# Change of Motion and Localization of Cholesterol Molecule during $L_\alpha$-$H_{II}$ Transition

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ABSTRACT Formation of the inverted hexagonal ($H_{II}$) phase from the lamellar ($L_\alpha$) phase of bovine brain-extracted phosphatidylcholine (BBPC) and phosphatidylethanolamine (BBPE) was investigated using $^{31}$P-NMR with or without cholesterol. When the ratio of BBPC to BBPE was 1:1, the $H_{II}$ formation was observed in the presence of 33 mol% cholesterol (i.e., BBPC:BBPE:cholesterol = 1:1:1) at 47°C. The fraction of the $H_{II}$ phase in the BBPC/BBPE/cholesterol system could be controlled by the addition of dioleoylglycerol. The change of molecular motion of cholesterol affected by the $H_{II}$ formation was measured at various ratios of the $L_\alpha$ to $H_{II}$ phase with the time-resolved fluorescence depolarization method, using dehydroergosterol as a fluorescent probe. It is observed that the motion of cholesterol became vigorous in the mixture state of the $L_\alpha$ and the $H_{II}$ phases compared to that in the $L_\alpha$ or the $H_{II}$ phase only. These facts show that cholesterol has the strong ability to induce the $H_{II}$ phase, probably by special molecular motion, which includes change of its location from the headgroup area to the acyl-chain area.

## INTRODUCTION

The lipid bilayer is a basic structure of the biological membrane that maintains the shape of cells and organelles, and rigidly divides inside and outside of these. In this sense, the lipid bilayer should be stable and not form nonbilayer structures at physiological temperatures. However, in several important cell functions involving membrane fusion, such as endocytosis and secretion, the lipid membrane must break the bilayer structure rapidly, and thus the bilayer structure should be unstable. By this point of view, the fusionable membrane must have two opposite properties: one is to maintain the bilayer structure, and the other is to destabilize the bilayer structure. To make switching between these two properties possible, it is expected that there are "functional lipids" that can stabilize both bilayer and nonbilayer structures. One of these functional lipids is cholesterol. It prevents the drastic change in membrane fluidity during the gel-liquid crystalline phase transition by increasing the fluidity in the gel phase and by decreasing it in the liquid-crystalline phase (Wu and Jacobson, 1977). Besides maintaining the membrane fluidity, cholesterol facilitates the conversion of the bilayer (lamellar; $L_\alpha$) structure to the inverted hexagonal ($H_{II}$) structure between closely apposed two membranes (Tilcock et al., 1982). The $H_{II}$ formation relates to membrane fusion, because one of the major processes of membrane fusion involves inverted micelle intermediate formation at the initial stage, and the intermediate has structure similar to that of the $H_{II}$ phase (Sieger et al., 1989). The membranes that form the $H_{II}$ phase have a strong tendency to form the inverted micelle intermediate, resulting in membrane fusion.

Physiologically, this type of membrane fusion is assumed to occur in neurotransmission at synapses. There are high concentrations of cholesterol in the lipids comprising the membranes of the synaptic vesicles (Breckenridge et al., 1973) and synaptsomes in the neuron system (Breckenridge et al., 1972). The major lipid components in synaptic vesicle and synaptosome membranes are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and cholesterol, in an approximate molar ratio of 1:1:1. The $H_{II}$ formation could be enhanced by increasing the temperature or by adding diacylglycerol (DG). It is reported that artificial membrane consisting of bovine brain-extracted PC, PE, and cholesterol (ratio = 1:1:1) forms the $H_{II}$ phase by adding small amounts of dioleoylglycerol (DOG) or increasing the temperature (Naganuma, 1994; Naganuma et al., 1996). Thus this artificial phospholipid membrane is of interest as a membrane fusion model, in examining the role of cholesterol in the $L_\alpha$-$H_{II}$ phase transition.

In this paper we report the results of $^{31}$P-NMR measurement for the $L_\alpha$-$H_{II}$ phase transition induced by the addition of various concentrations of cholesterol to multilamellar sheets composed of bovine brain-extracted lipids (BBPC:BBPE = 1:1) at various temperatures. We also report the molecular motion of cholesterol in the $L_\alpha$ or the $H_{II}$ phase alone and in mixtures of the $L_\alpha$ and the $H_{II}$ phases, using multilamellar vesicles composed of BBPC, BBPE, and cholesterol in the presence of various amounts of DOG. Measurement of the molecular motion was performed by the time-resolved fluorescence depolarization, with dehydroergosterol (DHE) as a fluorescent probe (Nemecz and Schroeder, 1988).

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## EXPERIMENTAL PROCEDURES

### Materials

Bovine brain phosphatidylcholine (BBPC), bovine brain phosphatidylethanolamine (BBPE), cholesterol, dioleoylglycerol (DOG), and dehydroergosterol (DHE) were purchased from Sigma Chemical Co. (St. Louis, MO). HEPES and ethylenediamine-*N,N,N′,N′*-tetraacetic acid disodium salt dihydrate (EDTA2Na) were purchased from Wako Junyaku Co. (Tokyo). All other chemicals were reagent grade.

### Sample preparation for $^{31}$P-NMR

Multilamellar sheets of BBPC/BBPE (1:1) were prepared as described previously (Gennis, 1990), with the inclusion of cholesterol concentrations of 0, 20, 33, and 45 mol%. BBPC and BBPE (100 mg each) were used to make one sample. Membranes composed of BBPC/BBPE/cholesterol (1:1:1) with various concentrations of DOG (0, 2.5, 5, 10, 15, 20 mol%) were also prepared. Chloroform solution of the mixture was evaporated by rotary evaporator. Vacuum drying was performed overnight. Buffer solution (10 mM HEPES, including 100 mM NaCl and 0.1 mM EDTA, pH 7.4) was added, and the solutions were kept at room temperature for 2 h. The mixture was vortexed softly, frozen in liquid $N_2$, and then melted spontaneously at room temperature (freeze and thaw process). This process was repeated three times, and the resulting suspension was centrifuged at 10,000 rpm for 30 min in a Hitachi ultracentrifuge (model himac CP 70G) at 4°C. The pellet, containing multilamellar sheets, was separated from the supernatant containing small micelles and stored at 4°C overnight.

### Preparation of liposomes

Multilamellar vesicles of phospholipids were prepared as described previously (Hong et al., 1988). Each phospholipid and fluorescent probe were dissolved in chloroform and stored at −80 before use. BBPC, BBPE, and cholesterol were mixed in a 1:1:1 mol% ratio, and then various concentrations of DOG (0, 2.5, 5, 10, 15, 20 mol%) were added into the mixture. We prepared a total of 1 $\mu$mol of lipid mixture, and added 0.2 mol% DHE. Chloroform solution was evaporated under a $N_2$ gas stream. Sodium phosphate buffer (70 mM, pH 7.4) was added and vortexed for ~10 min. Liposome solutions were preserved at 4°C overnight in light-proof containers to stabilize the liposomes.

### $^{31}$P-NMR measurements

$^{31}$P-NMR measurements were made with a Bruker MSL 400 spectrometer at 180 MHz. The spectra were accumulated 900-1200 times to improve the signal-to-noise ratio.

### Time-resolved fluorescence anisotropy measurement

DHE was used as a fluorescent probe to examine cholesterol molecular motion. The excitation and emission wavelengths of DHE were 325 nm and 390 nm, respectively. The temperature of the vesicle suspension was controlled by a circulating water system at 37°C within 0.2°C deviation.

The decay of polarized fluorescence of DHE was measured by a time-correlated single photon counting system with a synchronously pumped, cavity-dumped dye laser (Coherent, 700 dye laser) and a mode-locked Ar ion laser (Coherent, Innova 100).

The time dependence of the total fluorescence intensity, $TI(t)$, the intensity difference between vertical and horizontal components, $D(t)$, and the anisotropy ratio, $r(t)$, were obtained as follows:

$$TI(t) = I_{VV}(t) + 2f \cdot I_{VH}(t) \quad (1)$$

$$D(t) = I_{VV}(t) - f \cdot I_{VH}(t) \quad (2)$$

$$r(t) = D(t)/TI(t) \quad (3)$$

where $I_{VV}(t)$ and $I_{VH}(t)$ denote the fluorescence intensities of the vertical and horizontal components after excitation by vertically polarized light. Using excitation by horizontally polarized light, $f$ is expressed as follows:

$$f = \int_0^\infty I_{HV}(t)\mathrm{d}t \Big/ \int_0^\infty I_{HH}(t)\mathrm{d}t \quad (4)$$

$I_{VV}(t)$ and $fI_{VH}(t)$ correspond to the notations $I_{\parallel}(t)$ and $I_{\perp}(t)$, respectively, i.e., the parallel and perpendicular components of anisotropic fluorescent light. When the fluorophore is embedded in the lipid bilayers, they move with a wobbling motion. In this case, $r(t)$ may be expressed experimentally as follows (Kinosita et al., 1984):

$$r(t) = (r_0 - r_\infty)\exp(-t/\phi) + r_\infty \quad (5)$$

where $r$ is the residual limiting anisotropy ratio at infinite time and $\phi$ is the time constant of anisotropy decrease. To characterize the restricted motion of the fluorophore, the wobbling in-cone model is widely accepted. In this model, motion of the fluorophore is confined within a given cone area, which is expressed by a half cone angle ($\vartheta_c$), and the rate of the motion is expressed with the wobbling diffusion rate ($D_w$). These values were calculated from the following equations (Lipari and Szabo, 1980; Saito et al., 1991; Araiso and Koyama, 1995):

$$r_\infty/r_0 = \{\cos\vartheta_c(1 + \cos\vartheta_c)\}^2/4 \quad (6)$$

$$\begin{aligned} D_w\,\phi(r_0 - r_\infty)/r_0 &= -\chi^2(1+\chi)^2[\ln\{(1+\chi)/2\} + (1-\chi)/2]/\{2(1-\chi)\} \\ &\quad + (1-\chi)(6 + 8\chi - \chi^2 - 12\chi^3 - 7\chi^4)/24 \end{aligned} \quad (7)$$

where $\chi = \cos\vartheta_c$.

## RESULTS

### Effect of cholesterol on the $L_\alpha$-$H_{II}$ phase transition in a BBPC/BBPE system measured by $^{31}$P-NMR

$^{31}$P-NMR measurements were performed for BBPC/BBPE (1:1) multilamellar sheets with or without cholesterol. We examined the effect on the phase transition of adding cholesterol to phospholipid membrane sheets. Without cholesterol (Fig. 1) or with a 20 mol% concentration of cholesterol (Fig. 2) at temperatures ranging from 27°C to 57°C, almost the same spectra were observed. It means that only the $L_\alpha$ phase existed in the lipid system and the formation of the $H_{II}$ phase did not occur. Spectral peaks at 14 ppm in Fig. 2 correspond to inorganic phosphorus as a contaminant of phospholipids. In the presence of 33 mol% cholesterol (i.e., BBPC:BBPE:Chol = 1:1:1), at 27°C and 37°C we still observed only the $L_\alpha$ phase, but at 47°C the $H_{II}$ phase was observed in part as a spectral peak at 20 ppm (Fig. 3). At 57°C most of the lipid assembly formed the $H_{II}$ phase. The peak at 13 ppm represents formation of small micelles. Very small peaks at 15–16 ppm correspond to inorganic phosphorus. At higher concentrations of cholesterol (45 mol%), we observed the $L_\alpha$ phase only in the temperature range 37°C to 57°C (Fig. 4). At 67°C, the signal of the $H_{II}$ phase did not appear, but a new isotropic signal at 12 ppm appeared, suggesting that most of the $L_\alpha$ phase converted into small micelles.

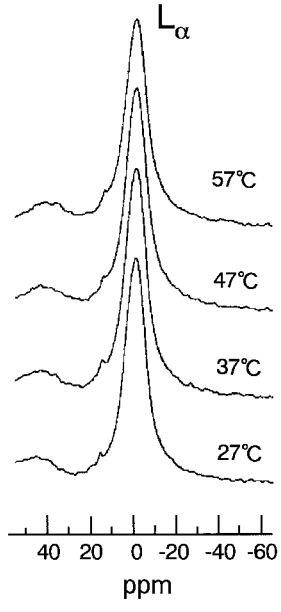


FIGURE 1 $^{31}$P-NMR spectra of membrane sheet composed of BBPC and BBPE (= 1:1). Only the $L_\alpha$ phase was observed at various temperatures (27–57°C).

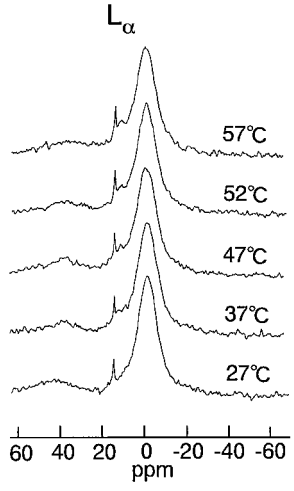


FIGURE 2 $^{31}$P-NMR spectra of BBPC/BBPE (1:1) with 20 mol% cholesterol. Only the $L_\alpha$ phase was observed at various temperatures (27–57°C). The inorganic phosphoric acid was observed at 14 ppm.

### Effect of DOG on the $L_\alpha$-$H_{II}$ phase transition in the BBPC/BBPE/cholesterol (1:1:1) system

We measured $H_{II}$ formation at various concentrations of DOG (0, 2.5, 5, 10, 15, 20 mol%) in BBPC/BBPE/cholesterol (1:1:1) membrane to investigate the effect of DOG with $^{31}$P-NMR. The temperature was fixed at 37°C as the physiological temperature. The result is shown in Fig. 5. Without DOG, only the $L_\alpha$ phase existed and the $H_{II}$ phase did not appear. But the $H_{II}$ phase was caused to occur in part by adding 5 mol% DOG. When the concentration of DOG was increased to 10 mol%, we observed increased $H_{II}$ phase formation. With 20 mol% DOG, the $L_\alpha$-$H_{II}$ phase transition was complete.

To analyze the ratio of the $L_\alpha$ phase to the $H_{II}$ phase, the amounts of the $L_\alpha$ phase were calculated by the peak height of $^{31}$P-NMR spectra at 0 ppm. The peak at 0 ppm is due mainly to the $L_\alpha$ phase; however, it has a few contributions

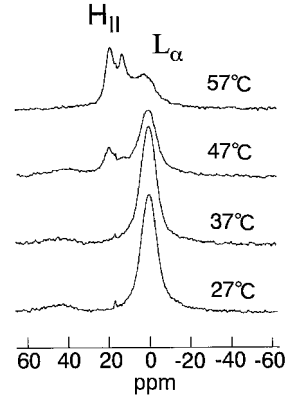


FIGURE 3 $^{31}$P-NMR spectra of BBPC/BBPE/cholesterol (1:1:1) membrane (the concentration of cholesterol is 33 mol%). The formation of the $H_{II}$ phase was confirmed as peaks at 20 ppm, at 47°C and 57°C. The inorganic phosphoric acid was observed at 1516 ppm.

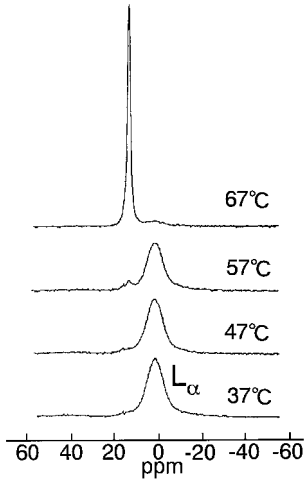


FIGURE 4 $^{31}$P-NMR spectra of BBPC/BBPE (1:1) with 45 mol% cholesterol. The $H_{II}$ phase did not appear at all temperatures. However, the isotropic and very narrow peak appeared at 12 ppm at 67°C, representing a micelle formation.

from the $H_{II}$ phase. To eliminate the contribution of the $H_{II}$ phase at 0 ppm, we must know the height of pure $L_\alpha$ and $H_{II}$ spectra at 0 ppm ($\epsilon(0)_L$ and $\epsilon(0)_H$, respectively). We can assume that all lipids formed the $L_\alpha$ phase in the absence of DOG, and all lipid assembly converted to the $H_{II}$ phase in the presence of 20 mol% DOG. Because the concentration of phospholipid and measuring conditions were the same for all measurements, the values of $\epsilon(0)_L$ and $\epsilon(0)_H$ could be obtained from experimental data shown in Fig. 5. Let the portion of the $L_\alpha$ phase be $X_L$, and that of the $H_{II}$ phase be $X_H$ ($X_L + X_H = 1$). The height of the 0 ppm spectra, $I(0)$, which corresponds to the coexistence of the $L_\alpha$ and the $H_{II}$ phases, may be represented as

$$I(0) = \epsilon(0)_L \cdot X_L + \epsilon(0)_H \cdot X_H \quad (8)$$

Then the portion of the $L_\alpha$ phase, $X_L$, may be calculated by

$$X_L = (I(0) - \epsilon(0)_H)/(\epsilon(0)_L - \epsilon(0)_H) \quad (9)$$

The values of $X_L$ of the BBPC/BBPE/cholesterol/DOG systems are shown as a function of DOG concentrations in Fig. 6.

## Molecular motion of cholesterol in the BBPC/BBPE/cholesterol/DOG system with formation of the $H_{II}$ phase

Molecular motion of cholesterol in BBPC/BBPE/cholesterol/DOG was examined by the time-resolved fluorescence depolarization measurements with DHE as the fluorescent probe. We could obtain the various mixture ratios of the $L_\alpha$ and the $H_{II}$ phases by changing the DOG concentrations. Under these experimental conditions, where the $L_\alpha$ and $H_{II}$ phases coexist, we measured the fluorescence depolarization of DHE. Fig. 7 *A* shows the anisotropic fluorescence decay of DHE in the BBPC/BBPE/cholesterol (1:1:1) system at 37°C (condition of 100% $L_\alpha$), and Fig. 7 *B* shows the results of the BBPC/BBPE/cholesterol (1:1:1)/DOG10 mol% system at 37°C (coexistence of $L_\alpha$ and $H_{II}$). Anisotropy changes in DHE in these two systems are shown in Fig. 8. A small but distinct difference is observed. Clearly, the extent of the anisotropy decay is large in the mixture of the $L_\alpha$ and $H_{II}$ phases, indicating that DHE can move over a wider range in the mixture of these phases than in the $L_\alpha$ phase only. The time course of anisotropy of DHE in the 100% $H_{II}$ phase was very similar to that in the 100% $L_\alpha$ phase (data not shown). We measured the DHE motion in the various mixture ratios of the $L_\alpha$ and $H_{II}$ phases. By using Eqs. 5–7, the wobbling cone angle ($\vartheta_C$) and the wobbling diffusion rate ($D_w$) were calculated as parameters for the wobbling motion of DHE. The results are shown in Figs. 9 and 10. When membranes were 100% in the $L_\alpha$ phase or

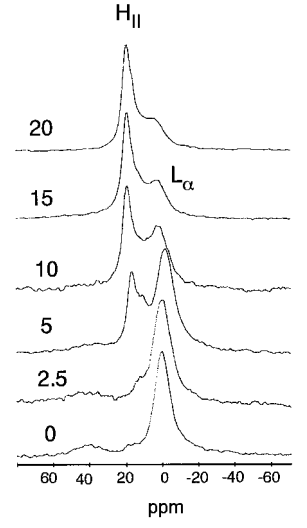


FIGURE 5 $^{31}$P-NMR spectra of BBPC/BBPE/cholesterol (1:1:1) in the presence of various concentrations of DOG (shown as mol% in the figure) at 37°C. The $H_{II}$ phase appeared over 5 mol% DOG. At 20 mol% DOG concentration, the $L_\alpha$-$H_{II}$ phase transition was complete.

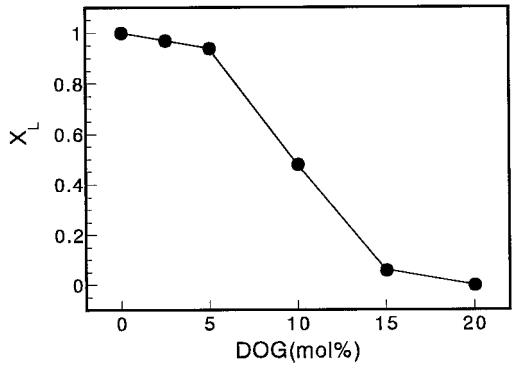


FIGURE 6 The relation between the concentration of DOG and the fraction of the $L_\alpha$ phase ($X_L$) in BBPC/BBPE/cholesterol (1:1:1) containing DOG at 37°C. The ratio of the $L_\alpha$ to $H_{II}$ phase was changed by the addition of DOG.

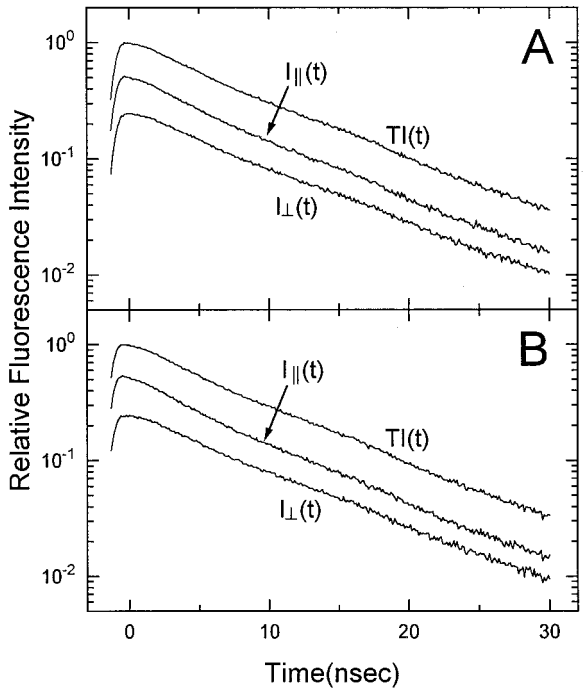


FIGURE 7 Decay of polarized fluorescence of DHE in BBPC/BBPE/cholesterol (1:1:1) systems at 37°C. $TI(t)$, $I_{\parallel}(t)$ and $I_{\perp}(t)$ indicate the total intensity and the vertically depolarized component and horizontally depolarized component of DHE fluorescence, respectively. (*A*) Without DOG. Under these conditions, the phase of membrane is $L_{\alpha}$ only. (*B*) With 10 mol% DOG. Mixture state of almost equal amounts of the $L_{\alpha}$ and the $H_{II}$ phases.

100% in the $H_{II}$ phase, $\vartheta_C$ showed small values of near 36° (Fig. 9). On the other hand, in situations where the $L_{\alpha}$ and $H_{II}$ phases coexisted, the value of $\vartheta_C$ increased. When the DOG concentration was 10 mol%, almost equal amounts of

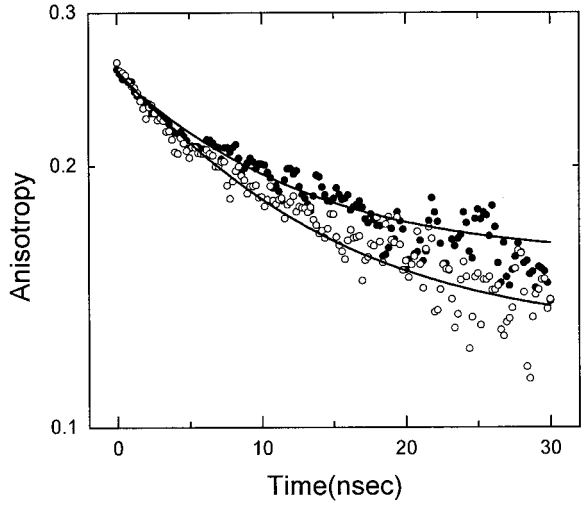


FIGURE 8 Decay of fluorescence anisotropy of DHE in BBPC/BBPE/cholesterol (1:1:1) in the absence of DOG (●) and in the presence (○) of 10 mol% DOG at 37°C. Solid lines are the best fit curves by least-squares analysis. The upper curve corresponds to 100% of the $L_{\alpha}$ phase; the lower curve corresponds to the mixture state of the $L_{\alpha}$ and the $H_{II}$ phases (~1:1).

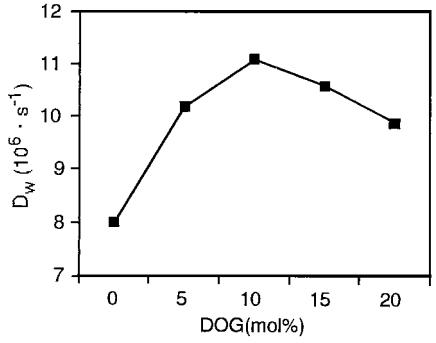


FIGURE 9 Amplitude of the molecular motion ($\vartheta_c$) of DHE in the BBPC/BBPE/cholesterol system in the presence of various concentrations of DOG. The wobbling cone angle ($\vartheta_c$) became large at 515 mol% DOG. In the $L_{\alpha}$ and the $H_{II}$ phases, the values of $\vartheta_c$ were similar.

the $L_{\alpha}$ and $H_{II}$ phases coexist. At this concentration of DOG, the wobbling cone angle had a maximum value (39°), showing that the amplitude of DHE motion became largest. Fig. 10 shows the results of calculating the wobbling diffusion rate, $D_w$, which indicates the frequency of molecular motion. When there was only the $L_{\alpha}$ phase, $D_w$ was small ($8 \times 10^6$ $s^{-1}$). Similar to changes seen in $\vartheta_C$, in the presence of 10 mol% DOG in BBPC/BBPE/cholesterol (1:1:1), $D_w$ took the largest value, $11 \times 10^{-6}$ $s^{-1}$. $D_w$ also decreased when the lipid structure was in the $H_{II}$ phase only. Because the molecular structure of DHE is almost the same as that of cholesterol, the molecular motion of DHE must represent that of cholesterol. Thus these results confirm that the molecular motion of cholesterol was restrained in a single phase, whereas in a mixture of both phases, where a transition between $L_{\alpha}$ and $H_{II}$ was occurring, the molecular motion of cholesterol increased in both frequency and amplitude.

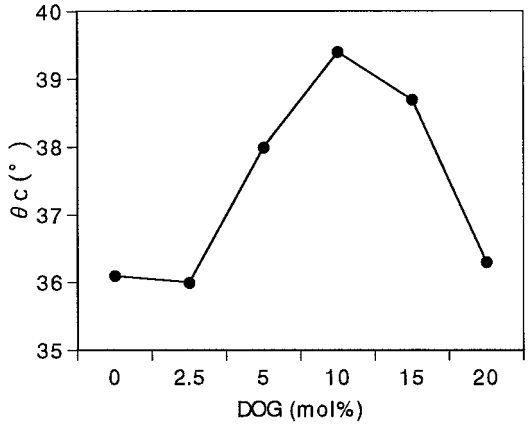


FIGURE 10 Frequency of the molecular motion ($D_w$) of DHE in the BBPC/BBPE/cholesterol system in the presence of various concentrations of DOG. The wobbling diffusion rate ($D_w$) had the maximum value at 10 mol% of DOG.

## DISCUSSION

Measurement of $H_{II}$ phase formation by using a BBPC/BBPE system with or without cholesterol (Fig. 1) showed that the $H_{II}$ phase appeared only when cholesterol concentration was 33 mol%. In 45 mol% cholesterol, the $H_{II}$ phase was not observed, although the $L_\alpha$ phase decreased slightly at 37°C to 57°C. Characteristically, an isotropic and very narrow peak appeared at 12 ppm at 67°C, and this peak is presumed to represent micelles or small irregular aggregates of the $L_\alpha$ structure that were destroyed by high temperature (Fig. 11). These results confirm that cholesterol has a very important role in the $L_\alpha$-$H_{II}$ phase transition in the PC/PE lipid system. It is meaningful that the concentrations of cholesterol that can induce the phase transition are similar to those of biological membranes. Therefore, it is clear that the concentrations of cholesterol in biological membrane are important in allowing the membrane fusion process to occur physiologically.

PC could not induce the $L_\alpha$-$H_{II}$ phase transition by itself. But when DG was added to the PC system, the $L_\alpha$-$H_{II}$ phase transition occurred. Furthermore, with 70 mol% DG, the cubic phase of PC was formed (Das and Rand, 1986). PE could induce the $H_{II}$ phase transition of PC/PE system by itself under certain conditions of temperature and pH. For the PC/PE system, DG strongly facilitated the $H_{II}$ phase transition. Two roles of DG in the membrane are worth noting. One is that DG, which is a hydrophobic molecule, imbedded itself in the crevices of the $H_{II}$ tubes to stabilize the $H_{II}$ phase. Another is that DG, because it has a small molecular volume of the headgroup, can decrease the curvature radius of the lipid-water interface. It causes the $H_{II}$ phase of the lipid system to stabilize. According to this, DG is also one of the functional lipids of membrane fusion.

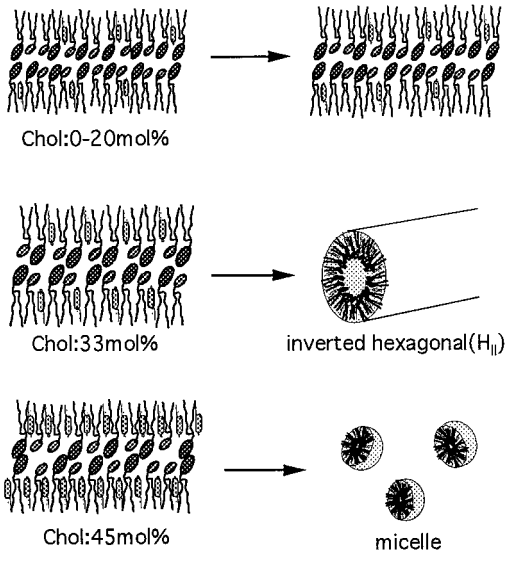


FIGURE 11 The effect of cholesterol on the formation of nonbilayer structure is deferred by cholesterol concentration. (*Top*) When the concentration of cholesterol was 0–20 mol% in the BBPC/BBPE (1:1) system, the $L_\alpha$-$H_{II}$ transition did not occur. (*Middle*) With 33 mol% of cholesterol, the $L_\alpha$ phase could transform into the $H_{II}$ phase. (*Bottom*) At 45 mol% cholesterol, the $H_{II}$ phase could not appear, but small vesicles or small micelles were formed.

The ratio of the $L_\alpha$ to the $H_{II}$ phase in a BBPC/BBPE/cholesterol membrane could be changed by adding various concentrations of DOG (Fig. 6). Under such conditions, we next measured the molecular motion of cholesterol in a mixture of the $L_\alpha$ and the $H_{II}$ phases with DHE, which is a fluorescent analog of cholesterol. Figs. 7 and 8 show data from DHE fluorescence depolarization measurements in BBPC/BBPE/cholesterol/0 mol% DOG and BBPC/BBPE/cholesterol/10 mol% DOG, respectively. Figs. 9 and 10 show $\vartheta_c$ and $D_w$, which were calculated from the $r(t)$ decay curve. It could be assumed that cholesterol also makes the wobbling motion with the same value of $\vartheta_c$ and $D_w$. The value of $\vartheta_c$ was larger at 515 mol% DOG than at 0 mol% and 20 mol% DOG. These results indicate that the amplitude of molecular motion in the mixture state of two phases became larger than in the $L_\alpha$ or the $H_{II}$ phase alone. It is also shown that the same tendency was found for $D_w$, the frequency of motion. This specifically increased motility in the mixture of the $L_\alpha$ and the $H_{II}$ phases was characteristic of DHE and was not seen in the polar headgroup (Hayakawa et al., 1997) or the lipid acyl chain (Naganuma, 1994). In a previous report (Hayakawa et al., 1997) we showed that the wobbling motion of polar headgroups was limited by the formation of the $H_{II}$ phase in the dioleoylphosphatidylcholin (DOPC)/dioleoylphosphatidylethanolamine (DOPE)/cholesterol system. This indicates that the headgroups of phospholipid molecules are more tightly packed in the $H_{II}$ phase than in the $L_\alpha$ phase of the membrane. During the $L_\alpha$-$H_{II}$ transition, the mobility of the headgroup decreased monotonously. In contrast to this headgroup motion, the mobility of cholesterol increased during the transition. This increased mobility of cholesterol in the mixed phases of $L_\alpha$ and $H_{II}$ leads to a new concept: that the cholesterol molecule moves from near the polar head area to the lipid acyl-chain area, and then cholesterol stabilizes the $H_{II}$ phase (Fig. 12). Because there is additional space between acyl chains in the partial formation of an $H_{II}$-like structure in the early stage of the transition, cholesterol movement is facilitated. The change of localization of cholesterol (polar head area to acyl-chain area) stabilizes the acyl-chain packing and makes the volume of polar head group area smaller. Both of these actions of cholesterol confer advantages on the $H_{II}$ formation.

In the PC/PE/cholesterol system, a mixed state of the $L_\alpha$ and the $H_{II}$ phases indicates the heterogeneous distribution of lipids in the $L_\alpha$ membrane just before the $H_{II}$ formation. It has been reported for the PC/cholesterol system that cholesterol interacts with PC and forms a cholesterol-rich domain that contributes to membrane stability (Imaizumi and Hatta, 1984; Hatta, 1985). Thus cholesterol domain formation is the most feasible way of making the heterogeneous structure in the PC/PE/cholesterol system. Cell membrane often shows an asymmetrical distribution of cholesterol between the inner and outer leaflets, which results in different manners of formation of a cholesterol-rich domain

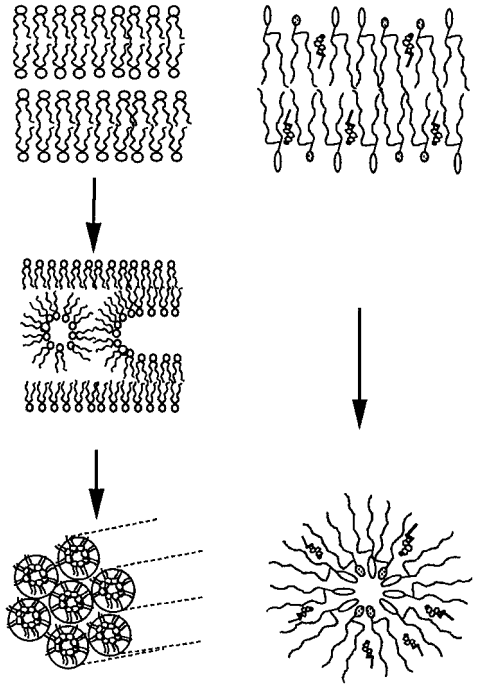

FIGURE 12 A model for the $L_\alpha$-$H_{II}$ transition and the role of cholesterol in this process. At an initial stage of this transition, an intermediate state that is a mixture of the $L_\alpha$ and the $H_{II}$ phases would appear. The molecular motion of cholesterol increases in this intermediate state, and finally cholesterol moves its position from near the polar head area to the lipid acyl-chain area, to stabilize the $H_{II}$ structure.

in the two leaflets. For example, in mouse brain synaptic plasma membrane, 88% of synaptic plasma membrane cholesterol was located in the inner leaflet (Schroeder et al., 1996). This may be related to facilitation of the membrane fusion between synaptic vesicle and synaptosome membrane, which must occur inside the synaptosome. Cholesterol is thought to perform its special roles in many biological membranes by changing the spatial density and/or by altering the hydrophobic forces of phospholipid assembly.

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