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Effects of solvents interacting favorably with hydrophilic segments of the membrane surface of phosphatidylcholine on their gel-phase membranes in water

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Abstract

We have investigated the effects of two kinds of solvents forming the lamellar liquid-crystalline (L_α) phase in phosphatidylcholine (PC) membranes in neat condition, such as formamide and 1,3-propanediol, on phase behaviors of multilamellar vesicle (MLV) of DPPC (DPPC-MLV). These solvents induced the interdigitated gel ($L_\beta I$) phase in DPPC-MLV in excess water above their critical concentrations. Solubility measurement indicates that these solvents interact favorably with the hydrophilic segment of the PC membrane but interact unfavorably with the alkyl chains. Based on these results, we propose the mechanism of the induction of the $L_\beta I$ phase by these solvents.

Keywords: Formamide; 1,3-propanediol; Excimer method; Phase transition; Interdigitated gel structure; Interaction free energy

1. Introduction

It is well known that diacylphosphatidylcholine (PC) such as DPPC (dipalmitoylphosphatidylcholine) can form the interdigitated gel ($L_\beta I$) phase in the presence of several alcohols such as ethanol [1–4]. Recently, we have shown that water-soluble organic solvents other than alcohols, such as acetone, acetonitrile, propionaldehyde, and tetrahydrofurane, also induce the $L_\beta I$ phase in DPPC-MLV at their low concentrations [5]. This result

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demonstrates that a specific interaction of alcohols with phospholipid membranes is not important in the induction of the $L_\beta I$ phase. Based on this result, we have proposed the mechanism of the induction of $L_\beta I$ phase in PC-MLV that water-soluble organic solvent molecules having two properties induce the $L_\beta I$ phase. One condition of this kind of solvent is a high solubility for alkane, and the other is its ability of penetration into the interfacial regions between the segments of the terminal alkyl chain and water in the $L_\beta I$ phases. In the presence of such solvent molecules (we shall call them 'type A' solvent), the interaction free energy between the terminal alkyl chain and solvents in the $L_\beta I$ phase decreases. Hence, it decreases the chemical potential of the lipid in the membranes at the $L_\beta I$ phase, and above the critical concentration, the $L_\beta I$ phase in the membrane is induced.

However, it is also known that different kinds of water-soluble solvents, such as glycerol and ethylene glycol, which have low solubilities for alkane, induce the $L_\beta I$ phase in PC membranes above critical concentrations [6–8]. These solvents don't meet the above condition for the induction of the $L_\beta I$ phase, and thereby, a different factor should be important in the induction of the $L_\beta I$ phase. These solvents are also known to form a lamellar liquid-crystalline (L_α) phase in soybean PC membrane at room temperature and in DPPC membrane at 54°C in neat condition (i.e. in 100% solvent without water) [9,10]. Do these apparently different phenomena have a connection?

Other solvents such as formamide and 1,3-propanediol are also known to form the L_α phase in egg PC and dioleoylphosphatidylcholine (DOPC) membranes in neat condition [11,12]. In this report, we have investigated effects of these solvents on phase behaviors of multilamellar vesicle (MLV) of DPPC (DPPC-MLV). We have found that these solvents induced the $L_\beta I$ phase above their critical concentrations, and also that they interact favorably with the hydrophilic segment of the PC headgroup. Based on these results, we propose the mechanism of the induction of the $L_\beta I$ phase by these solvents.

2. Materials and methods

1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) was purchased from Avanti Polar Lipids Inc. Formamide was purchased from Wako Chemical Co. 1,3-propanediol and phosphorylcholine (chloride calcium salt) were purchased from Sigma Chemical Co. 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phospho-choline (pyrene-PC) was purchased from Molecular Probes Inc. Multilamellar vesicles (MLVs) were prepared by adding the appropriate amounts of water-organic solvent (formamide or 1,3-propanediol) mixtures of various concentrations of the organic solvent to the dry lipids in excess mixture solvents (7 wt.% lipid), and the suspension was vortexed for 30 s, at approximately 55°C several times. X-ray diffraction experiments were performed by using Nickel filtered Cu Kα X-radiation ($\lambda = 0.154$ nm) from a rotating anode type X-ray generator (Rigaku, Rotaflex, RU-300). Detail of the experimental method was described in our previous paper [5,7].

The method and theory of the excimer method developed by us are described in detail in our previous paper [13]. For fluorescence measurement, a Hitachi F3000 spectrofluorimeter was used. The excitation wavelength of pyrene PC was 347 nm and emission wavelengths were 376 nm for monomer fluorescence and 481 nm for excimer fluorescence. The ratio of excimer to monomer fluorescence intensities (E/M) was calculated. The pyrene PC concentration in total phospholipids was 2.5 mol%. The concentrations for DPPC in the samples for the measurement of the fluorescence were 80 μM, determined by the standard phosphate analysis [14]. For measurement of the solubility of phosphorylcholine in 100% organic solvents other than formamide at 25°C, its concentrations in saturated solutions were determined [15] by the standard phosphorus analysis [14]. In the case of formamide, its solubility was determined by the weight of unsolubilized powder in saturated solution [16] to prevent the decomposition of formamide in the phosphorous method for safety. The solubility determined by the latter method is overestimated [16].

3. Results

At first, we have investigated effect of the presence of formamide in water on phase behavior and structure of DPPC-MLV by SAXS and WAXS. As shown in Fig. 1a, the spacing (d_1) of DPPC-MLV at 20°C rapidly decreased from 6.5 to 5.0 nm at 38% (v/v) formamide. A WAXS pattern of DPPC-MLV at 0% formamide at 20°C consisted of a sharp reflection at 0.42 nm and a diffuse broad reflection centered at 0.41 nm, indicating $L_{\beta'}$ structure. On the other hand, WAXS patterns above 42% formamide showed a symmetrical sharp peak at 0.41 nm showing that alkyl chains of the DPPC membrane were packed in a hexagonal arrangement without any inclination. Electron density profiles of DPPC-MLVs at high concentrations of formamide could not be determined due to weak intensities of the higher-order diffraction peaks in their SAXS patterns. Fluorescence intensity ratio E/M values of pyrene-PC in DPPC-MLV rapidly decreased at 40% (v/v) formamide, and became very low above 50% (v/v) (Fig. 1a). As indicated in the previous paper [5,13], the E/M value of pyrene-PC in phospholipid membranes in the $L_\beta I$ structure is much lower than that in the $L_{\beta'}$ structure, because structural restriction of the $L_\beta I$ phase largely decreases the frequency of the collision of pyrene molecules in the membrane. Fig. 1a shows that a rapid decrease of the E/M value occurred at the same concentration as the rapid decrease in the spacing, indicating that an $L_{\beta'}$ to $L_\beta I$ phase transition occurred in DPPC-MLV approximately 40% (v/v) formamide.

As another example, effects of the presence of 1,3-propanediol in water on phase behavior of DPPC-MLV by the same methods. As shown in Fig. 1b, the spacing (d_1) of DPPC-MLV at 20°C rapidly decreased from 6.6 to 5.2 nm at 35% (v/v) 1,3-propanediol. WAXS patterns of DPPC-MLV above 45% (v/v) 1,3-propanediol showed a symmetrical sharp peak at 0.41 nm. Electron density profiles of DPPC-MLVs at high concentrations of 1,3-propanediol could not be determined due to weak intensities of the higher-order diffraction peaks in their SAXS patterns. As shown in Fig. 1b, the E/M value rapidly decreased at 35% (v/v) 1,3-propanediol, and became very low above 50% (v/v). These results indicated that an $L_{\beta'}$ to $L_\beta I$ phase transition occurred in DPPC-MLV at 35% (v/v) 1,3-propanediol.

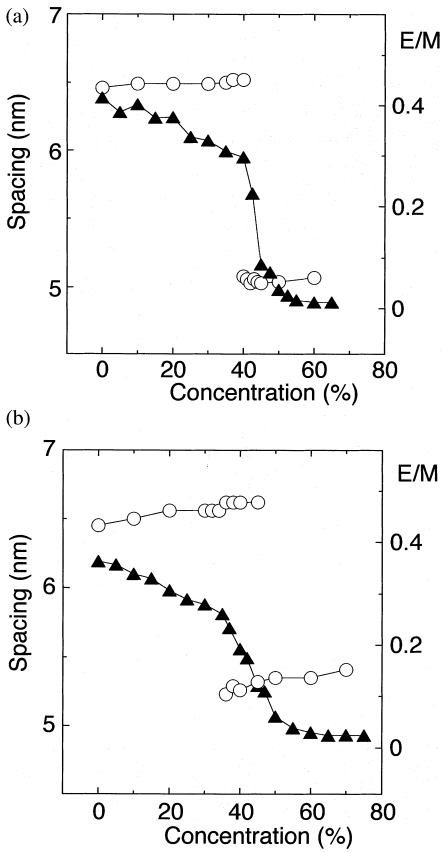

Fig. 1. Lamellar repeat period (spacing) of DPPC-MLV (O) and ratio of excimer to monomer fluorescence intensities (E/M) of pyrene-PC in DPPC-MLV (and) in various concentrations of (a) formamide [% (v/v)] and (b) 1,3-propanediol [% (v/v)] at 20°C.

To get information about the interaction free energy between the hydrophilic segments of PC membranes such as DPPC-MLV and these solvents, we measured the solubility of phosphorylcholine in pure solvents. The phosphorylcholine molecule has the same molecular structure as the hydrophilic segment of PC, and thereby, it represents the hydrophilic segment of the membrane surface of PC-MLV. High solubility means that the interaction free energy between the segment and solvent is low [5,15,16]. Table 1 shows that its

solubility in formamide was higher than that in water, and that its solubility in 1,3-propanediol was relatively high. Its solubilities in ethylene glycol or glycerol were almost the same as that in water. On the other hand, its solubility in acetone was much lower than that in water. These results indicate that formamide, ethylene glycol, glycerol, and 1,3-propanediol are good solvents for the hydrophilic segment of the PC membrane, and thereby, the interaction free energy between these solvents and the hydrophilic segments are low. On the other hand, solubilities of hexane, a kind of alkane, in these solvents were low (Table 1). For example, less than 0.6% (v/v) hexane completely dissolved in formamide and less than 1% (v/v) hexane completely dissolved in 1,3-propanediol. These values are extremely small compared with its solubility in acetone, ethanol, tetrahydrofurane, acetonitrile and propionaldehyde [5]. Free energies of transfer of hexane from type B solvents in Table 1 (formamide, 1,3-propanediol, ethylene glycol, and glycerol) to pure liquid hexane, ΔG_{tr}, are the same order as ΔG_{tr} (H_2O) from water to the liquid hexane, but their absolute values are a little smaller than that of ΔG_{tr} (H_2O).

4. Discussion

The results of Fig. 1 clearly show that formamide and 1,3-propanediol can induce the $L_\beta I$ phase in DPPC-MLV at high concentrations, as well as glycerol and ethylene glycol. These solvents are well known to form the L_α phase in PC membranes such as egg PC in neat condition [9–12]. Solubility measurements indicate that these are good solvents for the hydrophilic segment of the head group of PC membrane, and thereby, they interact favorably. On the other hand, these solvents have low solubility for alkane, and thereby, the interaction free energy between these solvents and alkane (or alkyl chains) is high. We shall call them 'type B' solvent. When PC lipids are suspended in neat type B solvents (100% pure solvents without water) under the condition forming L_α phase membranes, dry PC lipids swell in these liquids to increase the contact area between the hydrophilic segments and solvents. However, it also increases the contact of the alkyl chains with the solvents, increasing the chemical potential of the lipid, since the interaction free energy between these solvents and alkane (or alkyl chains) is high. In order to decrease the

Table 1
Solubility of phosphorylcholine and hexane in various kinds of solvents[a]

	Type	Solubility of phosphoryl-choline method (a)[b]	Solubility of phosphoryl-choline method (b)[c]	Solubility of hexane	Transfer free energy ΔG_{tr}[d]	Critical conc. for the induction of $L_\beta I$ phase[e]
Formamide	B	N.D.	2×10^3 mM	0.6% (v/v)	−20 kJ/mol	40% (v/v)
1,3-propanediol	B	8.2×10 mM	3×10^2 mM	1% (v/v)	−10	35% (v/v)
Ethylene glycol	B	7.5×10^2	1×10^3 mM	0.2% (v/v)	−20	36% (w/v) [7]
Glycerol	B	6.5×10^2	1×10^3 mM	< 0.1% (v/v)	< −20	82% (v/v) [6]
Water		8.2×10^2	1×10^3 mM	N.D.	−30 [17]	
Acetone	A	5.0×10^{-3} [15]	N.D.	Completely mix [5]	N.D.	9.4% (v/v) [5]
Ethanol	A	N.D.	N.D.	Completely mix [5]	N.D.	5.5% (v/v) [5]
Tetrahydrofuran	A	N.D.	N.D.	Completely mix [5]	N.D.	3.7% (v/v) [5]
Propionaldehyde	A	N.D.	N.D.	50% (v/v) [5]	N.D.	3.5% (v/v) [5]

[a] N.D.: not determined.
[b] Method (a): its concentrations in saturated solutions were determined by the standard phosphorus analysis.
[c] Method (b): its solubilities were determined by the weight of unsolubilized powder in saturated solution.
[d] ΔG_{tr}: free energy for transfer of hexane from various solvents to pure liquid hexane at 25°C based on the solubility measurement of hexane by $\Delta G_{tr} = RT \ln X$, where X is the saturated concentration of hexane in solvents (in mol fraction units), R the gas constant, and T the absolute temperature.
[e] Critical concentrations of the solvents for the induction of interdigitated gel phase in DPPC-MLV in excess water.

contact area between the alkyl chains and solvents, the lipids form the bilayer structure membranes in L_α phase (solvophobic interaction). This phenomenon is similar to the bilayer formation of the PC lipid in water. Tanford [17] already pointed out that glycerol and ethylene glycol have a strong solvophobic effect on the alkane. On the other hand, PC lipids such as DOPC dissolve in neat type A solvents, other than acetone due to their high solubilities of the liquid alkane and thereby they cannot form the membrane. Acetone has a very low solubility of DOPC, and thereby, a precipitation of DOPC occurs in neat acetone. The structure of the precipitation is not clear since its SAXS pattern consisted of one peak (unpublished results; Li et al.).

In the formation and stability of the $L_\beta I$ phase, the repulsive interaction between the head groups of phospholipid play an important role [18]. When DPPC lipids are suspended in a mixture of water and the type B solvents under the condition forming the gel ($L_{\beta'}$ or L_β) phase membranes, these solvent molecules can penetrate into the interfacial regions composed of the head groups and solvents to contact with the hydrophilic segments, since the interaction free energy between the solvents and the segment is low. As the concentration of type B solvents in bulk phase increases, the number of these solvent molecules interacting with the hydrophilic segments increases, inducing an increase in the effective volume of the head group region. As a result, the repulsive interaction between the headgroups of the PC lipids in the $L_{\beta'}$ phase increases. On the other hand, in the $L_\beta I$ phase, an increase in the repulsive interaction between the headgroups of the PC lipids is smaller than that in the $L_{\beta'}$ phase, because the area per head group in the $L_\beta I$ phase is much larger than that in the $L_{\beta'}$ phase. Above a critical concentration, the large repulsive interaction between the headgroups induces the $L_{\beta'}$ to $L_\beta I$ phase transition. Table 1 shows that the critical concentrations of the type B solvents for the induction of the $L_\beta I$ phase in DPPC-MLV are much higher than those of the type A solvents. Shchipunov and Shumilina [19] indicated that several solvents such as glycerol, formamide, and ethyleneglycol bind to the phosphate groups of the PC lipid via a hydrogen bond in egg PC — heptane — these solvents (low mol fraction) ternary systems by IR spectroscopy. This supports our results that type B solvents are good solvents for the hydrophilic segment of the PC, and thereby, increase the repulsive interaction between the DPPC headgroups in the $L_{\beta'}$ phase. Moreover, the solubilities of alkane in the type B solvents are a little higher that that of water, which decreases the interaction free energy between the terminal alkyl chain and solvents in the $L_\beta I$ phase. This may also contribute a little the formation of the $L_\beta I$ phase by the same mechanism for the type A solvent-induced $L_\beta I$ phase.

5. Nomenclature

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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