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Organic solvents induce interdigitated gel structures in multilamellar vesicles of dipalmitoylphosphatidylcholine

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Abstract

We have investigated the effects of several water-soluble organic solvents which also have a high solubility for alkanes, on the structure and phase behavior of multilamellar vesicles of dipalmitoylphosphatidylcholine (DPPC-MLV). Of these solvents, we selected five organic solvents; such as, acetonitrile, acetone, propionaldehyde, ethanol, and tetrahydrofuran. The main transition temperature of DPPC-MLV decreased with an increase in acetonitrile concentration from 0% to 6.0%(v/v) acetonitrile, and increased above 6.0%(v/v). X-ray diffraction data indicated that a phase transition from $L_{\beta'}$ to $L_{\beta}I$ phase (interdigitated gel phase) in DPPC-MLV, occurred at 5.0%(v/v) and DPPC-MLV were completely in the $L_{\beta}I$ phase above 6.0%(v/v) acetonitrile at 20°C. Results of the excimer method (Yamazaki, M. et al. (1992) Biochim. Biophys. Acta 1106, 94–98) supported the above results; the ratio of excimer to monomer fluorescence intensity (E/M) of pyrene-PC in DPPC-MLV rapidly decreased at 5.1%(v/v) and E/M became very low above 6.0%(v/v) acetonitrile. By the excimer method, we have found that other organic solvents; such as, acetone, propionaldehyde, and tetrahydrofuran, induced a phase transition from $L_{\beta'}$ to $L_{\beta}I$ phase in DPPC-MLV. Threshold concentrations of acetone, ethanol, propionaldehyde, and tetrahydrofuran for this phase transition at 20°C were 9.4%(v/v), 5.5%(w/v), 3.5%(w/v), and 3.7%(w/v), respectively. Substitution of H_2O by D_2O (deuterium oxide) increased the threshold concentrations of all the organic solvents. A mechanism of these phase transitions and the effect of the substitution of H_2O by D_2O is proposed and discussed; a concept of the χ parameter, which is an interaction energy parameter between the surface segments of DPPC-MLV and solvents, may explain these phenomena reasonably.

Keywords: Chi (χ) parameter; Excimer method; Phase transition; Phospholipid; Interdigitated gel structure; Deuterium oxide

1. Introduction

Biomembranes and phospholipid membranes usually form bilayer structures where two phospholipid-monolayers contact each other with their hydrophobic alkyl chains and their hydrophilic surfaces contact with water. These bilayer structures are very stable due to the hydrophobic interaction between phospholipid membranes and water [1]. Recently, interdigitated gel ($L_{\beta}I$) structures in phospholipid membranes have attracted much attention [2]. In these structures, lipid molecules from opposing monolayers are interpenetrated or interdigitated, and terminal segments of their alkyl chains face the aqueous phase and contact with water. In the presence of alcohol (such as, ethanol [3–5], ethylene glycol [6,7], oligomers of ethylene glycol [8], and short-chain alcohols [9,10]) and other small molecules (such as Tris buffer [11] and anions [12]) multilamellar vesicles (MLV) of phosphatidylcholine (PC) or phosphatidylglycerol can form $L_{\beta}I$ phases. High pressure also induces a $L_{\beta}I$ structure in PC-MLV [13].

In order to investigate $L_{\beta}I$ structures and elucidate the mechanism of phase transition from $L_{\beta'}$ to $L_{\beta}I$ structure, several physical techniques (such as, X-ray diffraction [14], high-resolution electron cryomicroscopy [5], scanning density meter [15], and fluorescence spectrometry [16–18]) have been used extensively. However, a mechanism of the induction of $L_{\beta}I$ structure in MLV by such molecules, is still unclear.

In this report, we have investigated effects of several water-soluble organic solvents – which also have a high solubility for alkane such as hexane – on structures and

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; MLV, multilamellar vesicle; $L_{\beta}I$ phase, interdigitated gel phase; $L_{\beta'}$ phase, tilted chain bilayer gel phase; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering.

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phase behavior of dipalmitoylphosphatidylcholine (DPPC)-MLV. Of these solvents, we selected five organic solvents; such as, acetonitrile, acetone, propionaldehyde, ethanol, and tetrahydrofuran. We expected that these solvents would induce the $L_\beta I$ structure in DPPC-MLV from a reason described below. In the $L_\beta I$ structure, the terminal segments of alkyl chains of the phospholipids – a kind of alkane – contact with water. This contact between the terminal alkyl segments and water is unstable (i.e., the free energy of the contact is large) because of low solubility of alkane in water [1], and hence increases the chemical potential of the membrane in the $L_\beta I$ structure. Consequently, the chemical potential of the membrane in the $L_\beta I$ structure is larger than that in the $L_{\beta'}$ structure. On the other hand, the contact of the terminal alkyl segments with these organic solvents is much more stable than that with water, because of the high solubility of alkane in these solvents. Thereby, the chemical potential of the membrane in the $L_\beta I$ structure would decrease with an increase in these organic solvents. By the X-ray diffraction and the excimer method, we have found that they could induce $L_\beta I$ structure in DPPC-MLV above their threshold concentrations. We have also investigated an effect of replacing water with D_2O (deuterium oxide) on the concentrations of the solvents that induce the $L_\beta I$ structure, since the substitution of H_2O by D_2O enhances the hydrophobic interaction and would be expected to increase the chemical potential of the membrane in the $L_\beta I$ structure. Based on these results, we propose a mechanism of the phase transition from the $L_{\beta'}$ to the $L_\beta I$ phase induced by these organic solvents.

Part of this research was presented at the 33rd of Annual Meeting of the Biophysical Society of Japan [19].

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) and propionaldehyde were purchased from Sigma. Acetone, acetonitrile, ethanol, tetrahydrofuran, and deuterium oxide (purity > 99.75%) were purchased from Wako. 1-Hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine (pyrene-PC) was purchased from Molecular Probes.

2.2. Sample preparations

Multilamellar vesicles (MLVs) were prepared by adding the appropriate amounts of water to dry lipids and an suspension was vortexed for about 30 s around 55°C several times. Solutions of high concentration organic solvent molecules in water were added to a preformed MLV solution, and this mixture was incubated for 15 min. at room temperature ($\approx$ 20°C). Pipes buffer (20 mM Pipes, pH 7.0 (or pD 7.0)) was used for propionaldehyde. For measurement of the X-ray diffraction, pellets after centrifugation (14 000 $\times$ *g*, 1 h at 20°C, Tomy, MR-150) of the suspensions were used.

2.3. X-ray diffraction

X-ray diffraction experiments were performed by using Nickel filtered Cu K_α X-radiation ($\lambda = 0.154$ nm) from 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 position sensitive proportional counter (Rigaku, PSPC-5) with a camera length of 350 mm and associated electronics (multichannel analyzer, etc., Rigaku). Wide-angle X-ray scattering (WAXS) patterns were recorded by a flat plate film cassette loaded with a high-sensitive X-ray film (Fuji Medical X-ray Film) with a camera length of 66.0 mm. Samples were sealed in a thin-walled glass capillary tube (outer diameter 1.0 mm) and mounted in a thermostatable holder whose stability was $\pm$0.2°C [7].

SAXS data were processed by a standard method [20]. Integrated intensities of various diffraction peaks, $I(h)$, where h is an 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 and a correction factor due to a geometry of PSPC, $P(h)$. Electron density distributions, $\rho(x)$, were calculated by use of the following formula:

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

where $j(h)$ is a 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.

2.4. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) experiments were performed using a Rigaku DSC-8230B instrument. Each sample was heated at a rate of 2.0 K/min. Main transition temperature was determined as an onset of an endothermic transition extrapolated to the baseline. A detailed method was described in our previous paper [7].

2.5. Excimer method

A method and theory of the excimer method developed by us are described in detail in our previous paper [16]. This method is based on spectroscopic properties of a fluorescent pyrene probe. Emission spectra of pyrene and its pyrene derivatives show two components; one is due to an excited pyrene monomer A^* and the other, at longer wavelength, is attributed to an excited dimer (excimer) $(AA)^*$ formed on a collision of an excited monomer A^* with a ground-state pyrene A. A ratio of the excimer to monomer fluorescence intensities (E/M) is proportional

to a collision frequency of pyrenes. In the excimer method, we use a pyrene-PC – a fluorescent lipid probe which has a pyrene ring at a terminus of an alkyl chain – in vesicle membranes. As indicated in the previous paper [16], the E/M value of pyrene-PC in DPPC vesicles in $L_{\beta}I$ structure is much lower than that in $L_{\beta'}$ structure, because structural restriction in $L_{\beta}I$ structure largely decreases the collision of pyrene molecules in the membrane. A threshold concentration of solvents where a phase transition from $L_{\beta'}$ to $L_{\beta}I$ structure occurs, is determined as a concentration where a rapid decrease of the E/M values occurs; which is almost the same as determined by the X-ray diffraction method [16]. This excimer method have been successfully applied to several systems [5,8].

For fluorescence measurement, a Hitachi F3000 spectrofluorimeter was used. An excitation wavelength of pyrene PC was 347 nm and emission wavelengths were 376 nm for monomer fluorescence and 481 nm for excimer fluorescence. Excitation bandpass and emission bandpass were 3 nm and 1.5 nm, respectively. A pyrene-PC concentration in total phospholipids (DPPC and pyrene-PC) was 5.0 mol%. At 15 min. after mixing the MLV with organic solvents in water, fluorescence intensities of samples due to monomer and excimer were measured at 20 ± 0.5°C by using a circulator (Refrigerated Circulator, RTE-110, NESLAB). Fluorescence intensities for calculation of E/M were obtained by their time averaging for 15 s, and by using these values, ratio of excimer to monomer fluorescence intensities (E/M) was calculated. Concentrations of the total phospholipids in the samples for the measurement of the fluorescence were 70 to 80 μM, which were determined by a standard phosphate analysis [21].

3. Results

3.1. Solubility of alkane in these organic solvents

To select several water-soluble organic solvents which also have high solubility of alkane, we at first investigated

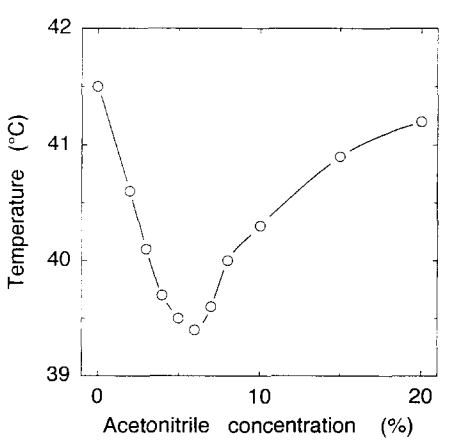

Fig. 1. Main transition temperature of DPPC-MLV in various concentrations of acetonitrile (%(v/v)). Heating rate was 2.0 K/min.

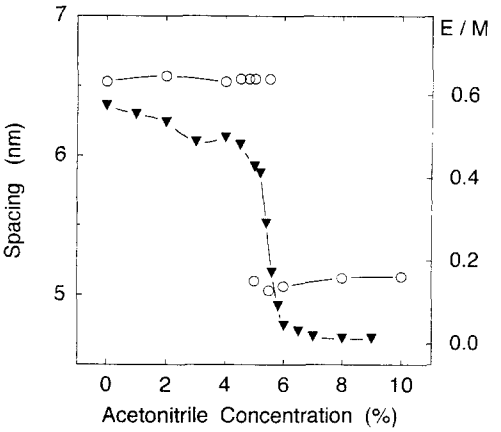

Fig. 2. Lamellar repeat periods (spacing) of DPPC-MLV (○) and ratio of excimer to monomer fluorescence intensities (E/M) of pyrene-PC in DPPC-MLV (▼) in various concentrations of acetonitrile (%(v/v)) at 20°C.

solubility of hexane – an alkane – in various water-soluble organic solvents. Hexane completely dissolved in acetone, ethanol, and tetrahydrofuran, in any ratio of hexane and these solvents at 20°C. These results mean that solubilities of hexane in these solvents were extremely high. Less than 10%(v/v) hexane completely dissolved in acetonitrile, and less than 50%(v/v) hexane completely dissolved in propionaldehyde at 20°C. These rough evaluations show that solubility of hexane in acetonitrile was 10%(v/v) (i.e., 0.8 M) and that in propionaldehyde was 50%(v/v) (i.e., 4 M). These results indicated that solubilities of hexane in these five organic solvents were high.

3.2. Interaction of acetonitrile with DPPC-MLV

At first, we have investigated effects of acetonitrile on a phase behavior and structure of DPPC-MLV. Main transition temperature of DPPC-MLV decreased with an increase in acetonitrile concentration from 0 to 6.0%(v/v), and increased above 6.0%(v/v) acetonitrile concentration (Fig. 1). This biphasic behavior of the change of main transition temperature is characteristic of an interaction between MLV and the substance such as ethanol and ethylene glycol which induce the $L_{\beta}I$ structure [3,4,7].

To get structural information, we have investigated DPPC-MLV in various acetonitrile concentrations by SAXS and WAXS. As shown in Fig. 2, the lamellar repeat period (spacing) (d_1) of DPPC-MLV at 20°C, rapidly decreased from 6.5 nm to 5.0 nm at 5.0%(v/v) acetonitrile. Between 5.0% and 5.8%, d_1 had two different values (6.5 nm and 5.0 nm), and above 6.0%(v/v), d_1 had only one value (5.0 nm).

Fig. 3 shows electron density profiles of DPPC-MLV in 0% and 10%(v/v) acetonitrile, which were determined by a set of phase $j(h)$, $(-1, -1, +1, -1, -1)$ for orders $h = 1$ to 5, and $(-1, -1, +1)$ for orders $h = 1$ to 3, respectively. They show that a distance between the head

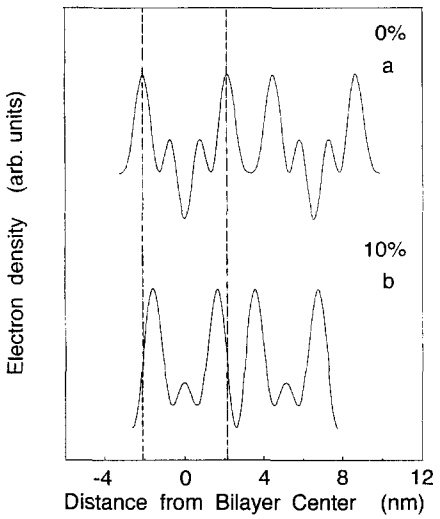

Fig. 3. Electron density profiles for DPPC-MLV in (a) 0%, (b) 10%(v/v) acetonitrile at 20°C. Abscissa is a distance from bilayer center (nm). For each profile, the geometric center of the bilayer is placed at the origin of the abscissa. Low density region in the center of the profile corresponds to the phospholipid hydrocarbon chains and the high density peaks on either side correspond to the lipid head groups.

group peaks across the bilayer, $d_{p\text{-}p}$, is 4.2 nm at 0% and 3.0 nm at 10%(v/v) acetonitrile. A WAXS pattern at 0% acetonitrile at 20°C consisted of a sharp reflection at 0.42 nm, and a diffuse reflection centered at 0.41 nm, which indicates $L_{\beta'}$ structure. On the other hand, a WAXS pattern above 6.0%(v/v) acetonitrile showed a single sharp peak at 0.41 nm, showing that alkyl chains were packed in a hexagonal arrangement without any inclination. The results of the WAXS and the value of the $d_{p\text{-}p}$ indicate that DPPC-MLVs in the high concentrations of acetonitrile above 6.0%(v/v) were in the interdigitated gel ($L_{\beta}I$) phase.

We have also investigated this phase transition by the

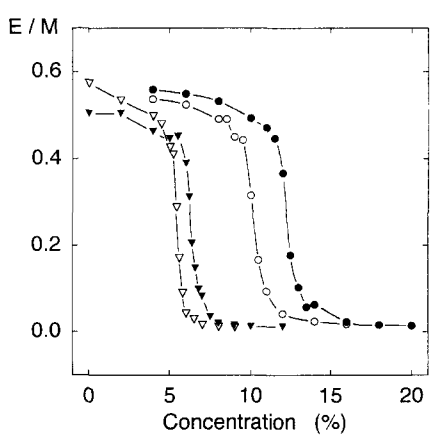

Fig. 4. Ratio of excimer to monomer fluorescence intensities (E/M) of pyrene-PC in DPPC-MLV in H_2O (▽) and D_2O (▼) in various concentrations of acetonitrile (%(v/v)) at 20°C, respectively, and that in DPPC-MLV in H_2O (○) and D_2O (●) in various concentrations of acetone (%(v/v)) at 20°C, respectively.

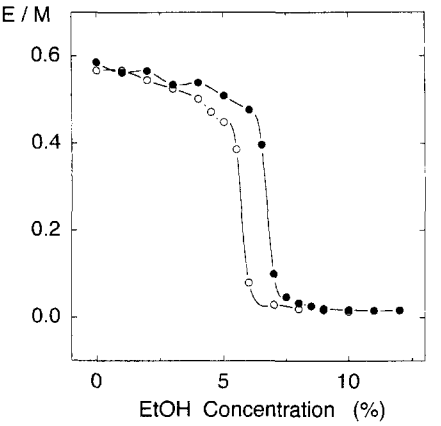

Fig. 5. Ratio of excimer to monomer fluorescence intensities (E/M) of pyrene-PC in DPPC-MLV in H_2O (○) and D_2O (●) in various concentrations of ethanol (%(w/v)) at 20°C, respectively.

excimer method. Fluorescence intensity ratio E/M of pyrene-PC in DPPC-MLV was plotted as a function of acetonitrile concentration (Fig. 2). As shown in Fig. 2, the E/M value rapidly decreased at 5.1%(v/v) acetonitrile, and above 6.0%(v/v), the E/M value became very low. As indicated in the previous paper [16], the E/M values of pyrene-PC in DPPC vesicles in $L_{\beta}I$ structure are much lower than those in $L_{\beta'}$ structure, because structural restriction of $L_{\beta}I$ structure largely decreases the collision of pyrene molecules in the membrane. Thereby, the results of the excimer method indicated that the transition from $L_{\beta'}$ to $L_{\beta}I$ structure occurred at 5.1%(v/v) acetonitrile, which was almost the same concentration as determined by the X-ray diffraction method. In other experiments in this report, we have used the excimer method; because it is so highly-sensitive that we can use much smaller amount of each sample in the excimer method than that in the X-ray diffraction method, and also that we can shorten a measurement time by this method [16,8,5].

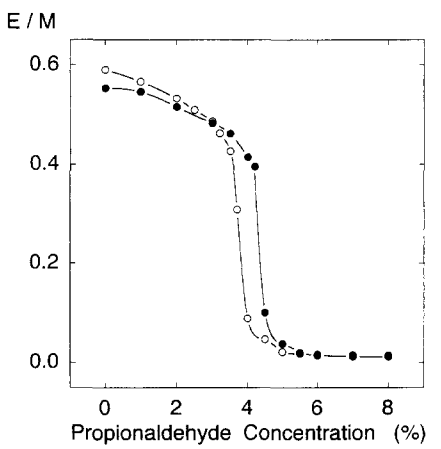

Fig. 6. Ratio of excimer to monomer fluorescence intensities (E/M) of pyrene-PC in DPPC-MLV in H_2O (○) and D_2O (●) in various concentrations of propionaldehyde (%(w/v)) at 20°C, respectively.

3.3. Effect of deuterium oxide on the induction of $L_\beta I$ structure in DPPC-MLV by organic solvents (acetonitrile, acetone, and ethanol)

By using the excimer method, we have investigated interactions of organic solvents; such as, acetonitrile, acetone, and ethanol in deuterium oxide with DPPC-MLV at 20°C, and compared results in deuterium oxide (D_2O) with those in water (H_2O). As shown in Fig. 4, the E/M values rapidly decreased at 5.1%(v/v) acetonitrile in H_2O; 6.0%(v/v) acetonitrile in D_2O. The E/M values became very low above 6.0%(v/v) in H_2O and 7.3%(v/v) in D_2O. These results show that the threshold concentration of acetonitrile for the induction of $L_\beta I$ structure in D_2O, is higher than that in H_2O.

Similar results were obtained in interactions of DPPC-MLV with acetone or ethanol (Figs. 4 and 5). As shown in Fig. 4, the E/M values rapidly decreased at 9.4%(v/v) acetone in H_2O; 12%(v/v) acetone in D_2O; the E/M values became very low above 12%(v/v) in H_2O and 14%(v/v) in D_2O. Results of X-ray diffraction indicate that a phase transition from $L_{\beta'}$ to $L_\beta I$ structure in DPPC-MLV occurred at the same acetone concentration as detected by the excimer method (manuscript in preparation, Kinoshita et al.) – which supports the result of the excimer method.

Ethanol is well known for a reagent which induces a $L_\beta I$ structure in DPPC-MLV [3–5]. As shown in Fig. 5, the E/M value rapidly decreased at 5.5%(w/v) ethanol in H_2O; 6.4%(w/v) ethanol in D_2O, and the E/M value became very low above 6.5%(w/v) in H_2O; 7.5%(w/v) in D_2O.

3.4. Interaction of propionaldehyde and tetrahydrofuran with DPPC-MLV in H_2O and D_2O

We have investigated the interaction of other organic solvents (such as, propionaldehyde and tetrahydrofuran) with DPPC-MLV by the excimer method. As shown in Figs. 6 and 7, the E/M values rapidly decreased at 3.5%(w/v) propionaldehyde in H_2O; 3.7%(w/v) tetrahydrofuran in H_2O. The E/M values became very low above 4.5%(w/v) propionaldehyde in H_2O; 5.0%(w/v) tetrahydrofuran in H_2O. These results indicate that a phase transition from $L_{\beta'}$ to $L_\beta I$ structure of DPPC-MLV in H_2O occurred at 3.5%(w/v) propionaldehyde and 3.7%(w/v) tetrahydrofuran. Figs. 6 and 7 clearly show that the threshold concentrations of propionaldehyde and tetrahydrofuran for the induction of $L_\beta I$ structure in D_2O were higher than those in H_2O.

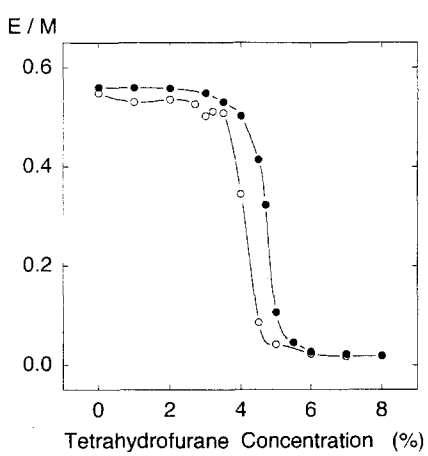

Fig. 7. Ratio of excimer to monomer fluorescence intensities (E/M) of pyrene-PC in DPPC-MLV in H_2O (○) and D_2O (●) in various concentrations of tetrahydrofuran (%(w/v)) at 20°C, respectively.

Table 1
Threshold concentrations of five organic solvents for the induction of interdigitated gel phase in DPPC-MLV in H_2O and in D_2O

	H_2O	D_20
Acetone	9.4%(v/v)	12%(v/v)
Acetonitrile	5.1%(v/v)	6.0%(v/v)
Ethanol	5.5%(w/v)	6.4%(w/v)
Propionaldehyde	3.5%(w/v)	4.2%(w/v)
Tetrahydrofuran	3.7%(w/v)	4.5%(w/v)

These values were determined by the results of the excimer method.

In Table 1, the threshold concentrations of five organic solvents for the induction of $L_\beta I$ structure in DPPC-MLV in H_2O and in D_2O are summarized.

4. Discussion

4.1. Mechanism of the phase transition from $L_{\beta'}$ to $L_\beta I$ phase in DPPC-MLV induced by organic solvents

The results of the X-ray diffraction and the excimer method, clearly indicate that several water-soluble organic solvents; such as, acetonitrile, acetone, propionaldehyde, and tetrahydrofuran, induce the phase transition from $L_{\beta'}$ to $L_\beta I$ phase in DPPC-MLV above their threshold concentrations. A mechanism of the induction of the $L_\beta I$ phase may be explained by using a concept of the 'χ parameter'.

The χ parameter is an interaction energy parameter, which can describe well thermodynamic properties of macromolecules in solvents [22,23]. It is a dimension-less parameter defining an interaction energy between solvents and solutes (or segments of polymer);

$$\chi = \Delta G / 2 k_B T \quad (1)$$

where ΔG is a free energy increase associated with the contact of segments with solvent, or a free energy decrease associated with the contact between segments, k_B and T are Boltzmann constant and absolute temperature, respectively. In good solvents where χ is small, solvent molecules interact with polymer segments favorably, and thereby, solubility of the polymer is large and the polymer in solution swells. On the other hand, in poor solvents where χ is large, the interaction energy between solvents

and segments of the polymer becomes large, and thereby, the solubility of the polymer is small, and the polymer in solution shrinks.

Terminal methyl groups of the alkyl chains of the phospholipids of DPPC-MLV are exposed to water in the $L_\beta I$ phase, and to alkane (alkyl chains) in the $L_{\beta'}$ phase. In the absence of such organic solvents, the χ parameter of the segment of the alkyl chain in the $L_\beta I$ structure – which is mainly determined by the terminal methyl group of the alkyl chain – is large (that is, water is a poor solvent for the segment of the alkyl chains), because the solubility of alkane in water is very low, and the Gibbs free energy of transfer of alkane from the same alkane into water is a large positive value [1]. Hence, the chemical potential of the membrane in the $L_{\beta'}$ phase ($\mu(L_{\beta'})$) is lower than that in the $L_\beta I$ phase ($\mu(L_\beta I)$) – that is, $\Delta\mu = \mu(L_\beta I) - \mu(L_{\beta'}) > 0$. This is why DPPC-MLV in water is not in the $L_\beta I$ phase but in the $L_{\beta'}$ phase.

In the presence of such organic solvents in water, these molecules (solvents) partition into an interfacial region between water and the segment of the terminal alkyl chains of the membrane in the $L_\beta I$ phase. The solubility data showed that these organic solvents have a high solubility of alkane such as hexane; which indicates that the χ parameter of the alkane is small in these organic solvents. Thereby, the χ parameter of the segment of the alkyl chain in the $L_\beta I$ phase may decrease with an increase in concentration of such solvents. Therefore, the difference in chemical potential of the membrane in the two phases ($\Delta\mu = \mu(L_\beta I) - \mu(L_{\beta'})$) would decrease with increasing concentrations of these solvents, and above critical concentrations, $\Delta\mu$ is negative, and thereby, the $L_\beta I$ phase may be induced. Hence, the decrease of χ parameter between the segments of the terminal alkyl chain of the phospholipid and solvents by addition of these solvents, is one of the main reasons of the induction of the $L_\beta I$ structure.

Partition of these substances into the head group regions of the membrane in the $L_{\beta'}$ phase (which may increase an excluded volume of the head group) may induce a mismatch between the areas (cross sections) of the head group and the alkyl chain [4,17,18,24]. This may increase a repulsive interaction between the head groups, and thereby, increase $\mu(L_{\beta'})$. This steric effect has been considered one of the main reasons of the induction of the $L_\beta I$ structure [4,17,18,24], and also, in our cases, may be one of the main reasons. However, contributions of these effects – the steric effect and the χ parameter effect – to the induction of the $L_\beta I$ structure, may depend on the type of interaction of phospholipids and substances. In the case of DPPC and ethanol, the steric effect may be a dominant factor of the induction of the $L_\beta I$ structure [24]. We believe, however, that further experimental and theoretical investigations to elucidate the contributions of these effects to the induction of the $L_\beta I$ structure, are necessary in each case.

Ethylene glycol (EG) induces the $L_\beta I$ phase in DMPC-, DPPC-, DSPC-MLVs [7]. The Gibbs free energy of transfer of alkane from the same alkane into EG is a smaller positive value than that from the same alkane into water [1]. This result suggests that the χ parameter between the segments of the terminal alkyl chain and EG is smaller than that between the segments and water. Consequently, a decrease in the χ parameter by addition of EG may be one of the main reasons of the induction of the $L_\beta I$ phase. We also investigated the effects of oligomers of EG on thermotropic phase transitions of DPPC-MLV, and showed that a diethylene glycol, a dimer of EG, induced a $L_\beta I$ phase, but oligomers of EG larger than the trimer (triethylene glycol) did not induce a $L_\beta I$ phase, and increased the main transition temperature [8]. Polymer of EG, i.e., poly(ethylene glycol), did not induce the $L_\beta I$ phase and increased main transition temperature [7]. The oligomers of EG have the same chemical properties as EG, and thereby, the χ parameters of alkane in the oligomers of EG may be almost the same values. However, the oligomers of EG – including poly(ethylene glycol) – cannot penetrate into the interfacial region between the segments of the terminal alkyl chains and water in the $L_\beta I$ phase, since they are large molecules. Hence, the χ parameter of the segments of the terminal alkyl chain of the membrane in the $L_\beta I$ phase, does not decrease with an increase in their concentrations, and thereby, they do not induce the $L_\beta I$ phase.

Based on these results, we propose a new hypothesis that water-soluble organic molecules (which have a high solubility for alkane, and also can penetrate the interfacial region between the segments of the terminal alkyl chain and water in the $L_\beta I$ phase or (and) the interfacial region between the head groups in the $L_{\beta'}$ phase) can induce the $L_\beta I$ phase. A small size of the hydrophilic part of the molecules and no repulsive interaction of the molecules with the interfacial region, may be important factors for the latter condition. Alcohols such as ethanol, EG, acetonitrile, acetone, propionaldehyde, and tetrahydrofuran, meet these conditions, and thereby, induce the $L_\beta I$ phase.

4.2. Effect of deuterium oxide (D_2O) on the phase transition from $L_{\beta'}$ to $L_\beta I$ phase in DPPC-MLV induced by organic solvents

Threshold concentrations of all the organic solvents used in this report for the phase transition from the $L_{\beta'}$ to the $L_\beta I$ phase in DPPC-MLV in deuterium oxide (D_2O), were higher than those in water (H_2O). These results may be explained by considering the chemical potential of the membrane in the $L_\beta I$ phase as follows.

As discussed previously, the Gibbs free energy of transfer of alkane from the same alkane into water, ΔG, is a large positive value [1]. Contributions of entropy change (ΔS) and enthalpy change (ΔH) of the transfer of alkane from the same alkane into water, to this ΔG, are strongly temperature-dependent. Around room temperature (25°C), ΔS has a large negative value and ΔH is negligibly small,

and thereby, $\Delta G \approx -T\Delta S$. This large negative entropy is considered to be due to an increase in the ordering of water molecules around the alkane by their hydrogen bonding [1,25]. This is the most important factor in the hydrophobic interaction around room temperature. As the temperature increases, the absolute values of ΔS decrease and those of ΔH increase. At high temperatures around 140°C, ΔS is negligibly small and ΔH has a large positive value, and thereby, $\Delta G \approx \Delta H$ [25–27].

Our experiments in this report were done at 20°C, and thereby, ΔG is mainly determined by the ΔS. Deuterium bond in deuterium oxide (D_2O) is stronger than hydrogen bond in water (H_2O) [28–32], and thereby, ΔG from the alkane to deuterium oxide is larger than ΔG from the alkane to water, and the substitution of H_2O by D_2O enhances the hydrophobic interaction [31]. Hence, the difference in chemical potential of the membrane in the two phases [$\Delta\mu = \mu(L_\beta I) - \mu(L_{\beta'})$] in D_2O is larger than that in H_2O. As discussed previously, $\Delta\mu$ decreases with an increase in concentrations of the organic solvents, and at the critical concentrations, $\Delta\mu$ becomes zero. The larger $\Delta\mu$ is, the higher concentrations of the organic solvents are necessary for the induction of the $L_\beta I$ phase. This is why the substitution of H_2O by D_2O increases the threshold concentrations for the induction of the $L_\beta I$ phase. Ohki observed that phase transition temperature of dihexadecylphosphatidylcholine between $L_\beta I$ phase and ripple phase, was lower in D_2O than in H_2O, and pointed out an important role of the hydrophobic interaction in the induction of the $L_\beta I$ phase [31]. His result supports our mechanism mentioned above.

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