyl chains of even- and odd-number carbons. We discuss also the effect of acyl chain-lengths 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) used in this study were as follows:

- 12:0-PC, didodecanoylphosphatidylcholine
- 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 (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 found to be 200~300 nm, which was determined by the light scattering method.

2.2. Differential scanning calorimetry

The phase transitions of phospholipid multilamellar vesicles under ambient pressure were observed by a MicroCal MCS high-sensitivity differential scanning calorimeter (Northampton, Mass, 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

The 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 Co. (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 Co.) and measured 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. The abrupt change of the transmittance accompanying the pressure-induced 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. Effect of pressure on the phase transition temperature

An example of the thermotropic phase-transition measurements for 15:0-PC bilayer membrane is depicted in Fig. 2-1. The DSC thermogram of heating scan at ambient pressure showed the main transition from the ripple gel phase to the liquid crystal phase, which involves the melting or disordering of the hydrocarbon chains of the lipid. The light transmittance also changed clearly at the same temperature. The midpoint between the beginning of the increase in transmittance and the point where the transmittance reached its plateau was taken as the phase-transition temperature. The transition temperatures by both methods were in good agreement with each other. As is seen from Fig. 2-1, the temperature of the thermotropic main-transition increased with an increase in pressure.

A typical measurement of isothermal barotropic phase transition for 15:0-PC bilayer membrane is shown in Fig. 2-2. A pressure-induced phase transition from the liquid crystal phase to the ripple gel phase was observed. The phase transition pressure was determined as a function of temperature from the transmittance-pressure profiles at various temperatures, which increased with an increase in temperature.

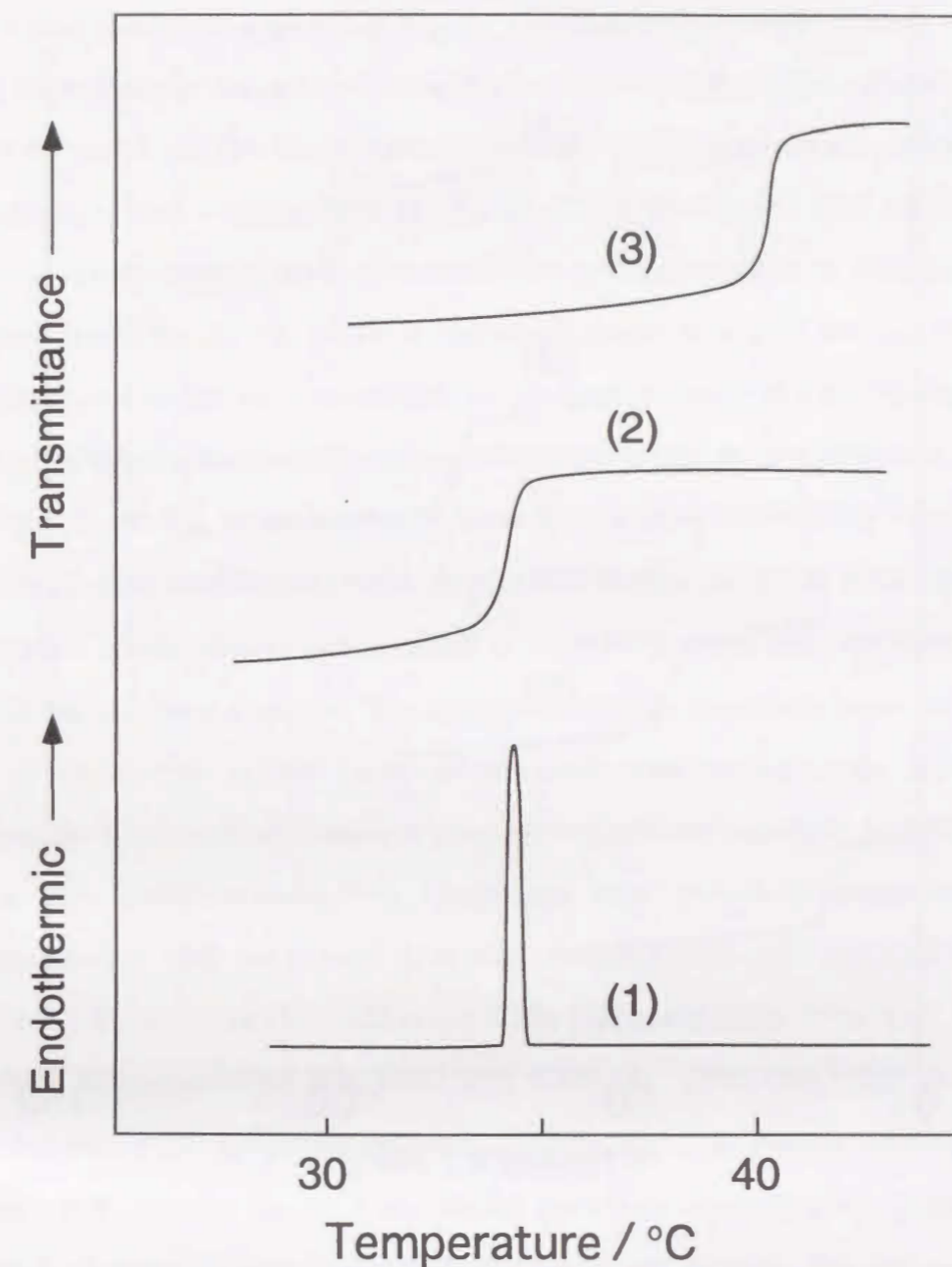


Fig. 2-1. Thermotropic phase-transitions of 15:0-PC bilayer membrane observed by (1) DSC at 0.1 MPa; (2) an optical method at 0.1 MPa; and (3) at 29.4 MPa.

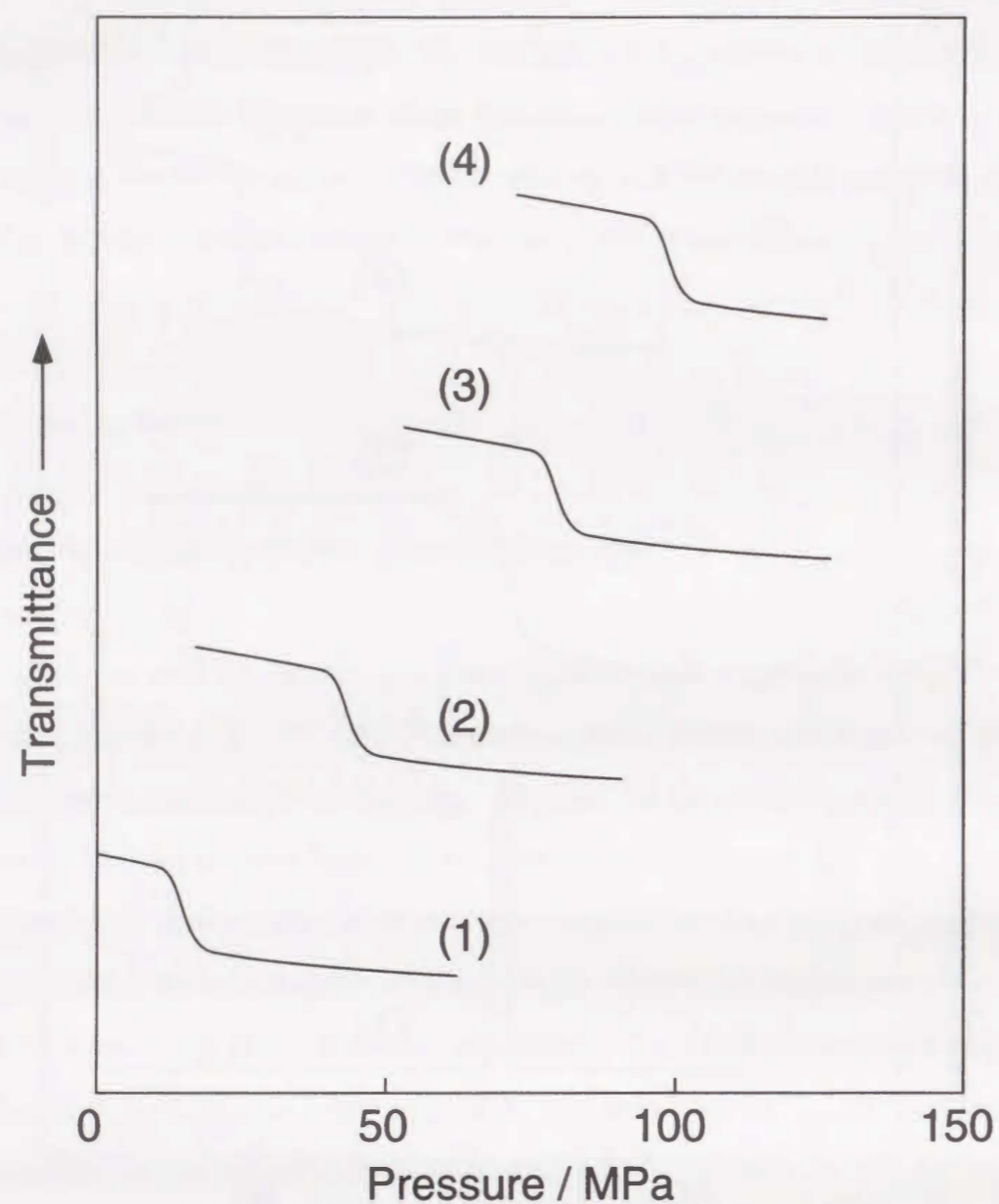


Fig. 2-2. Barotropic phase-transitions of 15:0-PC bilayer membrane observed by an optical method at various temperatures: (1) 38.0 °C; (2) 44.0 °C; (3) 51.0 °C; (4) 55.0 °C.

The temperature (T) - pressure (p) phase boundaries between the ripple gel and the liquid crystal phases are depicted in Fig. 2-3 for several lipids with different acyl chain-lengths. Both results of the isobaric and isothermal phase transitions give a single line on T - p diagram for each lipid. The temperatures of the main transition at ambient pressure were in good agreement with those in literatures [5, 28, 29]. With respect to the 12:0-PC bilayer membrane, extrapolation of the T - p line to ambient pressure suggests the temperature of the main transition to be -2.1 °C, which is in good agreement with previous data [5, 28]. The chain length dependence of the main transition temperature described a smooth curve with no evidence of odd/even discontinuities different from the melting point of fatty acids. As is seen from Fig. 2-3, the T - p curves are almost linear for the lipids studied. The values of slope, dT/dp , for the main transition are in the range of 0.20 K MPa⁻¹ to 0.23 K MPa⁻¹ depending on the acyl chain-length, which are summarized in Table 2-1 together with other thermodynamic quantities for the phase transition. The transitions of lipids containing linear saturated acyl chains of odd-number carbons have not previously been studied under high pressure. Regarding the lipids with acyl chains of even-number carbons (especially 14:0-PC and 16:0-PC), the phase transition under high pressure has been reported by several authors. The previous values of dT/dp for 14:0-PC have been reported to be 0.167 [30], 0.201 [15], 0.210 [22], 0.215 [18, 21], 0.218 [16], 0.224 [17], 0.230 [20] and 0.240 K MPa⁻¹ [31]. The values for 16:0-PC bilayer are 0.208 [15], 0.217 [22], 0.220 [21], 0.225 [18], 0.227 [8, 17], 0.230 [20], 0.237 [32], 0.243 [17], 0.244 [33] and 0.249 K MPa⁻¹ [16]. Present results are in good agreement with previous values. A few studies have been reported on the phase transition under high pressure for bilayer membranes of 12:0-PC and 18:0-PC. The reported values of dT/dp were 0.17 K MPa⁻¹ for 12:0-PC [34], 0.249 K MPa⁻¹ [16] and 0.280 K MPa⁻¹ [20] for 18:0-PC, respectively. The last value was determined by the method of neutron diffraction in D₂O, which seems to be somewhat large judging from the values for other lipid bilayer

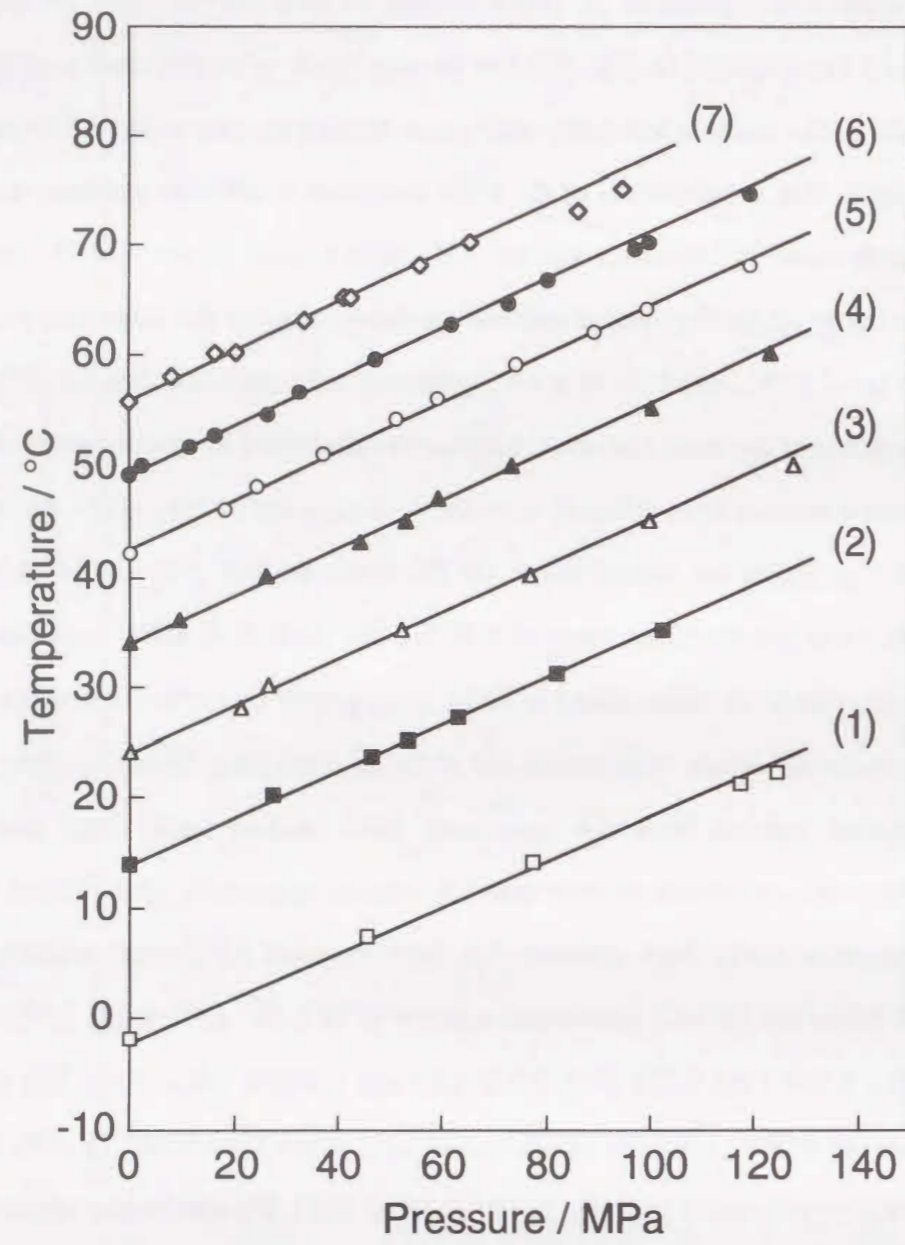


Fig. 2-3. Temperature-pressure phase boundaries between the ripple gel and liquid crystal phases for bilayer membranes of phospholipids with different acyl chain-lengths. (1) 12:0-PC, (2) 13:0-PC, (3) 14:0-PC, (4) 15:0-PC, (5) 16:0-PC, (6) 17:0-PC and (7) 17:0-PC.

Table 2-1. Thermodynamic properties of phase transition for the bilayer membranes of diacylphosphatidylcholines.

Lipid	Transition Temp.		dT/dP (K MPa ⁻¹)	ΔH (kJ mol ⁻¹) (kcal mol ⁻¹)	ΔS (J K ⁻¹ mol ⁻¹)	ΔV (cm ³ mol ⁻¹)
	(K)	(°C)				
12:0-PC	271.1	-2.1	0.200	7.5 ^a	28	5.5
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	48.9	0.224	41.4	129	28.8
18:0-PC	328.8	55.6	0.230	45.2	137	31.6

a) Lewis et al. [5]

membranes.

3. 2. Thermodynamic properties of the phase transition

The enthalpy (ΔH) and entropy ($\Delta S = \Delta H/T$) changes associated with the phase transition were determined by the DSC and are listed in Table 2-1. Present data are comparable with previous results. The transition enthalpies of the even acyl-chain lipid have been reported by many researchers. The reported value of ΔH of 14:0-PC scatters between 22.7 kJ mol⁻¹ [28] and 26.4 kJ mol⁻¹ [35]. With respect to 16:0-PC, most of the reported values lie between 34.7 kJ mol⁻¹ [36] and 37.2 kJ mol⁻¹ [37], and the majority reported 36.4 kJ mol⁻¹ [28, 38, 39]. The values of ΔH together with those in literatures are shown in Fig. 2-4 as a function of acyl chain-length. The chain length dependence of the main-transition enthalpy describes a smooth curve with no evidence of odd/even discontinuities which have been observed on a simple chain-melting process comparable to the fusion of straight-chain fatty acids [40] and soaps [41]. Lewis et al. [5] have reported by the DSC method that the enthalpy of the main transition increases with increasing acyl chain length and the data are best described by a smooth curve and not a linear function. As is seen from Fig. 2-4, however, there is discrepancy between data by Lewis et al. and by others, which seems to be out of the experimental error. Regarding the transition enthalpies of the odd acyl-chain lipids, only a few studies [5, 6] have been published. Lin et al. [6] have reported the value of ΔH of 17:0-PC bilayer membranes to be 40.6 kJ mol⁻¹, which is in good agreement with the present result.

The entropy changes associated with the phase transition are shown in Fig. 2-5 as a function of acyl chain-length. The dependence of chain length on the phase transition entropy also describes a smooth curve with no evidence of odd/even discontinuities as well as on the phase transition enthalpy.

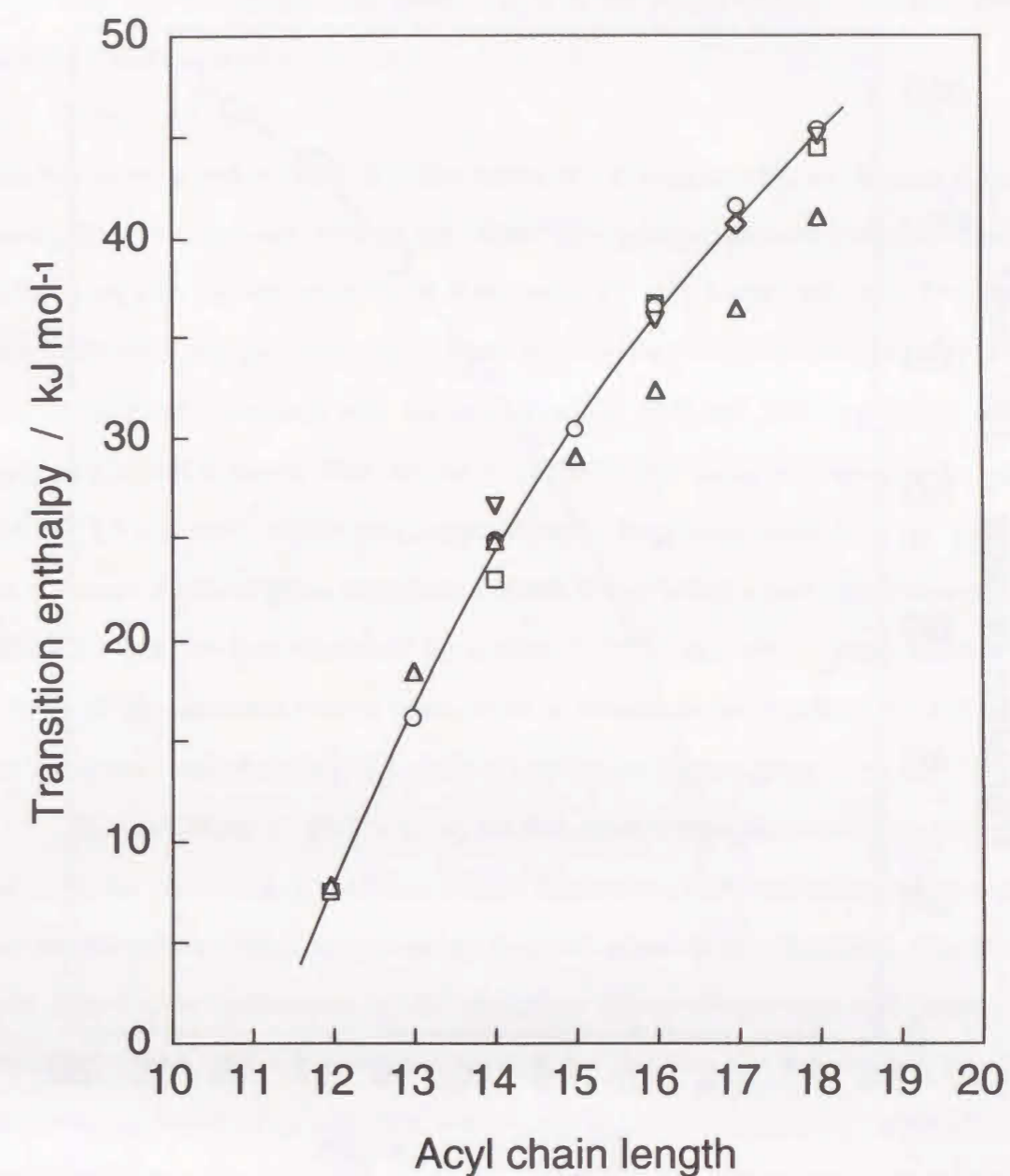


Fig. 2-4. Effect of acyl chain-length on the phase transition enthalpy of lipid bilayer membranes. (○) present result, (□) by Mabrey and Sturtevant [28], (▽) by Stümpel et al. [35], (△) by Lewis et al. and (◇) by Lin et al. [6].

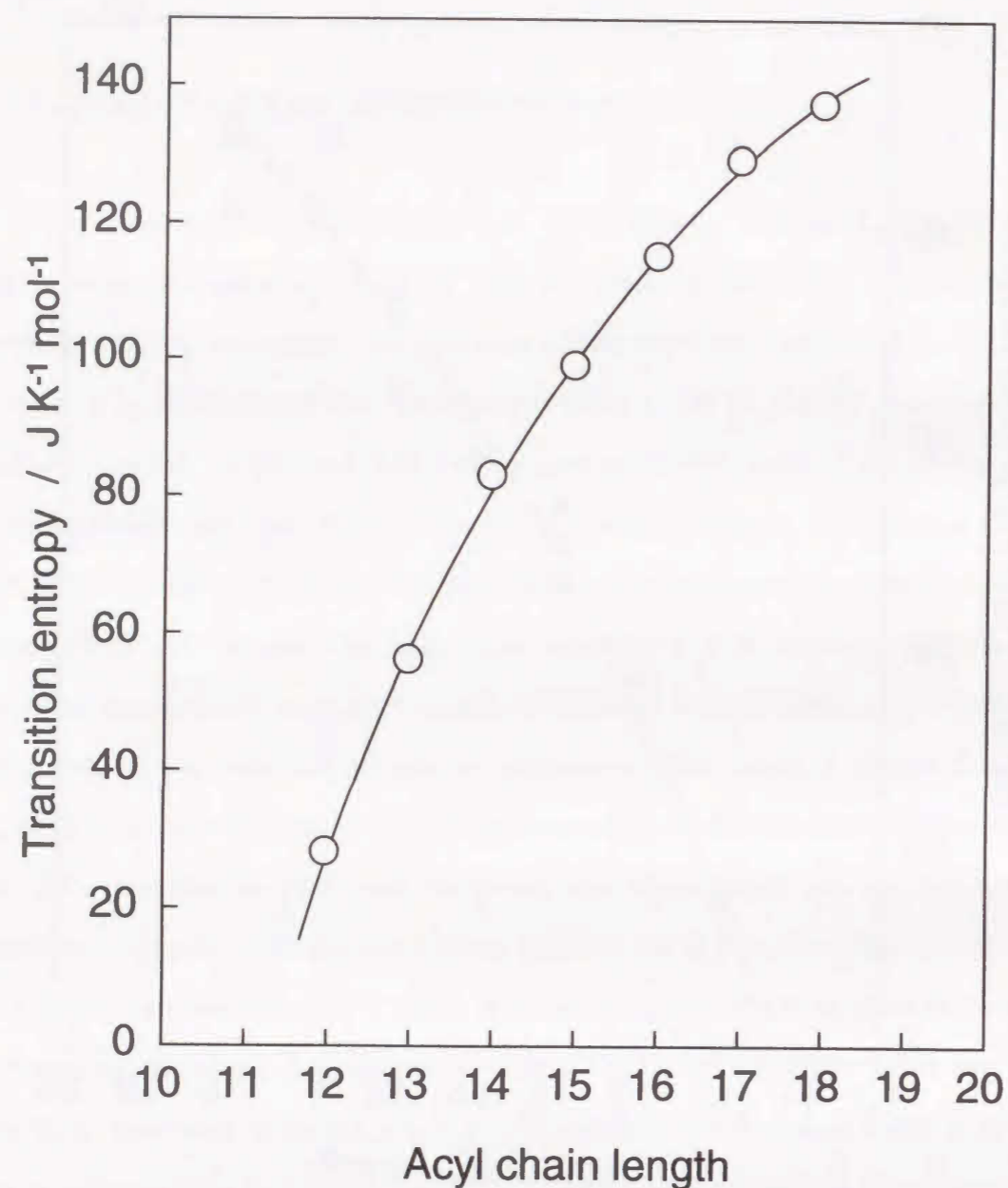


Fig. 2-5. Effect of acyl chain-length on the phase transition entropy of lipid bilayer membranes.

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

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

and is also summarized in Table 2-1. The values of ΔV increase with an increase in the acyl chain-length. Direct measurements of the volume change associated with the phase transition have been reported by the methods of dilatometry [8] and density [42, 43]. The reported values of ΔV were $24.2 \text{ cm}^3 \text{ mol}^{-1}$ [8], $27.9 \text{ cm}^3 \text{ mol}^{-1}$ [42] and $24.3 \text{ cm}^3 \text{ mol}^{-1}$ [43] for 16:0-PC, which are in good agreement with the present value, $25.4 \text{ cm}^3 \text{ mol}^{-1}$, estimated from the Clapeyron-Clausius equation. With respect to 18:0-PC, Ohki et al. [43] reported the value of ΔV to be $32.9 \text{ cm}^3 \text{ mol}^{-1}$, which are comparable with the present result, $31.6 \text{ cm}^3 \text{ mol}^{-1}$. The volume change of bilayer phase transition is shown in Fig. 2-6 as a function of the acyl chain-length. The data are best described by a smooth curve and not a linear function. The increment of the transition volume tends to be moderated as the length of the hydrocarbon chain is increased and amounts to $1.4 \text{ cm}^3 \text{ mol}^{-1}$ per one methylene group.

Thermodynamic properties (i. e., the transition temperature, enthalpy, entropy and volume) for the phase transition of lipid bilayer membranes were best described by a smooth curve and not a linear function with no evidence of odd/even discontinuities. The observed bilayer seems to be attributable to the end-group effects of the fatty acyl chains. Such considerations have not been thoroughly addressed in the literature. Mason and Huang [44] attempted an approach by an empirical perspective. In order to adequately explain the chain length dependent thermotropic phase behavior of saturated symmetric-chain phosphatidylcholine bilayers, it is necessary to consider not only the absolute chain length but also the relative distribution of the acyl chains. They consider that the bilayer interface region and conformationally inequivalent terminal ends of the fatty acyl chains perturb the packing associations of the rest of the hydrocarbon chains in the gel phase of the bilayer

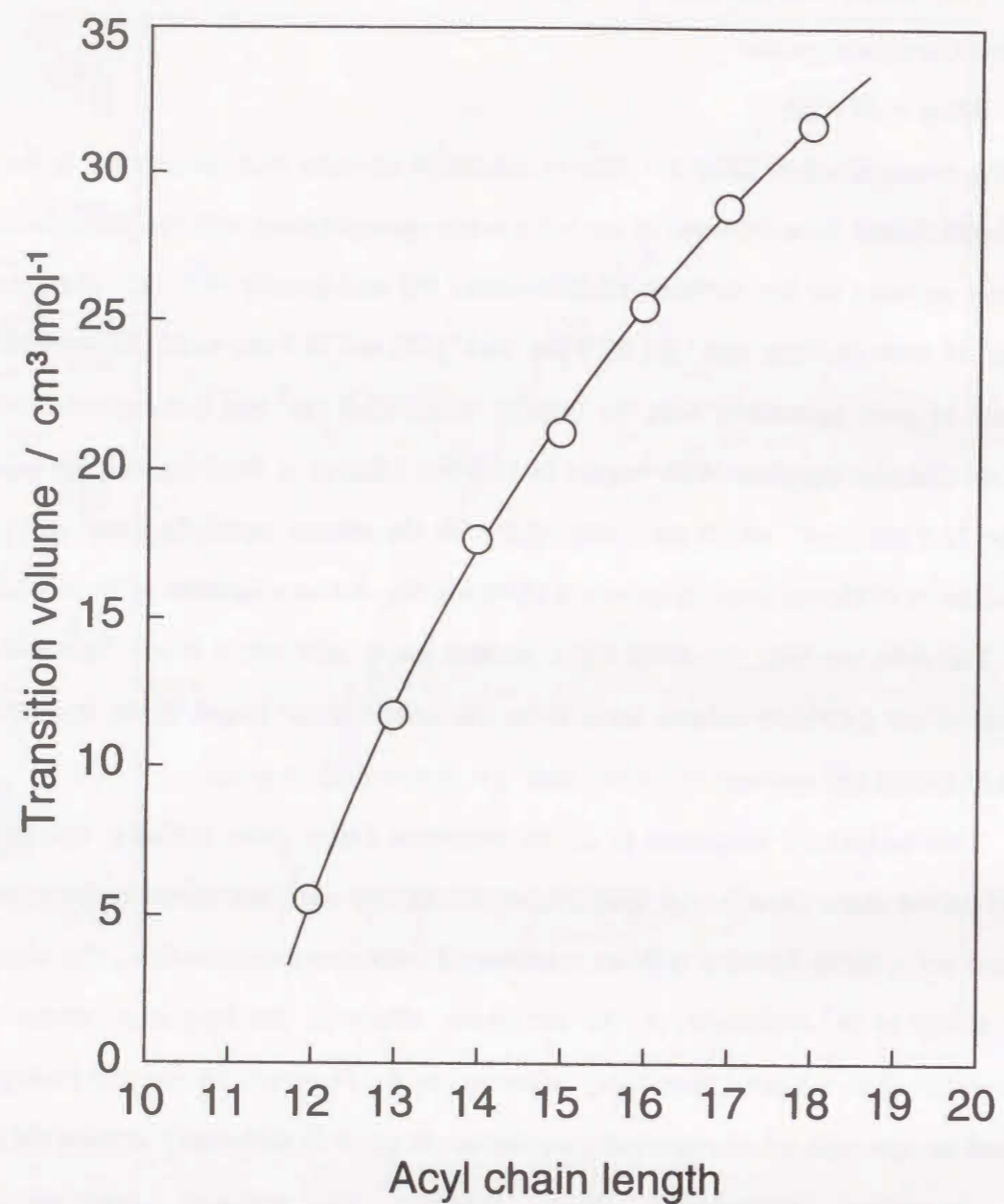


Fig. 2-6. Effect of acyl chain-length on the phase transition volume of lipid bilayer membranes.

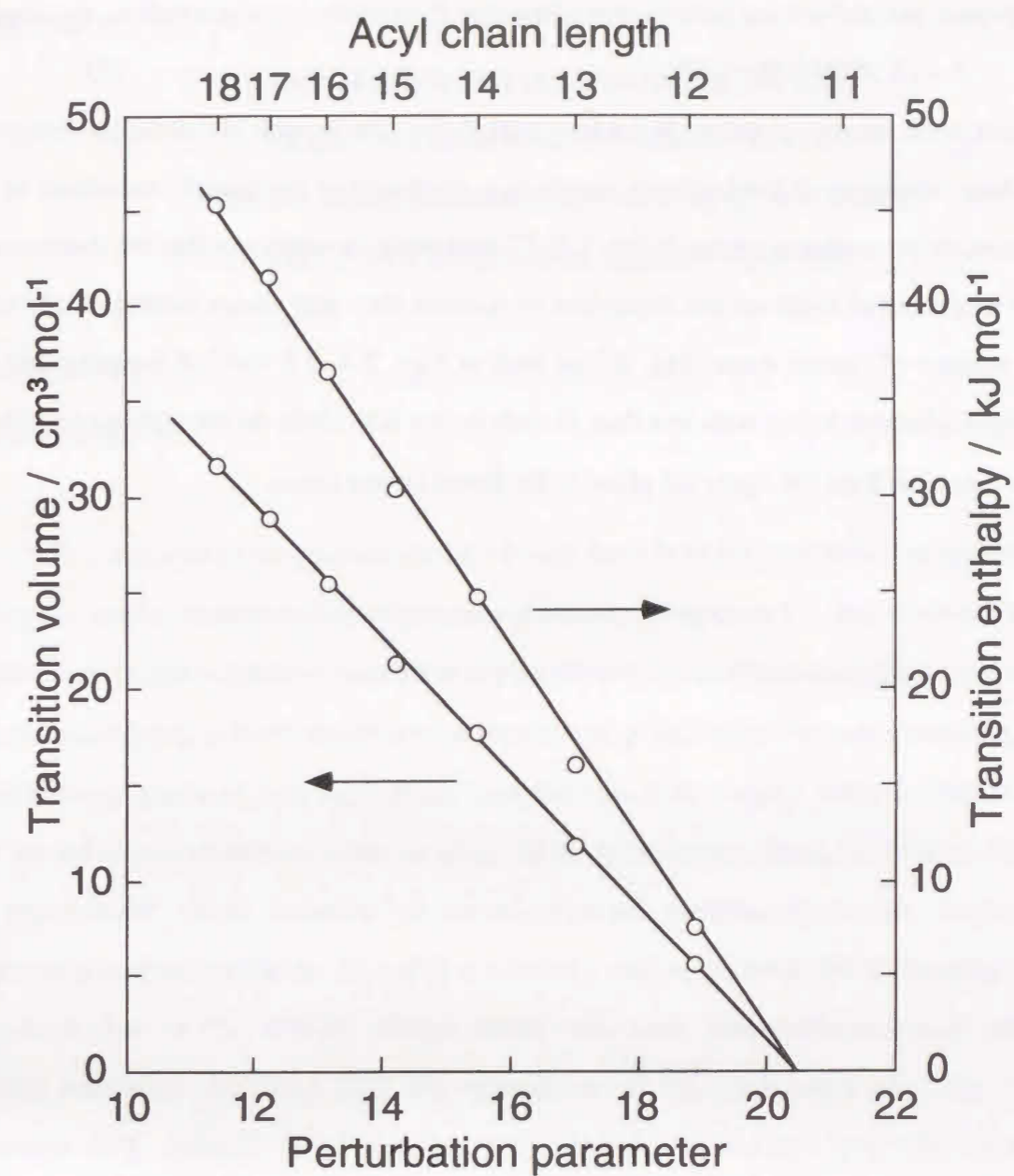


Fig. 2-7. Phase transition volume and enthalpy as a function of perturbation parameter defined by Mason and Huang [44].

membranes, and defined the perturbation parameter, P , expressed as a percentage, as follows:

$$P = \{3.5/(2N-5.5)\} \times 100 \quad (2)$$

where N is the number of carbon in the fatty acyl chains. The volume and enthalpy changes of the phase transition of lipid bilayer membranes are found to be linearly correlated to the perturbation parameter as shown in Fig. 2-7. 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. Fig. 2-7 as well as Figs. 2-4, 2-5 and 2-6 suggests that any diacylphosphatidylcholine with less than 11 carbons per acyl chain do not undergo the bilayer phase transition from the ripple gel phase to the liquid crystal phase.

CHAPTER 3.

**BAROTROPIC PHASE TRANSITIONS AND
PRESSURE-INDUCED INTERDIGITATION
ON BILAYER MEMBRANES OF PHOSPHOLIPIDS
WITH VARYING ACYL CHAIN-LENGTHS**

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 [7], dilatometry [8, 9], calorimetry [10, 11], X-ray diffraction [12], dynamic light scattering [13], Raman spectroscopy [14, 15], adiabatic compression [16], fluorescence [17, 18], FT-IR [19], neutron diffraction [20, 21], light transmittance [22, 23, 33] and NMR [24-26]. 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 [20, 21], light

transmittance [22, 23, 33] and NMR [26]. 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 [20-23, 33]. Regarding the slope of phase boundary between L_{β}' and $L_{\beta}I$ phases, there is still remaining a disagreement, which is positive or negative slope.

Our previous study [45] 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 an odd number of carbon atoms [5, 6, 46].

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 discuss also 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) were 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.

The phospholipid multilamellar vesicles were prepared by similar method in chapter

2.

2.2. Phase transition measurements

The phase transitions of phospholipid bilayer membrane were measured by DSC at ambient pressure and by light transmittance under high pressure. The detail information of the measurements was described in chapter 2.

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 Figure 3-1. The DSC thermogram of heating scan at ambient pressure showed two kinds of endothermic transitions (curve 1 in Fig. 3-1). 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}'

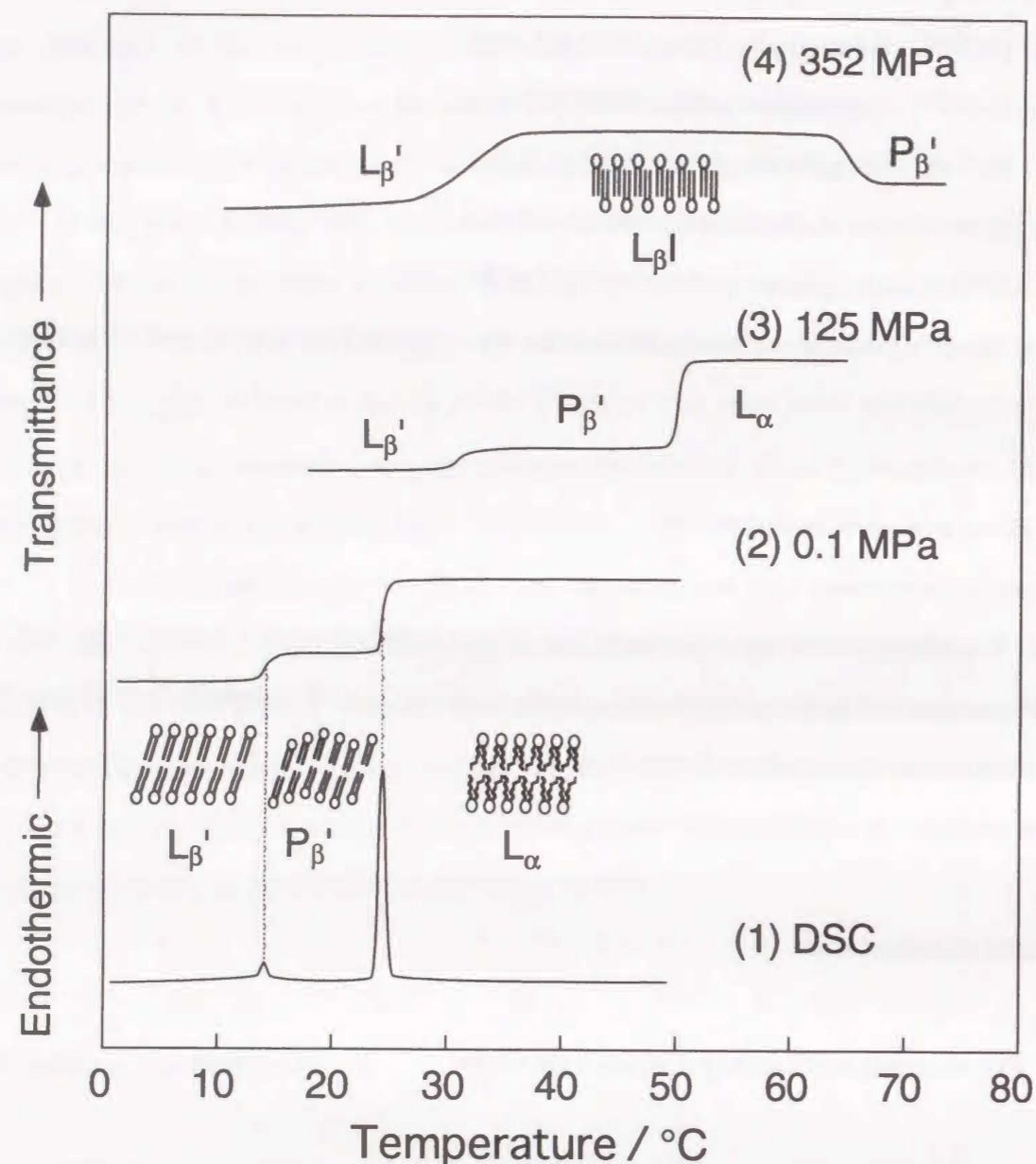


Fig. 3-1. Isobaric thermotropic phase transitions of 14:0-PC bilayer membrane. The main transition and pretransition by the methods of (1) DSC and (2) light transmittance at 0.1 MPa and (3) at 125 MPa. A pressure-induced interdigitation by the optical method (4) at 352 MPa.

phase to the P_{β}' phase. The light transmittance also changed clearly at two transition temperatures (curve 2 in Fig. 3-1). 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 are in good agreement with previously published data [5]. Both temperatures of the main transition and pretransition increased with an increase in pressure (curve 3 in Fig. 3-1). A difference in temperature between two transitions became wide as the pressure increases. At the pressure higher than 300 MPa, we observed a new pressure-induced phase transition (curve 4 in Fig. 3-1). It has been observed for 16:0-PC [33] and dihexadecylphosphatidylcholine (DHPC) [33,47] bilayer membranes that the transition from the L_{β}' phase to the $L_{\beta}I$ phase is accompanied with an increase in turbidity. More direct evidence for interdigitation has been observed from the spacing measurements by neutron diffraction [20, 21] or X-ray diffraction [48] 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 [23] and DHPC [33, 47].

Fig. 3-2 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 the increase in transmittance with increasing pressure. The middle point of the change in transmittance was taken as the transition pressure. A difference in pressure between two transitions becomes narrow as the temperature increases.

3. 2. Phase diagram of lipid bilayer membranes

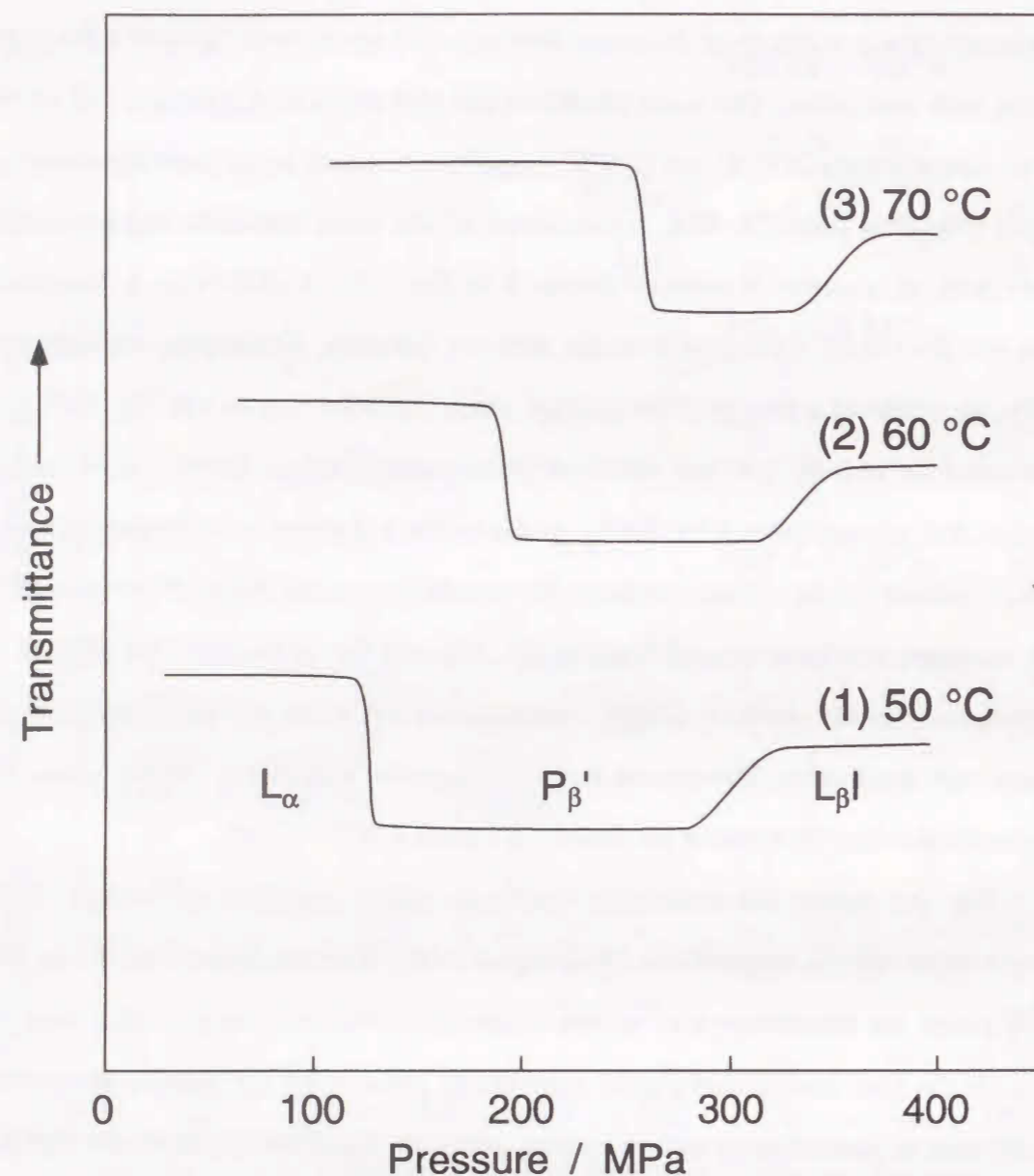


Fig. 3-2. Isothermal barotropic phase transitions of 14:0-PC bilayer membrane. The main transition and pressure-induced interdigitation by an optical method under various temperatures: (1) 50 °C, (2) 60 °C and (3) 70 °C.

The temperature (T) - pressure (P) phase diagram of 13:0-PC bilayer membranes is shown in Fig. 3-3. 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, dT/dP , were 0.210 K MPa⁻¹ for the main transition and 0.14 K MPa⁻¹ for the pretransition and are listed in Table 3-1. The main transition of 13:0-PC bilayer membranes under high pressure has been reported only by our laboratory [45], 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. Previous value of the pretransition temperature is -0.8 °C [5], which is in good agreement with the present value. We could not observe the L_{β} 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. 3-4. 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 13:0-PC. The values of dT/dP were 0.212 K MPa⁻¹ for the main transition and 0.13 K MPa⁻¹ for the pretransition, respectively. The main transition of 14:0-PC bilayer membranes under high pressure has been reported by several authors [15, 17, 18, 20-22, 31, 49]. The previous values of dT/dP 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 dT/dP to be 0.16 [49], 0.18 [31] and 0.12 K MPa⁻¹ [22], which are not consistent with each other. A detailed pressure study has been reported by Prasad and coworkers [22]. Their result, 0.12 K MPa⁻¹ is comparable to the present value, 0.13 K MPa⁻¹. As is seen from Fig. 3-4, a pressure-induced interdigitated gel phase, in which the hydrocarbon chains from apposing monolayers become interdigitated with the chains, was

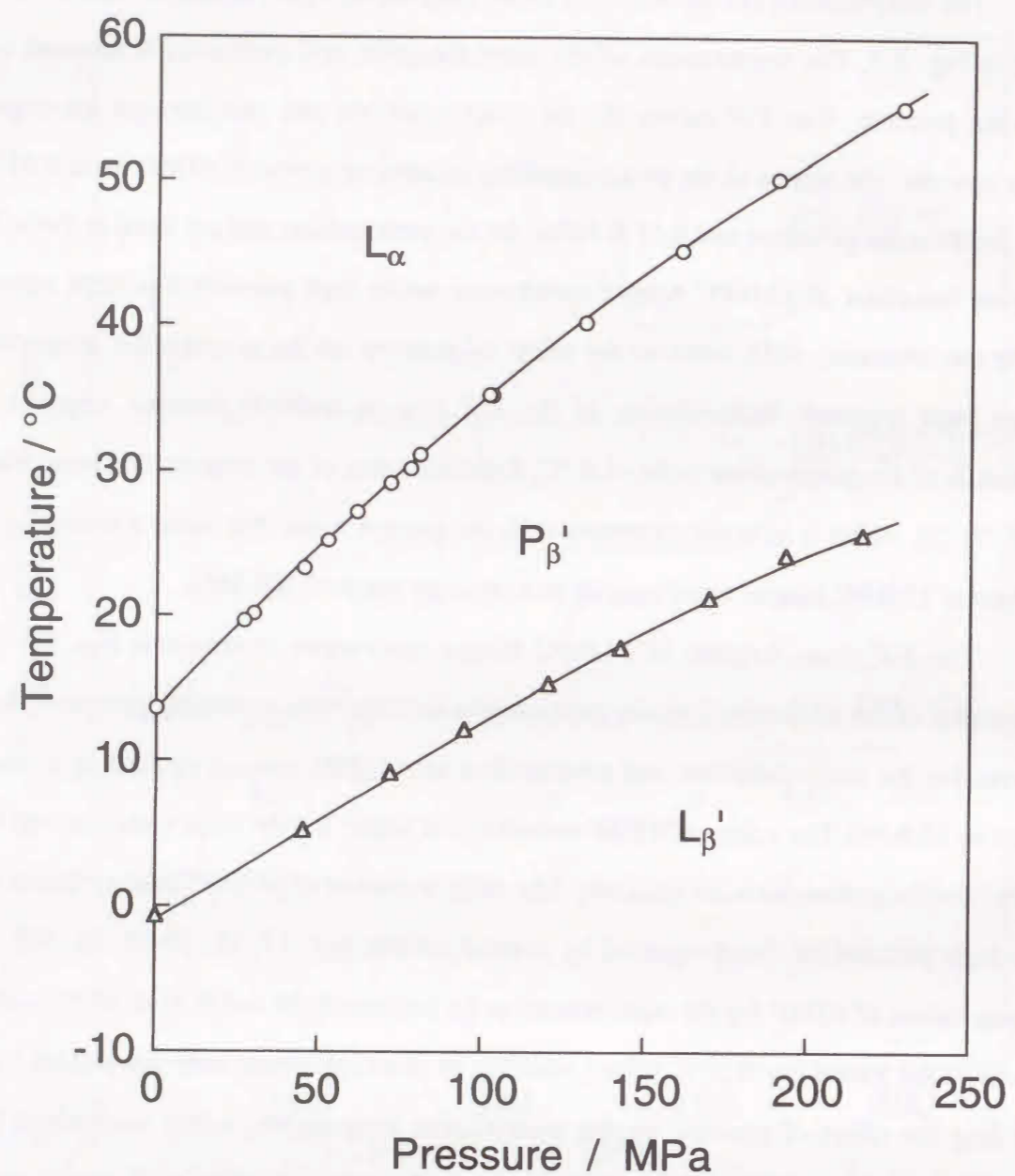


Fig. 3-3 Phase diagram of 13:0-PC bilayer membranes. The concentration of 13:0-PC was 1.0 mmol kg^{-1} . Phase transitions: (O) $P_{\beta'} \rightarrow L_{\alpha}$, (Δ) $L_{\beta'} \rightarrow P_{\beta'}$.

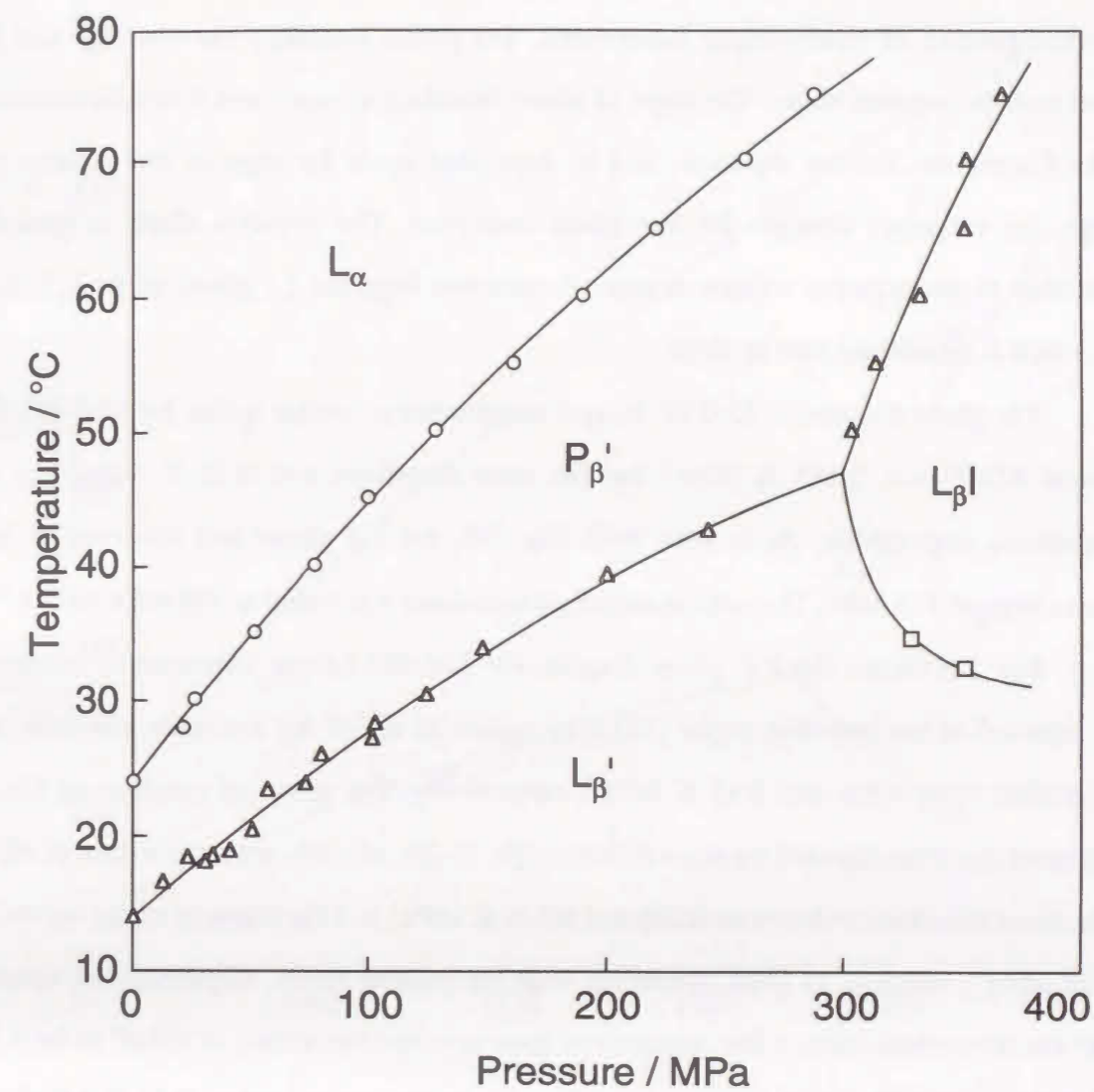


Fig. 3-4. Phase diagram of 14:0-PC bilayer membranes. The concentration of 14:0-PC was 2.0 mmol kg^{-1} . Phase transitions: (O) $P_{\beta'} \rightarrow L_{\alpha}$, (Δ) $L_{\beta'} \rightarrow P_{\beta'}$ or $L_{\beta I} \rightarrow P_{\beta'}$, (\square) $L_{\beta'} \rightarrow L_{\beta I}$.

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 the 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 slope is probably attributable to the negative volume change of transition from the L_{β}' phase to the $L_{\beta}I$ phase [43], which is mentioned later in detail.

The phase diagram of 15:0-PC bilayer membranes is similar to that for 14:0-PC. The value of dT/dP was 0.215 K MPa⁻¹ for the main transition and 0.12 K MPa⁻¹ for the pretransition, respectively. As is seen from Fig. 3-5, 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. 3-6 shows the T - P phase diagram for 16:0-PC bilayer membranes, which has been reported in our previous paper [33]. The values of dT/dP 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 [8, 15-23, 34]. The previous values of dT/dP 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 dT/dP for the pretransition, a few researchers have reported the values of dT/dP to be 0.162 [15], and 0.12 K MPa⁻¹ [22], 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 [20] and Winter and Pilgrim [21] reported the observation of the $L_{\beta}I$ phase in D₂O by the method of neutron diffraction at high pressure beyond 150 MPa and 160 MPa, respectively, which seems to be somewhat higher pressure than present result. Whereas Prasad et al. [22] and

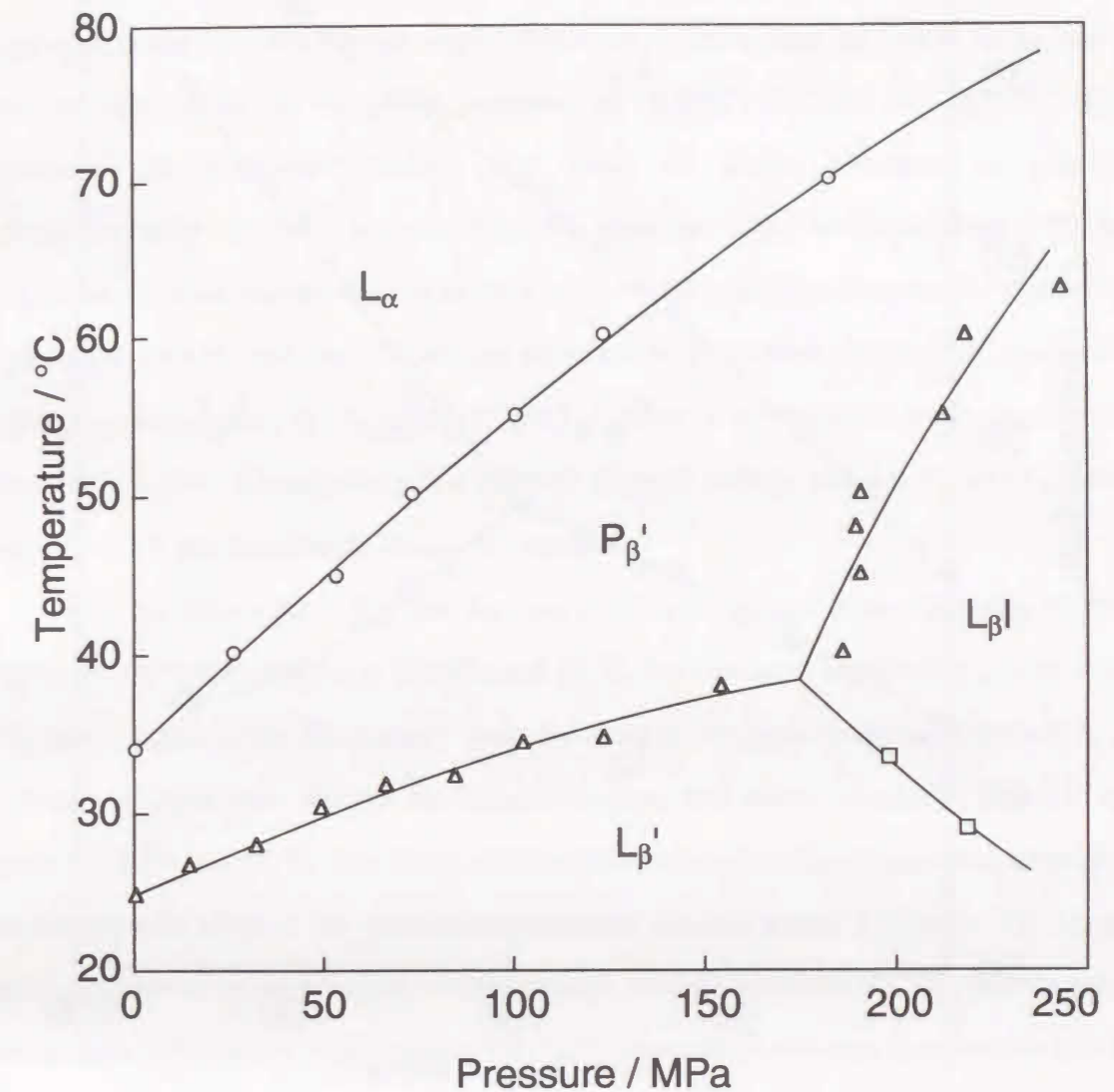


Fig. 3-5. Phase diagram of 15:0-PC bilayer membranes. The concentration of 15:0-PC was 1.0 mmol kg⁻¹. Phase transitions: (O) $P_{\beta}' \rightarrow L_{\alpha}$, (Δ) $L_{\beta}' \rightarrow P_{\beta}'$ or $L_{\beta}I \rightarrow P_{\beta}'$, (\square) $L_{\beta}' \rightarrow L_{\beta}I$.

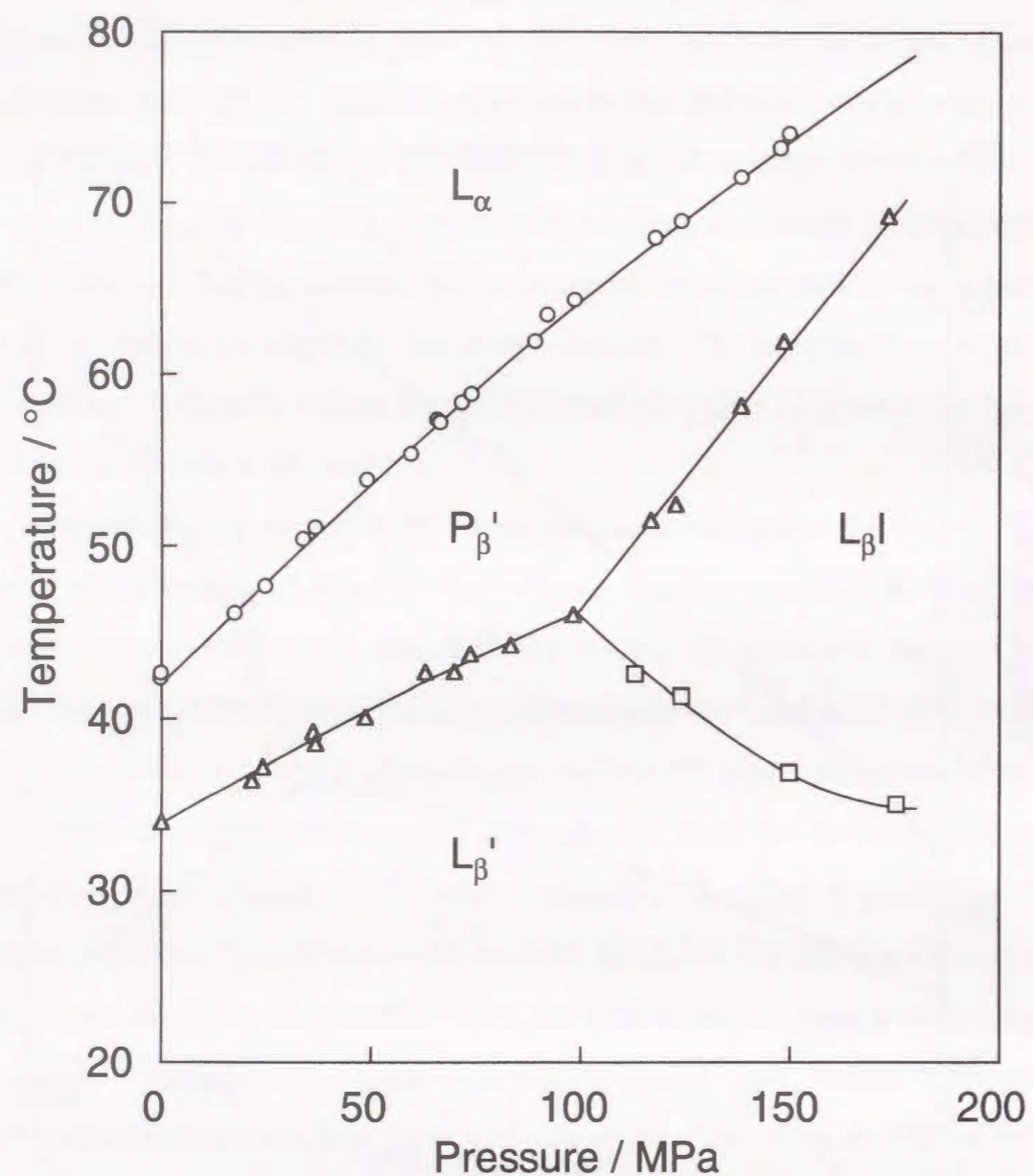


Fig. 3-6. Phase diagram of 16:0-PC bilayer membranes. The concentration of 16:0-PC was 2.0 mmol kg^{-1} . Phase transitions: (O) $P_{\beta}' \rightarrow L_{\alpha}$, (Δ) $L_{\beta}' \rightarrow P_{\beta}'$ or $L_{\beta}I \rightarrow P_{\beta}'$, (\square) $L_{\beta}' \rightarrow L_{\beta}I$.

Driscoll et al. [26] 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 [43] 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 ($\Delta H > 0$) [50]. Consequently, the slope of phase boundary between L_{β}' and $L_{\beta}I$ phases in these lipid bilayer membranes should be negative.

Fig. 3-7 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 intersect 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 the pressure 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 both 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 negative to positive value as the pressure increases. The pressure reversal of 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. 3-8. The

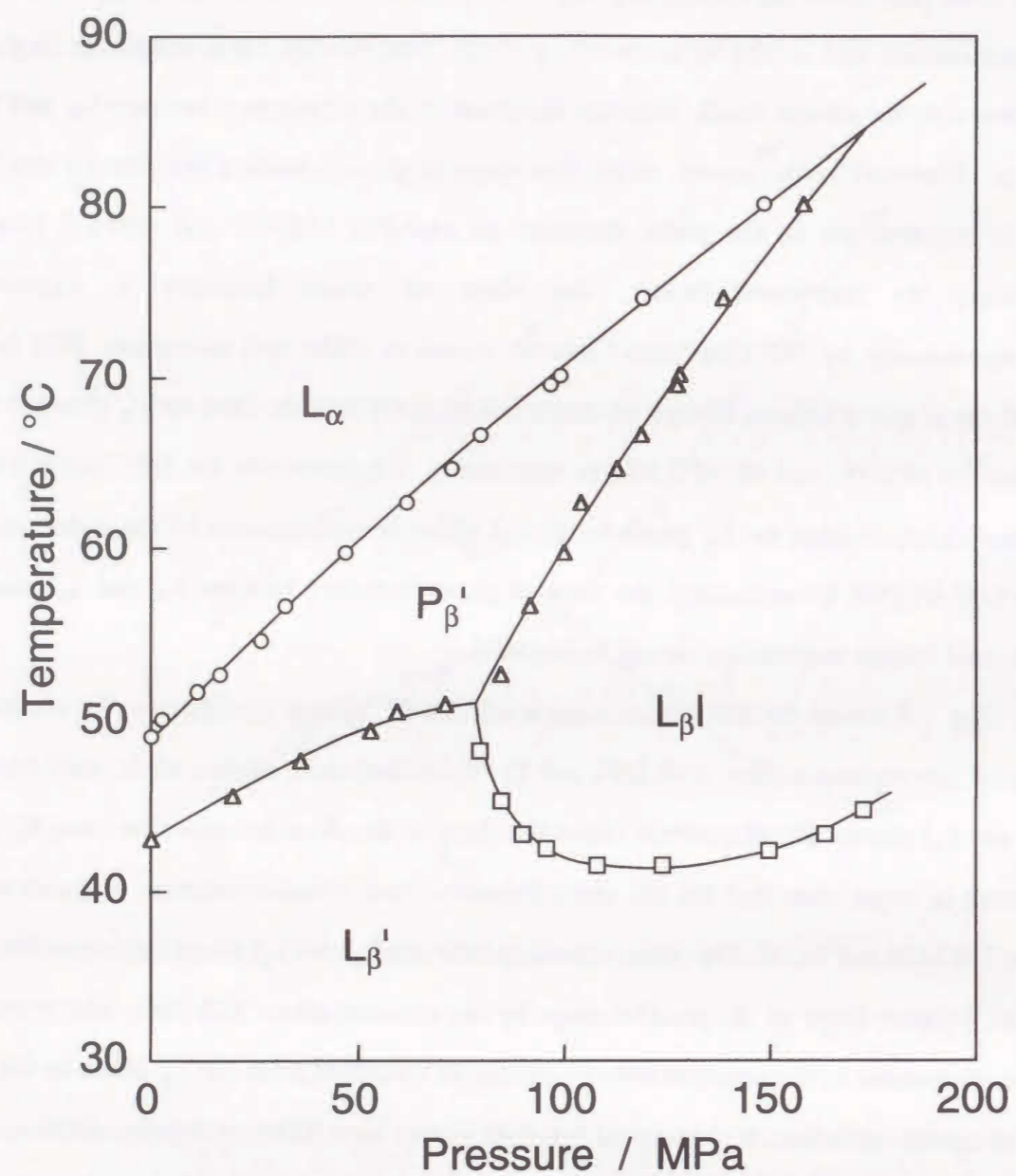


Fig. 3-7. Phase diagram of 17:0-PC bilayer membranes. The concentration of 17:0-PC was 1.0 mmol kg^{-1} . Phase transitions: (O) $P_{\beta'} \rightarrow L_{\alpha}$, (Δ) $L_{\beta'} \rightarrow P_{\beta'}$ or $L_{\beta I} \rightarrow P_{\beta'}$, (\square) $L_{\beta'} \rightarrow L_{\beta I}$.

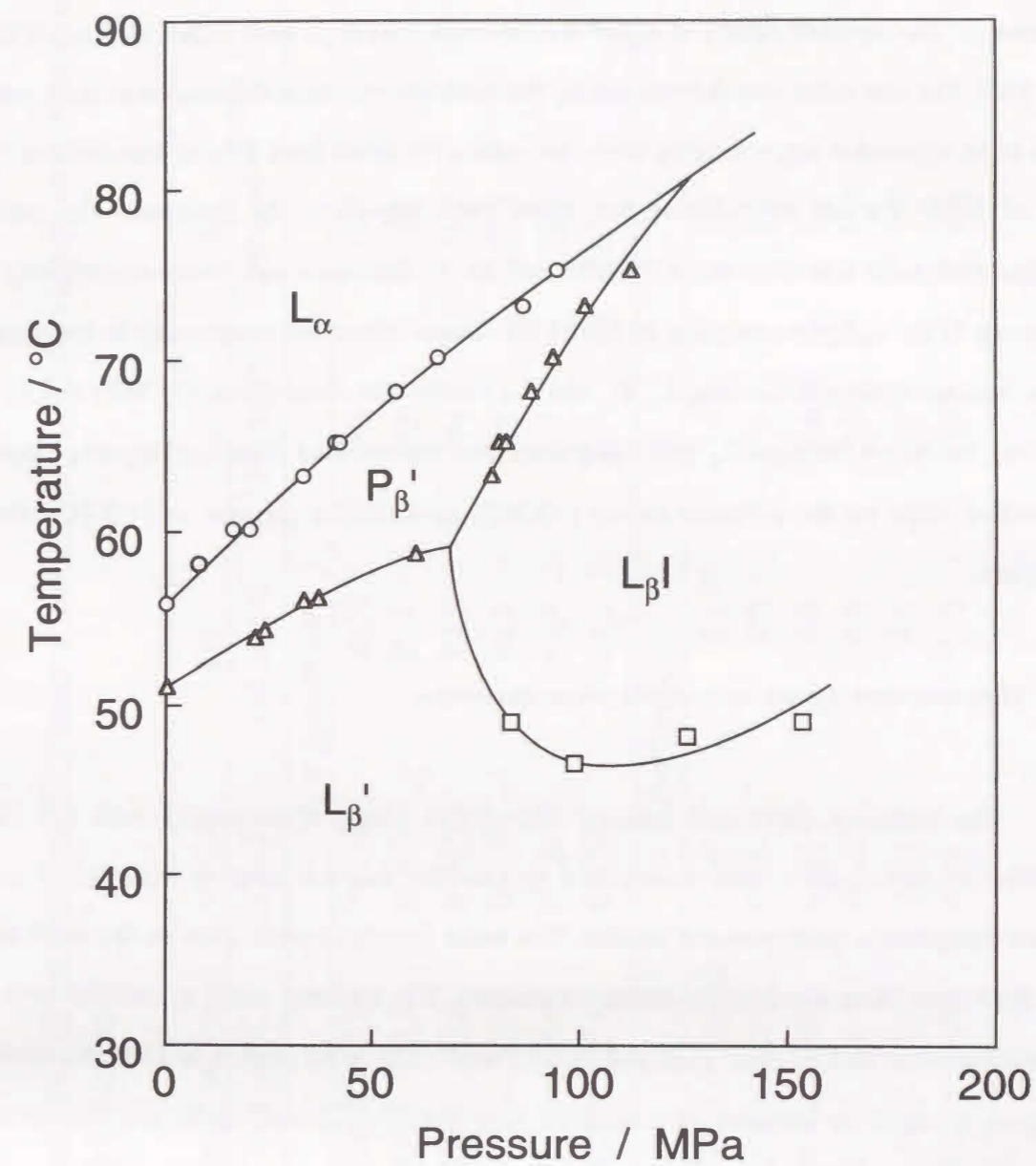


Fig. 3-8. Phase diagram of 18:0-PC bilayer membranes. The concentration of 18:0-PC was 2.0 mmol kg^{-1} . Phase transitions: (O) $P_{\beta'} \rightarrow L_{\alpha}$, (Δ) $L_{\beta'} \rightarrow P_{\beta'}$ or $L_{\beta I} \rightarrow P_{\beta'}$, (\square) $L_{\beta'} \rightarrow L_{\beta I}$.

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 [26] and 0.28 K MPa⁻¹ [20]. The last value was determined by the method of neutron diffraction in D₂O, 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_βI phase beyond 90 MPa [20]. These values are comparable to the present values. Another triple point among L_α, P_β' and L_βI phases was observed at 125 MPa and 80 °C. The phase boundary between L_β' and L_βI phases was transformed from the negative slope to the positive slope by the pressure above 110 MPa in a similar manner as 17:0-PC bilayer membrane.

3.3. Thermodynamic properties of the phase transition

The enthalpy (ΔH) and entropy ($\Delta S = \Delta H/T$) changes associated with the main transition and pretransition were determined by the DSC and are listed in Table 3-1. 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 [28] and 26.4 kJ mol⁻¹ [35]. With respect to 16:0-PC, most of the reported values lie between 34.7 kJ mol⁻¹ [36] and 37.2 kJ mol⁻¹ [37], and the majority reported 36.4 kJ mol⁻¹ [28, 38, 39]

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 3-1. The values of ΔV increased with an increase in the acyl

Table 3-1. Thermodynamic properties of phase transitions for the bilayer membranes of diacylphosphatidylcholines.

Lipid	Transition Temp.		dT/dP (K MPa ⁻¹)	ΔH (kJ mol ⁻¹)	ΔS (J K ⁻¹ mol ⁻¹)	ΔV (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) Lewis et al. [5]

chain-length. Direct measurements of the volume change associated with the phase transition have been reported by the methods of dilatometry [8, 51, 52] and density [42, 43, 53]. The reported values of ΔV were $19.1 \text{ cm}^3 \text{ mol}^{-1}$ [51], $24.2 \text{ cm}^3 \text{ mol}^{-1}$ [8], $24.3 \text{ cm}^3 \text{ mol}^{-1}$ [43], $27.2 \text{ cm}^3 \text{ mol}^{-1}$ [52], $27.9 \text{ cm}^3 \text{ mol}^{-1}$ [42] and $30.2 \text{ cm}^3 \text{ mol}^{-1}$ [53] for 16:0-PC, which are comparable with the present value, $25.4 \text{ cm}^3 \text{ mol}^{-1}$, estimated from the Clapeyron-Clausius equation. With respect to 18:0-PC, several authors reported the values of ΔV to be $28.4 \text{ cm}^3 \text{ mol}^{-1}$ [51], $32.9 \text{ cm}^3 \text{ mol}^{-1}$ [43] and $38.0 \text{ cm}^3 \text{ mol}^{-1}$ [53] which are comparable with the present result, $31.6 \text{ cm}^3 \text{ mol}^{-1}$.

The temperature and ΔH of pretransition were in good agreement with previously published data [5, 33, 53-55]. 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 the 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 owing 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 the pretransition there were no alterations in the structural 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 the phase transition

The temperatures of pretransition and main transition are plotted as a function of acyl chain length, which are shown in Fig. 3-9. Both temperatures of pretransition and main transition increased with an increase in acyl chain length. A difference in temperature between two transitions became narrow as the 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 researcher emphasized different considerations [54, 56]. Silvius et al. [56] and Dörfler and Brezesinski [54] 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 against acyl chain length. Results of Lewis et al. [5] and the present study showed no evidence of odd/even discontinuities. 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 [5], and that the structure of the subgel phases formed vary systematically with hydrocarbon chain length in a homologous series of linear, saturated diacylphosphatidylcholines [57].

The critical interdigitation points (T_i° , P_i°) for saturated diacylphosphatidylcholine bilayer membranes are plotted as a function of acyl chain length and shown in Fig. 3-10. The value of P_i° 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-,

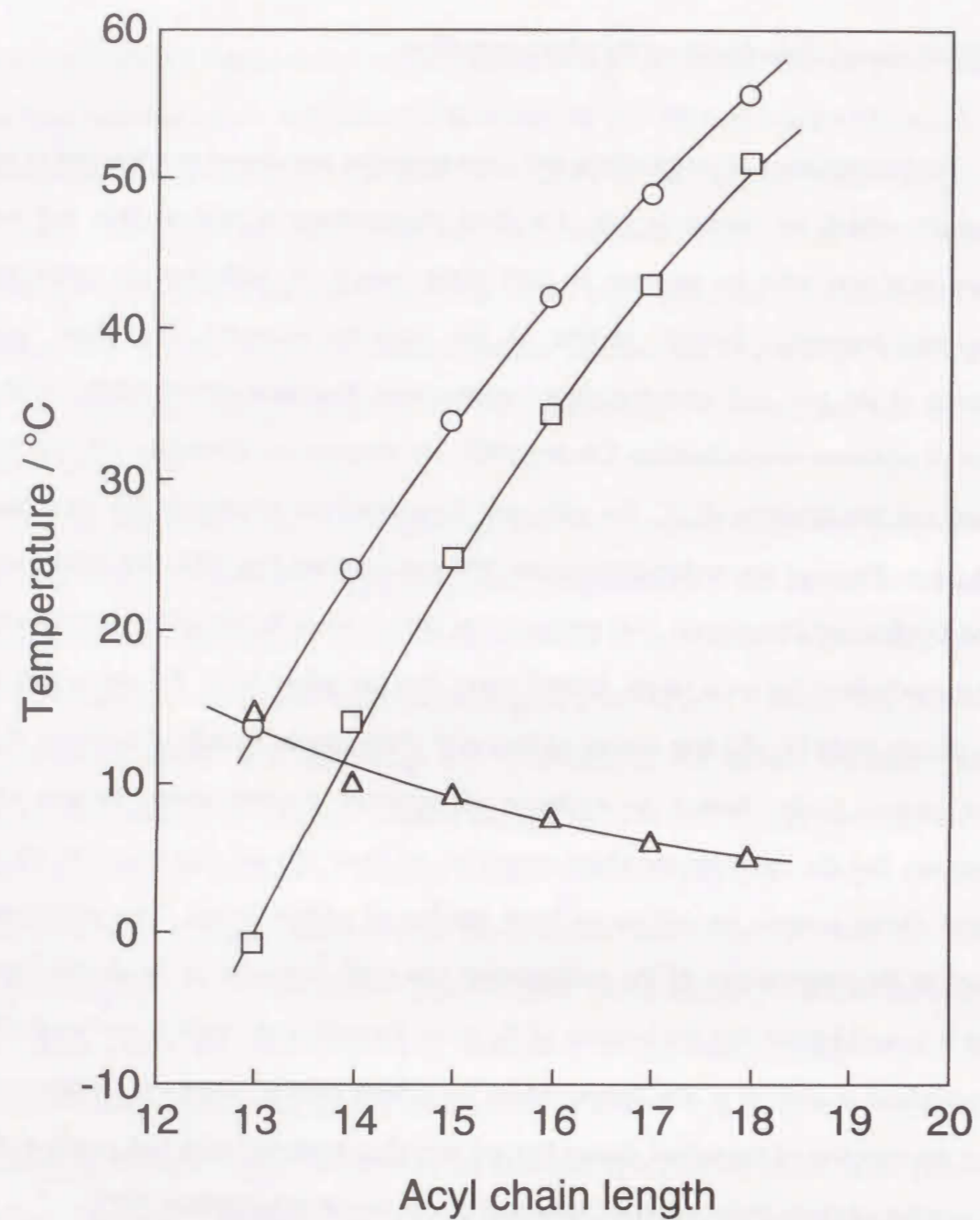


Fig. 3-9. Effect of acyl chain-length on the phase transition temperatures of lipid bilayer membranes: (O) Main transition, (□) pretransition and (Δ) the temperature difference between main transition and pretransition, $T_m - T_p$.

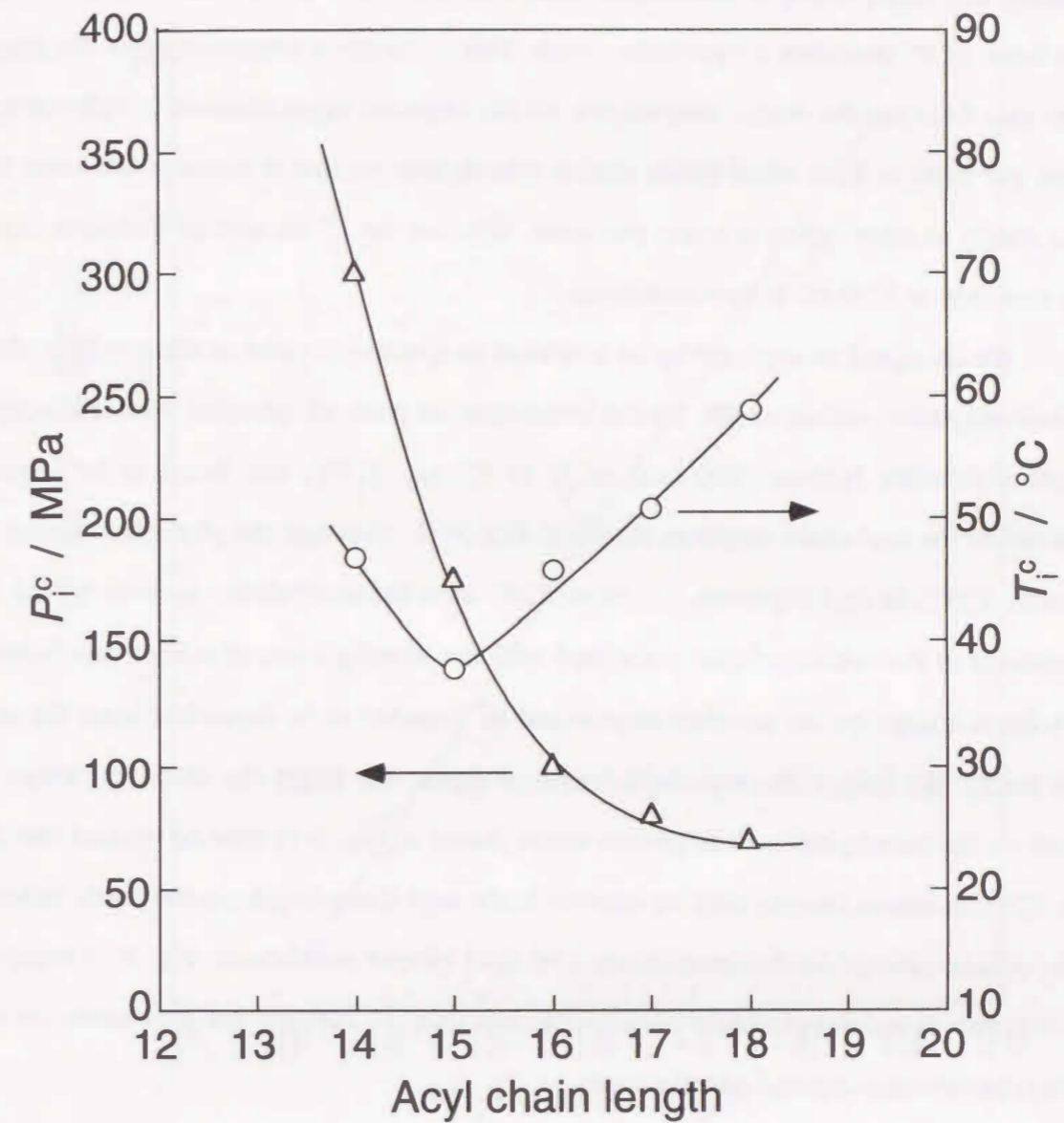


Fig. 3-10. Effect of acyl chain length on the critical interdigitation point (T_i^c , P_i^c) of lipid bilayer membranes: (O) T_i^c and (Δ) P_i^c .

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 nonlinear 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 membrane.

We attempted an approach by an empirical perspective in order to adequately explain the chain-length dependent 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. 3-11. 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. 3-11 may be suggest that the ratio, T_i^c/P_i^c increases linearly with an increase in the acyl chain-length via the linear increase of the volume change on the interdigitation of lipid bilayer membranes. Fig. 3-11 suggests also that any diacylphosphatidylcholines with less than 13 carbons per acyl chain do not undergo the pressure-induced interdigitation.

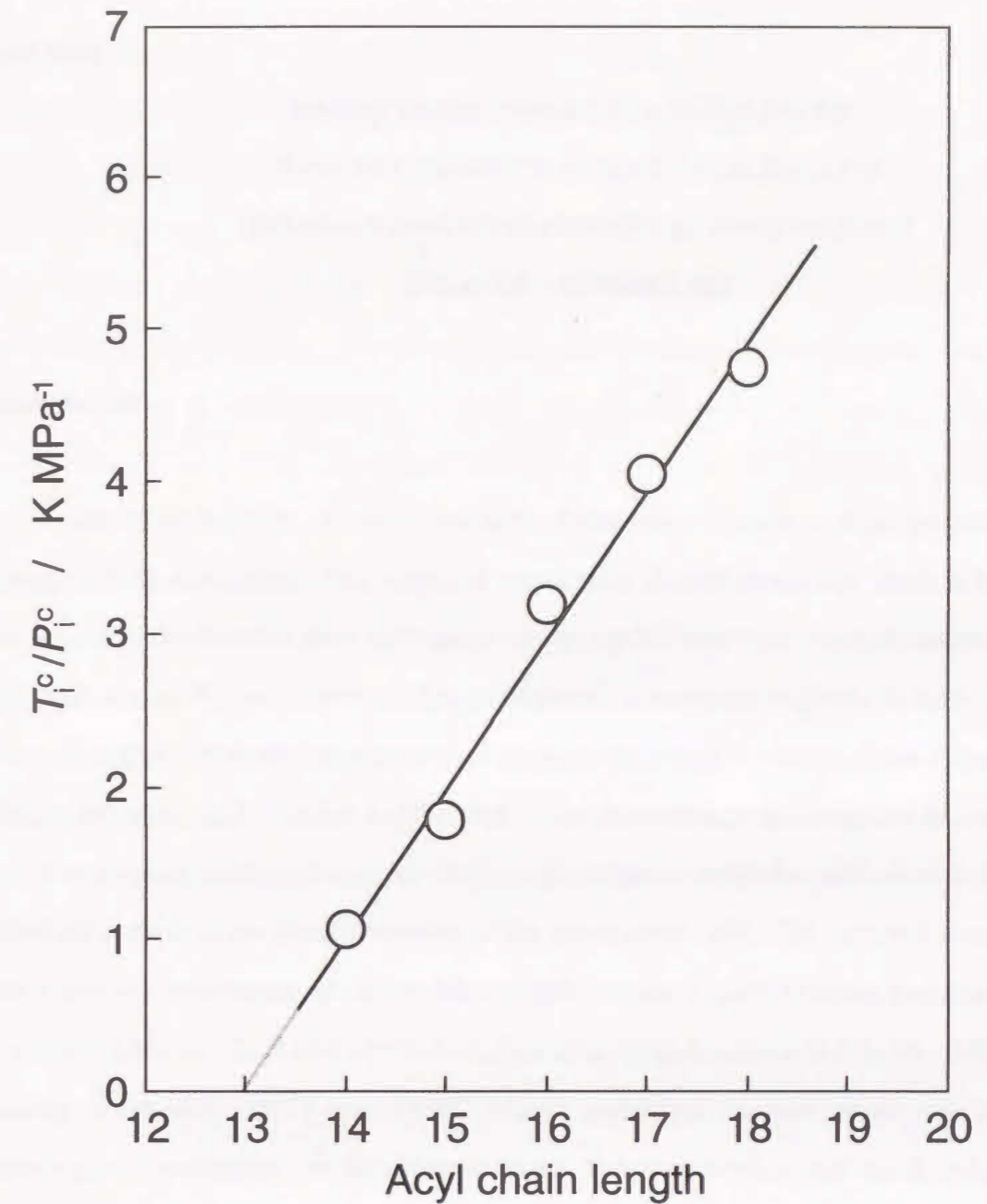


Fig. 3-11. A linear correlation between the ratio T_i^c/P_i^c and Acyl chain-length. The critical interdigitation point (T_i^c , P_i^c) refers the triple point among P_β' , L_β' and $L_\beta I$ phases on the phase diagram of bilayer membranes.

CHAPTER 4.

**BAROTROPIC PHASE TRANSITIONS OF
DIOLEOYLPHOSPHATIDYLCHOLINE AND
STEAROYL-OLEOYLPHOSPHATIDYLCHOLINE
BILAYER MEMBRANES****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 tends 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 investigators 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 [58-64] scatters between -23.4 and -11.8 °C. Lewis et al. [58] 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 [1], pressure adaptation in deep sea organism [2] and high-pressure sterilization in food processing [3]. Although bilayer membranes composed of phosphatidylcholines containing two identical linear saturated fatty

acyl chains have been most thoroughly studied under high pressure [7, 8, 11, 16, 18, 20, 22, 24, 26, 27, 37, 45], there have been few studies on the phase properties of unsaturated phosphatidylcholine bilayer membranes under high pressure [21, 65, 66]. Wong and Mantsch [65] 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 from 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

All of the phospholipids were purchased from Sigma (St. Louis, MO, USA).

Abbreviations of the phosphatidylcholines were as follows:

DOPC, 1,2-di[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine

SOPC, 1-octadecanoyl-2-[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine

DSPC, 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine

The phospholipid multilamellar vesicles were prepared by similar method in chapter 2.

2.2. Phase transition measurements

The phase transitions of phospholipid bilayer membrane were measured by DSC at ambient pressure and by light transmittance under high pressure. The detail information of the measurements was described in chapter 2. The average size of vesicles was determined by the light scattering method, which was found to be 320 ± 90 nm.

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. 4-1. The light transmittance of vesicle suspensions in the range of 540-580 nm was measured at 0 °C and various pressures (Fig. 4-1A). The transmittance at 560 nm was depicted in Fig. 4-1B as a function of pressure. The transmittance-pressure curve

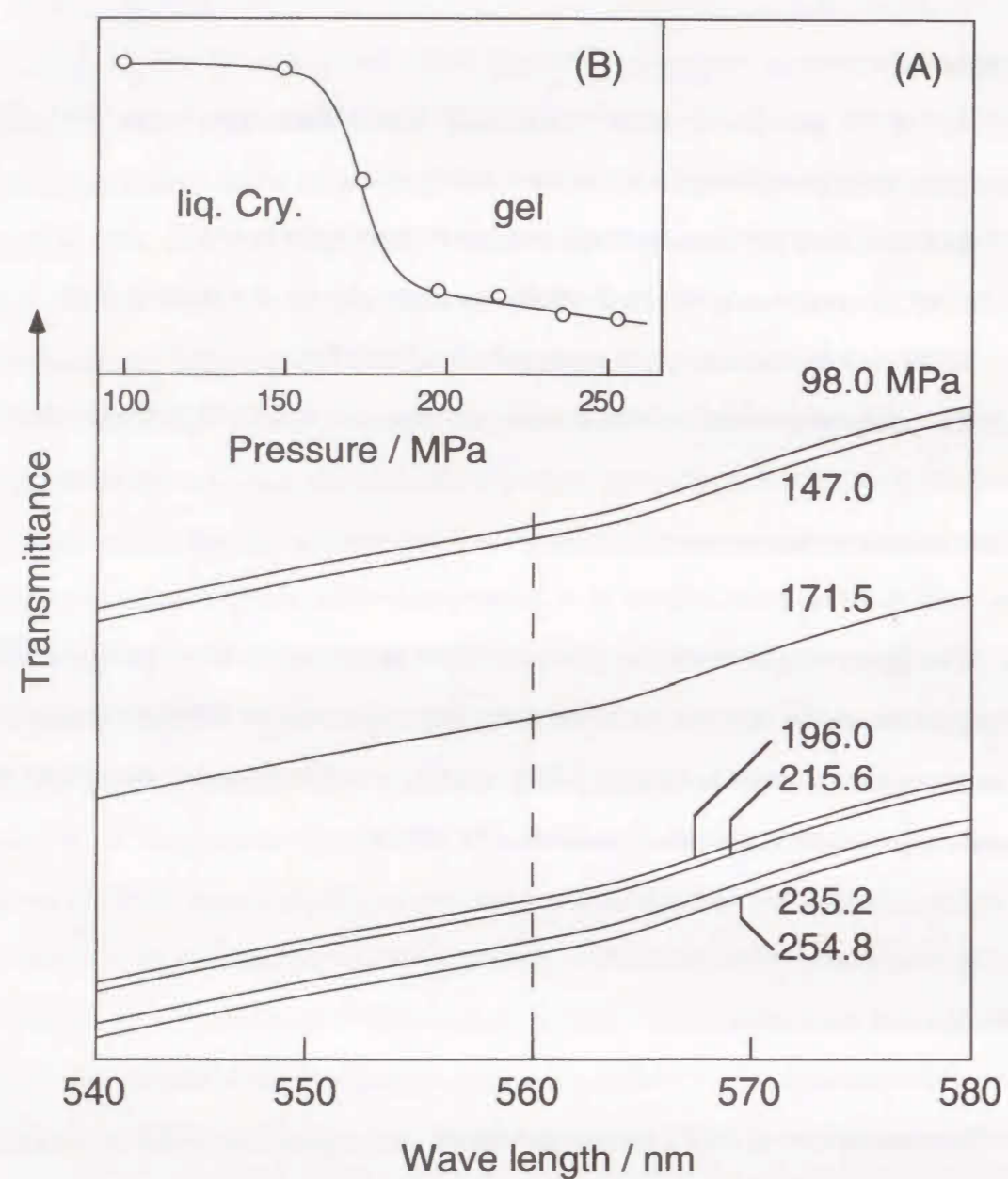


Fig. 4-1 Isothermal barotropic phase transition of DOPC bilayer membrane at 0°C. A) Transmittance-wave length curves at various pressures. B) A typical pressure-induced phase transition of DOPC bilayer membrane.

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. 4-2. 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.

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 [58-61]. The phase-transition temperature has been reported to be -11.8 , -14.0 , -15.0 and -15.8 °C in 50 % aqueous ethylene glycol [58-61] and -16.5 , -17.3 and -21.0 °C in water [58, 62, 63], respectively. The transition temperatures and associated enthalpy changes are significantly different in between water and aqueous ethylene glycol. Lewis et al. [58] 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 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. 4-3, which shows the existence of two kinds of transitions. When the vesicle suspension was heated at once after it was pressurized, a lower-

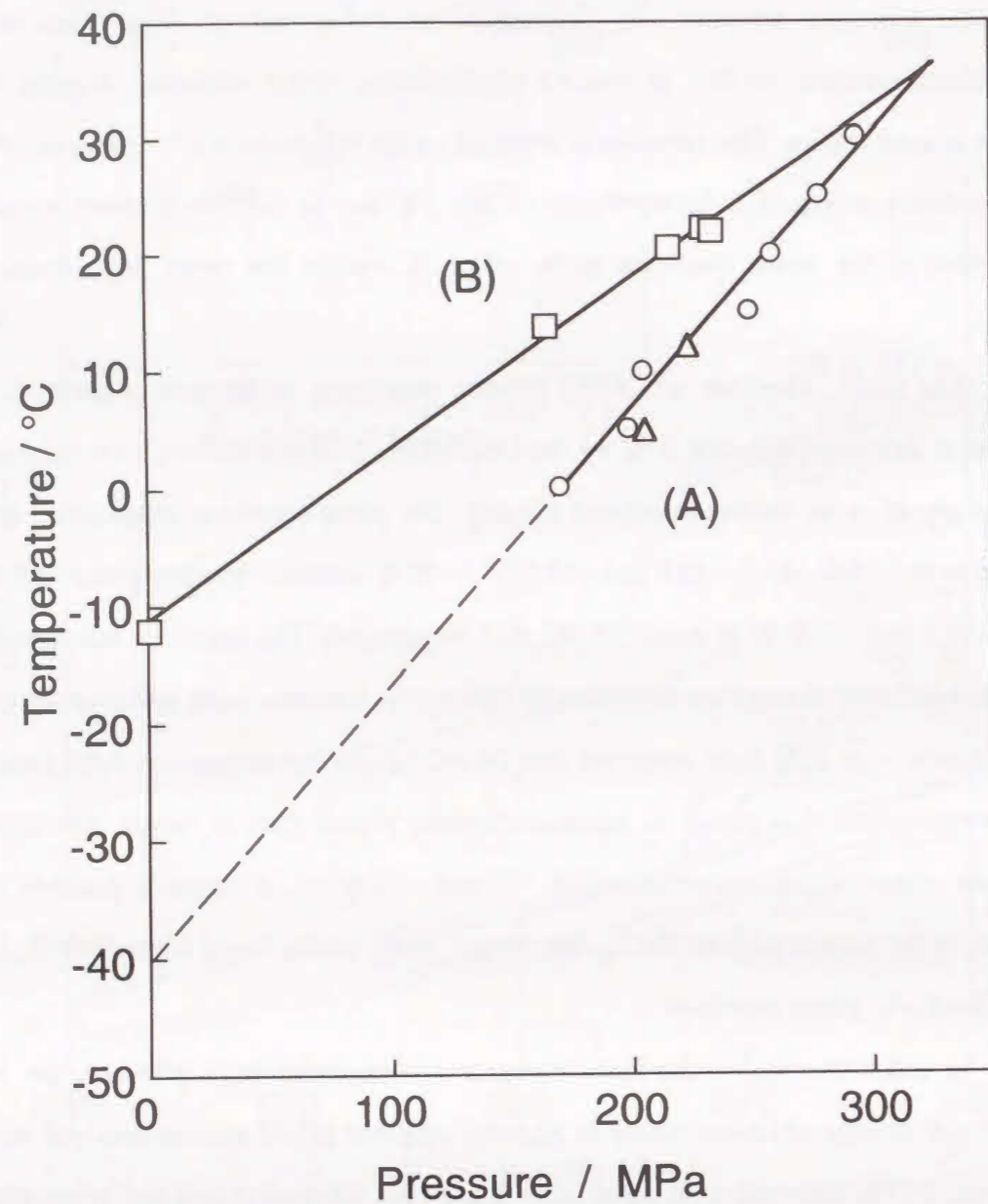


Fig. 4-2 A) Main transition in water (O) and in 50% aqueous ethylene glycol (Δ), (B) Subtransition in 50% aqueous ethylene glycol (\square).

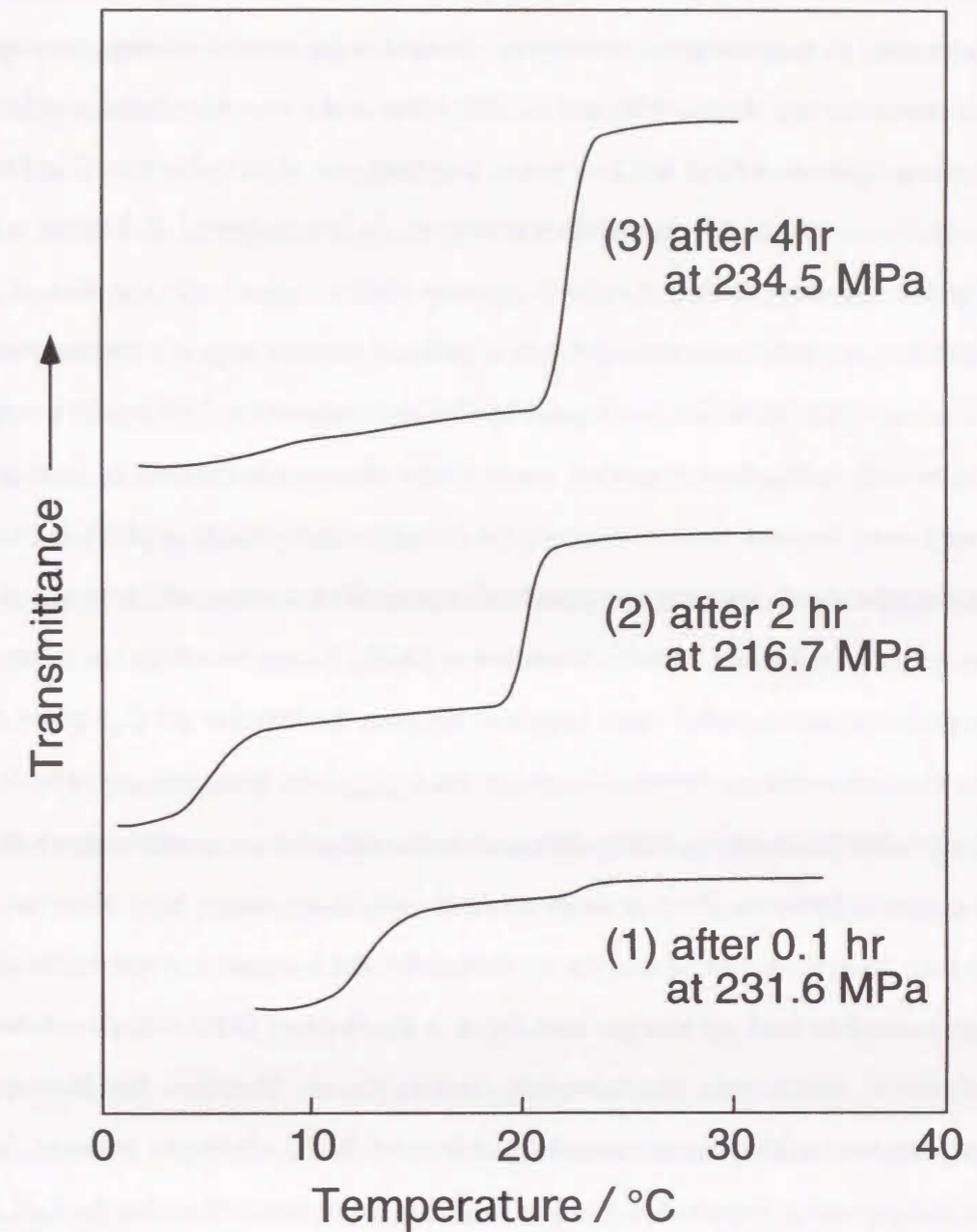


Fig. 4-3 Two kinds of phase transitions for DOPC vesicles in 50% aqueous ethylene glycol. DOPC vesicle suspension was pressurized first to about 200 MPa at 0 °C and then heated after it was allowed to stand for (1) 0.1 hr, (2) 2 hr and 4hr.

temperature transition was observed (curve A in Fig. 4-3). Curve B in Fig. 4-3 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. 4-3), which is consistent with higher-temperature transition. As is seen from T - P diagram in Fig. 4-2, 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. 4-3) 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, however, 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. 4-2, 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 [66] 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 [66] 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 increase 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 [68]. 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 increase with increasing pressure in the liquid crystalline phase at the 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. 4-4. The transmittance of SOPC vesicle suspensions in the range of 540-580 nm was measured at various pressures (Fig. 4-4A). Transmittance at 560 nm was depicted in Fig. 4-4B 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. 4-5 together with that for DOPC and DSPC bilayer membranes. Data for DSPC bilayer membrane were taken from our previous results [45]. 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

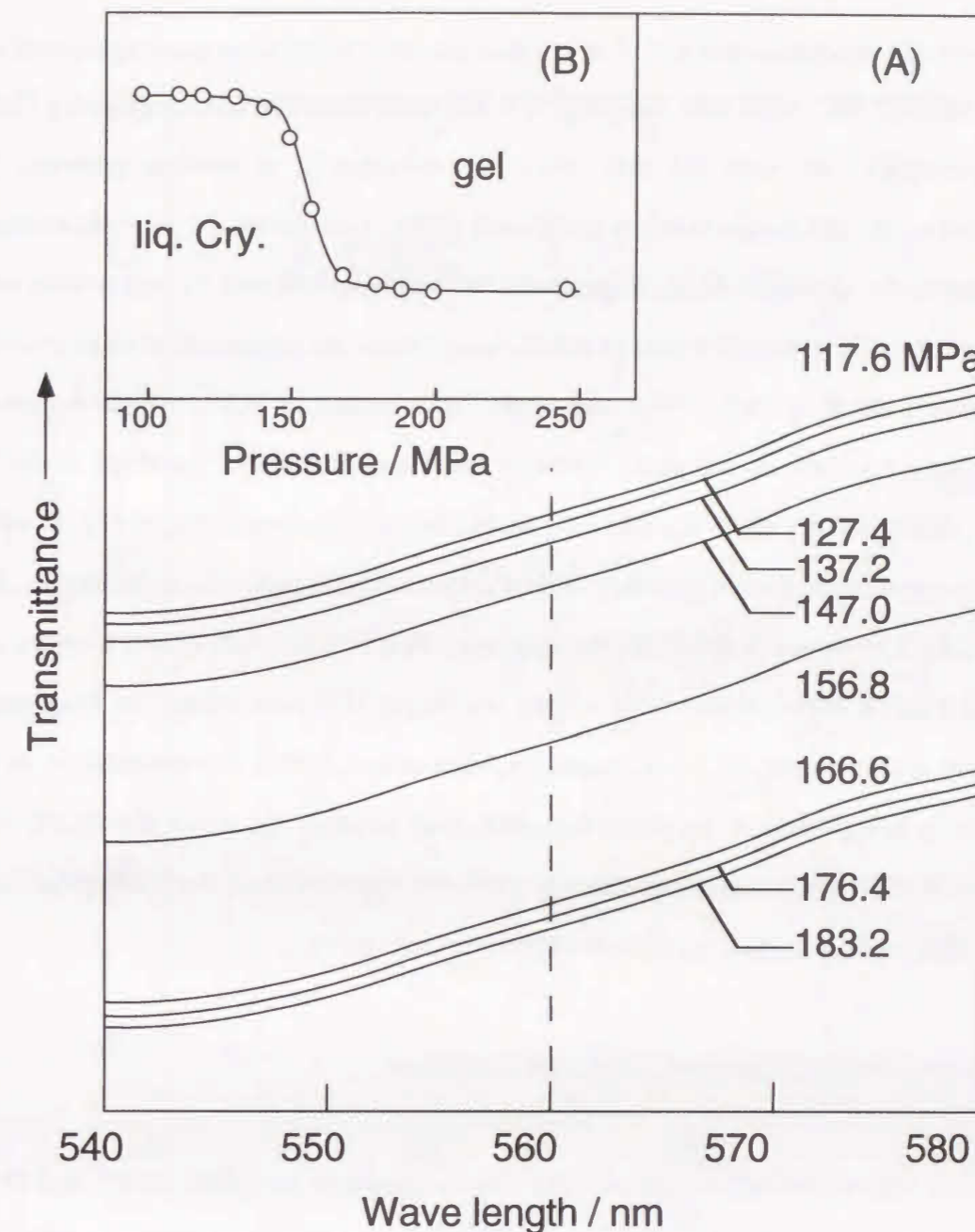


Fig. 4-4 Isothermal barotropic phase transition of SOPC bilayer membrane at 30°C. A) Transmittance-wave length curves at various pressures. B) A typical pressure-induced phase transition of SOPC bilayer membrane.

main-transition temperature was 6.7 °C at ambient pressure, which is in good agreement with previous data [63, 68]. As is seen from Fig. 4-5, 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, dT/dP , 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 [21]. The values of dT/dP for the main transition of DSPC and DOPC bilayers were 0.230 and 0.226 K MPa⁻¹, respectively. Chong and Weber [17] 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 dT/dP 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 dT/dP and phase-transition temperatures for DSPC, SOPC and DOPC bilayer membranes are listed in Table 4-1. The enthalpy (ΔH) and entropy ($\Delta S = \Delta H/T$) changes associated with the phase transition were determined by the DSC method. The values of ΔH for DSPC and SOPC were taken from our previous data [45] and results by Davis [63, 68], respectively, which are listed in Table 4-1. With respect 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

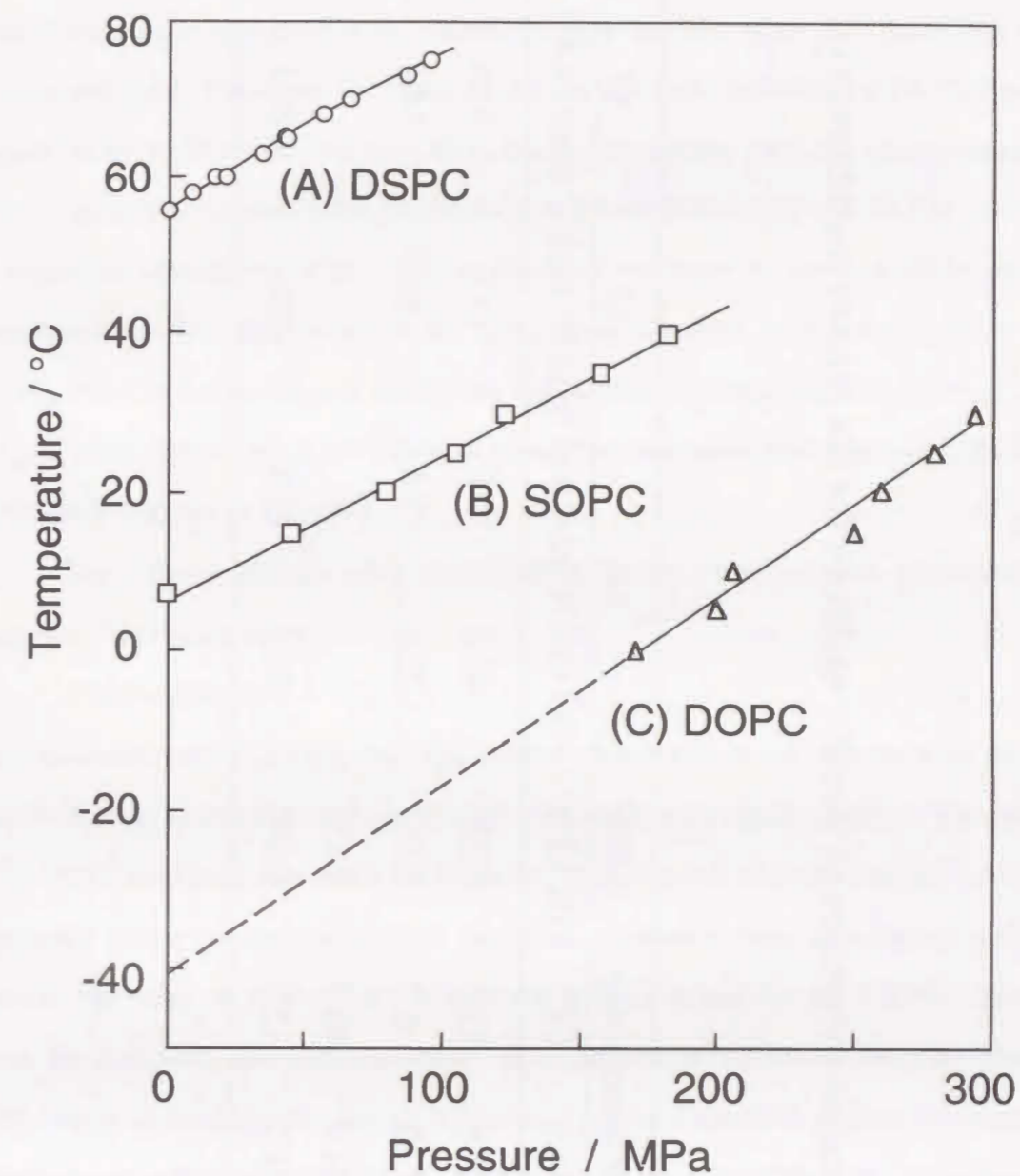


Fig. 4-5 Temperature-pressure phase diagrams for main transitions of phosphatidylcholine bilayer membranes. DSPC (O); SOPC (\square); DOPC (Δ).

Table 4-1. Thermodynamic properties of phase transitions for the bilayer membranes of DSPC, SOPC and DOPC.

Lipid	Transition	Transition Temp.		dT/dP (K MPa ⁻¹)	ΔH (kJ mol ⁻¹) (kcal mol ⁻¹)	ΔS (J K ⁻¹ mol ⁻¹)	ΔV (cm ³ mol ⁻¹)
		(K)	(°C)				
DSPC	(P β' / L α)	328.8	55.6	0.230	45.2 10.8	137	31.6
SOPC	(L β / L α)	279.9	6.7	0.181	22.6 5.4	80.7	14.6
DOPC	(L β / L α)	232.9	-40.3	0.233			
	(L c / L α)	261.2	-12.0	0.147	65.3 15.6	250	36.7

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 [58] and 32.2 kJ mol⁻¹ [62]. 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 [58]. The value of ΔH for the L c /L α phase transition of DOPC bilayer membrane was taken from literature [58], 65.3 kJ mol⁻¹, which is shown in Table 4-1.

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

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

and is also summarized in Table 4-1. 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 [51], 32.9 [43] and 39.0 cm³ mol⁻¹ [53], which are comparable to the present value, 31.6 cm³ 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.

CHAPTER 5.

**EFFECT OF UNSATURATED ACYL CHAINS
ON THE THERMOTROPIC AND BAROTROPIC PHASE TRANSITIONS
OF PHOSPHOLIPID BILAYER MEMBRANES**

1. Introduction

The effect of pressure on the gel to liquid-crystalline phase transition of lipid bilayer membranes is of particular interest to the studies of pressure reversal of anesthesia [1], pressure adaptation in deep-sea organisms [2], and high-pressure sterilization in food processing [3, 69]. Lipid bilayer membranes composed of phosphatidylcholines containing two identical linear saturated fatty acyl chains have been most thoroughly studied under high pressure [8, 15, 16, 18, 20-22, 30-32, 34, 45, 70]. Contrarily, there have been few studies on the phase properties of unsaturated phosphatidylcholine bilayers under high pressure [4, 21, 65-67, 71]. The phospholipids of most biological membranes are characterized by a high percentage of *cis* unsaturated 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 *cis* unsaturated acyl chains tends to have lower temperatures of transition from the gel phase to the liquid crystalline phase. Since the phase transition of the unsaturated lipids at ambient pressure has been observed at temperatures below 0 °C sometimes [62, 72-75], information on phase behavior has been difficult to obtain by conventional techniques, but such information is readily accessible from high pressure experiments.

Winter and Pilgrim [21] have reported by small-angle neutron scattering (SANS)

under high pressure up to 200 MPa that the effect of two *trans* double bonds in dielaidoylphosphatidylcholine (DEPC) and a *cis* double bond in the *sn*-2 chain of palmitoyl-oleoylphosphatidylcholine (POPC) enhances the conformational disorder in the hydrocarbon chains not only in the liquid crystalline, but also in the gel state. For DEPC and POPC the preferred structural conformation in the gel state is probably not tilted as it in the gel state of the saturated phospholipid dipalmitoylphosphatidylcholine (DPPC). Siminovitch et al. [65] measured infrared spectra of dioleoylphosphatidylcholine (DOPC) and DEPC bilayer membranes at increasing pressures up to 3600 MPa, and observed a phase transition from a disordered liquid crystalline phase to a highly ordered gel phase at 28 °C and 520 MPa for DOPC, and 28 °C and 70 MPa for DEPC. Siminovitch et al. (1988) also measured infrared spectra of palmitoyl-oleoylphosphatidylcholine (POPC) and oleoyl-palmitoylphosphatidylcholine (OPPC) bilayer membranes at 28 °C as a function of pressure up to 4700 MPa, in order to compare the effect of one and two monounsaturated (oleoyl) acyl chains on the structure and packing of phospholipid bilayers.

The present study demonstrates the pressure effect on the phase behavior of bilayer membranes of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC), 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (OSPC), and 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) as well as 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and reveals the influence of the *cis* unsaturated acyl chain in the *sn*-1 or *sn*-2 position and difference of *trans* and *cis* unsaturation on the phase behavior of lipid bilayer membranes. 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 *sn*-2 hydrocarbon chain on the phase behavior, the phase-transition temperatures of bilayer membranes of SOPC, 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (SAPC), and

1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (SDPC), as well as 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were measured by a high-pressure optical method.

2. Experimental procedures

2.1. Materials

Synthetic phosphatidylcholines used in this study were abbreviated as follows:

DSPC, 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine;

DOPC, 1,2-di[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine;

DEPC, 1,2-di[*trans*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine;

OSPC, 1-[*cis*-9-octadecenoyl]-2-octadecanoyl-*sn*-glycero-3-phosphocholine;

SOPC, 1-octadecanoyl-2-[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine;

SAPC, 1-octadecanoyl-2-[(*cis,cis,cis,cis*)-5,8,11,14-eicosatetraenoyl]-*sn*-glycero-3-phosphocholine;

SDPC, 1-octadecanoyl-2-[(*cis,cis,cis,cis,cis,cis*)-4,7,10,13,16,19]-docosahexaenoyl]-*sn*-glycero-3-phosphocholine;

POPC, 1-hexadecanoyl-2-[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphocholine.

The number of carbon atom and *cis* (or *trans*) double bond in acyl chain, as well as the abbreviation of phospholipids used, are shown in Table 5-1. All of the phospholipids were purchased from Sigma (St. Louis, MO, USA). The phospholipid multilamellar vesicles were prepared by similar method in chapter 2.

Table 5-1. Abbreviations of phospholipids and the number of carbon and cis (or trans) double bond in acyl chains.

Abb.	<i>sn</i> -1	<i>sn</i> -2
DSPC	18:0	18:0
DEPC	18:1 trans	18:1 trans
DOPC	18:1	18:1
OSPC	18:1	18:0
SOPC	18:0	18:1
SAPC	18:0	20:4
SDPC	18:0	22:6
POPC	16:0	18:1

2. 2. Phase transition measurements

The phase transitions of phospholipid bilayer membrane were measured by DSC at ambient pressure and by light transmittance under high pressure. The detail information of the measurements was described in chapter 2. The average size of vesicles was determined by the light scattering method, which was found to be $320 \pm 90 \text{ nm}$.

3. Results and discussion

3. 1. Influence of monounsaturated acyl chain containing eighteen carbons on the phase transition of PC bilayer membranes

The transmittance - pressure curve for DEPC bilayer membrane showed the main transition from the liquid crystalline (L_α) phase to the lamellar gel (L_β) phase, accompanying with an abrupt decrease in transmittance as described in the previous papers [23, 45, 76]. The phase-transition pressures were determined at various temperatures and increased linearly with an increase in temperature. The temperature (T) - pressure (P) phase diagram for DEPC bilayer membrane is shown in Fig. 5-1. The main transition temperature was $11.1 \text{ }^\circ\text{C}$ at ambient pressure which is in good agreement with previous data, $9.5 \text{ }^\circ\text{C}$ [59], $10 \text{ }^\circ\text{C}$ [77], $11 \text{ }^\circ\text{C}$ [21], $11.5 \text{ }^\circ\text{C}$ [78] and $12.9 \text{ }^\circ\text{C}$ [61]. The transition temperatures under high pressure reported by Winter and Pilgrim [21] are also shown in Fig. 5-1. The temperature of the main (L_β/L_α) transition increases linearly with increasing pressure giving a value of dT/dP to be 0.180 K MPa^{-1} , which is in good agreement with previous data, 0.195 K MPa^{-1} [21]. We found another phase transition by the thermotropic heating scan at $9.0 \text{ }^\circ\text{C}$ under ambient pressure.

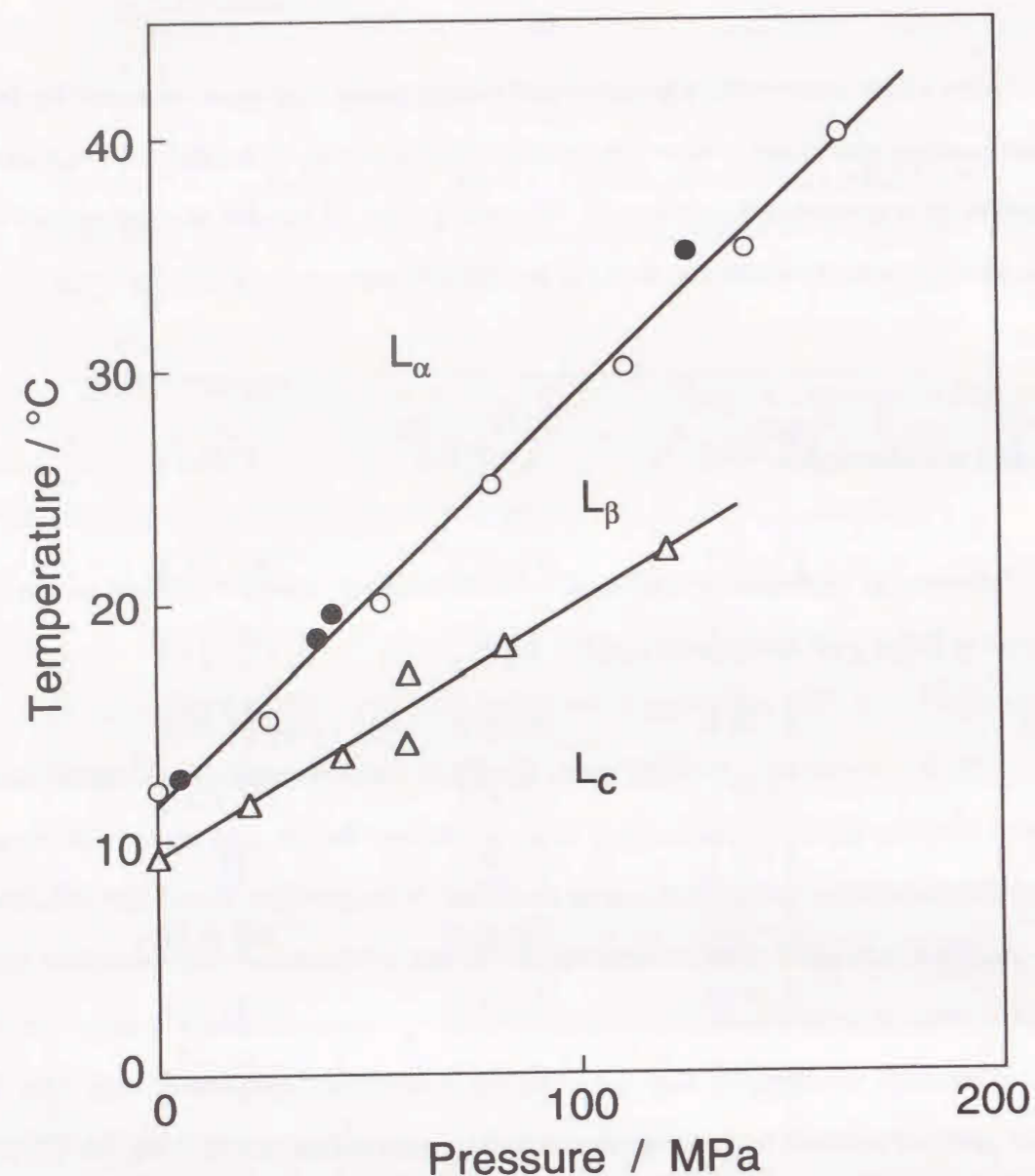


Fig. 5-1. Phase diagram of DEPC bilayer membrane. Concentration of DEPC was 1.0 mmol kg^{-1} . Phase transitions: (O) $L_\beta \rightarrow L_\alpha$, (Δ) $L_\gamma \rightarrow L_\beta$, and (●) by Winter and Pilgrim [21].

This new transition could not be observed in the cooling scan or in the pressurizing scan. The temperature of this new transition increased linearly with increasing pressure and the value of dT/dP was 0.108 K MPa^{-1} . In order to make clear the kind of phase transition, the DEPC bilayer suspensions prepared in 50 % aqueous ethylene glycol as an antifreeze solvent were incubated at $-20 \text{ }^\circ\text{C}$ for 1 week and analyzed by the DSC. The DSC thermograms shown in Fig. 5-2 demonstrate the DEPC bilayer in water without annealing (curve 1), initial heating in 50 % aqueous ethylene glycol after annealing (curve 2) and second time heating in 50% aqueous ethylene glycol (curve 3). The values of transition enthalpy (ΔH) for curves 1, 2 and 3 were $5.56 \text{ kcal mol}^{-1}$ at $11.9 \text{ }^\circ\text{C}$, $14.15 \text{ kcal mol}^{-1}$ at $10.8 \text{ }^\circ\text{C}$ and $5.56 \text{ kcal mol}^{-1}$ at $10.0 \text{ }^\circ\text{C}$, respectively. There was no significant difference in the transition temperature among these three thermograms, however, the ΔH obtained from the curve 2 was much larger than that from the curve 1, and the ΔH obtained from the thermogram of second time heating scan for the same sample (curve 3) is in good agreement with that from the curve 1. The DSC thermogram shown by the curve 1 refers to the main transition from the L_β phase to the L_α phase. The transition enthalpy is in good agreement with previously published data, $5.5 \text{ kcal mol}^{-1}$ [78]. The transition on the curve 2 can be regarded as the transition from the lamellar crystalline (L_γ) phase to the L_α phase, namely the L_γ/L_α phase transition, judging from the large enthalpy change and the formation of the L_γ phase being extremely slow. The temperature of the L_γ/L_α transition in aqueous ethylene glycol is known to be higher than that in water [58]. Regarding the DEPC bilayer membrane, the addition of ethylene glycol elevates the temperature of the L_γ/L_β transition, and then the phase transition undergoes directly from the L_γ phase to the L_α phase. The ΔH of the main (L_β/L_α) transition in water was the same value in 50% aqueous ethylene glycol. Therefore, there was no effect of ethylene glycol on the main-transition enthalpy. The ΔH of L_γ/L_β transition could be calculated to be $8.6 \text{ kcal mol}^{-1}$.

The T - P phase diagram of DOPC bilayer membrane is shown in Fig. 5-3. Two kinds

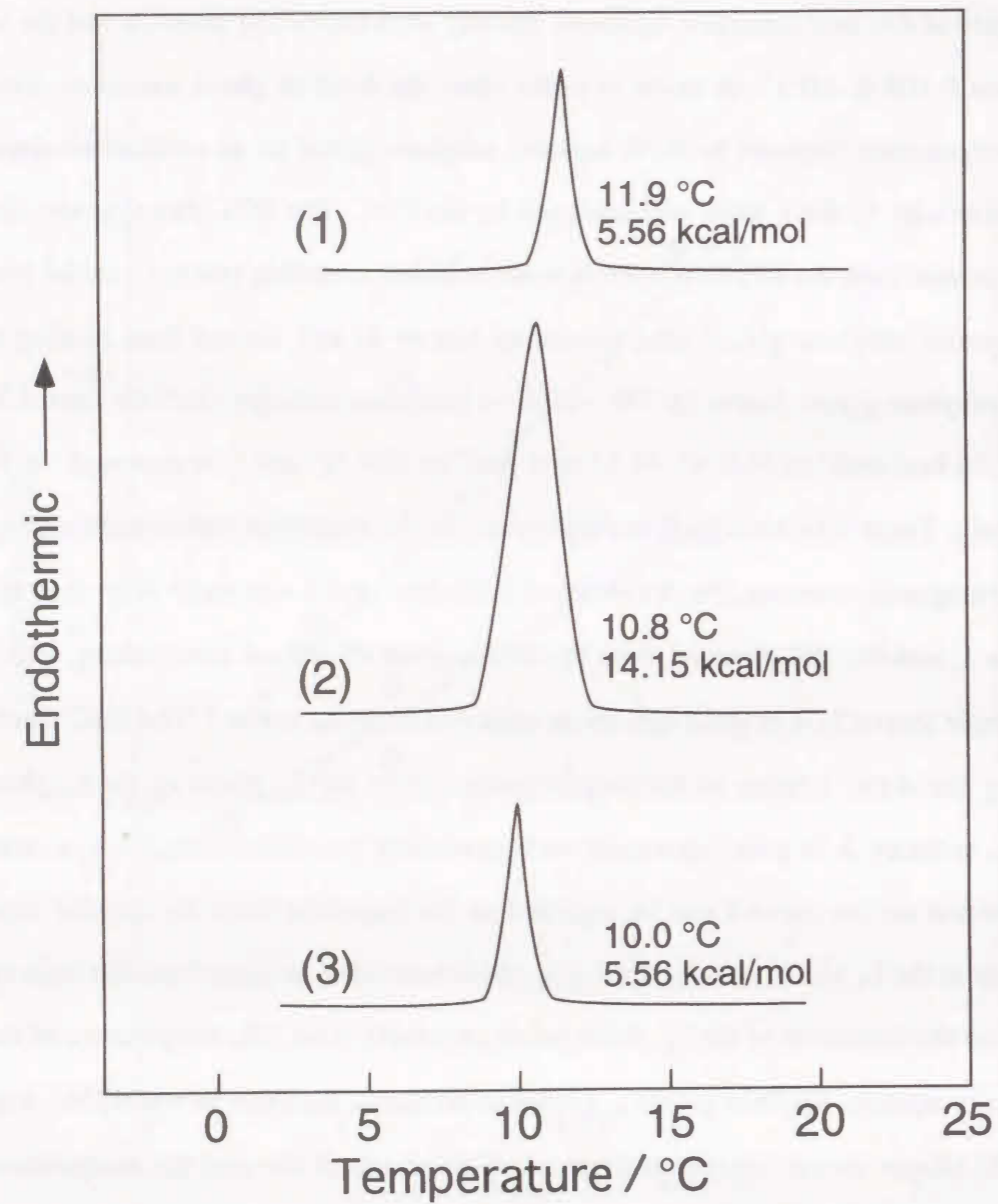


Fig. 5-2. DSC thermograms of DEPC bilayer membrane. (1) In water, (2) Initial heating in 50% aqueous ethylene glycol incubated at -20 °C for 1 week, (3) Second heating in 50% aqueous ethylene glycol.

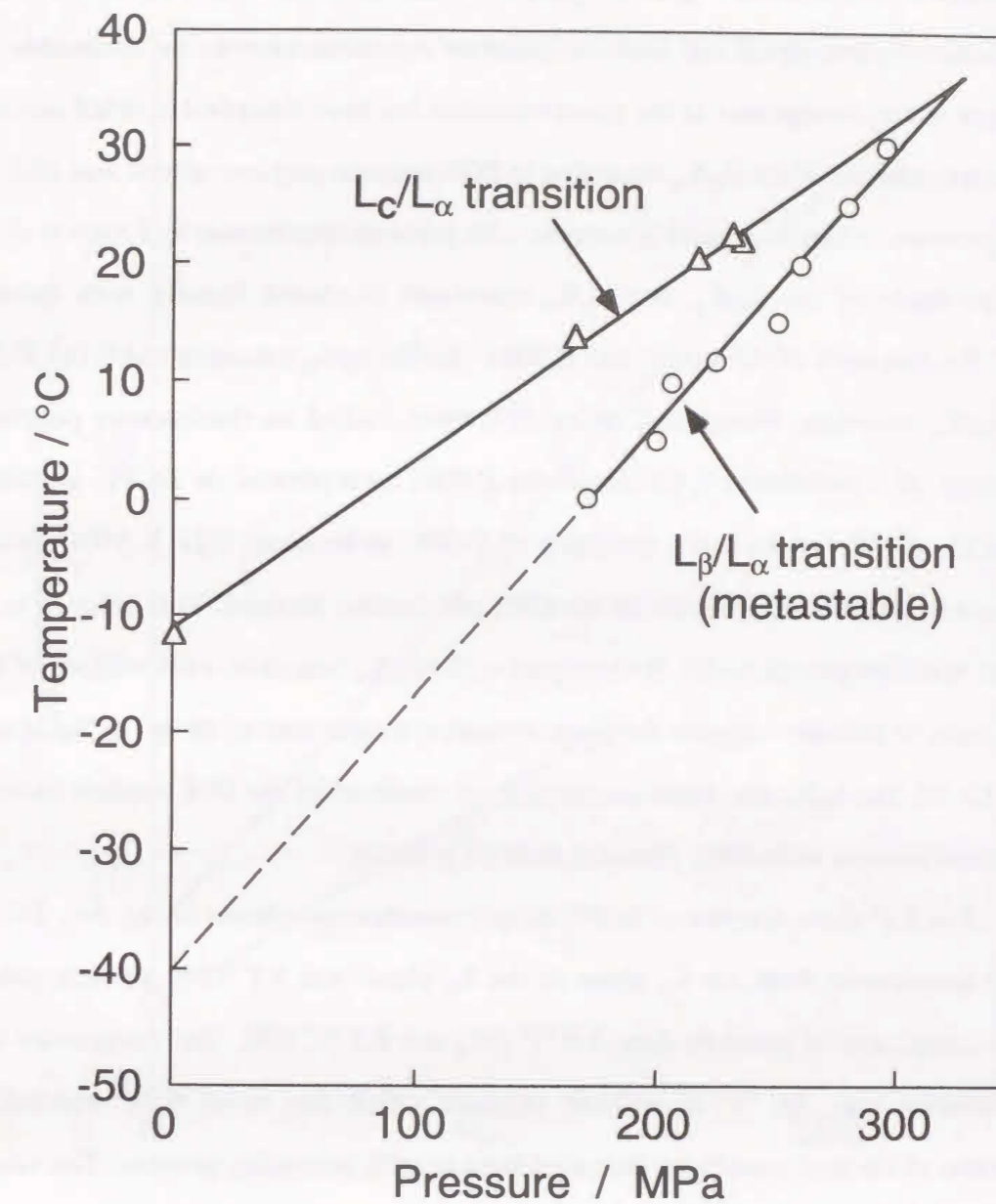


Fig. 5-3. Phase diagram of DOPC bilayer membrane. Concentration of DOPC was 1.0 mmol kg⁻¹. Phase transitions: (O) $L_\beta \rightarrow L_\alpha$ (metastable) in water, (Δ) $L_C \rightarrow L_\alpha$ in 50% aqueous ethylene glycol.

of transitions were observed. Higher-temperature transition refers to the L_C/L_α transition in 50% aqueous ethylene glycol and lower-temperature transition refers to the metastable L_β/L_α transition in water. Assignment of the phase transition has been described in detail previously [71]. The temperature of the L_C/L_α transition in 50% aqueous ethylene glycol was 12.0 °C at ambient pressure, which is in good agreement with previous observation by Lewis et al. [58]. The temperatures of the L_C/L_α and L_β/L_α transitions increased linearly with increasing pressure. The values of dT/dP were 0.233 K MPa⁻¹ for the L_β/L_α transition and 0.147 K MPa⁻¹ for the L_C/L_α transition. Chong and Weber [17] have studied on fluorescence polarization measurement of 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated in DOPC vesicle and estimated the dT/dP for the L_β/L_α transition of 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. With respect to the L_β/L_α transition, extrapolation of the T - P line to ambient pressure suggests the phase-transition temperature of the gel to liquid crystal to be -40.3 °C. The L_β/L_α transition has never been observed by the DSC method because of the transition between metastable phases at ambient pressure.

The T - P phase diagram of SOPC bilayer membrane is shown in Fig. 5-4. The main transition temperature from the L_β phase to the L_α phase was 6.7 °C at ambient pressure, which is comparable to previous data, 8.0 °C [63] and 8.3 °C [68]. The temperature of the L_C/L_β transition was 3.8 °C at ambient pressure which has never been reported. The temperatures of the both transitions increased linearly with increasing pressure. The values of the dT/dP were 0.181 K MPa⁻¹ for the main transition and 0.093 K MPa⁻¹ for the L_C/L_β transition.

The T - P phase diagram of OSPC bilayer membrane is shown in Fig. 5-5. The main transition temperature was 8.7 °C at ambient pressure, which is comparable to previous data, 10.0 °C [63] and 10.9 °C [68]. The temperature of the L_C/L_β transition was 4.9 °C at ambient

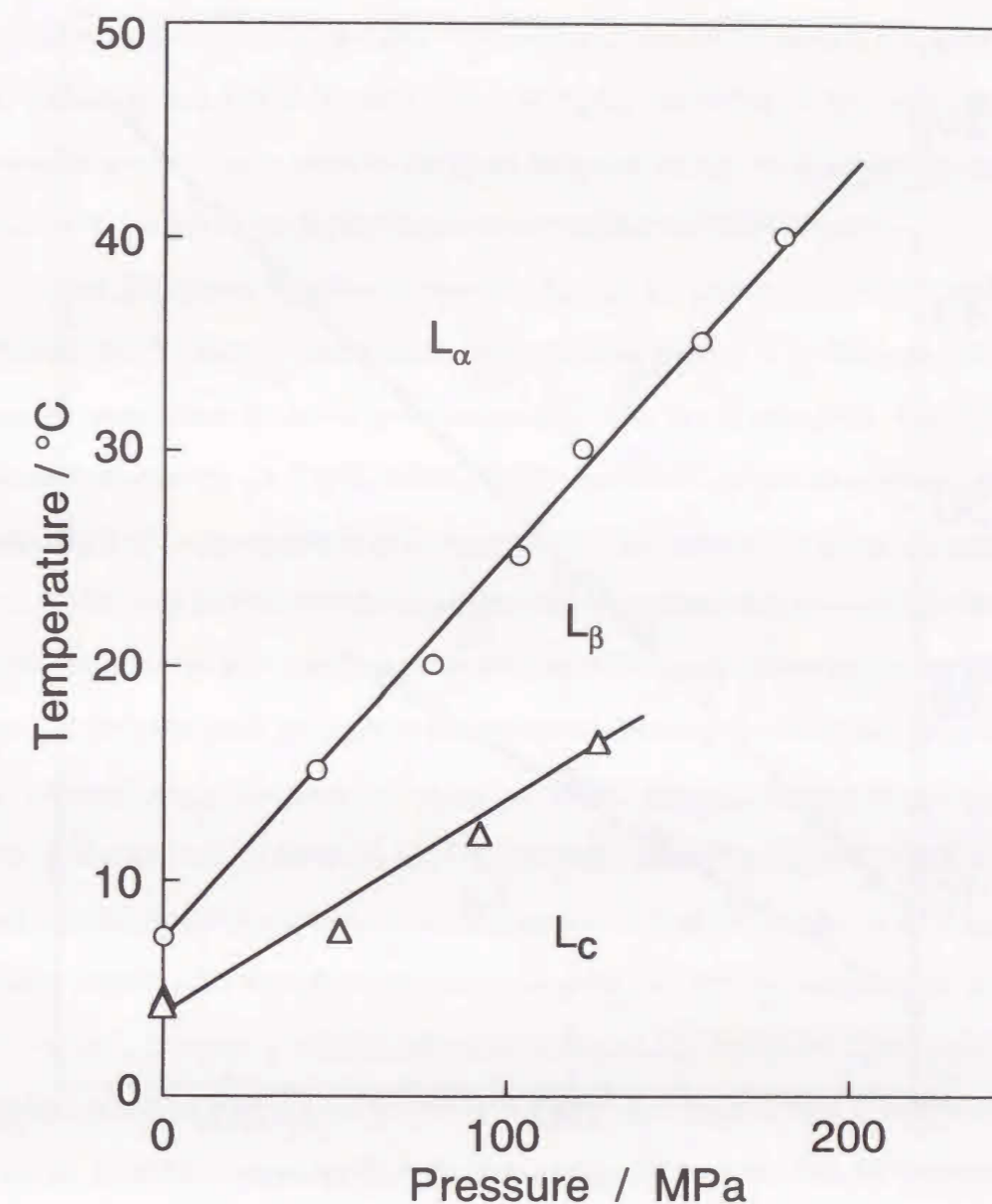


Fig. 5-4. Phase diagram of SOPC bilayer membrane. Concentration of SOPC was 1.0 mmol kg⁻¹. Phase transitions: (O) $L_\beta \rightarrow L_\alpha$, (Δ) $L_C \rightarrow L_\beta$.

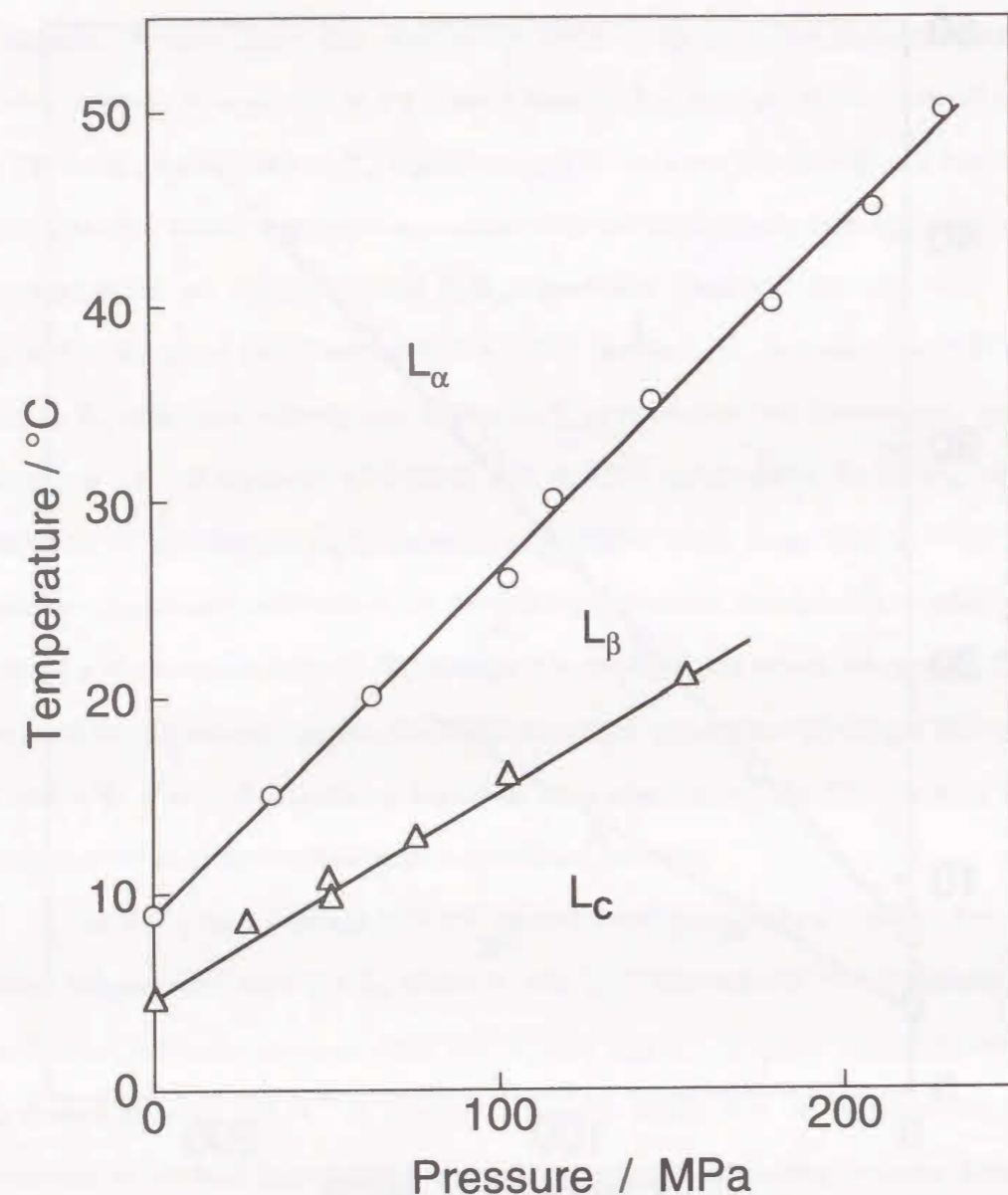


Fig. 5-5. Phase diagram of OSPC bilayer membrane. Concentration of OSPC was 1.0 mmol kg^{-1} . Phase transitions: (O) $L_{\beta} \rightarrow L_{\alpha}$, (Δ) $L_c \rightarrow L_{\beta}$.

pressure which has never been reported. The temperatures of the both transitions increased also linearly with increasing pressure. The values of the dT/dP were 0.175 K MPa^{-1} for the main transition and 0.105 K MPa^{-1} for the L_c/L_{β} transition. SOPC and OSPC bilayer membranes resemble each other in the phase behavior, except for the transition temperatures for the OSPC bilayer to be slightly higher than those for the SOPC bilayer.

The T - P phase boundaries between L_{β} and L_{α} phases for DSPC, DEPC, OSPC, SOPC and DOPC bilayer membranes were depicted in Fig. 5-6. Data for DSPC bilayer membrane were taken from our previous results [45]. As is seen from Fig. 5-6, the main transition temperatures for DSPC, SOPC, OSPC and DOPC bilayer membranes are 55.6 , 6.7 , 8.7 and -40.3 °C, respectively, at ambient pressure. The introduction of the *cis* double bond in SOPC, OSPC and DOPC thus decreases the main-transition temperature by 48.9 , 46.9 and 95.9 °C, respectively. It is interesting that the sum of the temperature depression by *cis* double bonds in SOPC and OSPC is equal to that in DOPC. 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 $48 (\pm 1)$ °C for each chain. With respect to SOPC and OSPC bilayer membranes, the presence of the unsaturated acyl group in *sn*-1 or *sn*-2 position does not affect significantly the phase transition temperatures, but the introduction of *cis* double bond into *sn*-2 position is slightly effective in depressing the phase transition temperature. The values of dT/dP for the main transition of SOPC and OSPC bilayer membrane were 0.181 and 0.175 K MPa^{-1} , respectively, which resemble each other. The temperature of main transition for DEPC bilayer membrane resembles that for SOPC and OSPC, and is much higher than that for DOPC. The substitution of *trans* unsaturated acyl chains for saturated acyl chains of DSPC brings about the depression of phase-transition temperature by 45 °C at ambient pressure. The temperature depression by *trans* double bond is less than half of the depression by *cis* double bond.

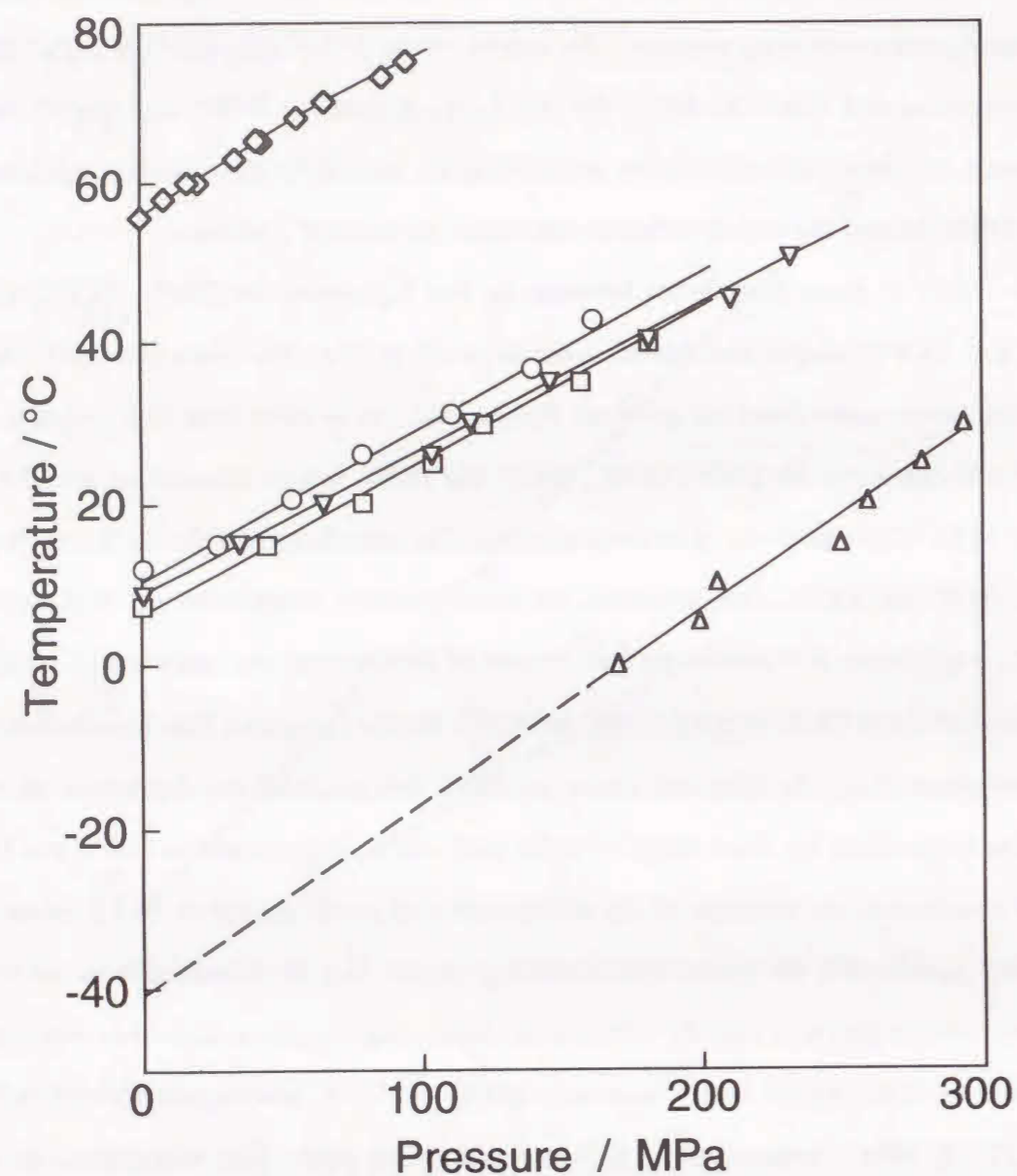


Fig. 5-6. Temperature – pressure phase diagram for bilayer membranes of phospholipids with saturated and unsaturated acyl chains. The concentration of lipid was 1.0 mmol kg^{-1} . Main transitions of phosphatidylcholine bilayer membranes. (\diamond) DSPC, (\circ) DEPC, (∇) OSPC, (\square) SOPC, (Δ) DOPC.

3. 2. Influence of Polyunsaturated acyl chain in *sn*-2 position on the phase transition of PC bilayer membranes

A significant feature of the structure of naturally occurring phospholipids concerns the nonrandom distribution of saturated and unsaturated fatty acids on the *sn*-1 and *sn*-2 carbons. The unsaturated fatty acid is predominantly found at the *sn*-2 position. The *T*-*P* phase diagrams for the bilayer membranes of SOPC, POPC, SAPC and SDPC, which are containing mono- or poly-unsaturated acyl chain in the *sn*-2 position, are shown in Fig. 5-7. The main (L_{β}/L_{α}) transition temperature of POPC bilayer membrane was $-1.6 \text{ }^{\circ}\text{C}$ at ambient pressure by linear extrapolation of *T*-*P* line to ambient pressure, which is in good agreement with previous data $0 \text{ }^{\circ}\text{C}$ [60], $-0.8 \text{ }^{\circ}\text{C}$ [68], $-2.2 \text{ }^{\circ}\text{C}$ [64] by the DSC method. The main transition temperature increased linearly with increasing pressure. Barotropic phase transition of POPC bilayer membrane has been observed by Winter and Pilgrim [21]. Their data are also shown in Fig. 5-7, which are in good agreement with our present result. The value of the dT/dP was 0.186 K MPa^{-1} at ambient pressure, which is comparable to previous data, 0.21 K MPa^{-1} .

Regarding SAPC bilayer membrane, extrapolation of the *T*-*P* line to ambient pressure suggests the temperature of the L_{β}/L_{α} phase transition to be $-13.0 \text{ }^{\circ}\text{C}$, which is in good agreement with previous data $-12.6 \text{ }^{\circ}\text{C}$ by the DSC method [79]. The main transition temperature increased linearly with increasing pressure. The value of the dT/dP was 0.134 K MPa^{-1} at ambient pressure.

The main transition temperature of SDPC bilayer membrane has not been studied at ambient pressure by the DSC method. Extrapolation was attempted to use the phase transition data under 200 MPa, because the *T*-*P* curve for the main transition of SDPC bilayer

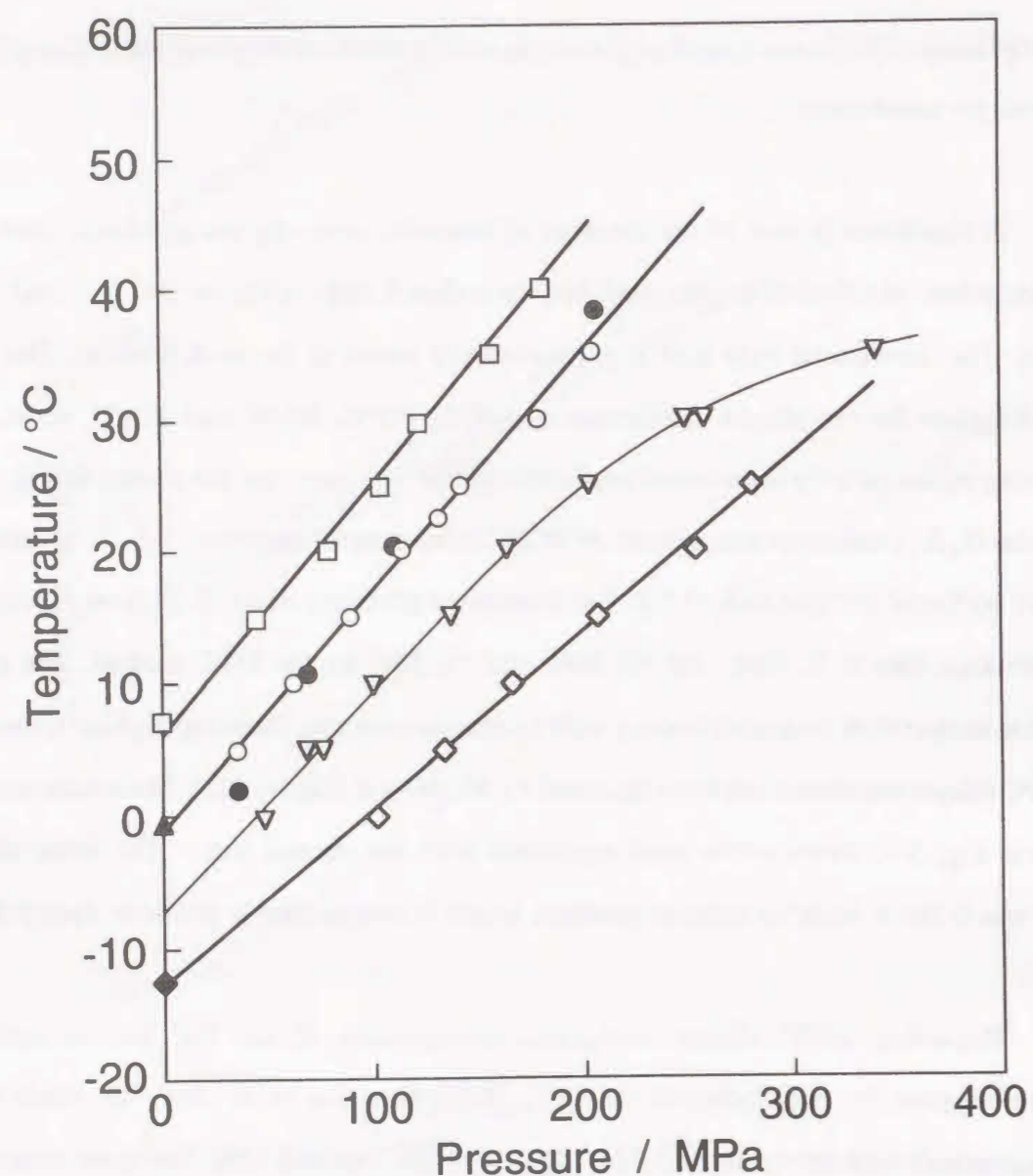


Fig. 5-7. Temperature – pressure phase diagram for bilayer membranes of phospholipids with unsaturated acyl chain in *sn*-2 position. The concentration of lipids was 1.0 mmol kg^{-1} . (\square) SOPC, (\circ) POPC, (∇) SDPC, (\diamond) SAPC. (\bullet) by Winter and Pilgrim [21]. (\blacktriangle) by Santaren et al. [64]. (\blacklozenge) by Coolbear et al. [79].

membrane convex upward over 200 MPa. The linear extrapolation of the T - P line to ambient pressure suggests the main transition temperature to be $-7.2 \text{ }^\circ\text{C}$. The value of the dT/dP was 0.165 K MPa^{-1} at ambient pressure.

We examined how the number of *cis* double bonds in the *sn*-2 chain affects the phase transition temperature of lipid bilayer membranes. The main transition temperatures of SOPC, SAPC and SDPC bilayer membranes were 6.7 , -13.0 and $-7.2 \text{ }^\circ\text{C}$, respectively. One might have predicted that the main transition for this highly unsaturated SDPC membrane would occur at a much lower temperature since the SAPC membrane occurs at $-13.0 \text{ }^\circ\text{C}$. The apparently large degree of cooperativity and the relatively high temperature of main transition suggest that in the gel state the SDPC bilayer membranes exist in a quite regular and relatively stable conformation. With regard to free fatty acids, melting points of stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), arachidonic acid (20:4) and docosahexaenoic acid (22:6) are 69.6 , 16.3 , -5.0 , -49.5 and $-44.5 \text{ }^\circ\text{C}$, respectively [80]. It can not be taken as axiomatic that there is simple linear relationship between physical properties (melting point) and the number of *cis* double bonds. Thus the phase transition temperatures of a series of phosphatidylcholine bilayer membranes reveal a somewhat similar influence of the number of *cis* double bond. A similar study [79] has shown that whilst introduction of the first *cis* double bond into DSPC (yielding SOPC) lowers the phase transition temperature by nearly $49 \text{ }^\circ\text{C}$, and two double bonds (18:0/18:2-PC) lowers it by a further $23 \text{ }^\circ\text{C}$, three or four bonds (18:0/18:3-PC or SAPC) bring about no further decrease, and in fact cause a slight increase. It is concluded that the main transition temperature of PC bilayer membranes did not decrease linearly with increasing degree of unsaturation [81, 82], like free fatty acids.

As is seen from Fig. 5-7, the T - P curve for the SDPC bilayer membrane is extremely convex upward over 200 MPa. Anomalous phase behavior of SDPC bilayer membrane may be attributable to the structure of docosahexaenoate chain in the bilayer membranes. Steady

state anisotropy data [83] are available for phosphatidylcholines containing docosahexaenoate. For 16:0/22:6-PC there is a further decrease in anisotropy compared to 16:0/22:4-PC. In a ^2H -NMR study [84], the phase transition of 16:0/22:6-PC was shown to be in the region of -10°C which is hardly different from the value of -12°C for 16:0/16:1-PC. It was suggested that, in the gel phase, 16:0/22:6-PC must exist in a relatively regular and stable configuration. It was also noted that in the phase behavior there was hysteresis (a difference between heating and cooling scan) which more resembled saturated lipids than the monounsaturated 16:0/16:1-PC examined. Stubbs and Smith [80] attempted to understand the structural role of docosahexaenoate using computer modeling. Bearing in mind that the acyl chain must presumably assume a conformation approximately parallel to the bilayer normal, and that motional freedom is severely limited by the lack of rotation at the six double bonds, allowed conformations are highly restricted. One of the possible conformations of SDPC in the gel state is as follows. One saturated chain is stretched, and the marked effect of the double bonds, including the reduction in the effective chain length, is clearly shown, yielding in this case an approximately helical conformation [85, 86].

Gawrisch and coworkers have studied extensively by ^2H -NMR and X-ray diffraction on the conformational order of the polyunsaturated chain of mixed- and saturated-unsaturated phosphatidylcholines [87-90]. Furthermore, Koenig et al. [91] determined the elastic area compressibility modulus of lamellar liquid crystalline bilayers by a new experimental approach ^2H -NMR order parameters of lipid hydrocarbon chains together with lamellar repeat spacings measured by X-ray diffraction. Combining NMR and X-ray results enables the determination of compressibility differences between saturated and unsaturated hydrocarbon chains. In mixed-chain SOPC both chains have similar compressibility moduli; however, in mixed-chain polyunsaturated SDPC, the saturated stearic acid chain appears to be far less compressible than the polyunsaturated docosahexaenoic acid chain.

The pretransition of phospholipids which contain unsaturated acyl chain was not observed, which means the substitution of unsaturated chain for saturated chain of DSPC brings about the disappearance of the ripple gel phase and the non-tilted bilayer structures in the gel state. The packing of bilayer membrane was disordered by the effect of unsaturated chain, because *cis* unsaturation induced a bend of acyl chain. Straight chain was one of the requirements to obtain a pressure-induced interdigitated gel phase, in which the hydrocarbon chains from apposing monolayers become interdigitated with the chains. Consequently, the interdigitated gel phase was not observed in unsaturated phospholipid bilayers.

3.3. Thermodynamic properties of the phase transitions

The values of the dT/dP and temperatures of phase transitions for various phospholipid bilayer membranes are listed in Table 5-2. With respect to the L_β/L_α phase transition, the bilayer membranes of DOPC showed the large value of dT/dP , 0.233 K MPa^{-1} , which is hardly different from the value of the saturated lipid DSPC, 0.230 K MPa^{-1} . The value of dT/dP for SOPC, OSPC and POPC, which are monounsaturated lipids, lie around 0.18 K MPa^{-1} . The polyunsaturated lipids SAPC and SDPC have smaller values of dT/dP , 0.134 and 0.165 K MPa^{-1} , respectively. However, the increase of the number of *cis* double bonds is not always the decrease of the value of dT/dP . With regard to the L_C/L_β phase transition, the values of dT/dP for DEPC, SOPC and OSPC were 0.108 , 0.093 and 0.105 K MPa^{-1} , respectively, which are almost the same.

The enthalpy (ΔH) and entropy ($\Delta S = \Delta H/T$) changes associated with the L_β/L_α transition have been determined by the DSC measurements and are summarized in Table 5-2. The values of ΔH for the L_β/L_α transition of DOPC and SDPC have never been obtained by the DSC. The enthalpy change for the L_C/L_α transition of DOPC bilayer membrane was

Table 5-2. Thermodynamic properties for phase transitions of unsaturated phospholipid bilayer membranes.

Lipid	Transition	Transition Temp.		dT/dP (K MPa ⁻¹)	ΔH (kJ mol ⁻¹) (kcal mol ⁻¹)	ΔS (J K ⁻¹ mol ⁻¹)	ΔV (cm ³ mol ⁻¹)
		(K)	(°C)				
DSPC	P β ' / L α	328.8	55.6	0.230	45.2	137	31.6
DEPC	L β / L α	284.3	11.1	0.180	23.3	82	14.7
	L c / L β	282.2	9.0	0.108	35.9	127	13.7
DOPC	L β / L α	232.9	-40.3	0.233	65.3 ^{a)}	250	36.7
	L c / L α	261.2	-12.0	0.147	28.0 ^{b)}	99	17.4
OSPC	L β / L α	281.9	8.7	0.175	29.3 ^{b)}	105	18.9
	L c / L β	278.1	4.9	0.105	22.2 ^{c)}	75	10.1
SOPC	L β / L α	279.9	6.7	0.181	22.6 ^{d)}	83	15.5
	L c / L β	277.0	3.8	0.093			
SAPC	L β / L α	260.2	-13.0	0.134			
SDPC	L β / L α	266.0	-7.2	0.165			
POPC	L β / L α	271.6	-1.6	0.186			

a) Lewis et al. [58]

b) Davis and Keough [63]

c) Coolbear et al. [79]

d) Davis et al. [68]

employed the value in 50% aqueous ethylene glycol, 65.3 kJ mol⁻¹ [58], because the L_c/L_α transition under high pressure was observed in 50% aqueous ethylene glycol. The value of ΔH for the L_c/L_β transition of DEPC bilayer membrane was calculated as 35.9 kJ mol⁻¹ from those for L_c/L_α and L_β/L_α transitions, which are 59.2 and 23.3 kJ mol⁻¹, respectively. The ΔH for the L_c/L_β transition of SOPC and OSPC bilayer membranes has not been determined yet.

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

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

and is also summarized in Table 5-2. The values of ΔV for the L_β/L_α transition of SOPC, OSPC and POPC bilayer membranes, which have a *cis* double bond in *sn*-1 or *sn*-2 chain, were 18.9, 17.4 and 15.5 cm³ mol⁻¹, respectively. These values are smaller than the transition volume for saturated lipid DSPC, 31.6 cm³ mol⁻¹, and larger than that for polyunsaturated lipid SAPC, 10.1 cm³ mol⁻¹. It seems that the volume changes for the L_β/L_α transition of lipid bilayer membranes are obviously dependent on the number of *cis* double bonds in the acyl chains. Monolayer study [92, 93] has shown that the introduction of double bonds in the acyl chains of phospholipids greatly increases the molecular area of lipid. The partial molar volume of lipids in the gel state of bilayer membranes increases probably with an increase in the number of double bonds, while in the liquid crystalline state relatively smaller effect of increasing the unsaturation on the volume is expected because of random packing of chains. Therefore, it may be concluded that the volume change for the L_β/L_α transition of lipid bilayer membranes decreases as the number of double bonds in the acyl chain increases.

We focus our attention here on differences in the phase transition properties of *cis* and *trans* unsaturated phospholipids, DOPC and DEPC. Since the values of ΔV for the L_c/L_β and L_β/L_α transitions are 14.7 and 13.7 cm³ mol⁻¹, respectively, the volume change for the L_c/L_α transition of DEPC bilayer membrane can be estimated as 28.4 cm³ mol⁻¹, which is

smaller than that for DOPC bilayer membrane, $36.7 \text{ cm}^3 \text{ mol}^{-1}$. The enthalpy change for the L_C/L_α transition of DEPC, 59.2 kJ mol^{-1} is also smaller than that for DOPC, 65.3 kJ mol^{-1} , with very similar tendency. This difference may be directly attributed to the different geometrical configurations of *cis* and *trans* double bonds; the bent oleoyl chains in the L_C state are closely packed with all chains oriented parallel to each other, and the *cis* double bonds of each chain are adjacent and at the same distance from the bilayer surface.

The most significant difference in the phase behavior between DEPC and DOPC bilayer membranes shown in Fig. 5-1 and Fig. 5-3 is as follows. The L_β/L_α (namely main) transition in the bilayer membrane of DEPC takes place at the temperature higher than the L_C/L_β transition does, and is the transition between stable phases. On the other hand, the L_β/L_α transition of DOPC bilayer membrane is observed as the lower-temperature transition between metastable L_β and L_α phases. It is apparent from Table 5-2 that the order of partial molar volumes and enthalpies of lipids among L_C , L_β and L_α states is lamellar crystal (L_C) < lamellar gel (L_β) < liquid crystal (L_α).

Partial molar volumes of lipids among the three states are demonstrated schematically in Fig. 5-8 as a function of pressure for example DEPC at 20°C and DOPC at 0°C . The gap in partial molar volumes between different phases refers to the volume change of the phase transition. The L_β phase of DEPC bilayer membrane is obtained by pressurizing the L_α phase up to 50 MPa at 20°C and is allowed to persist as a metastable state even at a pressure more than 100 MPa. The formation of the L_C phase is very slow. The transformation from the L_α to L_β phase and from the L_β to L_C phase brings about the reduction of volume. In the case of DOPC bilayer membrane, the L_α phase shows a direct transition to the L_C phase at 75 MPa and 0°C , but the transformation is extremely slow. The L_α phase is allowed to persist as a metastable phase even at a pressure as high as 170 MPa, and is transformed into the metastable L_β phase at 170 MPa.

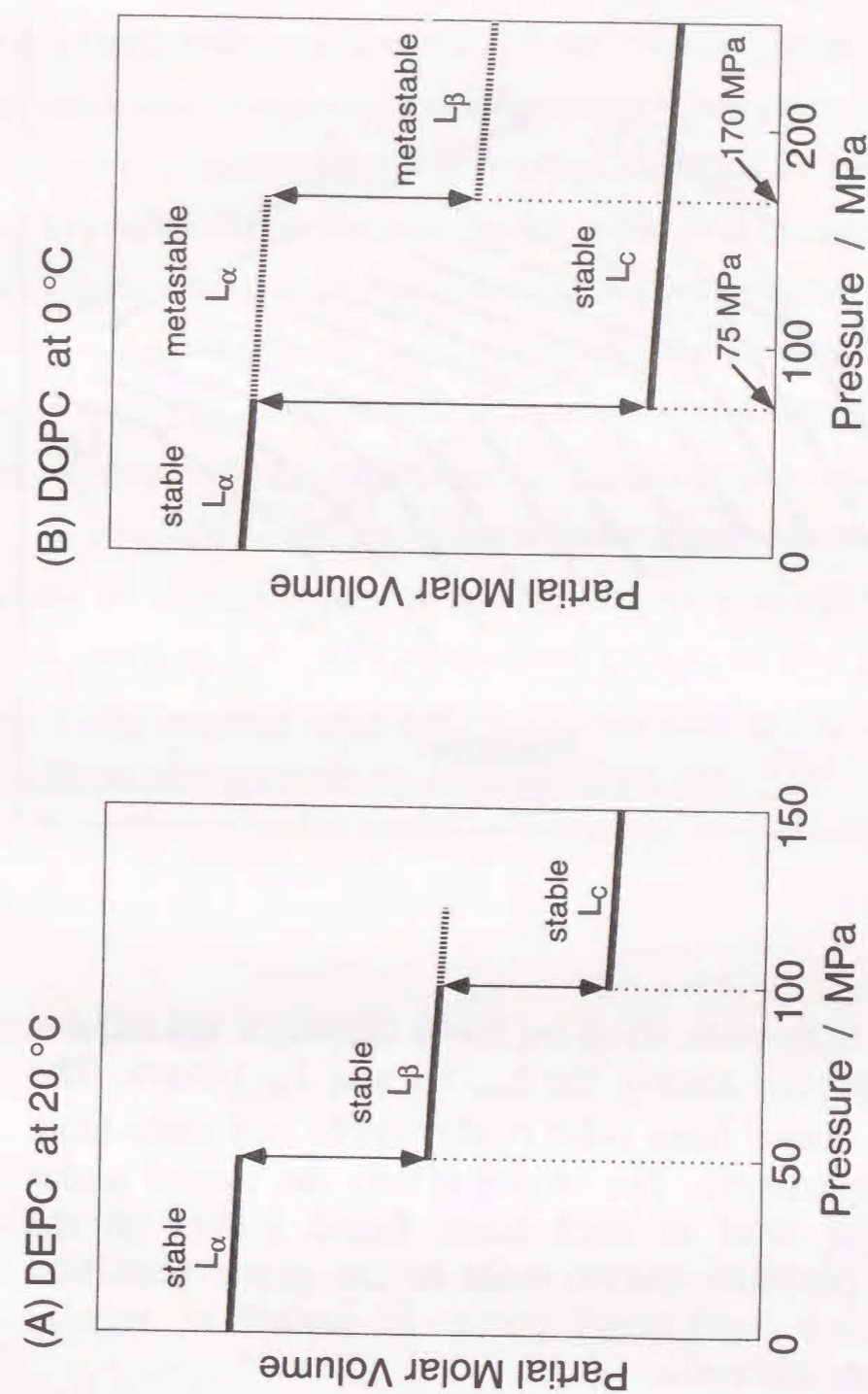


Fig. 5-8. Schematic diagrams showing the partial molar volume vs. pressure curves among three states of lipid bilayer membranes: (A) DEPC at 20°C and (B) DOPC at 0°C . The scale on the ordinate is arbitrary.

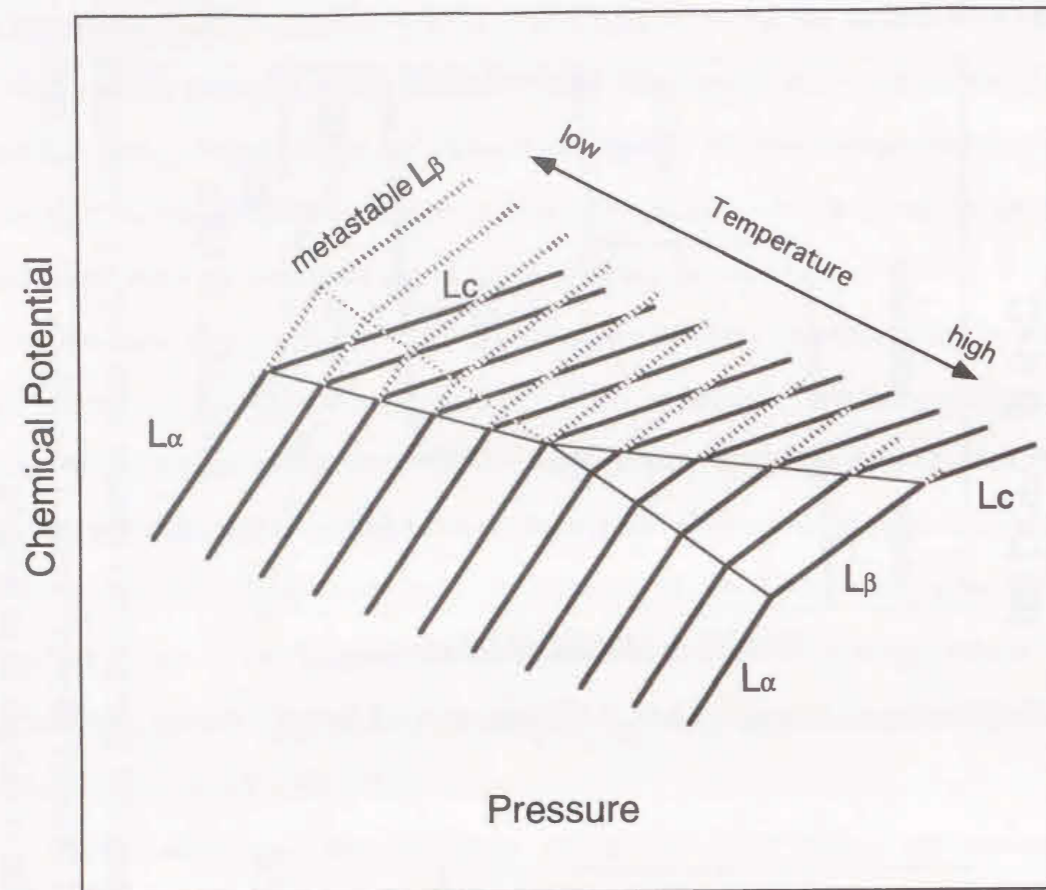


Fig. 5-9. Schematic diagram for a chemical potential – pressure profile among the L_α , L_β and L_C phases. The wide and dotted lines refer to the stable and metastable states, respectively. The slopes reflect the partial molar volumes of lipid in each state. Break points on the chemical potential curves refer to the phase transition points. Each isothermal curve is drawn at regular temperature intervals.

The partial molar volume and entropy are defined thermodynamically as the differential of chemical potential with respect to pressure and temperature, respectively. On the basis of the thermodynamic quantities for the phase transitions of lipid bilayer membranes, we can draw a chemical potential (μ) – pressure (P) profile among three (L_C , L_β and L_α) states of lipid bilayer as shown in Fig. 5-9. In this figure, isothermal $\mu - P$ curves are shown at regular temperature intervals. The slopes reflect the partial molar volumes of lipid in the states of L_α , L_β and L_C , which decrease in that order. In the region of lower temperature and pressure, the chemical potential of lipid in the L_β state is larger than that in the L_C state. The stable transition between L_α and L_C phases is thus observed. The transition between L_α and L_β phases is observed as the metastable transition at higher pressure (or lower temperature) than the L_C/L_α transition occurs. This is the case for DOPC bilayer membrane (Fig. 5-3). On the other hand, in the region of higher temperature and pressure we can observe two stable transitions, that is, the L_C/L_β and L_β/L_α transitions. The L_β/L_α transition is observed at lower pressure (or high temperature) than the L_C/L_β transition occurs as in the case for DEPC (Fig. 5-1), SOPC (Fig. 5-4) and OSPC (Fig. 5-5) bilayer membranes.

CHAPTER 6.

SUMMARY

1. In chapter 2, the bilayer phase-transitions of a series of 1, 2-diacylphosphatidylcholines containing linear saturated acyl chain of even- and odd-number carbons ($C=12,13,14,15,16,17$ and 18) 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. 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 linearly by pressure in the range of 150 MPa. The slope of the temperature-pressure diagram, dT/dP , was in the range of 0.20 - 0.23 K MPa $^{-1}$ depending on the acyl chain-length. The chain length dependence of the main transition temperature under ambient pressure described a smooth curve with no evidence of odd/even discontinuities. The phase transition enthalpy, ΔH , which was determined by the differential scanning calorimetry, increased with an increase in the acyl chain-length. The ΔH vs. chain-length curve was non-linear and convex upward. The volume change, ΔV , associated with the transition was calculated from the value of ΔH and dT/dP by means of the Clapeyron-Clausius equation. The values of ΔV increased with an increase in the acyl chain-length, which were best described by a smooth curve and not a linear function. The increment of the transition volume tends to be moderated as the length of the hydrocarbon chain is increased and amounts to 1.4 cm 3 mol $^{-1}$ per one methylene group. Non linear properties of thermodynamic quantities with respect to the acyl chain-length seems to be attributable to the end-group effects of the fatty acyl chains.

2. In chapter 3, 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 high-pressure optical methods. 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 $0.12-0.14 \text{ K MPa}^{-1}$. The temperatures of the pretransition under ambient pressure increased with an increase in the acyl chain-length. The chain-length dependence of the pretransition temperatures describe smooth curves with no evidence of odd/even discontinuities. Pressure-induced interdigitated gel ($L_{\beta}I$) phase was observed beyond 300 MPa for $14:0\text{-PC}$, 175 MPa for $15:0\text{-PC}$, 100 MPa for $16:0\text{-PC}$, 80 MPa for $17:0\text{-PC}$ and 70 MPa for $18:0\text{-PC}$, respectively. The minimum pressure for the interdigitation of lipid bilayer membranes decreased with an increase in the acyl chain-length in a manner of nonlinear relation. The slopes of phase boundary between L_{β}' and $L_{\beta}I$ phases transformed from the negative slope to the positive slope as the pressure increases.

3. In chapter 4. 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 L_{α} and the L_{β} phases was observed in water at above $0 \text{ }^{\circ}\text{C}$ under high pressure, in addition to the subtransition 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 $-40.3 \text{ }^{\circ}\text{C}$, 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 -

$12.0 \text{ }^{\circ}\text{C}$ at ambient pressure. The main transition temperatures for DSPC, SOPC and DOPC are $55.6, 6.7$ and $-40.3 \text{ }^{\circ}\text{C}$, 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 $48 (\pm 1) \text{ }^{\circ}\text{C}$ 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 (dT/dP) 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.

4. In chapter 5, In order to understand the effect of unsaturation on the thermotropic and barotropic phase behavior of phospholipid bilayer membranes, the phase transitions of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC), 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (OSPC), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC), 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (SAPC), 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (SDPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayer membranes were observed by high-pressure optical method and differential scanning calorimetry.

For all of the lipids studied, the temperatures of the so-called main transition between the L_{α} and the L_{β} phases were almost linearly elevated by pressure. The values of dT/dP for monounsaturated lipids (SOPC, OSPC and POPC) lie around 0.18 K MPa^{-1} , which are smaller than that for the saturated lipid DSPC, 0.23 K MPa^{-1} . The polyunsaturated lipids (SAPC and SDPC) have rather small values of dT/dP . The temperatures of L_{β}/L_{α} transition for DSPC,

SOPC, OSPC and DOPC were 55.6, 6.7, 8.7 and -40.3 °C, respectively. The substitution of *cis* unsaturated chain for saturated chains of DSPC brings about the depression of transition temperature by 47 °C for *sn*-1 chain and 49 °C for *sn*-2 chain. The volume change (ΔV) associated with the transitions were calculated from the transition enthalpy (ΔH) and the value of dT/dP by means of the Clapeyron-Clausius equation. The values of ΔV for SOPC, OSPC and POPC, which have an only *cis* double bond in *sn*-1 or *sn*-2 chain, were 18.9, 17.4 and 15.5 cm³ mol⁻¹, respectively. These values are smaller than that for the saturated DSPC, 31.6 cm³ mol⁻¹, and larger than that for the polyunsaturated SAPC, 10.1 cm³ mol⁻¹. It seems that the ΔV for the L_{β}/L_{α} transition is obviously dependent on the number of *cis* double bonds in the acyl chains.

With respect to the bilayer membranes of DEPC, SOPC and OSPC, the transition between the L_C and the L_{β} phases was observed; the values of dT/dP were 0.108, 0.093 and 0.105 K MPa⁻¹, respectively, which are almost the same. The values of ΔV for the L_C/L_{α} transition of *trans* unsaturated DEPC and *cis* unsaturated DOPC were 28.4 and 36.7 cm³ mol⁻¹, and the values of ΔH were 59.2 and 65.3 kJ mol⁻¹, respectively. This difference may be directly attributed to the different geometrical configurations of *trans* and *cis* double bonds.

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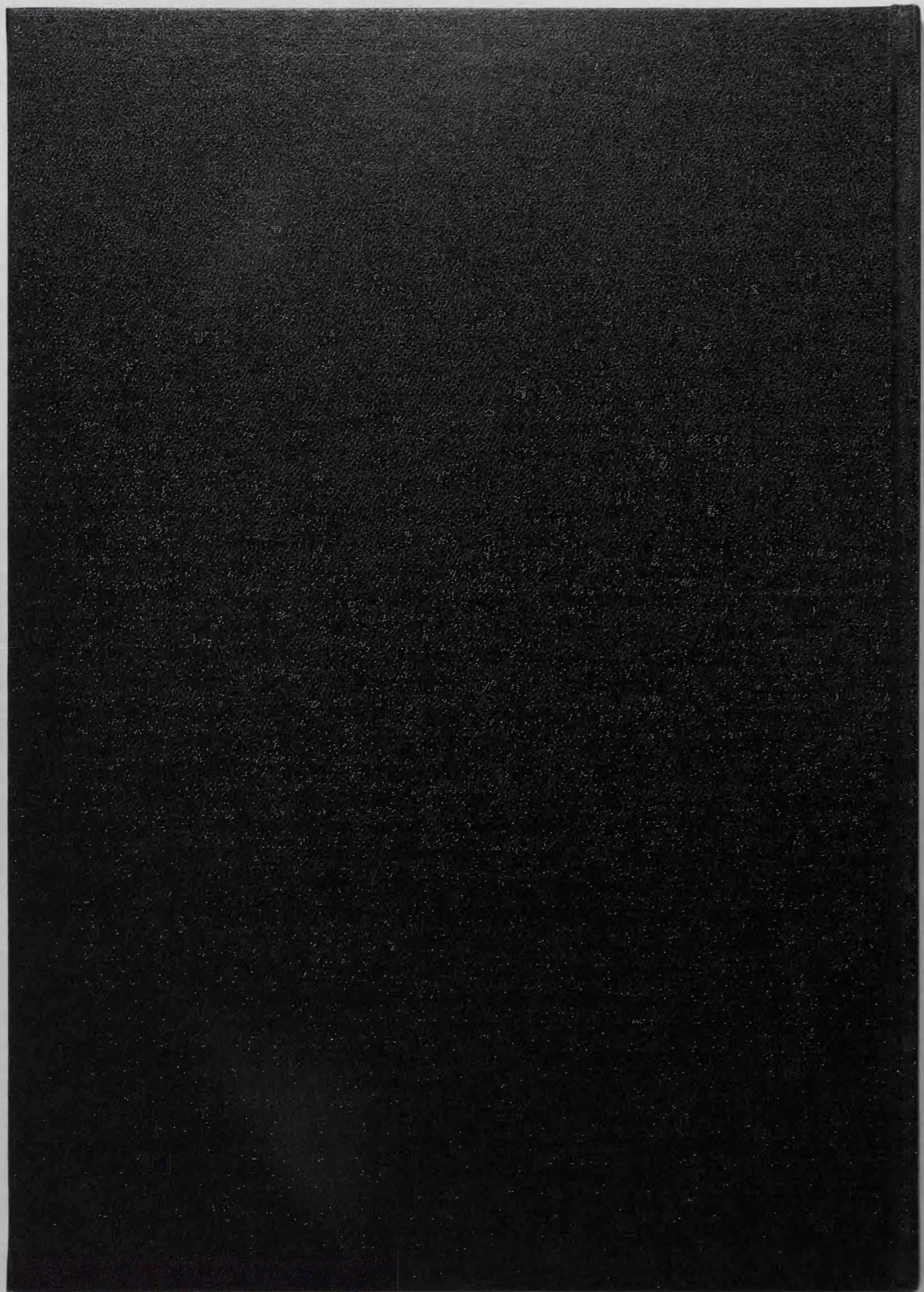
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様式9

論文審査の結果の要旨

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学位論文題目 BAROTROPIC AND THERMOTROPIC PHASE TRANSITIONS OF PHOSPHOLIPID BILAYER MEMBRANES			
審査結果の要旨 本研究は、リン脂質が水中で形成する二分子膜の分子集合系に対して特に圧力を実験変数として導入し、リン脂質分子構造を系統的に変化させながら、脂質二分子膜の相転移におよぼす圧力と温度の効果を明らかにしている。これまでリン脂質二分子膜の高圧力下における相転移の観測は世界で数例しか無く、脂質分子構造と関連付けた系統的研究は皆無であった。本研究では、まず、飽和アシル鎖をもつホスファチジルコリン (PC) の鎖長を炭素数 12 から 18 まで変化させ、リップルゲル相から液晶相への主転移熱力学量 (dT/dP , ΔH , ΔS , ΔV) の鎖長依存性を明らかにした。ラメラゲル相からリップルゲル相への前転移は炭素数 13 以上で観測でき、 dT/dP は鎖長によらずほぼ一定値を示すことが判明した。二分子膜が指組み構造となる interdigitated ゲル相は炭素数 14 以上の PC 二分子膜で圧力によって誘起され、アシル鎖が長くなると interdigitated ゲル相の現れる圧力は低下した。 次いで、不飽和アシル鎖をもつ PC 二分子膜の相挙動を明らかにした。不飽和リン脂質の二分子膜では、全般的に相転移温度は低下し、高圧力下においても interdigitated ゲル相は出現しない。リン脂質分子の二本のアシル鎖が共にシス型二重結合をもつオレイン酸から成るジオレオイルホスファチジルコリン (DOPC) 二分子膜では従来常圧下で -12°C に観測されていた相転移はサブゲル相 (或いはラメラ結晶相) から液晶相への転移であって、いわゆる主転移のゲル-液晶相転移は 0°C 以上の高圧力下で始めて観察された。トランス型二重結合をもつエライジン酸で置換した場合の二分子膜相図も示された。生体膜中に広く分布している不飽和リン脂質では <i>sn</i> -2 位のみが不飽和脂肪酸で置換されたものが多い。 <i>sn</i> -2 位のアシル鎖のシス型二重結合の数を変化させた場合の脂質二分子膜の相挙動も明らかにされている。 以上、本研究の成果は高圧力殺菌、食品の高圧処理加工、深海生物の環境適応などの機構解明にあたり有効に活用できる内容であり、本論文は博士(工学)の学位授与に値するものと判断する。			