Fluorescence depolarization study of lamellar liquid crystalline to inverted cylindrical micellar phase transition of phosphatidylethanolamine

Kwan Hon Cheng
Department of Physics, Texas Tech University, Lubbock, Texas 79409

ABSTRACT The orientational order and rotational dynamics of 2-[3-(diphenylhexatrienyl) propanoyl]-3-palmitoyl-L-α-phosphatidylcholine (DPH-PC) embedded in dioleoylphosphatidylethanolamine (DOPE) were studied by fluorescence depolarization technique. Upon increasing the temperature, the calculated wobbling diffusion constant $D_\perp$ of the fluorescent probe was found to decrease at the lamellar (L_α) to inverted cylindrical (H_{II}) phase transition (10°C). This suggested that the increased gauche rotamers of the alkene chains in the H_{II} phase imposes a constraint in the wobbling motion of the fluorophore. The calculated ratio of order parameter in the L_α phase to that in the H_{II} phase was 1.7 and different from the theoretical value of 2.0 as predicted from the change in packing symmetry. This result can be explained by a slightly higher local order parameter of the fluorophore or by the fast rotational diffusion motion of the fluorophore around the symmetry axis of the cylindrical tubes in the H_{II} phase.

INTRODUCTION

Among many thermotropic phase transitions exhibited by various lipid systems, the lamellar liquid crystalline (L_α) to the inverted cylindrical micellar (H_{II}) phase transition has attracted particular attention in recent years (Gruner et al., 1985; Small, 1986).

In the L_α phase, the molecules are arranged in a form of stacked bilayers with sheets of water between the polar surfaces of the lipids and form a one-dimensional lattice. The lipid molecules are free to diffuse laterally, and their hydrocarbon chains are in the form of a "melted" state which contain many gauche rotamers. In the H_{II} phase, the lipid molecules form long water-cored cylindrical tubes with the polar head groups facing the long symmetry axis of the tubes. The tubes stack together to form a two-dimensional hexagonal lattice. Unlike the commonly studied lamellar gel (L_β) to lamellar liquid crystalline (L_α) phase transition, the L_α-to-H_{II} transition involves abrupt changes in both the surface curvature and packing symmetry of the aggregates. The mechanisms governing this transition are still not well understood. Yet a promising thermodynamic model has recently been proposed by Gruner and co-workers (1985). This model provides a means to estimate the free energies of various interactions among the molecules due to the intrinsic curvature, hydration, and packing constraint of the lipids in both the L_α and H_{II} phases.

To understand the molecular orientation and dynamics of lipid phases, various spectroscopic techniques, such as nuclear magnetic resonance (NMR) (e.g., Seelig, 1977), fluorescence (e.g., Szabo, 1980, 1984), Raman (e.g., Levin, 1984), and infrared spectroscopy (e.g., Casal and Mantsch, 1984) are widely employed. The NMR technique (e.g., ^2H and ^{31}P NMR) provides molecular information in the long time region (10^{-3}–10^{-6} s). Both Raman and infrared deal with short time region (10^{-13}–10^{-15} s). The fluorescence technique falls in the range of 10^{-8}–10^{-12} s. Unlike other spectroscopic techniques, the approach of applying time-resolved fluorescence spectroscopy in studying the molecular mechanism of L_α-to-H_{II} phase transition is a relatively new development.

In the fluorescence time region, the slower motional contributions of the lateral diffusion and collective director fluctuation to the fluorescence depolarization can be neglected. For a cylindrical fluorophore in an anisotropic medium, many factors contribute to the depolarization. They are (*a*) the two independent modes of rotational diffusion as described by the two independent diffusion tensor elements, $D_\perp$ and $D_\parallel$, which correspond to the rotation along the directions perpendicular and parallel to the fluorophore long symmetry axis, respectively, (*b*) the steady-state orientational distributions of the fluorophores about the director symmetry axis of the lipids, and (*c*) the relative orientations of the emission and absorption dipoles of the fluorophore with respect to the fixed molecular frame of the fluorophore. With either, the absorption or emission dipole moment coincides with the long symmetry axis of the fluorophore; the only mode of diffusional motion that gives rise to fluorescence depolarization is $D_\perp$. In this case, the form of anisotropy decay follows a simple exponential decay at the short time domain and a nonzero residual anisotropy constant at the long time domain (Szabo, 1984; Van Der Meer et al., 1984). This motion is usually described as the wobbling motion of fluorophore.

Unfortunately, most of the commonly known lipid molecules are not fluorescent, and extrinsic fluorophores are therefore required. To avoid the perturbation of the physical states of the lipids, it is crucial to select a fluorophore to mimic as closely as possible the chemical structure of the host molecules that made up the lipids. The fluorophore 2-[3-(diphenylhexatrienyl)propanoyl]-3-palmitoyl-L-α-phosphatidylcholine (DPH-PC) is a fluorescent phospholipid which has a polar phosphatidylcholine head group but one of the two chains is replaced by a chromophore diphenylhexatriene (DPH). Using a space-filling model (Parente and Lentz, 1985), the stoichiometry of this fluorophore has been found to be identical to that of a saturated lipid. Furthermore, the photophysical properties of this DPH analogue are very similar to that of the DPH (Bisky et al., 1981). One distinct advantage of this amphiphilic DPH analogue as compared with other commonly used hydrophobic fluorophores is its known location in the lipids. Hydrophobic fluorophores have been known to exhibit nonuniform distribution within the lipids (Ameloot et al., 1984). The emission moment of the fluorophore in this study is located in the hydrophobic region of the lipid layers, spanning approximately from the third carbon position to the end of the alkene chains. It is therefore expected that the orientational order and rotational dynamics of this fluorophore can reflect the corresponding physical states of the host amphiphilic molecules.

MATERIALS AND METHODS

Sample preparation

1,2-Dioleoyl phosphatidylethanolamine (DOPE) in chloroform was obtained from Avanti Polar Lipids (Birmingham, AL). Dry powder of DPH-PC was obtained from Molecular Probes Inc. (Junction City, OR). The samples were all used without further purification. No fluorescence signal was detected for the pure DOPE sample. The DOPE and DPH-PC were dissolved in chloroform. The molar ratio of DPH-PC to DOPE was 2:1,000. The mixture was dried under nitrogen in a clean pyrex tube and further kept in vacuum for 4 h to ensure complete removal of chloroform. The thin film that formed on the tube was then hydrated in an aqueous buffer (100 mM NaCl, 10 mM TES, and 2 mM EDTA, pH = 7.4) at 4°C. The suspension thus formed was vortexed rigorously and under mild sonication in a bath sonicator for 3 min. The suspension was further incubated for 20 h at 4°C in the dark to ensure proper hydration of the sample. The lipids were found to be in a form of stable multilamellar dispersion as determined from freeze-fracture electron microscopy and P-31 NMR spectroscopy (results not shown). Upon further dilution to 50 μg/ml, the sample was further sonicated for a few seconds at 4°C and placed in a 10-mm UV quartz cuvette with a magnetic stirrer for proper mixing. The temperature of the sample was controlled by a water-jet circulator connected to a thermal-stat cell. The temperature of the sample was directly measured by inserting a microtip thermistor probe (YSI-427) into the cuvette at ~5 mm above the light path and recorded by a digital thermometer (VWR 500). After 15 min equilibration time between each measurement, the temperature variation during the experiment was found to be <0.05°C.

Instrument

Fluorescence measurements were performed on a frequency domain cross-correlation fluorometer (ISS Inc., Champaign, IL). The operational principle of this continuously variable frequency phase fluorometer has been described elsewhere (Gratton et al., 1984; Lakowicz and Maliwal, 1985).

Fluorescence decay measurement

Because the light exiting from the pockel cell (electrooptical device) is vertically polarized, a polarizer with polarization axis set at 35° with respect to the vertical was placed in the excitation beam. The excitation wavelength was at 350 nm. A nonfluorescent glycogen solution was used as a reference sample. The modulation ratio (M_F/M_S) and phase shift ($\delta_F - \delta_S$) were measured at different modulation frequencies. Here M_F and M_S represent the intensity modulation values of the fluorescent sample and that of the reference, and δ_F and δ_S represent the phase delay of the signal from the fluorescent sample and that from the reference sample. A lower wavelength cutoff filter (model 3-73, Corning Glass-Works, Corning, NY) was used to remove the excitation light from the fluorescent signal and no filter was used for the reference sample. Each fluorescence lifetime measurement required ~30 min.

Fluorescence emission anisotropy decay measurement

For the fluorescence emission anisotropy decay measurements, both excitation and emission polarizers were used. Identical excitation wavelength and emission filter as in the lifetime measurements were used in here. The ratio of polarized modulation amplitudes ($M_\parallel/M_\perp$) and differential polarized phase angle ($\delta_\perp - \delta_\parallel$) were measured at different modulation frequencies. The subscripts $\parallel$ and $\perp$ refer to the directions of the polarization axis of the emission polarizer that are parallel and perpendicular to the vertical axis, respectively. The polarization axis of the excitation polarizer was always set at the vertical direction. Each time-resolved anisotropic measurement required ~45 min.

Data analysis

Fluorescence decay

To simplify the analysis, a single lifetime decay profile was used to describe the fluorescence decay of the fluorophore.

$$I(t) = e^{-t/\tau}, \tag{1}$$

where τ represents the lifetime of the fluorophore.

By measuring the frequency domain data at different frequencies, the value of τ can be obtained by a nonlinear least square fit method.

Fluorescence emission anisotropy decay

For the case of cylindrically symmetric fluorophores in an uniaxial liquid crystalline environment, a model independent interpolation theory for macroscopically isotropic samples has been developed (Szabo, 1984; Van der Meer et al., 1984). According to this theory,

$$r(t) = r_0(\langle P_2(\Omega_{MD})\rangle^2 + [1 - \langle P_2(\Omega_{MD})\rangle^2] \exp\{-6D_\perp t/[1 - \langle P_2(\Omega_{MD})\rangle^2]\}) \tag{2}$$

$$r_0 = (2/5)P_2(\theta_\mu)P_2(\theta_r), \tag{3}$$

where θ_r and θ_μ refer to the polar angles of the absorption and emission dipole moments with respect to the fluorophore long symmetry axis

(M), Ω_{MD} represents the angle between the director symmetry axis (D) of the amphiphilic aggregates and M, and $D_\perp$ represents the average diffusion constant of the fluorophore with the rotational axis perpendicular to M. By expanding the steady-state orientational distribution function of the fluorophore in a series of even order Legendre polynomials, $\langle P_2(\Omega_{MD})\rangle$ represents the ensemble average of the second moment of the expansion. This first nontrivial term is usually called the order parameter. In the first order approximation, Eq. 2 is true only when either θ_μ or θ_r is very small. For the fluorophore containing a DPH chromophore, the value of θ_r has been known to be close to zero (see Discussion).

By measuring the frequency domain data, the three physical parameters, $\langle P_2(\Omega_{MD})\rangle$, $D_\perp$, and θ_μ, can therefore be obtained by using a nonlinear least square fit method.

Fitting criterion and errors

The best fitting of the data to the model mentioned above was based on the minimization of the reduced chi-square parameter. The fitting errors were calculated using the propagation of errors from the original frequency domain data to the derive parameters during the chi-square minimization process (Gratton et al., 1984).

RESULTS

Fluorescence lifetime

The modulation ratio (M_F/M_S) and relative phase delay (δ_F/δ_S) were measured for five different modulation frequencies (4, 10, 30, 60, and 90 MHz) and at different temperatures (0–40°C). As shown in Fig. 1, the values of (M_F/M_S) and (δ_F/δ_S) were plotted as a function of modulation frequency at 4°C. A single lifetime decay function was used to fit both the modulation and phase

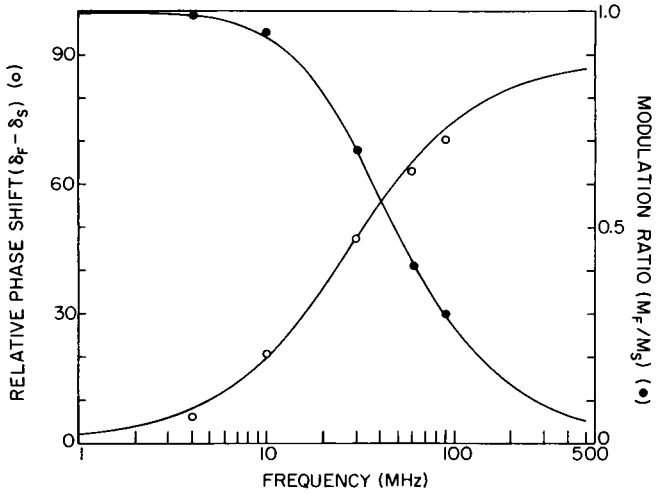

FIGURE 1 Phase ($\delta_F - \delta_S$) and modulation ratio (M_F/M_S) of the fluorescence signal with respect to the reference (glycogen) as a function of modulation frequency for DPH-PC in DOPE at 4°C. The lines were obtained from fitting the frequency domain data using a single exponential decay profile. The lifetime τ was 5.77 ± 0.09 ns. The reduced chi-square for the fit was 7.3.

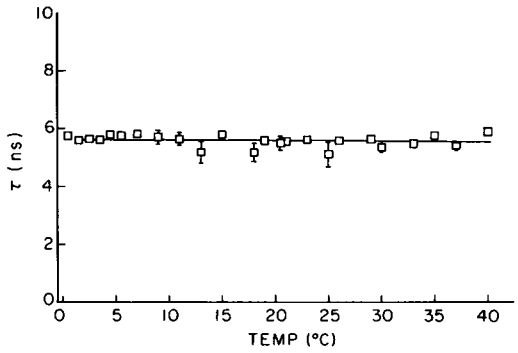

FIGURE 2 Fluorescence lifetime (τ) vs. temperature for DPH-PC in DOPE. Error bar represents estimated fitting error.

data for all the five frequencies. The value of lifetime (τ) was found to be 5.77 ± 0.09 ns at 4°C. As shown in Fig. 2, the values of τ were observed to be independent of temperature. A better fit was found for the data by using a double exponential decay profile rather than a monoexponential decay profile as evidenced by a decrease in the reduced chi-square value. With the double decay fitting, a long-lifetime component at ~6 ns and a short component at ~2 ns were obtained. The intensity fraction of the short component is around 0.05. A similar observation of the nonmonoexponential decay behavior of DPH-PC in phosphatidylcholine liposomes was also reported and an excellent description of the origins of this minor lifetime components was also given by Parente and Lentz (1985).

Fluorescence emission anisotropy decay

The ratio of polarized modulation amplitudes ($M_\parallel/M_\perp$) and differential polarized phase angle ($\delta_\perp - \delta_\parallel$) of the polarized fluorescence emission were measured for 10 different modulation frequencies (1, 2, 6, 10, 20, 40, 60, 90, 110, and 150 MHz) and at different temperatures (0–40°C). Fig. 3 showed the typical values of ($M_\parallel/M_\perp$) and ($\delta_\perp - \delta_\parallel$) as a function of modulation frequency at 4 and 30°C. Using the least square fit method as described in the previous section, the physical parameters θ_μ, $\langle P_2(\Omega_{MD})\rangle$ and $D_\perp$ were then calculated for different temperatures. Using the double lifetime decay profile in the anisotropic decay fitting, the values of θ_μ, $\langle P_2(\Omega_{MD})\rangle$ and $D_\perp$ were found to be similar to those obtained from a single lifetime decay profile within the fitting errors. Moreover, no improvement in the chi-square was found using the double lifetime decay profile. These results therefore suggested that the existence of the short lifetime component in the fluorescence intensity decay makes no significant contribution to the analysis of the depolarization behavior of the probe. As shown in Fig. 4, the values

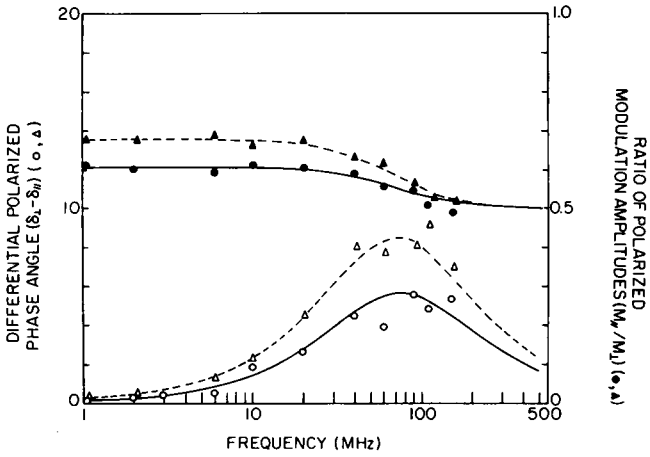

FIGURE 3 Differential polarized phase angle ($\delta_\perp - \delta_\parallel$) and ratio of polarized modulation amplitudes ($M_\parallel/M_\perp$) as a function of modulation frequency for DPH-PC in DOPE at 4°C (*circles*) and 30°C (*triangles*). The lines were obtained from fitting the frequency domain data using the interpolation theory (Eqs. 2 and 3 in Materials and Methods section). The fitted parameters were $\langle P_2(\Omega_{MD})\rangle = 0.75 \pm 0.03$, $D_\perp = (2.47 \pm 0.11) \times 10^7\ s^{-1}$ and $\theta_\mu = 29 \pm 0.1$ at 4°C, and $\langle P_2(\Omega_{MD})\rangle = 0.48 \pm 0.05$, $D_\perp = (3.3 \pm 0.10) \times 10^7\ s^{-1}$ and $\theta_\mu = 30 \pm 1$ at 30°C. The reduced chi-square of the fits were 8.6 and 6.4 at 4 and 30°C, respectively.

of θ_μ were found to be around 29° and independent of temperature. In Fig. 5, the order parameter $\langle P_2(\Omega_{MD})\rangle$ was found to be 0.79 at low temperature (0°C) and declined slightly as temperature increased from 0 to 8°C. From 8 to 15°C, the order parameter dropped appreciably from 0.72 to 0.42. For higher temperatures, the order parameter was found to remain constant at around 0.42. The above sigmoidal behavior of order parameter revealed a phase transition at around 10°C. This temperature range (8–15°C) corresponds to the L_α to H_{II} phase transition of DOPE as measured from x-ray diffraction and NMR methods (Gruner et al., 1985).

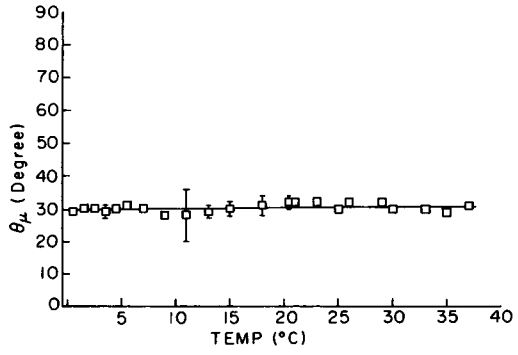

FIGURE 4 θ_μ vs. temperature for DPH-PC in DOPE. Error bar represents estimated fitting error.

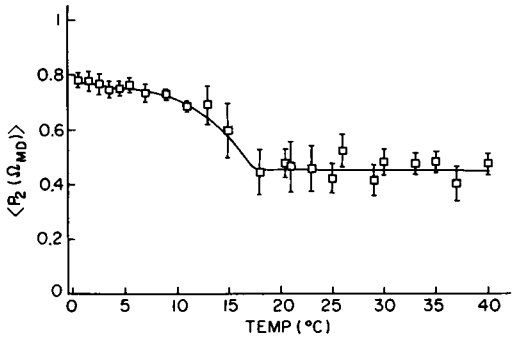

FIGURE 5 $\langle P_2(\Omega_{MD})\rangle$ vs. temperature for DPH-PC in DOPE. Error bar represents estimated fitting error.

The dependence of wobbling diffusion constant $D_\perp$ with temperature was shown in an Arrhenius plot (Fig. 6). An abrupt decline in $D_\perp$ was found at ~10°C, i.e., at the L_α and H_{II} phase transition. Similar to the rotational behavior of molecules in isotropic medium, the value of $D_\perp$ of fluorophore in amphiphilic aggregates increases with temperatures. The activation energies of the wobbling diffusion in the L_α and H_{II} phases were found to be 3.2 ± 0.5 and 2.2 ± 0.2 kcal/mol, respectively. Because diffusional motion is temperature sensitive, it is inappropriate to compare the values of $D_\perp$ at different temperatures. But upon extrapolating the values of $D_\perp$ in the L_α and H_{II} phases to the transition temperature (10°C), it was found that the value of $D_\perp$ in the L_α was $3.2 \times 10^7\ s^{-1}$ and that in the H_{II} phase was $1.7 \times 10^7\ s^{-1}$ as shown in Fig. 6.

DISCUSSION

The distribution of the fluorophore symmetry axis (M) with respect to the director axis (D) of the lipids is given

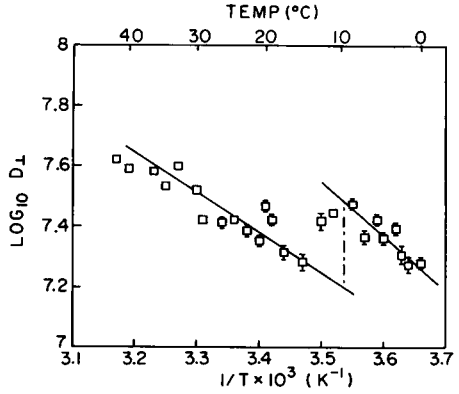

FIGURE 6 $\log_{10} D$ vs. inverse temperature (Arrhenius plot) for DPH-PC in DOPE. Error bar represents estimated fitting error.

by the order parameter $\langle P_2(\Omega_{MD})\rangle$. It is understood that a complete description of the orientational order of fluorophores in unaxially packed aggregates requires higher order expansion terms of the distribution function, such as $P_4(\Omega_{MD})$. The knowledge of higher order terms is important for understanding the existence of collective molecular tilt (Small, 1986) and nonuniform distribution of fluorophores within the lipids (Ameloot et al., 1984). From the x-ray diffraction study, the molecular tilt structure has only been found in some lower temperature phases. Moreover, those higher order terms were found to be negligible in those cases where the fluorophores are confined to distribute symmetrically and uniformly with respect to the local normal axis of the surface of the lipids (Deinum et al., 1988). Thus the higher order expansion of distribution for DPH-PC fluorophore was neglected in the first order approximation.

The decline in $\langle P_2(\Omega_{MD})\rangle$ as the lipids change from L_α to H_{II} phase can be explained by a change in the packing symmetry of the lipids. In both L_α and H_{II} phases the local packing symmetry axis of the molecules corresponds to the normal (N) of the surface of the lipids. In the one-dimensional lamellar L_α phase, N is parallel to the director axis D of the lipid layers. However in the two dimensional cylindrical H_{II} phase, the D axis, which corresponds to the symmetry axis of the cylinder, is always perpendicular to N. With this significant change in the aggregate symmetry, $\langle P_2(\Omega_{MD})\rangle$ is equal to $\langle P_2(\Omega_{MN})\rangle$ for the fluorophore in the L_α phase, but equal to $-(1/2)\ \langle P_2(\Omega_{MN})\rangle$ in the H_{II} phase (Johansson and Lindblom, 1983). Similar to the definition of $\langle P_2(\Omega_{MD})\rangle$ (see Materials and Methods), $\langle P_2(\Omega_{MN})\rangle$ represents the order parameter of the fluorophore symmetry axis with respect to the local N axis and is therefore defined as the local order parameter. If the local order parameter remains constant in both phases, the theoretically predicted ratio of $\langle P_2(\Omega_{MD})\rangle$ in L_α to that in H_{II} should therefore be 2.0. In this study the ratio was 1.7 ± 0.2. Based on the above theory of packing symmetry, the lower order parameter ratio therefore reflects an increase in the local order packing of the fluorophore in the H_{II} phase. A simple calculation revealed that the ratio of local order parameter in the L_α phase to that in H_{II} phase is 0.9. It is believed that the higher local order parameter of fluorophore in H_{II} phase is due to the enhanced packing constraint of the molecules in the hydrocarbon region of the lipids. The origin of this higher packing constraint may be due to a change in the local geometry of the lipids, i.e., a reduction of the surface curvature of the lipids in the tubular H_{II} phase.

There is however an alternative explanation for the discrepancy of the measured order parameter ratio. Noted that the validity of the symmetry packing expression mentioned above is based on two implicit assumptions: (a) At sufficiently long time (t_∞), the fluorophores excited at time zero (photo-selection) can reorient themselves to an equilibrium distribution with respect to the director axis and the local packing of the fluorophore depends on the packing symmetry of the lipids, and (b) the correlation time for the reorientation of fluorophores around the long symmetry axis of the cylindrical tubes in H_{II} phase is much shorter than t_∞. An expression for the anisotropic decay of fluorophore in H_{II} phase that included the motional contribution of rotation of the fluorophore around the long symmetry axis of the cylindrical tubes (hopping motion) has recently been determined (van der Meer, B.W., private communication). A lower limit of this hopping motion was found to be $\sim 3 \times 10^8\ s^{-1}$ which was ~10-fold higher than the wobbling diffusion constant of the fluorophore (see Appendix). A more detailed discussion of the above theory will be presented elsewhere.

The rotational diffusion constant $D_\perp$ measures the rate of wobbling motion of the fluorophore symmetry axis around the N. This parameter is therefore sensitive to the dynamic interactions (Hu and Zwanzig, 1974) of the fluorophores with the nearby host molecules in an intrinsically anisotropic environment. The wobbling diffusion motion in the H_{II} phase was found to be slower than that in the L_α phase at the transition temperature. From vibrational spectroscopic measurements (Wong et al., 1986), an increase in the amount of gauche rotamers of the alkene chains was detected as the lipids changed from the L_α to H_{II} phase. The increased amount of gauche rotamers in the hydrophobic region of the lipids may alter the interactions between the fluorophore and its neighbor and impose a constraint in the rotational motion of the fluorophore. It is likely that the more disordered alkene chains cause an increase in the "sticking" rotational mode and a concomitant decrease in the "slipping" rotational mode of the fluorophores at the phase transition. This alteration of rotation mode of fluorophore has also been reported previously (e.g., Chong and Cossins, 1983; Cheng and Lepock, 1985). Whether a similar conclusion can be drawn regarding the fluorophore rotation along its long symmetry axis (i.e., the behavior of $D_\parallel$) in lipids is now under investigation.

There is a possibility that the observed decline in both the $\langle P_2(\Omega_{MD})\rangle$ and $D_\perp$ in the H_{II} phase can be explained by a phase separation of DPH-PC probes from the DOPE lipids at temperature above 10°C. This possibility appears less likely because of the following explanations. Firstly, at low PC/PE ratio, both PE and PC exhibit ideal mixing behavior in the H_{II} phase as determined from the P-31 and H-2 NMR measurements (Tilcock et al., 1982). Secondly, if most of the DPH-PC probes are excluded from the DOPE lipids in the H_{II} phase, one should observe a quenching of the fluorescence lifetime of DPH-PC. This

fluorescence lifetime quenching phenomenon of DPH-PC was used to study the phase-separation behavior of liposomes (Parente and Lentz, 1986). However, as shown in Fig. 2, it is clear that no change in the fluorescence lifetime of DPH-PC occurs at temperature above 10°C. Lastly, the change of $D_\perp$ at the $L_\alpha - H_{II}$ transition is quite different from that at the transition involving lamellar-to-lamellar form, particularly the order-to-disorder transition of the fatty acyl chains. In the latter case, the value of $D_\perp$ increases, instead of decreases, at the transition (Parente et al., 1985). Therefore, the changes in the physical parameters reported by DPH-PC in this study reflect the $L_\alpha - H_{II}$ transition.

In the analysis of fluorescence depolarization, it is commonly assumed that the value of $r(0)$, the limiting anisotropy at zero time, is a fixed number (Ameloot et al., 1984). From the theory (Eq. 3), $r(0)$ is related to θ_μ and θ_ν. From angle-resolved fluorescence depolarization measurements (Deinum et al., 1988) on DPH and its analogue trimethyammonium DPH (TMA-DPH) in macroscopically oriented samples, θ_ν was found to be close to 0, whereas θ_μ varies from 10 to 40°. The exact value of θ_μ was known to depend on the chemical structure of the fluorophore and also the composition of the lipids. In this study, the DPH is covalently attached to the *sn*-2 position of the glycerol backbone of the lipid and θ_μ was found to be 29° and independent of temperature. It is interesting to mention that even if the $r(0)$ value is confined to a fixed value (e.g., 0.38 [see Parente et al., 1985]) the calculated values of diffusion constants and order parameters showed exactly the same temperature dependence as discussed above.

One other point that deserves further clarification is the photophysics of the DPH-PC probe used in this study. The validity of the simple anisotropy decay formula mentioned in Materials and Methods is based on the assumptions that the probe is cylindrically symmetric and the absorption dipole moment of the probe is parallel to the molecular long axis. Yet the above two assumptions have never been well established. Even though the chromophore (DPH) has a plane of symmetry, an inversion center and a twofold rotation axis perpendicular to the molecular plane (Zannoni, 1980), a twofold symmetry operation may not exist to establish the "cylindrical" symmetry assumption of the whole DPH-PC molecule. Hence the direction of the absorption dipole may not be exactly parallel to the long axis of the molecule. It may be that the possible rapid rotation around the molecular long axis (Pastor et al., 1988) that is unresolved in these measurements results in an effective cylindrical symmetry of the whole molecule. Therefore the simple first order approximation of the anisotropy decay formula will still be valid. A further investigation of the photophysics of this lipid probe is now in progress in this laboratory.

APPENDIX

It can be shown (van der Meer, B. W., private communication) that at sufficiently long time t_∞ and under the same first order approximation, the ratio of residual anisotropy of the fluorophore in the H_{II} phase to that in the L_α phase can be written as follows:

$$[r(t_\infty)]_H/[r(t_\infty)]_L = (1/4)[\langle P_2(\Omega_{MN})\rangle_H^2 / \langle P_2(\Omega_{MN})\rangle_L^2][1 + 3\exp(-4D_c t_\infty)]. \quad (4)$$

Here D_c represents the rotational diffusion of the fluorophore around the long symmetry axis of the cylindrical tubes. The subscripts L and H represent the lamellar and hexagonal phase, respectively. It is clear that this expression agrees with the simple symmetry argument described above by taking the value of D_c to be infinite, and the discrepancy in the measured order parameter ratio is then attributed solely to the local order parameter difference in the two phases. Because one has no prior knowledge of the magnitude of $D_c t_\infty$ in the exponential term, an average of this term over the fluorescence lifetime τ is therefore used. The last term of Eq. 4 therefore can be rewritten as $[1 + 3/(1 + 4D_c\tau)]$. With this result, one can further make an estimate of the magnitude of D_c. Assuming the ratio of the order parameter (second term in the expression) is one and using the measured ratio of residual anisotropy (=0.34), the value of D_c was found to be $3 \times 10^8\ s^{-1}$.

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