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Low concentration of DMSO stabilizes the bilayer gel phase rather than the interdigitated gel phase in dihexadecylphosphatidylcholine membrane

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Abstract

We have investigated effects of dimethylsulfoxide (DMSO) on the phase stability of multilamellar vesicles of the ether-linked 1,2-dihexadecyl-*sn*-glycero-3-phosphatidylcholine (DHPC-MLV), which is known to be in the interdigitated gel ($L_\beta I$) phase in excess water at 20°C. The results of X-ray diffraction experiments indicate that the DHPC membrane was in the $L_{\beta'}$ phase at $X \geq 0.12$ (X = mole fraction of DMSO in DMSO/water mixture). The result of differential scanning calorimetry indicate that the gel to liquid-crystalline phase transition temperature increased, but the $L_\beta I$ to $P_{\beta'}$ phase transition temperature decreased with an increase in DMSO concentration. These results show that DMSO stabilizes the bilayer gel phase rather than the $L_\beta I$ phase at its low concentration. The solubility of phosphorylcholine, which is the same structure as the headgroup of DHPC, decreased with an increase in DMSO concentration, indicating that the interaction free energy of the hydrophilic segments of the membrane with solvents increases with an increase in DMSO concentration. On the basis of the thermodynamic analysis, the mechanism of the stabilization of the bilayer gel phase of DHPC-MLV by DMSO is discussed. The decrease in the repulsive interaction between the headgroups of the phospholipid induced by the low concentrations of DMSO in water plays an important role in this stabilization.

Keywords: Interdigitated gel phase; Phase transition; Dimethylsulfoxide; Interaction free energy; Phospholipid membrane interface; Hydration

1. Introduction

Phosphatidylcholine (PC) membranes such as dipalmitoylphosphatidylcholine (DPPC) and 1,2-dihexadecyl-*sn*-glycero-3-phosphatidylcholine (DHPC) can form two kinds of gel phases, the bilayer gel phase such as that with tilted hydrocarbon chains ($L_{\beta'}$ phase) and the interdigitated gel ($L_\beta I$) phase, depending on conditions, and a phase transition between both the phases occurs at a critical condition. In diacylphosphatidylcholine membranes such as DPPC, the $L_{\beta'}$ phase is stable in water. On the contrary, above the critical concentrations of ethanol and other short-chain alcohols in aqueous solution, the $L_\beta I$ phase becomes more stable than the $L_{\beta'}$

Abbreviations: DHPC, 1,2-dihexadecyl-*sn*-glycero-3-phosphatidylcholine; $L_\beta I$ phase, interdigitated gel phase; $L_{\beta'}$ phase, bilayer gel phase with tilted hydrocarbon chains; $P_{\beta'}$ phase, ripple phase; L_α phase, liquid-crystalline phase; DSC, differential scanning calorimetry; SAXS, small angle X-ray scattering; WAXS, wide angle X-ray scattering

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phase [1–8]. Recently, we have shown that water-soluble organic solvents such as acetone, acetonitrile, propionaldehyde, and tetrahydrofurane also induce the $L_\beta I$ phase in DPPC-multilamellar vesicles (MLV) [9]. These results demonstrate that a specific interaction of alcohols with phospholipid membranes is not important in the formation of the $L_\beta I$ phase.

Dialkylphospholipids and alkyl-acyl-phospholipids that contain ether-linkages have attracted much attention as a platelet-activating factor and as having antitumor activity [10,11], and also as major lipids of archaebacterial membranes [12]. For an ether-linked dialkylphosphatidylcholine, DHPC, the $L_\beta I$ phase is stable in excess water at 20°C [13,14]. The large repulsion between the headgroups has been considered as a main reason for the formation of the $L_\beta I$ phase in DHPC-MLV [15]. When the repulsive interaction between the headgroups decreases to the critical value due to the decrease in pH of the bulk solution or osmotic stress, the $L_\beta I$ to bilayer gel phase transition occurs [15,16].

Dimethylsulfoxide (DMSO) has been used as a cryoprotectant for cells, tissues and isolated proteins, and also has been known to induce membrane fusions between cells and phospholipid vesicles [17]. Several groups have studied the effects of DMSO on structures and phase stabilities of PC membranes [18–21]. The intermembrane distances of PC-MLVs with a saturated alkyl chain in the gel phase decreased with an increase in DMSO concentration. Moreover, the temperatures of the pre- and main transitions of these PC-MLVs increased with an increase in DMSO concentration. However, their mechanisms are not clear yet.

In this report, we have investigated the effects of DMSO on the stability of the $L_\beta I$ phase of DHPC-MLV. Since the $L_\beta I$ phase has not been found in cells and other biological organs to date, it may not be considered an important phase from a biological point of view. However, investigation of the induction of the $L_\beta I$ phase and the stability of this phase in phospholipid membranes has been considered a very useful method to elucidate the mechanism of the interaction between substances and the membrane interface ([2,3,9] and references therein). As mentioned above, the mechanism of the $L_\beta I$ to bilayer gel phase transition of the DHPC-MLV is well understood [15,16] and we can expect to elucidate the general mechanism of the effects of DMSO on biomembranes and various kinds of phospholipid membranes in this report. We have found that an induction of a bilayer gel phase occurs at 20°C above a low concentration of DMSO in water, which indicates that DMSO stabilizes the bilayer gel phase rather than the $L_\beta I$ phase under these conditions. The mechanism of this stabilization is discussed. The interaction free energy between the segments of the membrane surface and solvents increases with an increase in DMSO concentration. This induces a decrease in the repulsive interaction between the headgroups of the DHPC in the membrane interface, which plays an important role in this stabilization. This conclusion suggests that in other phospholipid membranes and biomembranes the low concentration of DMSO in water induces a decrease in the repulsive interaction between the headgroups in the membrane interface. This new concept can be helpful in understanding the mechanism of DMSO as a cryo-protectant for various cells, and also, in understanding the effects of DMSO on the physical properties of various kinds of phospholipid membranes.

2. Materials and methods

2.1. Materials and sample preparation

DHPC was purchased from Fluka Chemie AG. Phosphorylcholine (chloride calcium salt) was purchased from Sigma.

MLVs were prepared by adding appropriate amounts of water containing a given concentration of DMSO to dry lipids (in excess water) and the suspensions were vortexed for about 30 s several times, at around 65°C. For measurements of X-ray diffraction, pellets ($\sim$50 wt% lipid) obtained after centrifugation of the suspensions (14 000$\times g$, 20 min at 20°C; Tomy, MR-150) were used.

2.2. X-ray diffraction

X-ray diffraction experiments were performed by using a nickel-filtered Cu K_α-radiation ($\lambda = 0.154$ nm) from the rotating anode type X-ray generator (Rigaku, Rotaflex, RU-300, 50 kV$\times$300 mA). Small-angle X-ray scattering (SAXS) data were recorded

using a linear (1D) position sensitive proportional counter (PSPC) (Rigaku, PSPC-5) [22] with a camera length of 350 mm and associated electronics (multichannel analyzer, etc., Rigaku). Wide-angle X-ray scattering (WAXS) patterns were recorded by using a 1D PSPC with a sample-to-detector distance of 250 mm, and diffraction spacings were calibrated by using polyethylene [23]. In all cases, samples were sealed in a thin-walled glass capillary tube (outer diameter 1.0 mm) and mounted in a thermostatable holder.

SAXS data were processed by a standard method [25,26]. Integrated intensities of various diffraction peaks, $I(h)$, where h is the order number, were determined after background subtraction. Measured intensities are corrected by multiplying by the square of the order number (h^2) for a powder pattern (unoriented samples) and a correction factor, $P(h)$, due to the geometry of the 1D PSPC [22]. Hence, the structure amplitude, $F(h)$, equals $\sqrt{h^2 I(h) P(h)}$. Electron density distributions, $\rho(x)$, were calculated by using the following formula:

$$\rho(x) \propto \sum \sqrt{h^2 I(h) P(h)}\, j(h) \cos(2\pi h x/d) \qquad (1)$$

where $j(h)$ is the phase information for each order h, and d is a spacing. For a centrosymmetric $\rho(x)$ function, $j(h)$ must be either +1 or −1 for each order h. To calculate the electron density profile, it is necessary to determine the phase, $j(h)$. For DHPC-MLV in water, $j(h)$ is known as (−1, −1, +1) for orders $h=1$ to 3, which was determined by the standard swelling method [13]. For DHPC-MLV in a high concentration of DMSO ($X \geq 0.12$), we made a systematic check of all the combinations of phases. As a result, only one combination of phase, $j(h)$ at high concentrations of DMSO ($X \geq 0.12$) can give a plausible electron density profile for phospholipid membranes; $j(h)$ is (−1, −1, +1, −1) for orders $h=1$ to 4. This phase set is the same as that of the $L_{\beta'}$ phase of DHPC-MLV under several conditions [3,15,27]. By using these phases, electron density profiles of DHPC-MLV were obtained.

2.3. Differential scanning calorimetry (DSC)

DSC experiments were performed using a Rigaku DSC-8230B instrument. 50 mM DHPC-MLV or DPPC-MLV dispersions were heated at a rate of 2.0°C/min. The main transition temperature and pre-transition temperature of DHPC-MLV or DPPC-MLV were determined as the onset of the endothermic transition extrapolated to the baseline. The details were described in our previous paper [24].

2.4. Measurement of solubility

Appropriate amounts of phosphorylcholine were mixed with various concentrations of DMSO aqueous solution, in order to make saturated solutions, and centrifuged. Concentrations of phosphorylcholine in the supernatant of these solutions were determined by the standard phosphorus analysis [28].

3. Results

3.1. Structural changes of DHPC-MLV induced by DMSO

DHPC-MLV in excess water at neutral pH at 20°C is known to be in the $L_\beta I$ phase [13–15]. To investigate the effects of DMSO on the structures of DHPC membrane, we have carried out the X-ray diffraction experiments such as SAXS and WAXS for DHPC-MLVs at various DMSO concentrations in water, X (or X_{DMSO}, mole fraction of DMSO in DMSO/water mixture solvent). Fig. 1 shows SAXS patterns of DHPC-MLV at $X=0$, 0.06 and 0.17 at 20°C. The lamellar patterns with a set of SAXS peaks in the ratio of 1:2:3:4 were observed at $X=0$ and 0.17, and the spacings (lamellar repeat period) (d) of DHPC-MLV were 4.8 nm at $X=0$ and 6.0 nm at $X=0.17$. However, at $X=0.06$, only one broad peak was observed around 7.2 nm. Fig. 2 shows a detailed dependence of the spacing on DMSO concentration. The spacing gradually decreased with an increase in DMSO concentration at $0 \leq X < 0.05$. For DHPC-MLV suspensions at the intermediate DMSO concentrations ($0.05 \leq X < 0.12$), only one broad peak was observed for each and the spacings estimated by the peaks were much larger than those at $0 \leq X < 0.05$ and also a little larger than those at $X \geq 0.12$. At $X \geq 0.12$, a new set of SAXS peaks with a larger spacing in the ratio of 1:2:3:4, and the spacing gradually decreased with an increase in DMSO

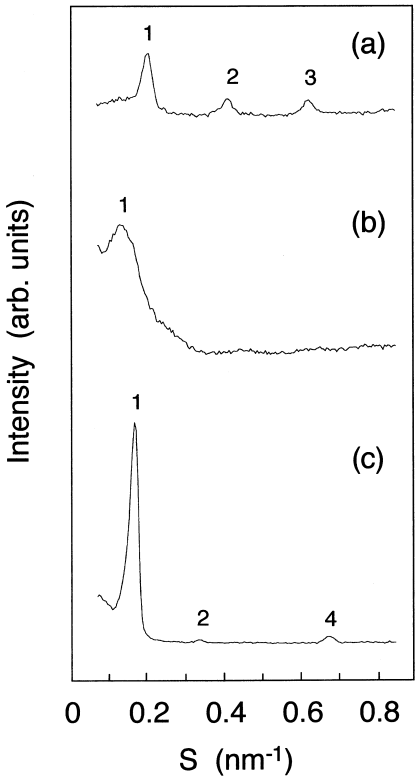

Fig. 1. SAXS profiles of DHPC-MLV at 20°C at various concentrations of DMSO (mole fraction), X_{DMSO} or X. (a) $X=0$, (b) $X=0.06$ and (c) $X=0.17$.

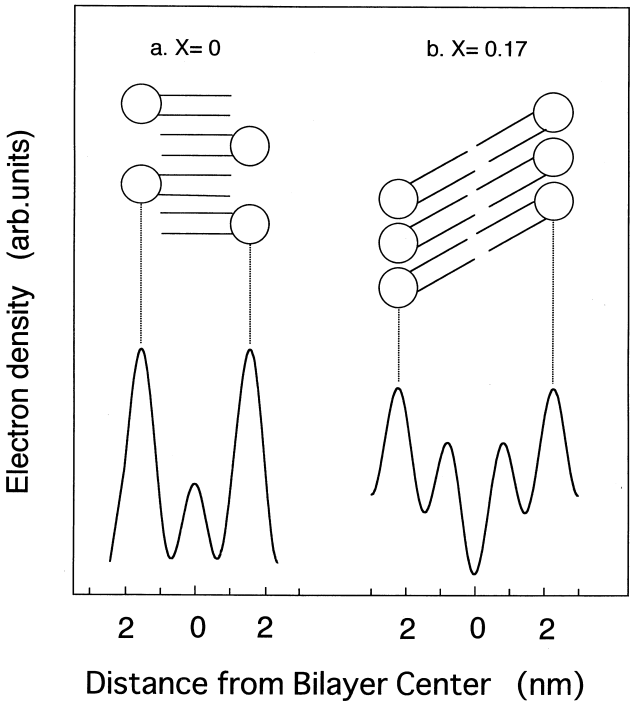

Fig. 3. Electron density profiles for DHPC-MLV in (a) water ($X=0$), (b) $X=0.17$ at 20°C. Abscissa is a distance from membrane center (nm). For each profile, the geometric center of the membrane is placed at the origin of the abscissa. Low density regions in the center of the profile correspond to the phospholipid hydrocarbon chains and the high density peaks on either side correspond to the lipid headgroups.

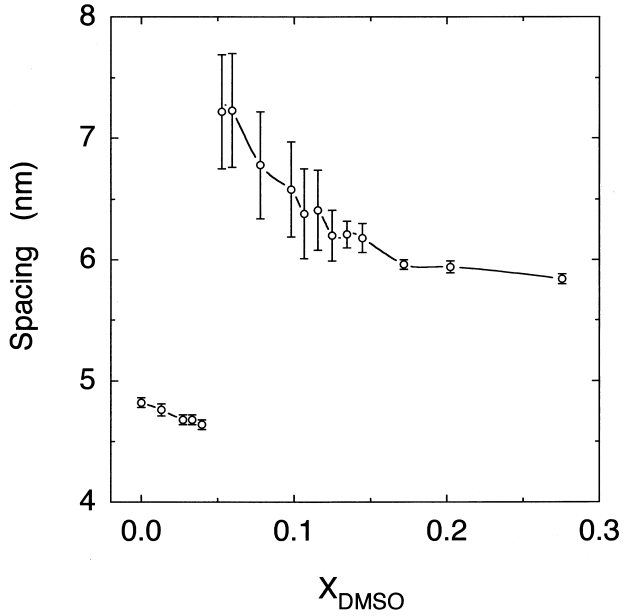

Fig. 2. The spacing of DHPC-MLV at 20°C at various concentrations of DMSO (mole fraction), X_{DMSO}.

concentration from 6.4 nm ($X=0.12$) to 5.8 nm ($X=0.28$).

We have determined the electron density profiles of the DHPC-MLVs by using Eq. 1. A set of phases, $j(h)$, in water is known to be (−1, −1, +1) for orders $h=1$ to 3 [13], and $j(h)$ at high concentrations of DMSO ($X \geq 0.12$) was determined to be (−1, −1, +1, −1) for orders $h=1$ to 4, which is the same as that of the $L_{\beta'}$ phase of DHPC-MLV under several conditions [13,15,27]. By using these phases, the electron density profiles of DHPC-MLV in water ($X=0$) and in $X=0.17$ were obtained (Fig. 3). They show that the distances between headgroup peaks across the bilayer, d_{p-p}, are 3.1 nm at $X=0$, and 4.5 nm at $X=0.17$. A WAXS of phospholipid membranes can give us information on the arrangement and distance of the alkyl chains in the membrane [29]. A WAXS pattern of DHPC-MLV at $X=0$ at 20°C consisted of a sharp reflection at 1/0.409 nm^{-1}, corresponding to a real-space periodicity at 0.409 nm (Fig. 4). It shows

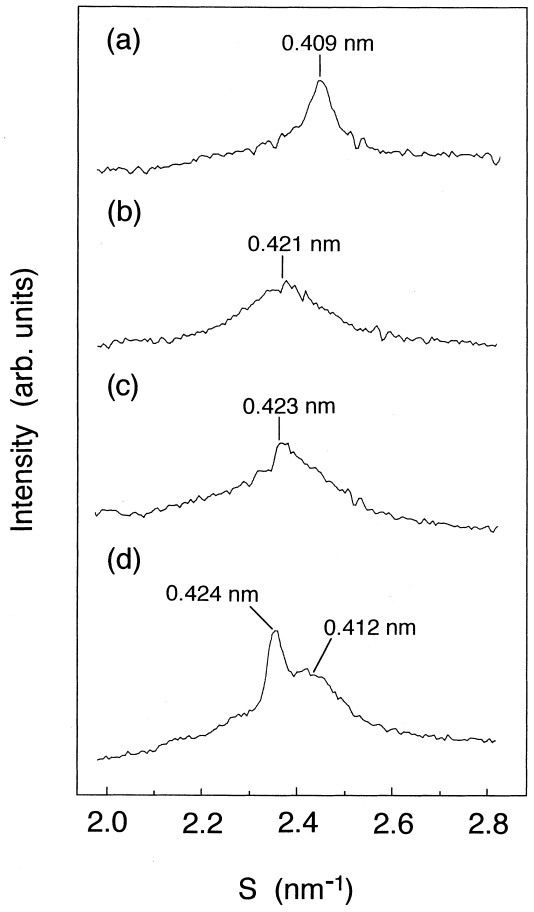

Fig. 4. WAXS profiles of DHPC-MLV at 20°C at various concentrations of DMSO. (a) $X=0$, (b) $X=0.06$ and (c) $X=0.17$. For comparison, a WAXS profile of DPPC-MLV in water at 20°C ($L_{\beta'}$ phase) is shown in (d).

that alkyl chains were in the gel phase and packed in a 2D hexagonal lattice without any chain tilt with respect to the normal to the membrane surface [29]. On the other hand, at $X=0.06$, it consisted of a broad reflection around 1/0.421 nm^{-1}, suggesting that the alkyl chains were in the gel phase and packed in a 2D hexagonal lattice with the chain tilt with the normal to the membrane surface. At $X=0.17$, it consisted of a sharp reflection at 1/0.423 nm^{-1} and a diffuse reflection around 1/0.41 nm^{-1}, which means that alkyl chains were in the gel phase and packed in a 2D quasi-hexagonal lattice, tilted with the normal to the membrane surface. This is characteristic of the $L_{\beta'}$ phase.

The results of the electron density profile and the WAXS pattern indicate that DHPC-MLVs at $X \geq 0.12$ are in the $L_{\beta'}$ phase. On the other hand, the SAXS and WAXS results suggest that DHPC-MLVs at an intermediate concentration of DMSO ($0.05 \leq X < 0.12$) are in the bilayer gel phase, which cannot be assigned to a more specific phase, such as the $L_{\beta'}$ phase or ripple phase ($P_{\beta'}$ phase), at present. A similar situation was reported in the trehalose-induced $L_{\beta}I$ to the bilayer gel phase transition in DHPC-MLV [27] and the low pH-induced $L_{\beta}I$ to the bilayer gel phase transition in DHPC-MLV [15].

3.2. Dependence of phase transition temperatures of DHPC-MLV on DMSO concentration

We have investigated the dependence of the phase transition temperatures of DHPC-MLV on DMSO concentration by using DSC. Fig. 5 shows DSC heating curves of DHPC-MLV in the presence of various concentrations of DMSO. At $X=0$, there were two endothermic peaks, which correspond to an $L_{\beta}I$ to $P_{\beta'}$ phase transition and a gel to liquid-crystalline phase transition (a chain-melting phase transition). As shown in Fig. 6, the chain-melting phase transition temperature (T_m) increased with an increase in DMSO concentration. On the other hand, the phase transition temperature from $L_{\beta}I$ to $P_{\beta'}$ phase de-

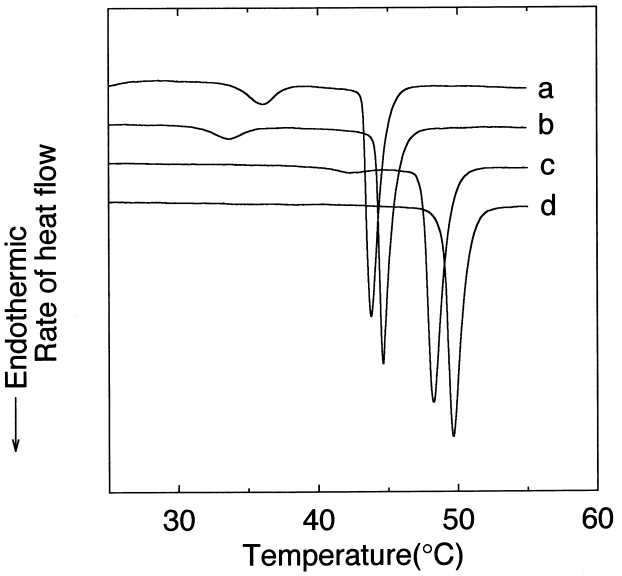

Fig. 5. DSC heating curves of DHPC-MLV at various concentrations of DMSO (mole fraction). (a) $X=0$, (b) $X=0.027$, (c) $X=0.14$ and (d) $X=0.28$. Heating rates were 2.0°C/min.

creased with an increase in DMSO concentration and this transition was not detected at high concentrations of DMSO ($X \geq 0.05$). At the same concentration ($X \geq 0.05$), the $L_\beta I$ phase was not detected in the DHPC-MLV at 20°C (Fig. 2). At $X = 0.14$ and 0.20, a small endothermic peak was observed at 41°C, whose origin is not identified at present.

3.3. Solubility of phosphorylcholine

To obtain information on the interaction free energy between the hydrophilic segments of the DHPC membranes with solvents, we have investigated the dependence of the solubility of a phosphorylcholine on DMSO concentration in a DMSO–water mixture by the same method described in our previous paper [26,30]. The phosphorylcholine molecule has the same molecular structure as the headgroup of PC and, thereby, it represents a hydrophilic segment of the membrane interface of DHPC-MLV. Fig. 7 shows that the solubility of the phosphorylcholine in water at 20°C decreased with an increase in DMSO concentration. This result indicates that DMSO is a poor solvent for the hydrophilic segments of the PC membrane and the interaction free energy between solvents and the hydrophilic segments of the PC membrane increases with an increase in DMSO concentration.

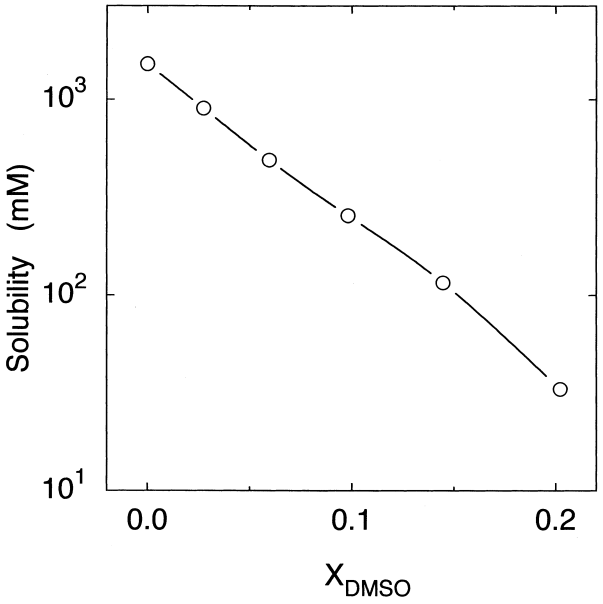

Fig. 7. Solubility of phosphorylcholine at various concentrations of DMSO (mole fraction) in DMSO–water mixture at 20°C.

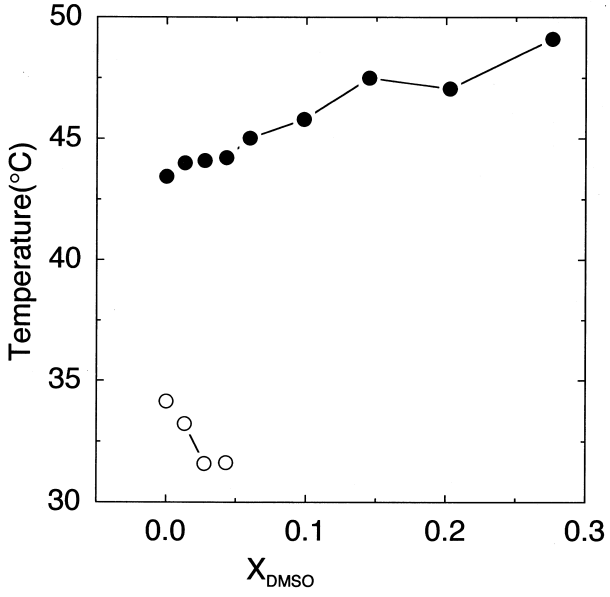

Fig. 6. Phase transition temperatures of DHPC-MLV at various concentrations of DMSO (mole fraction) determined by DSC. (●) shows gel to liquid-crystalline phase transition temperatures and (○) shows $L_\beta I$ to $P_{\beta'}$ phase transition temperatures. Heating rates were 2.0°C/min.

4. Discussion

4.1. The mechanism of the increase in the gel to liquid-crystalline phase transition temperature with an increase in DMSO concentration

In our previous paper [9], we indicated that several water-soluble organic solvents such as acetone, acetonitrile, propionaldehyde, ethanol and tetrahydrofurane induced the $L_{\beta'}$ to $L_\beta I$ phase transitions in DPPC-MLV. On the contrary, DMSO cannot induce this phase transition in DPPC-MLV [18]. Our results in this report indicate that DHPC-MLV was in the bilayer gel phase at $0.05 \leq X < 0.12$ and was in the $L_{\beta'}$ phase at $X \geq 0.12$. This phenomenon is similar to the low pH-induced phase transition in DHPC-MLV [15]; the $L_\beta I$ to bilayer gel phase transition occurred at pH 3.9 with a decrease in pH, and at lower pH values ($pH \leq 2.2$) the DHPC membranes were in the $L_{\beta'}$ phase. As shown in Fig. 6, the decrease in the $L_\beta I$

to $P_{\beta'}$ phase transition temperature with an increase in DMSO concentration indicates that DMSO stabilizes the $P_{\beta'}$ phase rather than the $L_\beta I$ phase. These results indicate that DMSO stabilizes the bilayer gel phase rather than the $L_\beta I$ phase in PC membranes. Hence, DMSO has the opposite effect on PC membranes compared with other organic solvents such as ethanol and acetone.

Recently, we have proposed that the interaction free energy of the surface segments of phospholipid membranes with solvents, ΔG_i, plays an important role in the structure and phase behaviors of these membranes [9,26,30,31]. ΔG_i is defined as the free energy increase associated with the contact of segments with solvent. In good solvents, where the interaction between the segments of the membrane surface and the solvents is favorable (i.e. ΔG_i is small), the segments swell to contact the solvents; on the other hand, in poor solvents, where their interaction is unfavorable (i.e. ΔG_i is large), the segments shrink or associate with each other to prevent contact with the solvents. We have to consider two kinds of segments of the membrane surface, hydrophilic segments of the headgroup and hydrophobic segments of the alkyl chains and, thereby, two kinds of interaction free energy between the surface segments of the phospholipid membrane and solvents; one is a free energy of interaction between the hydrophilic segments of the headgroups and solvents (ΔG_{i1}) and the other is a free energy of interaction between the hydrophobic segments of the alkyl chains and solvents (ΔG_{i2}). This new concept can explain reasonably the induction of the $L_\beta I$ phase in DPPC-MLV and also the H_{II} to L_α phase transition in the DOPE membrane by water-soluble organic solvents such as acetone, acetonitrile and ethanol, and also the intermembrane distance in MLV [9,30,31].

DMSO has a relatively low solubility for alkanes such as hexane (2% (v/v) hexane completely dissolved in DMSO) at 20°C and the oil–water partition coefficient of DMSO was low (0.0030) [32]. Several investigations have shown that a DMSO molecule strongly interacts with two water molecules by hydrogen bonding at low concentrations around 20°C and, thereby, DMSO has the effect of rigidifying the water structure, whereas at higher concentrations, DMSO breaks the water structure ([33], and references therein). In this paper, we have investigated effects of low concentrations of DMSO in water at 20°C where the property of DMSO is the former. The DMSO molecule associated with water molecules may have a more hydrophilic character than the DMSO itself and, thereby, it is a poorer solvent for alkane than the DMSO itself. Therefore, ΔG_{i2} of the hydrophobic segments of the membrane interfaces with DMSO at low concentrations in water is large. On the other hand, other water-soluble organic solvents such as acetone, acetonitrile and ethanol, have the high solubility of alkane and ΔG_{i2} of the hydrophobic segments of the membrane interfaces with these solvents is small [9]. Moreover, the result of Fig. 7 indicated that DMSO was a poor solvent for the phosphorylcholine, which has the same structure as the headgroup segments of PC membranes. This result means that DMSO is a poor solvent for the hydrophilic segments of the surface of the PC membrane and also that ΔG_{i1} of the hydrophilic segments of the PC with solvents increased with an increase in DMSO concentration. Hence, DMSO is a poor solvent for both the hydrophilic segment and hydrophobic segment of PC membrane surface under these conditions (low concentrations of DMSO at 20°C). The increase in ΔG_i induces the shrinkage of the membrane interface consisting of the surface segments and solvents, which decreases the amount of solvents in the membrane interface and, thereby, reduces the effective cross-sectional area of the phospholipid headgroup regions, or induces the conformational change of the hydrophilic segments. This change decreases the repulsive interaction between the headgroups, which decreases the headgroup pressure, Π_{head}. In equilibrium, three kinds of lateral pressures in the membrane have to balance, i.e. $\Pi_{head}+\Pi_{chain}=\gamma$, where Π_{chain} is the repulsive chain pressure and γ is the attractive interfacial pressure due to the hydrophobic interaction between the alkyl chains and water at the membrane surface [35]. We can assume that γ is almost constant in the presence of various concentrations of DMSO, because DMSO molecules are preferentially excluded from the headgroup region of these membranes under these conditions. Therefore, the lateral compression pressure in the hydrophobic core of the membrane, $\gamma-\Pi_{head}$, increases with an increase in DMSO concentration, which induces the increase in the chain-melting phase transition temperature, T_m (Fig. 6) of the DHPC

membrane. Similar situations where the increase in the lateral compression pressure induces an increase in T_m were observed in other systems [15,24,30]. Tristram-Nagle et al. reported that DMSO has a dehydrating effect on the lipid headgroup of DPPC membranes at low concentrations in water [19], which supports the above discussion.

Fig. 2 indicates that the spacing of DHPC-MLV at the $L_\beta I$ phase ($0 \leq X < 0.05$) and the $L_{\beta'}$ phase ($X \geq 0.12$) decreased with an increase in DMSO concentration. Yu and Quinn reported a similar result that the intermembrane distance of DPPC-MLV decreased with an increase in DMSO concentration, and indicated that the repulsive force between the membrane must decrease because van der Waals attractive force between the membranes decreases with an increase in DMSO concentration [18]. As discussed above, the increase in ΔG_i with an increase in DMSO concentration induces the shrinkage of the membrane interface consisting of the surface segments and solvents, which decreases the amount of solvents in the membrane interface. The steric overlap force between the headgroup segments is considered to induce the repulsive force between the membranes in the gel phase, while the undulation force is a main repulsive force at long distance for the liquid-crystalline phase membrane [35,30]. The increase in ΔG_i decreases the steric overlap forces between the headgroups and, thereby, induces a decrease in the repulsive force between the membranes. Therefore, it decreases the intermembrane distance.

4.2. The mechanism of stabilization of the bilayer gel phase rather than the $L_\beta I$ phase in DHPC-MLV induced by DMSO

The difference in the chemical potential of the DHPC in the membrane at the $L_\beta I$ phase (μ^{int}) and in that at the $L_{\beta'}$ phase (μ^{bil}), $\Delta\mu$, is expressed as follows (see e.g. [15,16]):

$$\Delta\mu = \mu^{int} - \mu^{bil} = (\mu^{int}_{hd} - \mu^{bil}_{hd}) + (\mu^{int}_{ch} - \mu^{bil}_{ch}) + (\mu^{int}_{th} - \mu^{bil}_{th}) = \Delta\mu_{hd} + \Delta\mu_{ch} + \Delta\mu_{th} \quad (2)$$

where $\Delta\mu_{hd}$ is a term due to the membrane interface, $\Delta\mu_{ch}$ is a term due to the hydrophobic interior zone of the membrane and $\Delta\mu_{th}$ is a term due to the interaction of the terminal methyl groups of the alkyl chains with surroundings. The terminal methyl groups are exposed to water in the $L_\beta I$ phase and to the opposite monolayer in the bilayer gel phase. The contact between the segments of the alkyl chains and water is unfavorable due to the hydrophobic interaction, i.e. $\Delta\mu_{th} > 0$ [36]. As discussed in Section 4.1, we can assume that γ is almost constant in the presence of various concentrations of DMSO, because DMSO molecules are preferentially excluded from the headgroup region of these membranes. Thereby, $\Delta\mu_{th}$ is almost constant in various concentrations of DMSO. The second term $\Delta\mu_{ch}$ is determined by the van der Waals interaction between the alkyl chains in the membrane [1], which is proportional to $1/r^5$ (where r is the separation between the alkyl chains) [37,38]. Our X-ray diffraction data indicated that an average value of r of the DHPC membrane in water (in the $L_\beta I$ phase) was smaller than that in high concentrations of DMSO (in L_β phase), i.e. $\Delta\mu_{ch} < 0$ (see Section 3). However, we can reasonably assume that the van der Waals interaction between the alkyl chains doesn't depend on DMSO concentration. Hence, this term, $\Delta\mu_{ch}$, does not play a significant role in this phase transition. On the other hand, the chemical potential of the membrane interface, μ_{hd}, is determined mainly by interactions between the headgroups of the phospholipids due to a steric hindrance and the interface hydration and, thereby, they depend significantly on the solution conditions. Therefore, $\Delta\mu_{hd}$ in Eq. 2 plays an important role in this phase transition. Generally, μ_{hd} can be described as the sum of a term due to attractive interaction γA and that due to repulsive interaction R/A [39,40]:

$$\mu_{hd}(T, A) = \gamma(T)A + R(T)/A \quad (3)$$

where R is a repulsive parameter and A is the area per lipid head. The main contribution to the attractive term (γA) is the hydrophobic interaction. The repulsive term (R/A) in Eq. 3 is determined mainly by the interaction between the headgroups of the phospholipids due to the steric hindrance, an electrostatic interaction and the interfacial hydration. The physical origin of the attractive parameter $\gamma(T)$ is the hydrophobic interaction and, thereby, we can assume that $\gamma(T)$ is almost constant in the presence of various concentrations of DMSO.

Assuming that $\mu^{bil}_{th} = 0$ and $\mu^{int}_{th} = \gamma_{th}(2A^{int}_{ch})$, we can

now represent the chemical potentials μ^{int} and μ^{bil} as [15]:

$$\mu^{int}(R) = 2\gamma A_{ch}^{int} + \frac{R}{4A_{ch}^{int}} + \mu_{ch}^{int} + \gamma_{th}(2A_{ch}^{int}) \quad (4)$$

$$\mu^{bil}(R) = 2\gamma A_{ch}^{bil} + \frac{R}{2A_{ch}^{bil}} + \mu_{ch}^{bil} \quad (5)$$

where A_{ch} is the cross-area per chain in the membrane. If γ is constant during the change of the external effects on the phospholipid membrane, at large values of R where $R > R^*$ (R^* is the critical repulsive parameter where $\mu^{int}(R^*) = \mu^{bil}(R^*)$, the energetically favorable phase of the membrane is the $L_\beta I$ phase, but at small values of R where $R < R^*$ the bilayer gel phase becomes energetically favorable. When we can use the approximation that $A_{ch}^{int} \approx A_{ch}^{bil} = A/2$ and, thereby, $R^* = 2\gamma_{th}A^2$, where $\mu^{int}(R^*) = \mu^{bil}(R^*)$ [15]. The surface density of the polar headgroups of lipids in the $L_\beta I$ phase is almost a half of that in the bilayer gel phase. This lowered density reduces the steric overlap of headgroup regions that consist of the hydrophilic segments and water molecules, and also increases conformational and mixing entropy of the headgroup regions. Therefore, the repulsive interaction free energy in the headgroup regions in the $L_\beta I$ phase is lower than that in the bilayer gel phase, which is expressed in Eqs. 4 and 5.

For DHPC-MLV in water, the repulsive interaction between the headgroups is large, i.e. $R > R^*$ and, thereby, the energetically favorable phase is the $L_\beta I$ phase, contrary to the exposition of the terminal methyl groups in water. As discussed in Section 4.1, with an increase in DMSO concentration the headgroup pressure, Π_{head}, decreases and, thereby, the repulsive parameter R decreases. At the critical concentration of DMSO, R becomes equal to the critical value R^* and, thereby, the $L_\beta I$ to bilayer gel phase transition occurs. Now the profit of head–head repulsive energy at the $L_\beta I$ phase cannot compensate the energetic loss due to exposition of the terminal methyl groups to aqueous solution at the $L_\beta I$ phase. The bilayer gel phase of DHPC-MLV at high concentrations of DMSO becomes energetically favorable. Therefore, the decrease of the repulsion between the polar headgroups induces the $L_\beta I$ to bilayer gel phase transition.

It is worth emphasizing that the mechanism of the DMSO-induced phase transition of DHPC-MLV is almost the same as that of the $L_\beta I$ to bilayer gel phase transition of DHPC-MLV due to the increase in poly(ethylene glycol)-6K (PEG 6K) concentration [16] or the decrease in pH [15]. The former can be explained on the basis of the osmoelastic coupling theory [24,34]. The exclusion of PEG molecules from the region adjacent to the membrane surface induces an osmotic stress onto the membranes. To lower the chemical potential of water in the exclusion layer, the membrane is compressed to produce elastic pressure (osmoelastic coupling). This compression induces the decrease of the repulsion between the DHPCs' headgroups. The low pH-induced phase transition can also be explained by the decrease in the repulsive interaction between the headgroups due to the protonation of the phosphate group, because it increases the interaction free energy between the hydrophilic segments and water and, thereby, decreases the effective polarity of the membrane interface [15].

It is important to note that the effects of DMSO on phospholipid membranes or biomembranes is strongly dependent on the concentration of DMSO in water and the temperature, because these conditions affect the physical property of DMSO in water. As discussed in Section 4.1, a DMSO molecule strongly interacts with two water molecules by hydrogen bonding at low concentrations around 20°C and, thereby, DMSO has an effect of rigidifying the water structure, whereas at higher concentrations, DMSO breaks the water structure [33]. At higher temperatures, the hydrogen bonding between DMSO and water molecules tends to break and, thereby, DMSO has a more hydrophobic character and easily associates with the phospholipid membrane due to the hydrophobic association ([41] and references therein). This property of DMSO aqueous solution can explain the phenomenon that DMSO can be used as a cellular cryoprotectant at low temperatures while it is toxic for cells at higher temperatures [41–43]. Therefore, under these conditions (higher concentrations of DMSO in water or higher temperatures), ΔG_{i2} of the hydrophobic segments of the membrane interfaces with DMSO is small. This is the same property as the other organic solvents such as ethanol or acetone display [9] and, thereby, DMSO under these conditions stabilizes the $L_\beta I$

phase rather than the bilayer gel phase. This speculation can reasonably explain the result of Gordeliy et al. that DPPC-MLV was in the $L_\beta I$ phase at very high concentrations ($X \geq 0.9$) of DMSO [20].

One of the main conclusions of this report is that the decrease in the repulsive interaction between the headgroups of the DHPC induced by low concentrations of DMSO in water plays an important role in the stabilization of the bilayer gel phase rather than the $L_\beta I$ phase. This conclusion can be applied to other phospholipid membranes (in L_α and other phases) and biomembranes; the presence of low concentrations of DMSO in water induces a decrease in the repulsive interaction between the headgroups of these membranes. Moreover, as discussed in Section 4.1, this induces an increase in the lateral compression pressure in the hydrophobic core of the membrane, $\gamma - \Pi_{head}$, with an increase in DMSO concentration. Recently, lipids forming non-bilayer membranes such as hexagonal II phase and cubic phases are considered to preserve the functional structure of membrane proteins by increasing the lateral compression pressure in the hydrophobic core of the membrane [44]. Therefore, DMSO may be used to stabilize the membrane proteins embedded in the membranes composed of bilayer-preferring lipids such as PC. These characteristics can be helpful in understanding the mechanism of the role of DMSO as a cryoprotectant for various cells and, also, of effects of DMSO on other physical properties of various kinds of phospholipid membranes and biomembranes.

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