Phase transition between hexagonal II (H_{II}) and liquid-crystalline phase induced by interaction between solvents and segments of the membrane surface of dioleoylphosphatidylethanolamine

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Received 6 May 1997; revised 27 June 1997; accepted 4 July 1997

Abstract

We have investigated effects of several water-soluble organic solvents such as acetone, acetonitrile, and ethanol, which also have high solubility in alkane, on the structure and phase behavior of dioleoylphosphatidylethanolamine (DOPE) dispersion. X-ray diffraction data indicated that a phase transition from hexagonal II (H_{II}) to liquid-crystalline (L_{a}) phase in DOPE dispersion, occurred at 13% (v/v) acetone in H_{2}O at 20°C. The temperature of the L_{a}-H_{II} phase transition of DOPE dispersion increased with an increase in acetone concentration, and it was 37°C at 20% (v/v) acetone. These results indicated that acetone stabilized L_{a} phase relative to H_{II} phase. Similar results were obtained in interactions of DOPE dispersions in H_{2}O with acetonitrile or ethanol. X-ray diffraction data indicated that the H_{II}-L_{a} phase transition occurred at 9.0% (v/v) acetonitrile or at 9.8% (v/v) ethanol in water at 20°C. The L_{a}-H_{II} phase transition temperature of DOPE dispersion increased with an increase in acetonitrile or ethanol concentration, and it was 66°C at 20% (v/v) acetonitrile. Substitution of H_{2}O by D_{2}O (deuterium oxide) increased their threshold concentrations of the H_{II}-L_{a} phase transition induced by these organic solvents. A mechanism of these phase transitions and the effect of the substitution of H_{2}O by D_{2}O is proposed and discussed; an interaction free energy between solvents and the hydrophobic segments of the alkyl chains in the membrane surface, and also a packing parameter of the phospholipid may be main factors to explain these phenomena reasonably. © 1997 Elsevier Science B.V.

Keywords: Hexagonal II phase; Liquid-crystalline phase; Phase transition; Dioleoylphosphatidylethanolamine; Deuterium oxide; χ Parameter

1. Introduction

The inverted hexagonal (H_{II}) phase has attracted much attention, not only because this nonbilayer phase has been postulated to play an important biological role in membrane fusion and transport functions, but also the phase transition between H_{II} phase and liquid-crystalline (L_{a}) phase (Fig. 1) in itself has been a very interesting subject for understanding mechanisms of the lipid polymorphism [1-8]. Espe-
Fig. 1. Schematic representation of $H_{II}$ (a) and $L_{α}$ (b) phospholipid phases. Lipid headgroups are represented by black circles attached to two melted hydrocarbon chains. The distance, $d$, is the ordinate for Figs. 2 and 4.

Specially, the $H_{II}$ phases of various kinds of phosphatidylethanolamine (PE) have been extensively studied. As temperature increases, they undergo gel-$L_{α}$ phase transitions, and after that $L_{α}-H_{II}$ phase transition [9,2]. Several substances have been shown to influence their polymorphic phase behavior; high concentration of NaCl [9], sugars such as sucrose and trehalose [10], and alkanes [11] decreased the temperature of the $L_{α}-H_{II}$ phase transition. On the other hand, short-chain alcohols such as methanol [12], chaotropic ions such as NaSCN [13], increased the $L_{α}-H_{II}$ phase transition temperature, and stabilize the $L_{α}$ phase.

Recently, we have proposed that the interaction free energy of the segments of the membrane surface of biomembranes and phospholipid membranes with solvents, played an important role in structures and phase behaviors of these membranes [14,15]. In good solvents, where the interaction between the segments and the solvents is favorable, the segments swell to contact with the solvents, and the structure where they contact each other is stable; on the other hand, in poor solvents, where their interaction is unfavorable, the segments shrink or associate each other to prevent the contact with the solvents, and the structure where the segments contact with the solvents is unstable. The induction of interdigitated gel phase in dipalmitoylphosphatidylcholine (DPPC)-MLV by several water-soluble organic solvents such as acetone, acetonitrile, and ethanol may be explained by this interaction free energy [14].

In this report, we have investigated effects of several water-soluble organic solvents— which also have high solubility in alkane such as hexane—on structures and phase behavior of dioleoylphosphatidylethanolamine (DOPE) dispersion. DOPE in excess water at $20^°C$ is in $H_{II}$ phase, and its $L_{α}-H_{II}$ phase transition temperature is $5^°C$ [11]. We have selected three organic solvents; acetone, acetonitrile, and ethanol. We expect that these solvents are good solvents for the alkyl chains, and thereby, they may stabilize the liquid-crystalline ($L_{α}$) phase, because a recent physical picture of the $L_{α}$ phase gives a dynamic motion of the membrane surface such as protrusion and undulation, and thereby, the alkyl chains contact with water [16–18]. By using the X-ray diffraction method, we have found that these organic solvents induced $H_{II}-L_{α}$ phase transitions. We propose a mechanism of the $H_{II}-L_{α}$ phase transitions induced by these organic solvents. This research was presented at the 34th Annual Meeting of the Biophysical Society of Japan [19].

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) was purchased from Avanti. Acetone, acetonitrile, ethanol, and deuterium oxide (purity > 99.75%) were purchased from Wako.

2.2. Sample preparations

DOPE suspensions were prepared by adding the appropriate amounts of water (or $D_2O$) containing a given concentration of organic solvents (such as ace-
tone, acetonitrile, and ethanol) to dry lipids in excess solvents (7 wt% lipids), and a suspension was vortexed for about 30 s at RT (~ 20°C) several times, and after that it was sonicated in a bath-type sonicator for 10 s twice. For measurement of X-ray diffraction, pellets after centrifugation (14000 × g, 1 h at 20°C, Tomy, MR-150) of the suspension were used.

2.3. X-ray diffraction

X-ray diffraction experiments were performed by using Nickel filtered CuKα X-radiation (λ = 0.154 nm) from rotating anode type X-ray generator (Rigaku, Rotaflex, RU-300, 50 kV × 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 m 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 ± 0.2°C [20, 15].

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°C min⁻¹. Main transition temperature was determined as an onset of an endothermic transition extrapolated to the baseline. A detail method was described in our previous paper [20].

3. Results

3.1. Interaction of aqueous organic solvents (acetone, acetonitrile, and ethanol) with DOPE dispersion

In our previous paper [14], we indicated that water-soluble organic solvents such as acetone, acetonitrile, and ethanol have high solubility in alkane such as hexane. In this paper, we have investigated an interaction of these organic solvents with DOPE dispersion.

At first, we have investigated effects of acetone on a phase behavior and structure of DOPE (di-oleoylphosphatidylethanolamine) dispersions by X-ray diffraction. SAXS data of the DOPE dispersion in water at 20°C showed that a set of SAXS peaks had spacings in the ratio of 1:√3:2:√7:3···, indicating that it was in two-dimensional hexagonal (H₃) phase (Fig. 1(a)) [11, 5]. Below 16% (v/v) acetone concentration, DOPE dispersions had a similar set of SAXS peaks and, thus, they were in H₃ phase. The basis vector length of the H₃ phase (center-to-center distance of adjacent cylinders), d, calculated by $d = (2/√3) \times x$ (x is the spacing in the SAXS), gradually decreased from 7.8 to 7.4 nm with an increase in acetone concentration from 0 to 16% (v/v) (Fig. 2(a)). At 13% (v/v) acetone, a new set of SAXS peak with a shorter spacing (5.1 nm) was superimposed on the H₃ peaks; the new set had spacings in the ratio of 1:2:3···, which is consistent with a lamellar liquid-crystalline (Lₐ) phase (Fig. 1(b)). By 18% (v/v), the H₃ peaks was essentially gone, and the Lₐ peaks had grown in intensity. Wide-angle reflections of DOPE dispersions in high concentration (above 18% (v/v)) of acetone consisted of a diffuse broad band around 0.44 nm, which supports that they were in Lₐ phase. Hence, this structural

![Fig. 2. Basis vector length, d, of DOPE dispersion in various concentration (%(v/v)) of acetone (a) and acetonitrile (b) at 20°C. d is defined in Fig. 1.](image-url)
change is due to a phase transition from \( H_{II} \) to \( L_{\alpha} \) phase. Between 13\% and 16\%, both the phases coexisted. Above 16\%, only the \( L_{\alpha} \) phase existed and the spacing gradually decreased with an increase in acetone concentration.

Next, we have investigated effects of other water-soluble organic solvents such as acetonitrile and ethanol on a phase behavior and structure of DOPE dispersions by X-ray diffraction. SAXS data of DOPE dispersions in water-acetonitrile mixture at 20°C showed that below 9.0\% (v/v) acetonitrile concentration, a set of SAXS peaks had spacings in the ratio of 1:3:2:3:2:3:1:2, indicating that the MLVs were in \( H_{II} \) phase. The basis vector length of the \( H_{II} \) phase \((d)\) gradually decreased from 7.8 to 7.6 nm with an increase in acetonitrile concentration from 0 to 9.0\% (v/v) (Fig. 2(b)). At 9.0\% (v/v) acetonitrile, a new set of SAXS peak with a shorter spacing (5.2 nm) was superimposed on the \( H_{II} \) peaks; the new set had spacings in the ratio of 1:2:3:1:2, which is consistent with the \( L_{\alpha} \) phase. Above 9.0\% (v/v) acetonitrile, only the \( L_{\alpha} \) phase existed and the spacing gradually decreased from 5.2 to 4.7 nm with an increase in acetone concentration. Hence, this structural change at 9.0\% (v/v) is due to a \( H_{II} \)-\( L_{\alpha} \) phase transition. Similarly, ethanol induced a \( H_{II} \)-\( L_{\alpha} \) phase transition in DOPE suspension at 20°C at 9.8\% (v/v) (data not shown).

The dispersion of DOPE in excess water is known to display a phase sequence of \( L_{\alpha} \rightarrow L_{\alpha} \rightarrow H_{II} \) during heat scans [11,1]. DOPE in excess water at 20°C is in the \( H_{II} \) phase, and its \( L_{\alpha} \)-\( H_{II} \) phase transition temperature is 5°C [11]. We have investigated a dependence of the \( L_{\alpha} \)-\( H_{II} \) phase transition temperature \( (T_h) \) on these organic solvents concentration by DSC. As shown in Fig. 3, \( T_h \) of DOPE rapidly increased with an increase in their concentrations. For example, \( T_h \) was 37°C at 20\% (v/v) acetone, and 66°C at 20\% (v/v) acetonitrile, which was more than 60°C higher than \( T_h \) in \( H_2O \).

### 3.2. Effect of deuterium oxide on the \( H_{II} \)-\( L_{\alpha} \) phase transition in DOPE dispersion induced by these organic solvents

We have investigated interactions of these organic solvents (acetone, acetonitrile, and ethanol) in deuterium oxide with DOPE dispersions at 20°C by SAXS, and compared results in deuterium oxide \((D_2O)\) with those in water \((H_2O)\). As shown in Fig. 4, a \( H_{II} \)-\( L_{\alpha} \) phase transition at 19\% (v/v) acetone in \( D_2O \) occurred, and it also occurred at 11.0\% (v/v) acetonitrile in \( D_2O \). Similarly, ethanol induced a
Table 1
Threshold concentrations of three organic solvents for the induction of L\textsubscript{\alpha} phase in DOPE dispersions in H\textsubscript{2}O and D\textsubscript{2}O.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>H\textsubscript{2}O (%)</th>
<th>D\textsubscript{2}O (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>13%</td>
<td>19%</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>9.0%</td>
<td>11.0%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>9.8%</td>
<td>11.0%</td>
</tr>
</tbody>
</table>

These values were determined by the SAXS.

H\textsubscript{II}-L\textsubscript{\alpha} phase transition in DOPE suspension in D\textsubscript{2}O at 20°C at 11.0% (v/v) (data not shown). In Table 1, the threshold concentrations of three organic solvents for the H\textsubscript{II}-L\textsubscript{\alpha} phase transition in DOPE suspension in H\textsubscript{2}O and in D\textsubscript{2}O are summarized.

These results show that the threshold concentrations of these three organic solvents for the H\textsubscript{II}-L\textsubscript{\alpha} phase transition in DOPE suspension in D\textsubscript{2}O, are higher than those in H\textsubscript{2}O.

4. Discussion

4.1. Mechanism of the H\textsubscript{II}-L\textsubscript{\alpha} phase transition in DOPE suspension induced by organic solvents

The results of the X-ray diffraction clearly indicate that several water-soluble organic solvents such as acetone, acetonitrile, and ethanol, induce the H\textsubscript{II}-L\textsubscript{\alpha} phase transition in DOPE suspension above their threshold concentrations. The results of DSC indicates that the L\textsubscript{\alpha}-H\textsubscript{II} phase transition temperatures ($T_\text{h}$) of DOPE increase with an increase in these organic solvents concentrations. These results indicates that these organic solvents stabilize the L\textsubscript{\alpha} phase relative to the H\textsubscript{II} phase. This stabilization may be explained as follows.

The $\chi$ parameter is an interaction free energy parameter, which can describe well the thermodynamic properties of macromolecules in solvents [21,22]. It is a dimensionless parameter defining an interaction energy between solvents and solutes (or segments of polymer):

$$\chi = \Delta G / 2k_B T,$$

where $\Delta G$ 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 $T$ the Boltzmann constant and absolute temperature, respectively. In good solvents where $\chi$ 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 $\chi$ is large, the interaction energy between solvents and segments of the polymer becomes large, and thereby, solubility of the polymer is small, and the polymer in solution shrinks. In our previous paper [14,15], we showed that the concept of this $\chi$ parameter could be applied to the physics of the biomembrane and the phospholipid vesicles, where the $\chi$ parameter is defined between solvents and segments of the membrane surface. In good solvents ($\chi$ is small), where the interaction between the segments and the solvents is favorable, the segments swell to contact with the solvents, and the structure where they contact each other is stable; on the other hand, in poor solvents ($\chi$ is large), where their interaction is unfavorable, the segments shrink or associate each other to prevent the contact with the solvents, and the structure where the segments make contact with the solvents is unstable. The induction of interdigitated gel phase in DPPC-MLV by several water-soluble organic solvents such as acetone, acetonitrile, and ethanol may be explained by the $\chi$ parameter [14].

In the phospholipid membrane in the L\textsubscript{\alpha} phase, the local out-of-plane motions of lipids, protrusion (amplitude of 0.2–0.3 nm [16,17]) cause roughness of the membrane surface, and thermal fluctuations can excite pronounced bending undulation of the membrane [16]. By using the joint refinement method of X-ray and neutron diffraction data, Wiener and White [18] clearly indicated that the interfaces of the phospholipid membrane of dioleoylphosphatidylcholine (DOPC), defined by the distribution of the water associated with the headgroups, are each about 1.5 nm thick and consist of a complex and thermally disordered mixture of water and headgroups and methylenes from the edges of the hydrocarbon core, supporting the dynamic structures of biomembranes described here. Hence, in the L\textsubscript{\alpha} phase, a small fraction of hydrophobic segments of the alkyl chains, as well as the hydrophilic segments of the head groups, faces water or solvents. Therefore, in the phospholipid membrane in L\textsubscript{\alpha} phase, we have to consider two kinds of segments of the membrane surface, and thereby, two kinds of interaction free energy between...
the surface segments of the phospholipid membrane and solvents (χ); one is a free energy of interaction between the hydrophilic segments of the head groups and solvents (χ1), and the other is a free energy of interaction between the hydrophobic segments of the alkyl chain and solvents (χ2). On the other hand, in the phospholipid membrane in HII or Lα phase, most of the hydrophobic segments of the alkyl chain do not face the aqueous phase, and thus, the χ parameter is determined by only χ1. As discussed in our previous paper [14], the χ parameter between the segment of the alkyl chain and water has a large positive value (that is, water is a poor solvent for the segment of the alkyl chain), because the solubility of alkane is very low, and the Gibbs free energy of transfer of the alkane into water has a large positive value [23]. In the presence of organic solvents such as acetone, acetonitrile, and ethanol, these molecules (solvents) partition into the interfaces of the membrane, which consist of a complex and thermally disordered mixture of water and headgroups and methylenes from the edges of the hydrocarbon core [18]. The solubility data showed that these organic solvents have a high solubility in alkane such as hexane; which indicates that the χ parameter of the alkane is small in these organic solvents [14]. Therefore, the χ2 parameter, representing a free energy of interaction between the hydrophobic segments of the alkyl chain and solvents, may decrease with an increase in concentration of such solvents, which may stabilize the Lα phase relative to the HII and Lβ phases. Hence, the difference of the chemical potential of the membrane in two phases; Δμ(Lα - HII) = μ(Lα) - μ(HII) or Δμ(Lα - Lβ) = μ(Lα) - μ(Lβ) may decrease with increasing concentrations of these solvents, and above critical concentrations, Δμ may decrease with increasing concentrations of these solvents, and above critical concentrations, Δμ is negative, and thus, the HII-Lα or the Lβ-Lα phase transition may be induced. Hence, the decrease of χ parameter between the segments of the alkyl chain of the phospholipid in the Lα phase and solvents by addition of these solvents, is one of the main reasons of the stabilization of the Lα phase and the HII-Lα phase transition.

Geometric packing properties have been considered an important factor for morphology and structures of phospholipid membranes [24-27,7]. Israelachvili has treated the lipid packing problem in terms of a critical packing parameter (v/a0l̄c) of the alkyl chains of the phospholipids, where v is the volume of their alkyl chains, l̄c the critical chain length, and a0 the optimal surface area per molecule, defined at the hydrocarbon-water interface, the size of which is derived from thermodynamic considerations. When v/a0l̄c = 1, lamellar structures such as Lα and Lβ phases are formed, whereas inverted curved structures such as HII phase and inverted micelle when v/a0l̄c > 1 [24,25]. Gruner et al. [26,28] have considered that the Lα-HII phase transition behavior was dominated by the competition between the tendency to curl the lipid monolayers to an intrinsic (or spontaneous) radius of curvature and opposing hydrocarbon packing constraints. Recently, Marsh [7] proposed a new geometric packing parameter (V/Al) of the whole phospholipid molecule, where V is the volume of the entire lipid molecule, l its length, and A the area of the lipid headgroup at the lipid–water interface, and showed that the intrinsic radius of curvature of lipid monolayer was expressed in terms of this new parameter. The advantage of this parameter is that it can be directly determined by structural measurements from X-ray diffraction, and also that dependence of the Lα-HII transition temperature (TII) on this packing parameter can be described, which shows that a decrease in packing parameter may elevate TII. In our cases, the partition of these organic solvents into the interface of the membrane may increase the contact of the hydrophobic segments of the alkyl chains with solvents, inducing an increase in A (the area of the lipid headgroup at the lipid–water interface), and as a result, the packing parameter may decrease. According to the Marsh theory, this decrease in the packing parameter may elevate TII. Therefore, the change in the packing parameter of the phospholipid induced concomitantly by the interaction of these organic solvents with the surface segments of the phospholipid membrane may also stabilize the Lα rather than the HII phase and increase TII.

Rowe et al. [12] showed that short-chain alcohols such as methanol shift the Lα-Lβ phase transition of DEPE (dielaidoylphosphatidylethanolamine) to lower temperatures and also its Lα-HII phase transition to higher temperature, which indicates these alcohols stabilize Lα phase relative to the HII and Lβ phases. Their results may be explained by our hypothesis described above.
4.2. Effect of deuterium oxide (D\textsubscript{2}O) on the H\textsubscript{n}-L\textsubscript{α} phase transition in DOPE dispersion induced by these organic solvents

Threshold concentrations of all the organic solvents used in this report for the H\textsubscript{n}-L\textsubscript{α} phase transition in DOPE dispersion in deuterium oxide (D\textsubscript{2}O), were higher than those in water (H\textsubscript{2}O). These results may be explained by considering the chemical potential of the membrane in the L\textsubscript{α} phase as follows.

As discussed previously, the Gibbs free energy of transfer of alkane from the same alkane into water, $\Delta G$, is a large positive value [23]. Contributions of entropy change ($\Delta S$) and enthalpy change ($\Delta H$) of the transfer of alkane from the same alkane into water, to this $\Delta G$, are strongly temperature dependent. Around room temperature (25°C), $\Delta S$ has a large negative value and $\Delta H$ 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 [29,23]. This is the most important factor in the hydrophobic interaction around room temperature. $\Delta G$ increases with an increase in temperature from 0°C to 140°C, whereas absolute values of the $\Delta S$ decreases and those of $\Delta H$ increases. At high temperatures around 140°C, $\Delta G$ has a maximum value and $\Delta S$ is negligibly small, and thereby, $\Delta G \approx \Delta H$ [29-31].

Our experiments in this report were done at 20°C, and thereby, $\Delta G$ is mainly determined by the $\Delta S$. Deuterium bond in deuterium oxide (D\textsubscript{2}O) is stronger than hydrogen bond in water (H\textsubscript{2}O) [32-35], and thereby, $\Delta G$ from the alkane to deuterium oxide is larger than $\Delta G$ from the alkane to water. Therefore, the substitution of H\textsubscript{2}O by D\textsubscript{2}O may increase the interaction free energy between the hydrophobic segments of the alkyl chain in the membrane and solvents. As discussed in the previous section, in the L\textsubscript{α} phase, a small fraction of hydrophobic segments of the alkyl chains, as well as the hydrophilic segments of the head groups, contacts with water or solvents. Hence, the substitution of H\textsubscript{2}O by D\textsubscript{2}O may increase the chemical potential of the phospholipid membrane in L\textsubscript{α} phase, and thereby, the difference of the chemical potential of the membrane in two phases; $\Delta \mu(L_\alpha - H_{II}) = \mu(L_\alpha) - \mu(H_{II})$ in D\textsubscript{2}O and $\Delta \mu(L_\alpha - L_\beta) = \mu(L_\alpha) - \mu(L_\beta)$ in D\textsubscript{2}O are larger than those in H\textsubscript{2}O. As discussed in the previous section, $\Delta \mu$ 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 H\textsubscript{n}-L\textsubscript{α} phase transition or the L\textsubscript{α}-L\textsubscript{β} phase transition. This is why the substitution of H\textsubscript{2}O by D\textsubscript{2}O increases the threshold concentrations for the H\textsubscript{n}-L\textsubscript{α} phase transition.

Epand [36] observed that substitution of H\textsubscript{2}O by D\textsubscript{2}O raise the L\textsubscript{β} to L\textsubscript{α} transition temperature but lowered L\textsubscript{α}-H\textsubscript{II} transition temperature of POPE (1-palmitoyl-2-oleoyl-phosphatidylethanolamine) and DEPE (dielaidoylphosphatidylethanolamine). His result may be explained by our mechanism mentioned above.

In our previous paper [14], we indicated that the chemical potential of the interdigitated gel phase, where the terminal alkyl chains face water or solvents, of DPPC-MLV in D\textsubscript{2}O was higher than that in H\textsubscript{2}O, because the substitution of H\textsubscript{2}O by D\textsubscript{2}O increase the interaction free energy between alkane and the solvents (H\textsubscript{2}O or D\textsubscript{2}O), which explained the experimental results reasonably. This result supports the above discussion on the H\textsubscript{n}-L\textsubscript{α} phase transition in H\textsubscript{2}O and D\textsubscript{2}O.

Based on these results, we propose a new hypothesis that water-soluble organic molecules (which have a high solubility in alkane and also can penetrate into the interface of the membrane) can stabilize the L\textsubscript{α} phase relative to the H\textsubscript{II} phase and increase the L\textsubscript{α}-H\textsubscript{II} phase transition temperature. 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. Moreover, it is important that these molecules mainly exist in the interface of the membrane, not in the center of the hydrophobic core region; because if they did not meet the above condition and would directly relieve hydrocarbon packing stress by partitioning into relatively inaccessible regions (the contact regions of the terminal alkyl chains) of the H\textsubscript{n} phase, they could decrease the L\textsubscript{α}-H\textsubscript{II} phase transition temperature as like as alkane could [11]. We also propose a new hypothesis that substances which decrease an interaction free energy between water and the hydrophobic segments of the alkyl chains can stabilize the L\textsubscript{α} phase relative to the H\textsubscript{II} phase and...
increase the $L_n - H_1$ phase transition temperature. Further investigations are necessary to confirm the validity of these new hypotheses.

Acknowledgements

This work was supported partly by Special Research Project Grant from Shizuoka University (to M.Y.).

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