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Biochimica et Biophysica Acta 1712 (2005) 92 – 100

BIOCHIMICA ET BIOPHYSICA ACTA

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Effect of deuterium oxide on the thermodynamic quantities associated with phase transitions of phosphatidylcholine bilayer membranes

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Received 21 January 2005; received in revised form 11 March 2005; accepted 12 March 2005

Abstract

The bilayer phase transitions of three kinds of phospholipids, dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC) and dihexadecylphosphatidylcholine (DHPC), in deuterium oxide (D_2O) and hydrogen oxide (H_2O) were observed by differential scanning calorimetry (DSC) under ambient pressure and light-transmittance measurements under high pressure. The DSC measurements showed that the substitution of H_2O by D_2O affected the pretransition temperatures and the main-transition enthalpies of all PC bilayers. The temperature–pressure phase diagrams for these PC bilayer membranes in both solvents were constructed by use of the data of light-transmittance measurements. Regarding the main transition of all PC bilayer membranes, there was no appreciable difference between the transition temperatures in D_2O and H_2O under high pressure. On the other hand, the phase transitions among the gel phases including the pretransition were significantly affected by the solvent substitution. The thermodynamic quantities of phase transitions for the PC bilayer membranes were evaluated and the differences in thermodynamic properties by the water substitution were considered from the difference of interfacial-free energy per molecule in the bilayer in both solvents. It was proved that the substitution of H_2O by D_2O causes shrinkage of the molecular area of phospholipid at bilayer interface due to the difference in bond strength between deuterium and hydrogen bonds and produces the great influence on the bilayer phase with the smaller area. Further, the induction of bilayer interdigitation in D_2O turned out to need higher pressures than in H_2O.

Keywords: Deuterium oxide; Differential scanning calorimetry; Interdigitation; Phase transition; Phospholipid bilayer; Pressure

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1. Introduction

Phospholipids form molecular aggregates of bilayer membranes called vesicles or liposomes in aqueous solutions. These bilayer membranes change the aggregate state in response to temperature, pressure, kinds of solvents and concentrations of additives, hence, they exhibit various phase behavior depending on such thermodynamic variables. We have studied the effects of the thermodynamic variables, especially focusing our attention on pressure, on bilayer membranes of phospholipids from the viewpoint of the change of membrane states [1–6]. The temperature–pressure phase diagrams constructed for various bilayer membranes of phospholipids have been proved to be useful for a comprehensive understanding of the phase behavior for phospholipid bilayers.

The phase behavior of bilayer membranes for diacylphosphatidylcholine (diacyl-PC) containing linear saturated acyl chains has been most extensively examined by several physico-chemical techniques under ambient and high pressures. We also have reported the pressure effect on the bilayer transitions of a series of diacyl-PCs with different acyl chain-lengths [2,4]. In these high-pressure studies of PC bilayer membranes, we found that the temperature–pressure phase diagrams obtained from methods of neutron diffraction and nuclear magnetic resonance in deuterium oxide (D_2O) [7–10] were not consistent with those obtained from methods of spectroscopy as light-transmittance [4,11],

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doi:10.1016/j.bbamem.2005.03.005

fluorescence [12], infrared [13–15] and Raman scattering [16] in hydrogen oxide (H_2O). Braganza and Worcester [7] and Winter and Pilgrim [8] have reported observations of pressure-induced, interdigitated gel phase of dipalmitoylphosphatidylcholine (DPPC) bilayer membranes in D_2O by neutron diffraction measurements at high pressures beyond 150 MPa. On the other hand, we observed the same gel phase in H_2O at a lower pressure around 100 MPa. The finding apparently indicates that substitution of a solvent from H_2O to D_2O produces marked differences of phase boundaries between two phases in the temperature–pressure phase diagram.

D_2O is similar in many properties like dielectric constants, refractive indices and chemical reactivities, etc. to H_2O. However, the several physical properties like vapor pressures and boiling and melting points, etc. differ from those of H_2O slightly but definitely [17,18]. It is used as a solvent in experimental process of several physico-chemical measurements. Because D_2O shows the stabilization for immobilized catalyst of enzymes and growth inhibition for microorganisms, it is interesting to investigate how the substitution of H_2O by D_2O influences cell–membrane properties. Some comparative studies of bilayer properties in D_2O and H_2O have been made [19–26], but they are confined to those performed under ambient pressure. In the present study, we extend previous measurements and thermodynamic analysis for the PC bilayer membranes to the systems including D_2O solvent in order to clarify the difference in solvent effect between D_2O and H_2O. Three kinds of PCs, DPPC, distearoylphosphatidylcholine (DSPC) and dihexadecylphosphatidylcholine (DHPC), were selected for this purpose. The differential scanning calorimetry (DSC) and high-pressure light transmittance measurement were performed on these PC bilayer membranes and the thermodynamic properties for the bilayer phase-transitions were calculated. The substitution effect of H_2O by D_2O on the phase-transition properties and the phase behavior is discussed in terms of the interfacial-free energies of PCs in the bilayers in these aqueous environments.

2. Materials and methods

Three kinds of synthetic phospholipids, DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine) and DHPC (1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine), were purchased from Sigma-Aldrich Co. (St. Louis, MO) and Avanti Polar Lipids, Inc. (Alabaster, AL). They were directly used as received. Deuterium oxide (99.8% incl.) was obtained from Merck KGaA (Darmstadt, Germany) and used without further purification. Water (hydrogen oxide) was distilled twice after a deionized treatment, where the second step was done from dilute alkaline permanganate solution.

The multilamellar vesicle solutions of three PCs were prepared by suspending each PC in H_2O or D_2O using a vortex mixer at a concentration of 1.0 mmol kg^{-1} for calorimetric experiments and at 2.0 mmol kg^{-1} for high-pressure experiments, respectively. The suspensions were sonicated for a short time (ca. 3 min) by using a Branson model 185 sonifier at a temperature several degrees above the main-transition temperature of each lipid. Then, the sample solutions were allowed to stand at about 5 °C for a few days.

The phase transitions of three PC bilayer membranes under ambient pressure were observed by using a Microcal MCS high-sensitivity differential scanning calorimeter (Northampton, MA, USA) with a heating rate of 0.75 K min^{-1}. The temperatures and enthalpies of phase transitions were obtained from endothermic peaks in the DSC thermograms by use of a software ORIGIN from MicroCal. The phase transitions under high pressure were observed by two kinds of light-transmittance measurements developed in our laboratory; one is an isothermal barotropic observation by scanning pressure at constant temperature and the other is an isobaric thermotropic observation by scanning temperature at constant pressure. The general arrangements of high-pressure apparatus and the measurement procedures in both methods were described elsewhere [1,3].

3. Results

DPPC and DSPC are representative PCs having two saturated fatty acyl chains linked to the glycerol backbone by ester bonds. It is well known that bilayer membranes of these ester-linked PCs undergo the pretransition from the lamellar gel (L'_β) phase to the ripple gel (P'_β) phase and sequentially the main transition from the P'_β phase to the liquid crystal (L_α) phase. In addition, a new type of gel phase with non-bilayer structure, interdigitated gel ($L_\beta I$) phase appears in the DPPC and DSPC bilayers under high pressure [4,7,8]. Although the ester-linked PCs are main constituents of cell membranes for mammalians, dialkyl-PCs with two saturated alkyl chains linked by ether bonds are also found abundantly in cell membranes of archaebacteria. In the case of bilayer membranes of the ether-linked PC, namely, DHPC, two phase transitions are observed with heating scan like the DPPC bilayer, however, the DHPC bilayer already forms the $L_\beta I$ phase under ambient pressure and the pretransition corresponds to the phase transition from the $L_\beta I$ phase to the P'_β phase [27–29]. The polymorphism observed in the gel states is a characteristic of PC bilayers and originated from the bulky choline head group in the PC molecule.

The DSC thermograms of three PC bilayer membranes in a heating scan are demonstrated in Fig. 1. All PC bilayer membranes in both solvents showed two endothermic peaks: a small peak observed at a lower temperature corresponds to the pretransition and a large peak at a higher temperature corresponds to the main transition. Both the temperatures in H_2O were compatible with

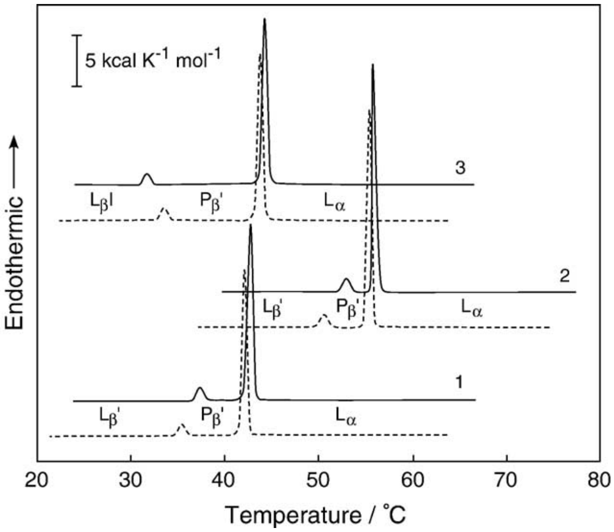

Fig. 1. DSC heating thermograms for solutions of PC bilayer membranes in D_2O and H_2O: (1) DPPC, (2) DSPC, (3) DHPC. Solid and broken lines indicate the thermograms in D_2O and H_2O, respectively.

literature values reported previously [27–32]. By solvent substitution of H_2O by D_2O, a noticeable shift of the pretransition peak for each lipid was observed while the main-transition peak for all lipids moved toward a higher temperature slightly. The temperatures and enthalpies (ΔH) of phase transitions for three PC bilayers were determined from the DSC thermograms and the results are listed together with the standard deviations in Table 1. The raise of the main-transition temperatures of three PC bilayer membranes in D_2O is about 0.4 °C. Unlike the main transition, the variation of pretransition temperature differed in the ester-PC and ether-PC: the pretransition temperatures of DPPC and DSPC bilayer membranes in D_2O increased by 1.6 and 1.8 °C, respectively, as compared in H_2O while that of DHPC bilayer membrane conversely decreased by 3.0 °C. The ΔH values showed the same trends as the transition temperatures; they increased in all the transitions except for the pretransition of the DHPC bilayer by substitution of H_2O by D_2O. Only a ΔH value for the pretransition of DHPC bilayer decreased by substitution of H_2O by D_2O. It is noticed from Table 1 that substitution of H_2O by D_2O affected rather the pretransition temperatures and the main-transition enthalpies.

Fig. 2 shows one of results for the isobaric thermotropic phase transitions of the DPPC bilayer membranes in D_2O at various pressures. Two abrupt increases of the transmittance accompanying the pre- and main transitions were observed in the transmittance–temperature curve under ambient pressure (curve 1). Both transition temperatures determined by the DSC and optical methods were in good agreement with each other. They increased by applying pressure and a new pressure-induced phase transition appeared at pressures higher than 160 MPa (curve 3). The result in H_2O under high pressure is also included in the figure (curve 4). Unlike the case in D_2O, the pressure-induced transition in H_2O started from lower pressures. The phase-transition temperature (T) was determined as a function of pressure from the transmittance-pressure profiles in the isobaric thermotropic observations. Similarly, the phase-transition pressure (p) was also determined as a function of temperature in the isothermal barotropic observations [4].

The T–p phase diagrams of three PC bilayer membranes in D_2O are compared with the corresponding results obtained in H_2O in Figs. 3–5. All the PC bilayer membranes exhibited the similar phase behavior in D_2O and H_2O. The pre- and main-transition temperatures increased with increasing pressure and the values of pressure dependence of both transition temperatures (dT/dp) in D_2O became the same values as those in H_2O. In both solvents, the bilayer interdigitation was induced for the ester-linked PC membranes under high pressure, and a triple point on the phase diagram among P'_β, L'_β and $L_\beta I$ phases for the ester-linked PC bilayers and among P'_β, $L_\beta I$ and L_α phases for the DHPC bilayer was observed. Regarding the main transition (P'_β/L_α transition) of all the PC bilayer membranes, there was no appreciable difference between the transition temperatures in D_2O and H_2O under high pressure. On the other hand, the phase transitions among the gel phases including the pretransition, L'_β/P'_β, $L'_\beta/L_\beta I$ and $L_\beta I/P'_\beta$ transitions, were significantly affected by the solvent substitution. The substitution of H_2O by D_2O at constant pressure brought about the elevation of the L'_β/P'_β and $L'_\beta/L_\beta I$ transition temperatures and the depression of the $L_\beta I/P'_\beta$ transition temperature. In other words, the phase boundaries

Table 1
Temperatures and enthalpies for the phase transitions of PC bilayer membranes in D_2O and H_2O

Lipid	Transition	In D_2O		In H_2O	
		T (°C)	ΔH (kcal mol^{-1})	T (°C)	ΔH (kcal mol^{-1})
DPPC	L'_β/P'_β	37.4 ± 0.13 (9)	1.4 ± 0.11 (11)	35.8 ± 0.16 (8)	1.3 ± 0.07 (9)
	P'_β/L_α	42.0 ± 0.06 (16)	8.8 ± 0.35 (10)	41.6 ± 0.09 (9)	8.3 ± 0.18 (8)
DSPC	L'_β/P'_β	52.6 ± 0.64 (19)	1.5 ± 0.12 (17)	50.8 ± 0.14 (15)	1.3 ± 0.08 (12)
	P'_β/L_α	55.4 ± 0.05 (19)	11.1 ± 0.43 (13)	55.0 ± 0.04 (16)	10.7 ± 1.00 (16)
DHPC	$L_\beta I/P'_\beta$	31.2 ± 0.18 (10)	1.0 ± 0.06 (11)	34.2 ± 0.39 (11)	1.3 ± 0.16 (11)
	P'_β/L_α	44.0 ± 0.03 (12)	8.7 ± 0.16 (9)	43.6 ± 0.05 (15)	8.1 ± 0.16 (15)

Values are indicated with the standard deviation.
A numerical value in parenthesis represents the number of measurements.

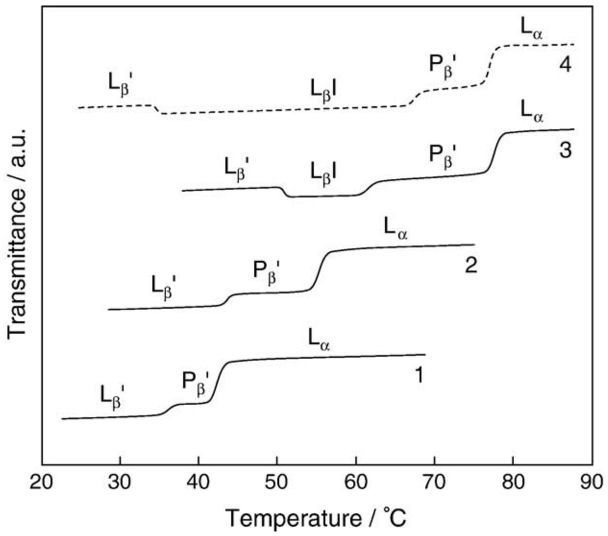

Fig. 2. Typical curves for the isobaric thermotropic phase transitions of the DPPC bilayer membranes in D_2O at various pressures: (1) 0.1 MPa, (2) 56 MPa, (3) 179 MPa, (4) 175 MPa in H_2O.

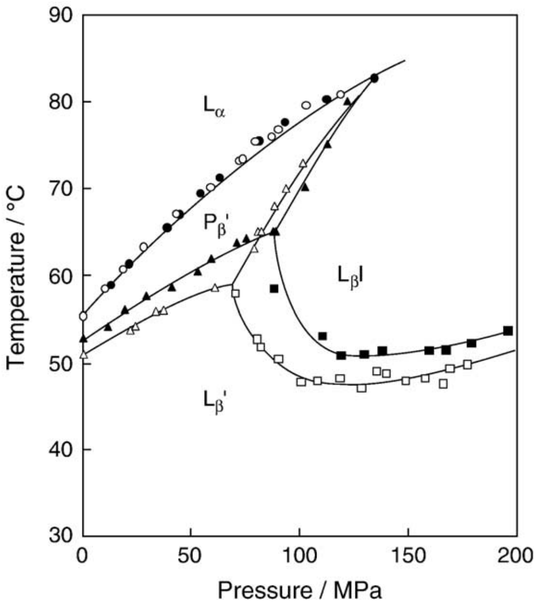

Fig. 4. Temperature–pressure phase diagram of DSPC bilayer membrane in D_2O and H_2O. Phase transitions: (● and ○) P'_β/L_α (main transition), (▲ and △) L'_β/P'_β (pretransition) and $L_\beta I/P'_\beta$, (■ and □) $L'_\beta/L_\beta I$. Closed and open symbols indicate the results in D_2O and H_2O, respectively.

between the L'_β and P'_β phases and between the L'_β and $L_\beta I$ phases in the diagrams shifted upward while that between the $L_\beta I$ and P'_β phase conversely sifted downward. The pressures and temperatures of the triple point on the phase diagram in H_2O were 100 MPa and 46 °C for DPPC, 70 MPa and 59 °C for DSPC and 110 MPa and 69 °C for DHPC, respectively. They were all raised in D_2O: 160 MPa and 56 °C for DPPC, 90 MPa and 65 °C for DSPC and 150 MPa and 78 °C for DHPC. Combination of these boundary shifts resulted in observations for the region of the $L_\beta I$ phase at higher pressure in D_2O than in H_2O. The pressures at which the $L_\beta I$ phase appears in the ester-PC bilayer membranes in D_2O were comparable with the reported pressures by Braganza and Worcester [7] (155 MPa for DPPC and 88 MPa for DSPC) and Winter and Pilgrim [8] (160 MPa for DPPC) although they could not observe the pretransition due to a small-angle neutron scattering method under high pressure.

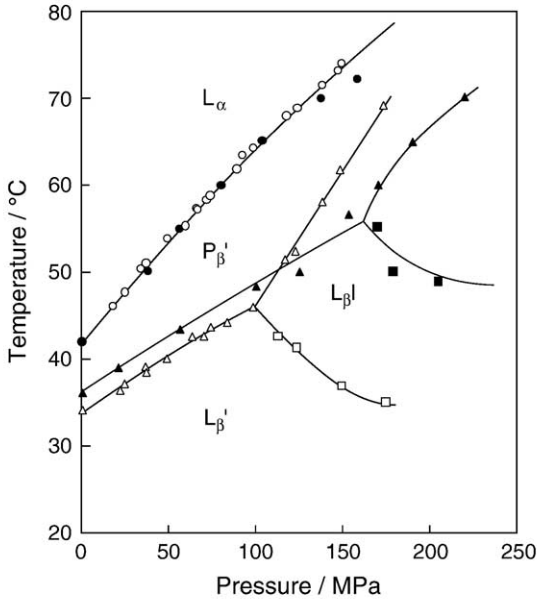

Fig. 3. Temperature–pressure phase diagram of DPPC bilayer membrane in D_2O and H_2O. Phase transitions: (● and ○) P'_β/L_α (main transition), (▲ and △) L'_β/P'_β (pretransition) and $L_\beta I/P'_\beta$, (■ and □) $L'_\beta/L_\beta I$. Closed and open symbols indicate the results in D_2O and H_2O, respectively.

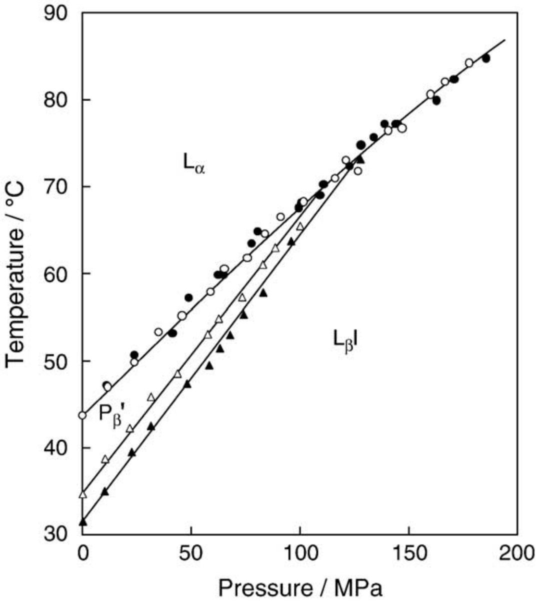

Fig. 5. Temperature–pressure phase diagram of DHPC bilayer membrane in D_2O and H_2O. Phase transitions: (● and ○) P'_β/L_α (main transition), (▲ and △) $L_\beta I/P'_\beta$ (pretransition). Closed and open symbols indicate the results in D_2O and H_2O, respectively.

Table 2
Thermodynamic properties for the phase transitions of PC bilayer membranes in D_2O and H_2O

Lipid	Transition	In D_2O					In H_2O				
		T (K)	dT/dp (K MPa^{-1})	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})	ΔV (cm^3 mol^{-1})	T (K)	dT/dp (K MPa^{-1})	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})	ΔV (cm^3 mol^{-1})
DPPC	L'_β/P'_β	310.6	0.13	5.7	18.3	2.4	308.9	0.13	5.4	17.5	2.3
	P'_β/L_α	315.2	0.22	37.0	117.3	25.8	314.8	0.22	34.5	109.7	24.1
DSPC	L'_β/P'_β	325.7	0.14	6.2	18.9	2.6	324.0	0.14	5.5	17.0	2.4
	P'_β/L_α	328.5	0.23	46.3	140.9	32.4	328.1	0.23	44.8	136.5	31.4
DHPC	$L_\beta I/P'_\beta$	304.4	0.31	4.3	14.0	4.3	307.3	0.31	5.3	17.2	5.3
	P'_β/L_α	317.2	0.22	36.3	114.5	25.5	316.8	0.22	33.7	106.4	23.7

Other thermodynamic quantities associated with the phase transitions, the entropy (ΔS) and volume (ΔV) changes, were evaluated. They are obtained by applying the following equations held at phase equilibrium

$$\Delta S = \Delta H/T \tag{1}$$

and

$$\Delta V = \Delta S(\mathrm{d}T/\mathrm{d}p) \tag{2}$$

to the T and ΔH values obtained from the DSC data and the dT/dp values from T–p phase diagrams. The thermodynamic properties determined in this study for the phase transitions of three PC bilayer membranes in D_2O and H_2O are summarized in Table 2. The behavior of ΔS and ΔV values in D_2O and H_2O had a great resemblance to the ΔH values as expected from Eqs. (1) and (2) and the same dT/dp values in both solvents. They increased in the both transitions of the ester-linked PC bilayers and the main transition of the DHPC bilayer in D_2O when compared with those in H_2O while decreased in the pretransition of the DHPC bilayer in D_2O. Since the DHPC bilayer membrane lies a state of the $L_\beta I$ phase at temperatures below the pretransition, the decreases in thermodynamic quantities for the pretransition of the DHPC bilayer in D_2O are closely related to the difference in stability of the $L_\beta I$ phase in between both solvents.

4. Discussion

The driving force for bilayer formation of phospholipids is cohesive force between hydrophobic acyl chains and the repulsive force between polar head groups. Therefore, the chemical potential of the phospholipid in bilayer membranes (μ) can be regarded as the sum of above two contributions,

$$\mu = \mu_{\mathrm{CH}} + \mu_{\mathrm{HG}}, \tag{3}$$

where μ_{CH} is the chemical potential of hydrophobic core due to the van der Waals interaction between alkyl chains in the membrane and μ_{HG} is the chemical potential of interfacial region due to the interaction between head groups of the phospholipid, respectively. In the case of the non-bilayer $L_\beta I$ phase, the contribution of interaction of terminal methyl groups of the alkyl chains with solvents must be taken into account in the chemical potential [33,34]:

$$\mu = \mu_{\mathrm{CH}} + \mu_{\mathrm{HG}} + \mu_{\mathrm{TH}}, \tag{4}$$

where μ_{TH} is the chemical potential of terminal methyl groups due to the interaction of the methyl groups with surroundings (H_2O or D_2O). Present study showed that the substitution of H_2O by D_2O did not affect the temperature of main transition but affected the temperatures of phase transition between gel phases under ambient and high pressures. Judging from the situation, it seems that the water substitution is effective at the bilayer– or non-bilayer–water interface rather than at the hydrocarbon-chain domain.

Now let us consider the interactions at the bilayer– or nonbilayer–water interface in phospholipid membranes, that is, μ_{HG} and μ_{TH}. They include effects of hydrophobic interaction, steric hindrance, electrostatic interaction and interface hydration. The μ_{HG} can be generally written in the form [35–37]

$$\mu_{\mathrm{HG}} = \gamma A + C/A, \tag{5}$$

where γ is an interfacial-free energy per unit area characteristic of a liquid hydrocarbon–water interface, A is the molecular area of phospholipid in the membrane and C is the free energy constant of repulsion, respectively. For the $L_\beta I$ phase, we obtain the relation

$$\mu_{\mathrm{TH}} = \gamma_{\mathrm{TH}} A, \tag{6}$$

where γ_{TH} is an interfacial-free energy of terminal methyl groups with the surroundings [34,38]. The optimal A value at minimum free energy (A_0) can be obtained from the differential of μ_{HG} with respect to A being zero in Eq. (5):

$$A_0 = (C/\gamma)^{1/2}. \tag{7}$$

The γ value has a larger one in D_2O than in H_2O because deuterium bonds in D_2O are stronger than hydrogen bonds in H_2O, of which dissociation energies of deuterium bonds in D_2O and hydrogen bonds in H_2O are 6.53 kJ mol^{-1} and 5.52 kJ mol^{-1}, respectively [17,40,41]. In practice, various physico-chemical properties such as melting and boiling points, density and viscosity are known to be higher in D_2O

than in H_2O [17,18]. According to Eq. (7), the inequality of γ (in D_2O) > γ (in H_2O) gives that of A_0 (in D_2O) < A_0 (in H_2O). The substitution of H_2O by D_2O causes a shrinkage of the molecular area of phospholipid at membrane interface. Since the smaller A value the larger γ value becomes, the phase with a small A value is influenced greatly by the D_2O substitution. Furthermore, it should be noted that the γ_{TH} value seems to be approximately equal to the γ value given in Eq. (5) [34,38,39], then γ_{TH} (in D_2O) A (in D_2O) $\approx$ γ_{TH} (in H_2O) A (in H_2O), the main contribution by the solvent substitution in the $L_\beta I$ phase may also result in the change of μ_{HG}. Molecular area of phospholipid in the bilayer gel phases increases in the order of L'_β (0.39 $\mathrm{nm}^2\ \mathrm{mol}^{-1}$) < P'_β (0.41 $\mathrm{nm}^2\ \mathrm{mol}^{-1}$) < $L_\beta I$ (0.78 $\mathrm{nm}^2\ \mathrm{mol}^{-1}$) [29,42], hence, the substitution effect by D_2O enhances in reverse order, $L_\beta I$ < P'_β < L'_β.

The effect of substitution of H_2O by D_2O on the thermodynamic properties for the phase transitions of three PC bilayer membranes was examined from the correlation between the molecular area of each phase in the bilayer and the magnitude of the D_2O substitution effect on the phase transitions. Table 3 shows the differences in thermodynamic properties (ΔT, $\Delta(\Delta H)$, $\Delta(\Delta S)$ and $\Delta(\Delta V)$) for the pre- and main transitions of three PC bilayers in D_2O and H_2O. Here, the differences were obtained by subtraction of the properties in H_2O from those in D_2O. The differences in thermodynamic properties for the main transition of all the PC bilayer membranes had positive values. Because the molecular area occupied by a phospholipid molecule in the P'_β phase is smaller than the corresponding area in the L_α phase (0.45–0.47 $\mathrm{nm}^2\ \mathrm{mol}^{-1}$) [29,42], the stabilization of the P'_β phase rather than the L_α phase is enhanced in D_2O in comparison with in H_2O. The large stabilization of the P'_β phase in D_2O contributes to the increase in the thermodynamic quantities associated with bilayer phase transitions. Thus, we can say for the main transition in D_2O that the transition temperatures of all the PC bilayers are elevated by about 0.4 °C and extra heat and volume are required to expand the suppressed molecular area owing to the stabilized P'_β phase. Further, it is noticed that the differences in thermodynamic properties of the DPPC and DHPC bilayers seem to be similar values in magnitude, while the DSPC bilayer has a smaller value than both bilayers. This means that the substitution effect on the main transition does not depend on the linkage type between acyl chains and glycerol backbone. The bilayer membrane of DSPC with longer acyl chains undergoes larger *trans-gauche* conformational changes in the acyl chains than those of DPPC and DHPC, accordingly, the substitution effect of D_2O at bilayer interface of the DSPC membrane seems to be reduced.

The differences in thermodynamic properties for the pretransition of three PC bilayer membranes are also given in Table 3. The differences in thermodynamic properties of the ester-linked PC bilayers had all positive values as seen in the main transition. The pretransition from the L'_β phase to the P'_β phase in the bilayer membranes of ester-linked PCs leads to expand the molecular area at bilayer interface. The L'_β phase rather than the P'_β phase in the ester-linked bilayers was stabilized in D_2O. This situation is essentially the same as the case of the main transition although the difference in the transition temperature is markedly small for the main transition. Comparing the differences in thermodynamic properties for the pretransition of the ester-linked PC bilayers with those for the main transition, the former was much smaller than the latter. We may speculate that the substitution effect of D_2O on the gel phases is considerably larger than that on the L_α phase and the difference between the substitution effect on the L'_β and P'_β phases is small. On the contrary, the corresponding results of the DHPC bilayers became all negative. In the DHPC bilayer membrane, the pretransition from the $L_\beta I$ phase to the P'_β phase leads to shrink the area at bilayer interface in contrast with the case of the ester-linked PCs. The smaller stabilization of the $L_\beta I$ phase in D_2O contributes to the decrease in the thermodynamic properties since the relative stabilization of the $L_\beta I$ phase to the P'_β phase is reduced in D_2O in comparison with in H_2O. Ohki [23] has reported the thermotropic phase transitions of the same PC bilayer membranes as used in this study in D_2O and H_2O by the DSC method and Kinoshita and Yamazaki [24] have also reported the organic solvent-induced interdigitation of DPPC bilayer membrane in both solvents by the excimer method of pyrene-PC. Their results were in good agreement with the present ones although they were restricted to measurements carried out under ambient pressure.

Table 3
Differences between thermodynamic properties for the phase transitions of PC bilayer membranes in D_2O and H_2O

Lipid	Main transition				Pretransition			
	ΔT (K)	$\Delta(\Delta H)$ ($\mathrm{kJ\ mol^{-1}}$)	$\Delta(\Delta S)$ ($\mathrm{J\ K^{-1}\ mol^{-1}}$)	$\Delta(\Delta V)$ ($\mathrm{cm^3\ mol^{-1}}$)	ΔT (K)	$\Delta(\Delta H)$ ($\mathrm{kJ\ mol^{-1}}$)	$\Delta(\Delta S)$ ($\mathrm{J\ K^{-1}\ mol^{-1}}$)	$\Delta(\Delta V)$ ($\mathrm{cm^3\ mol^{-1}}$)
DPPC	0.4	2.5	7.6	1.7	1.6	0.3	0.8	0.1
DSPC	0.4	1.5	4.4	1.0	1.8	0.7	1.9	0.2
DHPC	0.4	2.6	8.1	1.8	−3.0	−1.0	−3.2	−1.0

The differences were obtained by subtraction of the properties in H_2O from those in D_2O.

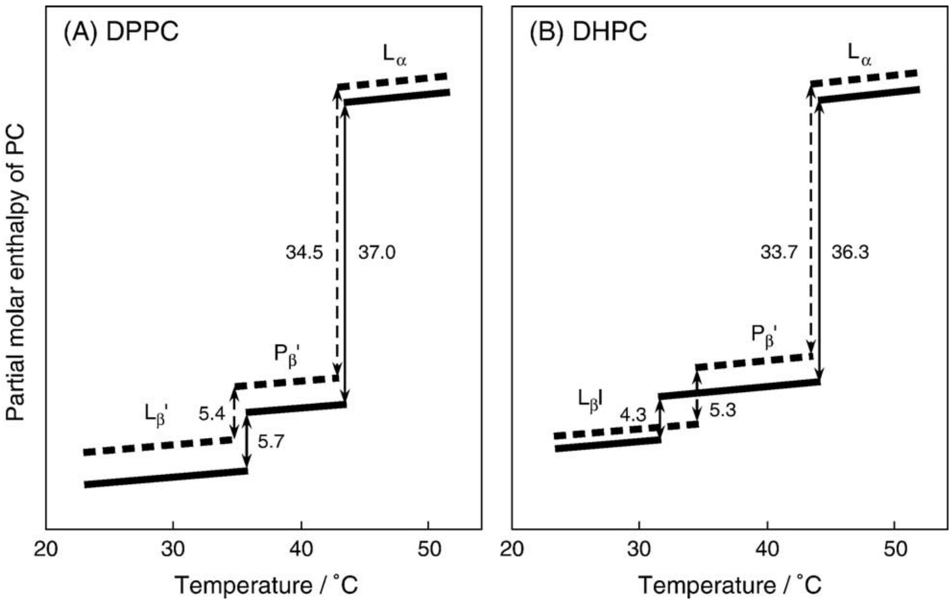

Fig. 6. Schematic diagram for partial molar enthalpies of PC bilayer membrane in D_2O and H_2O under ambient pressure: (A) DPPC, (B) DHPC. Solid and broken lines indicate the enthalpy values in D_2O and H_2O, respectively. The scale on the ordinate is arbitrary and the values of enthalpy changes associated with phase transitions are given in kJ mol^{-1}.

The variations in partial molar enthalpies of the DPPC and DHPC bilayer membranes in both solvents under ambient pressure are schematically demonstrated in Fig. 6. Here, the sudden changes associated with phase transitions in partial molar enthalpies are drawn on the basis of the ΔH values given in Table 2. Since we can determine only the enthalpy changes of phase transitions in this study, the differences between partial molar enthalpies in D_2O and H_2O are arbitrary and their exact determination requires an evaluation of the absolute values of heat capacities, for example, by Blume [43]. However, judging from the behavior of their DSC thermograms and T–p phase diagrams, it seems reasonable to assume that the partial molar enthalpies in D_2O are smaller than those in H_2O, and that the thermal stabilization of the L_α phase by D_2O concerning DPPC and DHPC bilayer membranes is nearly the same as each other. From both the figures, we immediately notice that the magnitude of difference in partial molar enthalpy of each phase in D_2O and H_2O is well correlated to the stabilization order of the bilayer phases as mentioned above, i. e., $L_\beta I < L_\alpha < P'_\beta < L'_\beta$. This fact supports the view that the substitution of H_2O by D_2O provides significant influence on the interfacial region of the membrane and the influence is in inverse proportion to the molecular area of phospholipid.

Extending the above consideration to the phase transitions among the gel phases under high pressure, we can explain qualitatively that the substitution of H_2O by D_2O brings about the elevation of temperature of the L'_β/P'_β and $L'_\beta/L_\beta I$ transitions and the depression of temperature of the $L_\beta I/P'_\beta$ transition. From the combination of these changes among phase boundaries, the region of $L_\beta I$ phase on the phase diagram in D_2O shifts to the direction of higher pressure. Consequently, it can be said that the induction of bilayer interdigitation in D_2O is needed higher pressures than in H_2O. Although the main-transition temperatures of all the PC bilayers in D_2O and H_2O under ambient pressure were observed with a slight difference by the DSC, we could not recognize any difference of the transition temperature in both solvents under high pressure. This fact confirms that the solvent effect of D_2O acts on interfacial region of PC bilayer membranes and the effect on the chain melting of the bilayers under high pressure is extremely small.

5. Conclusions

The present study revealed the solvent effect of D_2O and H_2O on the phase behavior and the thermodynamic properties of PC bilayer membranes. The difference observed in this study intrinsically is originated from the difference in bond strength between deuterium and hydrogen bonds. The interfacial region of bilayer membranes was proved to be more effective by the substitution. The deviations found in the T–p phase diagrams of PC bilayer membranes in D_2O and H_2O support the validity of our previous results accumulated in H_2O and they also suggest that the phase behavior of phospholipid bilayer membranes obtained from the experiments performed in D_2O must be treated with special care.

Acknowledgements

This study was supported in part by a Grant-in Aid for Scientific Research (B) (2) (11440206) and (C) (2)

(15550122) from Japan Society for the Promotion of Science.

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