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Thermotropic and barotropic phase transitions of *N*-methylated dipalmitoylphosphatidylethanolamine bilayers

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Abstract

In order to understand the effect of polar head group modification on the thermotropic and barotropic phase behavior of phospholipid bilayer membranes, the phase transitions of dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidyl-*N*-methylethanolamine (DPMePE), dipalmitoylphosphatidyl-*N*,*N*-dimethylethanolamine ($DPMe_2PE$) and dipalmitoylphosphatidylcholine (DPPC) bilayer membranes were observed by differential scanning calorimetry and high-pressure optical methods. The temperatures of the so-called main transition from the gel (L_β) or ripple gel (P'_β) phase to the liquid crystalline (L_α) phase were almost linearly elevated by applying pressure. The slope of the temperature–pressure boundary, dT/dp, was in the range of 0.220–0.264 K MPa^{-1} depending on the number of methyl groups in the head group of lipids. The main-transition temperatures of *N*-methylated DPPEs decreased with increasing size of head group by stepwise *N*-methylation. On the other hand, there was no significant difference in thermodynamic quantities of the main transition between the phospholipids. With respect to the transition from the subgel (L_c) phase to the lamellar gel (L_β or L'_β) phase, the transition temperatures were also elevated by applying pressure. In the case of DPPE bilayer the L_c/L_β transition appeared at a pressure higher than 21.8 MPa. At a pressure below 21.8 MPa the L_c/L_α transition was observed at a temperature higher than the main-transition temperature. The main (L_β/L_α) transition can be recognized as the transformation between metastable phases in the range from ambient pressure to 21.8 MPa. Polymorphism in the gel phase is characteristic of DPPC bilayer membrane unlike other lipid bilayers used in this study: the L'_β, P'_β and pressure-induced interdigitated gel (L_βI) phases were observed only in the DPPC bilayer. Regarding the bilayers of DPPE, DPMePE and $DPMe_2PE$, the interdigitation of acyl chain did not appear even at pressures as high as 200 MPa.

Keywords: Bilayer membrane; *N*-methylated phosphatidylethanolamine; Phase transition; Phospholipid; Pressure; Vesicle

1. Introduction

The effect of pressure on lipid bilayer membranes and cellular membranes is of particular interest to studies of pressure–anesthetic antagonism [1], pressure adaptation in deep sea organisms [2], and high-pressure sterilization in food processing [3–6]. Lipid bilayer membranes composed of phosphatidylcholines (PCs) containing two identical linear saturated fatty acyl chains have been most thoroughly studied under high pressure [7–10]. PCs in vivo can be derived from sequential methylation of the amino group of phosphatidylethanolamines (PEs) by methyltransferases. Therefore, there exist two intermediates which contain one and two methyl groups in the ethanolamine moiety, respectively. Since the state of biological membranes is regulated not only through changes in the nature of the lipid acyl chains but also through changes in the head group, it is plausible that the partially methylated PE may play a role in the regulation of membrane state [11,12]. So far, bilayer membranes of *N*-methylated dipalmitoylphosphatidylethanolamines (DPPEs) have been studied with regard to the thermotropic phase behavior [13–15], sodium and glucose permeabilities [16], membrane fluidity [14,17], volume changes associated with the gel to liquid crystalline phase transition [18] and miscible behavior of *N*-methylated DPPE

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mixtures in the bilayers [15]. However, the effect of pressure on bilayer phase behavior of *N*-methylated DPPEs has not yet been confirmed. With respect to dipalmitoylphosphatidylcholine (DPPC), pressure studies on the bilayer phase transition have been reported by various physical techniques including ESR [19], dilatometry [20,21], calorimetry [22,23], X-ray diffraction [24], dynamic light scattering [25], Raman spectroscopy [26,27], adiabatic compression [28], fluorescence [29,30], FT-IR [31], neutron diffraction [32,33], light transmittance [34,35], and NMR [36–38]. These measurements have revealed the phase behavior of DPPC bilayer membranes. A new pressure-induced gel phase, i.e., the interdigitated gel phase, as well as the liquid crystal, ripple gel and lamellar gel phases has been observed under high pressure [7–10,32,33,35].

The present study demonstrates the pressure effect on the phase behavior of bilayer membranes of DPPE, dipalmitoylphosphatidyl-*N*-methylethanolamine (DPMePE), dipalmitoylphosphatidyl-*N*,*N*-dimethylethanolamine ($DPMe_2PE$) and DPPC, and reveals the effect of polar head group modification on the barotropic phase behavior of lipid bilayer membranes.

2. Experimental

2.1. Materials

Highly pure phospholipids, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (DPPE) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC, i.e., $DPMe_3PE$), were obtained from Sigma Chemical Co. (St. Louis, MO). Other lipids, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyl-*N*-methylethanolamine (DPMePE) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyl-*N,N*-dimethylethanolamine ($DPMe_2PE$), were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). These phospholipids were used without further purification. Water was distilled twice from dilute alkaline permanganate solution. The phospholipid multilamellar vesicles were prepared by suspending each phospholipid in water at 0.5–1.0 mmol kg^{-1}, using a Branson model 185 sonifier at a temperature several degrees above the main transition for a short time (approximately 3 min) in order to prepare the multilamellar vesicle suitable for the optical measurements of phase transition. The average size of vesicles was found to be 200–300 nm, which was determined by a light scattering method.

2.2. Differential scanning calorimetry

Calorimetric scans were performed with a MicroCal MCS (Northampton, MA) highly sensitive differential scanning calorimeter at a heating rate of 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 pressure

In order to transform completely into the subgel phase of lipid bilayer, vesicle suspensions were kept in a refrigerator (at about 5 °C) for 2 or 3 days and then transferred to a freezer (at about −20 °C). The thermo-cycle was repeated five times or more. The sample was periodically shaken by vortex during the storage to prevent from precipitating. Phase transitions under high pressure 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 supplied by Hikari High Pressure Instruments (Hiroshima, Japan), was connected to a spectrophotometer through an optical fiber. The light transmittance of the vesicle suspension was determined at a suitable interval of pressure (or temperature) by a Photal model IMUC 7000 spectrophotometer equipped with a photodiode array of 512 ch. (Otsuka Electronics, Osaka).

Pressures were generated by a hand-operated KP-3B hydraulic pump (Hikari High Pressure Instruments) 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. In the isobaric thermotropic phase transition measurements, the abrupt change in transmittance accompanying the phase transition was followed at 560 nm. The heating rate at a given pressure was 0.33 K min^{-1}. In the isothermal barotropic phase transition, vesicle suspension was compressed slowly and stepwise, i.e., the pressure was increased by approximately 5 MPa in each step in the vicinity of the phase transition, and allowed to stand for 15 min. in each step.

3. Results and discussion

3.1. Phase transitions of DPPE bilayer membrane

The heating DSC thermograms of DPPE bilayer membrane showed two kinds of endothermic transitions (curve 1 in Fig. 1). Higher-temperature transition obtained by the first scan can be assigned as the transition from the subgel or lamellar crystal (L_c) phase to the liquid crystalline (L_α) phase. On the other hand, lower-temperature transition observed by the second scan can be assigned as the main transition from the gel (L_β) phase to the L_α phase. In the figure are also included the results of *N*-methylated DPPE bilayers. The DSC thermograms of DPMePE and $DPMe_2PE$ bilayer membranes showed two endothermic transitions (curves 2 and 3 in Fig. 1). Higher-temperature transition can be assigned as the main transition from the L_β phase to the L_α phase, which was in good agreement with previous observation [11–14]. Lower-temperature transition was observed newly after cold storage and was not observed

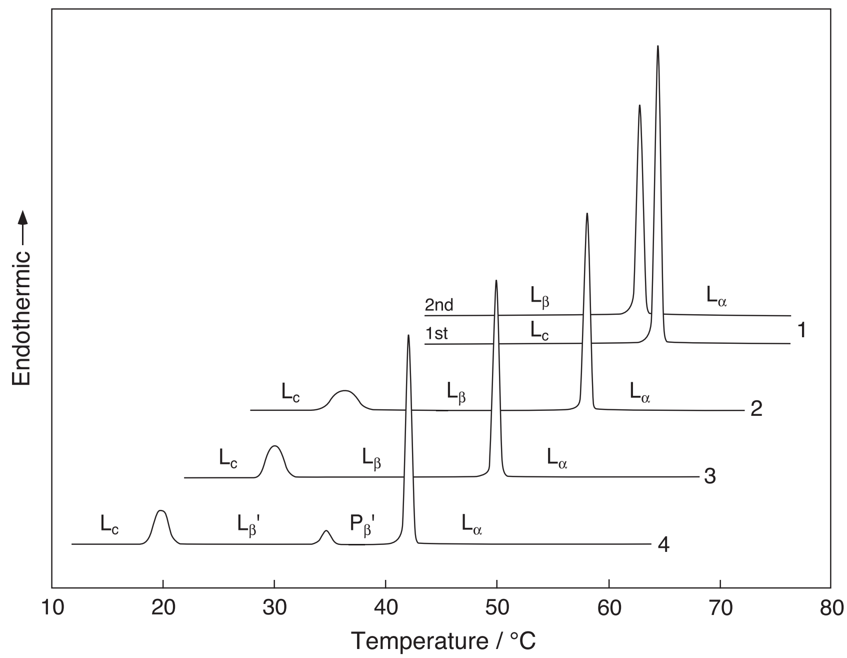

Fig. 1. DSC thermograms for the bilayer membranes of (1) DPPE, (2) DPMePE, (3) $DPMe_2PE$ and (4) DPPC.

in the second scan, which could be assigned as the transition from the L_c phase to the L_β phase. The DSC thermogram of DPPC bilayer membrane showed three endothermic transitions (curve 4 in Fig. 1). The DPPC bilayer undergoes three phase transitions with increasing temperature: the subtransition from the L_c phase to the lamellar gel (L'_β) phase, the pretransition from the L'_β phase to the ripple gel (P'_β) phase, and finally the main transition from the P'_β phase to the L_α phase, in turn.

Fig. 2 shows examples of phase transition measurements by an optical method for DPPE bilayer membrane. Light transmittance increased abruptly at a certain phase-transition temperature, which was in good agreement with the phase-transition temperature determined by the DSC method. At ambient pressure, the L_c/L_α transition temperature, which was observed by the first scan after cold storage, was found to be 64.3 °C. The temperature of main (L_β/L_α) transition, which was obtained by the second scan, was 63.1 °C. Observation of the L_β/L_α transition could be repeated immediately but the L_c/L_α transition was observed only in the first heating scan after cold storage because the rate of transformation into the L_c phase was extremely slow [39]. Light transmittance under high pressure showed a different profile from that at ambient pressure. As is shown in Fig. 2B, the first scan at 82.0 MPa showed stepwise two transitions, of which temperatures were 77.6 and 83.0 °C, respectively. The second scan at 82.0 MPa showed only one transition at the latter

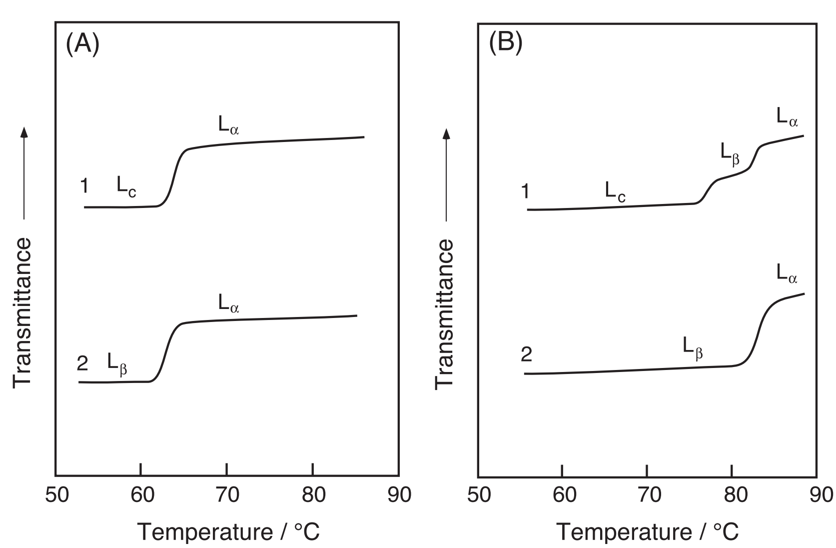

Fig. 2. Two kinds of phase transitions for DPPE bilayer membrane at (A) 0.1 MPa and (B) 82.0 MPa. Curves: (1) first heating scan, (2) second heating scan.

temperature (i.e., 83.0 °C). Since the L_β/L_α transition can be observed at the second scan, in turn two transitions observed by the first heating scan can be assigned as the L_c/L_β and L_β/L_α transitions, respectively.

The temperature (T)–pressure (p) phase diagram of DPPE bilayer membrane is shown in Fig. 3A. The temperatures of the L_c/L_α and L_β/L_α transitions increased with increasing pressure. Since the slope of the T–p phase boundary for the L_β/L_α transition, 0.264 K MPa^{-1}, is larger than that for the L_c/L_α transition, 0.230 K MPa^{-1}, the T–p curves for the L_β/L_α and L_c/L_α transitions intersect each other on the phase diagram at 21.8 MPa. Here the phase behavior in the vicinity of the intersection pressure is magnified in the inset. Under high pressure above 21.8 MPa, the L_c/L_β transition instead of the L_c/L_α transition was observed at the temperature lower than the L_β/L_α transition temperature. The slope of the phase boundary between the L_c and L_β phases was found to be 0.160 K MPa^{-1}. Thus, the L_β phase as a stable phase of bilayer membrane can be observed under high pressure above 21.8 MPa. The L_β/L_α transition at pressures below 21.8 MPa can be recognized as the transformation between metastable phases.

3.2. Phase diagrams of N-methylated DPPE bilayer membranes

The T–p phase diagram of DPMePE bilayer membrane is shown in Fig. 3B. The temperatures of L_c/L_β transition and L_β/L_α transition at ambient pressure were 35.5 and 58.0 °C, respectively, and increased with increasing pressure. Two phase boundary curves for the L_c/L_β and L_β/L_α phase transitions of DPMePE bilayer membrane did not intersect

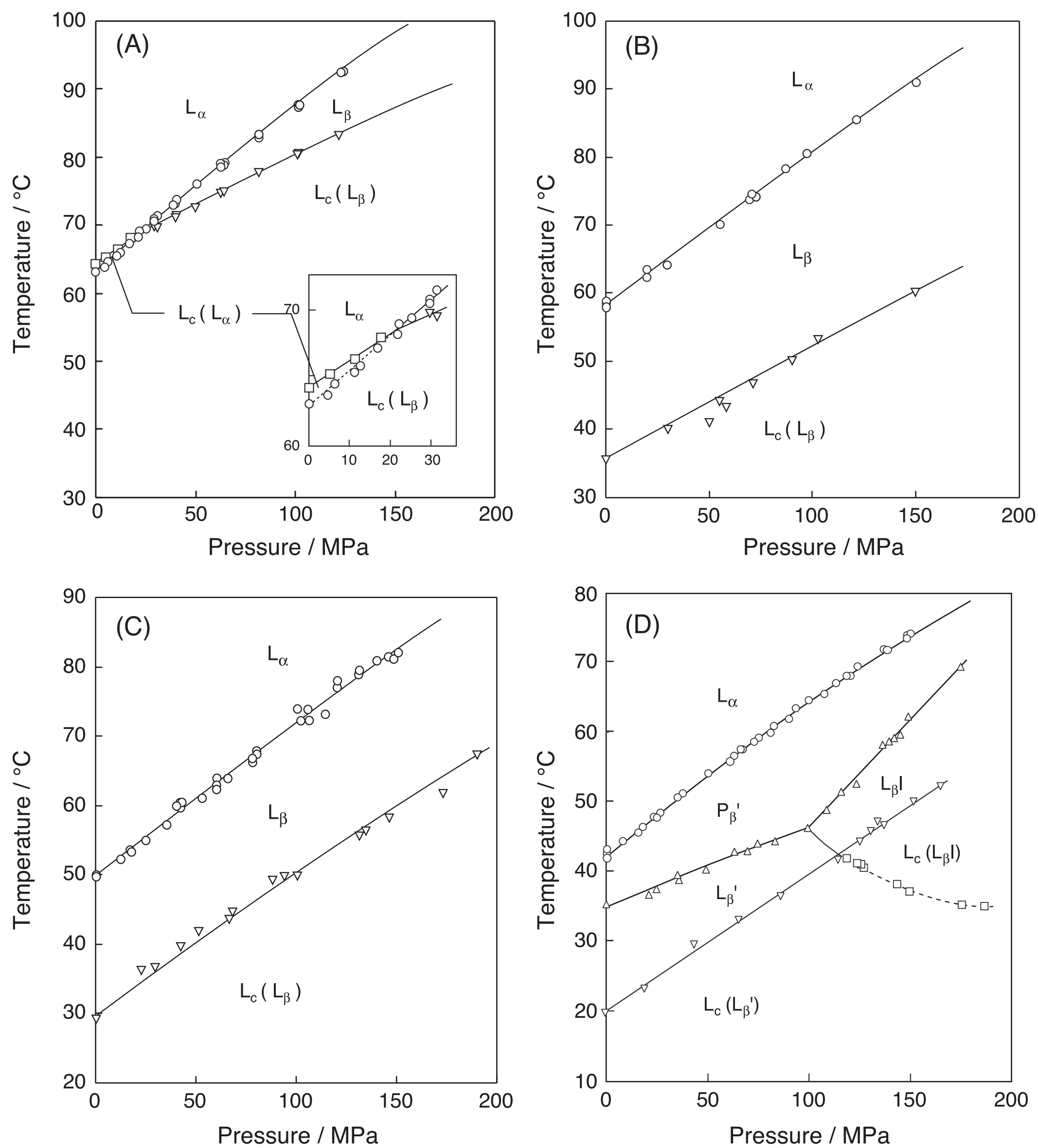

Fig. 3. Temperature–pressure phase diagrams for the bilayer membrane of (A) DPPE, (B) DPMePE, (C) $DPMe_2PE$ and (D) DPPC. Bilayer phases are assigned as liquid crystalline (L_α), lamellar gel (L_β or L'_β), ripple gel (P'_β) and subgel (L_c). Bilayer phase in parentheses refers to the metastable phase.

each other in the pressure range studied unlike that of DPPE bilayer membrane. In other words, the main transition of DPMePE bilayer membrane can be recognized as the transformation between stable L_β and L_α phases in the whole range of pressure. The slopes of the *T–p* phase boundary at ambient pressure were 0.160 K MPa^{-1} for the L_c/L_β transition and 0.246 K MPa^{-1} for the L_β/L_α transition, respectively. The *T–p* phase diagram of $DPMe_2PE$ bilayer membrane is shown in Fig. 3C. The bilayer membrane of $DPMe_2PE$ exhibited a similar phase behavior to that of DPMePE. The temperatures of L_c/L_β transition and L_β/L_α transition at ambient pressure were 29.4 and 49.2 °C, respectively, which were lower than the corresponding temperatures to the DPMePE bilayer membrane. The slopes of the *T–p* phase boundary at ambient pressure were 0.170 K MPa^{-1} for the L_c/L_β transition and 0.230 K MPa^{-1} for the L_β/L_α transition, respectively.

Fig. 3D shows the *T–p* phase diagram of DPPC bilayer membrane. The effect of pressure on the subtransition temperature is also included in Fig. 3D. The temperatures of sub-, pre- and main-transition at ambient pressure were 20.4, 35.8 and 41.6 °C, respectively, which are in good agreement with the previous results [40,41]. Pressure dependences on the pre- and main-transition temperatures were taken from our previous data [10]. The slopes of the phase boundary at ambient pressure were 0.220 K MPa^{-1} for the P'_β/L_α phase transition, 0.130 K MPa^{-1} for the L'_β/P'_β phase transition and 0.180 K MPa^{-1} for the L_c/L'_β phase transition, respectively. When the bilayer suspension was heated at once after cooling, the subtransition did not appear since the formation of the subgel phase required too much time. Under high pressure above 100 MPa, the pressure-induced interdigitated gel ($L_\beta I$) phase appeared. The bilayer interdigitation was assigned on the basis of the previous results of neutron diffraction method [32,33]: the bilayer periodicities and the derived electron density profiles indicated significantly smaller bilayer thickness. The slope of phase boundary between L'_β and $L_\beta I$ phases is negative, which is attributed to the negative volume change accompanied by the transition from the L'_β phase to the $L_\beta I$ phase for DPPC bilayer membrane [7,42]. Therefore, the *T–p* curves for the L_c/L'_β and $L'_\beta/L_\beta I$ transitions intersect each other on the phase diagram at about 115 MPa: we can observe the $L_c/L_\beta I$ transition at the first scan after cold storage and the $L'_\beta/L_\beta I$ transition at the repeated heating scan. The $L'_\beta/L_\beta I$ transition at higher pressure than 115 MPa can be recognized as the transformation between metastable phases, of which phase boundary is shown as a dotted line in Fig. 3D. In this phase diagram the symbols of bilayer phase in parenthesis refer to the metastable phase.

Polymorphism in the gel phase is characteristic of DPPC bilayer membrane unlike other lipid bilayers used in this study: the lamellar gel (L'_β), ripple gel (P'_β) and $L_\beta I$ phases are observed. With respect to the bilayers of DPPE, DPMePE and $DPMe_2PE$, the interdigitation of acyl chains is not observe even at pressures as high as 200 MPa. Because the choline head group is bulky, the acyl chains are tilted away from the perpendicular. The larger phosphatidylcholine head-group prevents chains from coming close enough together to minimize the van der Waals interactions. However, as a result of tilting, the distance between the all-*trans* chains is reduced and the van der Waals interactions are minimized. It is thought that the chain tilting is essentially responsible for the polymorphism in the gel phase of DPPC bilayer membrane.

3.3. Thermodynamic properties of phase transition

The values of d*T*/d*p* for the main (L_β/L_α or P'_β/L_α) transition are in the range of 0.220–0.264 K MPa^{-1} depending on the head group methylation, which were taken from the slopes of phase boundary curves shown in Fig. 3 and are listed in Table 1. With respect to the transition from the L_c phase to the L_β (or L'_β) phase, the values of d*T*/d*p* are in the range of 0.160–0.180 K MPa^{-1}, which are smaller than those for the main transition.

The enthalpy (ΔH) and entropy ($\Delta S=\Delta H/T$) changes associated with phase transitions of *N*-methylated DPPE bilayer membranes were determined by DSC. The main-transition enthalpies of DPPE, DPMePE, $DPMe_2PE$ and DPPC bilayers were 34.7, 35.6, 36.8 and 36.4 kJ mol^{-1}, which are comparable with previous results [11,18]. The

Table 1
Thermodynamic properties of phase transitions for the bilayer membranes with different head groups

Lipid	Transition	Transition temperature		d*T*/d*p*	ΔH	ΔS	ΔV
		(K)	(°C)	(K MPa^{-1})	(kJ mol^{-1})	(J K^{-1} mol^{-1})	(cm^3 mol^{-1})
DPPE	L_c/L_α	337.5	64.3	0.230	74.3±5.09	220±15	50.6±3.5
	L_β/L_α	336.3	63.1	0.264	34.7±1.68	103±5	27.2±0.8
	L_c/L_β			0.160			
DPMePE	L_c/L_β	308.7	35.5	0.160	25.4±1.15	82±4	13.1±0.6
	L_β/L_α	331.2	58.0	0.246	35.6±0.63	107±2	26.4±0.5
$DPMe_2PE$	L_c/L_β	302.6	29.4	0.170	24.5±3.16	81±10	13.8±1.7
	L_β/L_α	322.4	49.2	0.230	36.8±1.02	114±3	26.2±0.7
DPPC	L_c/L'_β	293.6	20.4	0.180	22.5±1.41	77±5	13.9±0.9
	L'_β/P'_β	309.0	35.8	0.130	5.4±0.66	17±2	2.2±0.3
	P'_β/L_α	314.8	41.6	0.220	36.4±0.32	116±1	25.5±0.2

volume change (ΔV) associated with the phase transitions was calculated from the Clapeyron equation.

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

The reported values of ΔV associated with the main transition were comparable with the present values: 27.2 $cm^3\ mol^{-1}$ for DPPE and 25.5 $cm^3\ mol^{-1}$ for DPPC. With respect to DPMePE and $DPMe_2PE$, there was only a report by Mason and O'Leary [18]. They reported the values of ΔV to be 21.1 $cm^3\ mol^{-1}$ for DPMePE and 17.7 $cm^3\ mol^{-1}$ for $DPMe_2PE$, which were significantly smaller than the present results. The thermodynamic properties of *N*-methylated DPPE are summarized in Table 1.

The striking resemblance as a whole in thermodynamic quantities (i.e., ΔH, ΔS and ΔV) of the main (L_β/L_α or P'_β/L_α) transition among *N*-methylated DPPEs was observed. This finding seems to be attributable to the same hydrophobic part in the lipid molecules. Because the main transition occurs from the *trans-gauche* conformational change of saturated fatty acyl chain due to the chain melting in the bilayer membranes, it is expected that the *N*-methylated DPPE molecules with the same dipalmitoyl-chain occur with similar chain melting in the membrane, and they show similar thermodynamic behavior of the main transition. Contrarily, there existed a significant difference in the main-transition temperatures among *N*-methylated DPPEs: the transition temperature increased in the order of DPPE, DPMePE, $DPMe_2PE$ and DPPC bilayers. It was suggested from the previous study [11] that the difference in the main-transition temperatures between PE and PC bilayer membranes might be caused by the polar head group interaction in the gel phases. As is well known, the acyl chains in the gel phase of DPPC bilayer are tilted away from the perpendicular because of the bulky head-group choline; whereas the acyl chains in the gel phase of DPPE bilayer are oriented perpendicular to the bilayer plane. One likely explanation for the difference in the main-transition temperature between DPPC and DPPE bilayers is the presence of transient hydrogen bonding between the protons of the quaternary nitrogen of the PE head group and the phosphate group on adjacent PE head group. Such hydrogen bonding might inhibit lateral expansion of the bilayer, so that the temperature of main transition rises. In the case of DPMePE and $DPMe_2PE$, they exhibited intermediate transition temperatures between DPPC and DPPE. The cross-sectional area of the molecules increases and the interchain distances expand with increasing number of methyl groups in the head group. A remarkable difference in the phase-transition temperatures among *N*-methylated DPPE bilayers may be attributed to the different hydrogen bonding capabilities of *N*-methylated head groups.

Similar thermodynamic quantities (i.e., ΔH, ΔS and ΔV) among *N*-methylated DPPEs, except for DPPE, were obtained regarding the bilayer transition from the L_c phase to the L_β (or L'_β) phase, which means that the states of subgel phase for these lipid bilayers are the same as each other. On the other hand, since the ΔH of L_c/L_β transition for the DPPE bilayer can be estimated to be 39.6 kJ mol^{-1} from the difference in the enthalpy changes between L_c/L_α and L_β/L_α

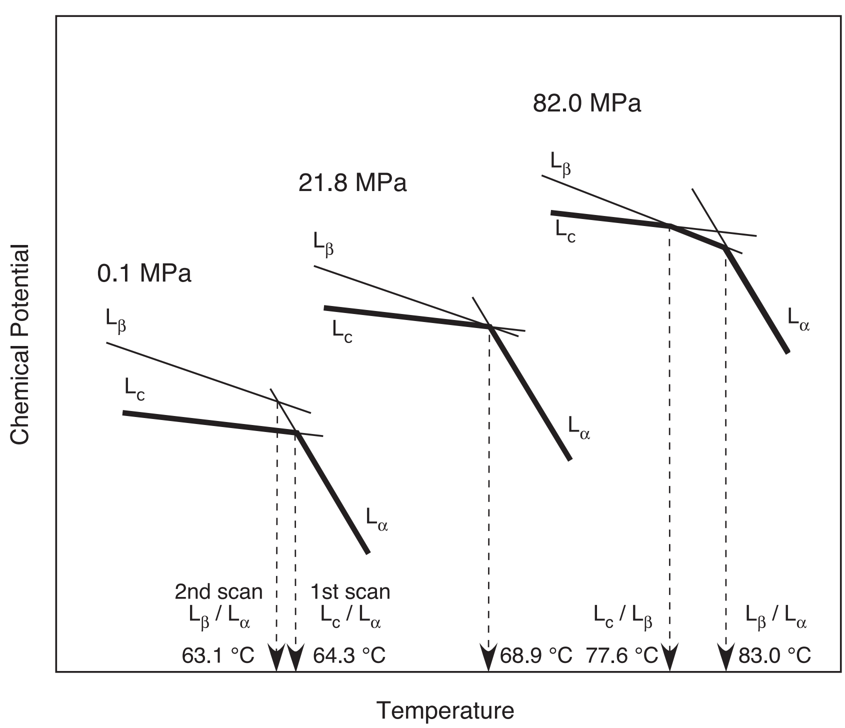

Fig. 4. Schematic diagram for a chemical potential–temperature profile among the L_α, L_β and L_c phases. The wide and narrow lines refer to the stable and metastable states, respectively. The slope reflects the partial molar entropy of lipid in each state. Break points on the chemical potential curves refer to the phase-transition points.

phase transitions and the values of ΔH for the other lipid bilayers are around 24 kJ mol^{-1}, the subgel phase of DPPE bilayer seems to have lower level of partial molar enthalpy than that of other lipid bilayers. The enhancement of the ΔH value for the L_c/L_β transition of DPPE may be responsible for the difference in head group hydration. Kodama et al. [43] demonstrated from a DSC study that in the case of the subgel phase of dimyristoylphosphatidylethanolamine bilayer, the transformation into the subgel phase took place through dehydration at the head group of PE and almost no interlamellar water was found in intrabilayer regions between the head groups of PE subgel phase. The situation of dehydration for PE head group significantly differs from that for PC head group [44].

Finally, we consider the stability of bilayer phases and the phase transition in the DPPE bilayer membrane given in Fig. 3A thermodynamically. On the basis of the entropy change associated with the phase transitions of lipid bilayer membrane, we can draw a chemical potential (μ)–temperature (T) profile among three (L_c, L_β and L_α) states of DPPE bilayer and the profile is shown in Fig. 4. Some isobaric μ–T curves at different pressures are included in this figure. An intersection point of chemical potential curves shows the phase transition point and the slopes indicate the partial molar entropies of lipid in the states of L_α, L_β and L_c, which decrease in that order judging from the values of entropy change in Table 1. The chemical potential of lipid in the L_β state is larger than that in the L_c state at ambient pressure. The stable phase transition from L_c to L_α phase is thus observed if the transformation into the subgel phase goes essentially to completion. In the case of the second heating scan, the DPPE bilayer undergoes the phase transition from the metastable L_β phase to the L_α phase because the transformation into the L_c phase is extremely slow. The chemical potential curves for three states intersect at 21.8 MPa and 68.9 °C, and a triple point among L_c, L_β and L_α phases was found under the same condition. At higher pressure than 21.8 MPa, we can observe two stable transitions, namely, the L_c/L_β and L_β/L_α phase transitions. If the transformation into the L_c phase does not undergo completion, we can observe only a L_β/L_α phase transition in this pressure region.

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