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Rotational Diffusion of a Steroid Molecule in Phosphatidylcholine–Cholesterol Membranes: Fluid-Phase Microimmiscibility in Unsaturated Phosphatidylcholine–Cholesterol Membranes†

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ABSTRACT: Rotational diffusion of androstane spin-label (ASL), a sterol analogue, in various phosphatidylcholine (PC)–cholesterol membranes was systematically studied by computer simulation of steady-state ESR spectra as a function of the chain length and unsaturation of the alkyl chains, cholesterol mole fraction, and temperature for a better understanding of phospholipid–cholesterol and cholesterol–cholesterol interactions. Special attention was paid to the differences in the cholesterol effects on ASL motion between saturated and unsaturated PC membranes. ASL motion in the membrane was treated as Brownian rotational diffusion of a rigid rod within the confines of a cone imposed by the membrane environment. The wobbling rotational diffusion constant of the long axis, its activation energy, and the cone angle of the confines were obtained for various PC–cholesterol membranes in the liquid-crystalline phase. Cholesterol decreases both the cone angle and the wobbling rotational diffusion constant for ASL in all PC membranes studied in this work. The cholesterol effects are the largest in DMPC membranes. An increase of cholesterol mole fraction from 0 to 30% decreases the rotational diffusion constant by a factor of 9–15 (depending on temperature) and the cone angle by a factor of about 2. In dioleoyl-PC membranes, addition of 30 mol % cholesterol reduces both the rotational diffusion constant and the cone angle of ASL by factors of $\approx$2.5 and $\approx$1.3, respectively, while it was previously found to cause only modest effects on the motional freedom of phospholipid analogue spin probes [Kusumi, A., Subczynski, W. K., Pasenkiewicz-Gierula, M., Hyde, J. S., & Merkle, H. (1986) *Biochim. Biophys. Acta 854*, 307–317]. It is proposed that fluid-phase microimmiscibility takes place in dioleoyl-PC–cholesterol membranes at physiological temperatures, which induces cholesterol-rich domains in the membrane, partially due to the steric nonconformability between the rigid fused-ring structure of cholesterol and the 30° bend at the C9–C10 cis double bond of the alkyl chains of dioleoyl-PC. The mechanism by which cholesterol influences the lipid dynamics in the membrane is different between saturated and unsaturated PC membranes.

Phosphatidylcholine (PC)[1]–cholesterol membranes have been extensively utilized as model membranes to study a variety of biochemical and biophysical processes involving cellular plasma membranes because PC and cholesterol are the major constituents of eukaryotic plasma membranes [for reviews on cholesterol effects on membranes, see Demel and de Kruyff (1976), Schroeder (1984), Yeagle (1985, 1988) and Presti (1985)]. For example, recent studies using PC–cholesterol membranes include the following subjects: protein–lipid and protein–protein interactions in reconstituted membrane vesicles of band 3–PC–cholesterol (Kusumi et al., 1990), adsorption and fusion of vesicular stomatitis virus (Yamada & Ohnishi, 1986) and Sendai virus (Asano & Asano, 1988), and oxygen transport in the membrane (Subczynski et al., 1989). A requirement of cholesterol was found for pressure-induced expulsion of a local anesthetic from the model membranes (Auger et al., 1987). Inclusion of cholesterol was found to be essential for functional reconstitution of fusion activities of influenza virus in artificial membranes (Kawasaki et al., 1983). For further advancement of these types of investigations on plasma membranes, a more comprehensive understanding of lipid organization, lipid mobilities, and PC–PC, PC–cholesterol, and cholesterol–cholesterol interactions in PC–cholesterol membranes is needed.

In the course of our recent studies of molecular diffusion in PC–cholesterol membranes, we found striking differences in the effect of cholesterol between saturated and unsaturated PC membranes:

(1) Lateral diffusion of a fluorescently labeled phosphatidylethanolamine is decreased by a factor of 4 in the presence of 30 mol % cholesterol in L-α-dimyristoylphosphatidylcholine (DMPC, a saturated PC) membranes. However, incorporation of cholesterol shows little effect on lateral diffusion in L-α-dioleoylphosphatidylcholine (DOPC, an unsaturated PC) membranes [see Table III in Kusumi et al. (1986)].

(2) Gauche–trans isomerization of alkyl chains as observed with stearic acid spin-labels (SASL) is decreased with incorporation of cholesterol up to 33 mol % at 45 °C and then slightly increased from 33 to 50 mol % (cholesterol mole

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[1] Abbreviations: PC, phosphatidylcholine; DMPC, L-α-dimyristoylphosphatidylcholine; DOPC, L-α-dioleoylphosphatidylcholine; SASL, doxylstearic acid spin-label; CSL, cholestane spin-label; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; ASL, androstane spin-label; DLPC, L-α-dilauroylphosphatidylcholine; DPPC, L-α-dipalmitoylphosphatidylcholine.

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fraction that gives minimal gauche–trans isomerization increases with temperature). Unsaturation of alkyl chains moderates, or in some cases abolishes, the cholesterol effects on alkyl chain motion (Kusumi et al., 1986; Merkle et al., 1987).

(3) Oxygen permeability of the membrane as measured with SASL is decreased in the presence of cholesterol in DMPC membranes. The cholesterol effect is much less in DOPC membranes. Cholesterol *increases* oxygen transport in the central region of DOPC membranes (Subczynski et al., 1989).

(4) Translational diffusion of a square-planar copper complex, 3-ethoxy-2-oxabutyraldehyde–bis(N^4,N^4-dimethylthiosemicarbazonato)copper(II), in the membrane as measured by the collision rate with SASL is decreased in the presence of cholesterol in saturated PC membranes but not in unsaturated PC membranes (Subczynski et al., 1990).

In the investigations above, we utilized phospholipid analogue probes to study cholesterol effects on PC membranes. All the results above indicated that, with phospholipid analogue probes, the cholesterol effect is strong in saturated PC membranes and weak in unsaturated PC membranes.

Recently, however, we have made an apparently contradictory observation using the cholestane spin-label (CSL), a spin-labeled *cholesterol analogue* (Kusumi & Pasenkiewicz-Gierula, 1988). A strong effect of cholesterol on CSL motion in *unsaturated* PC membranes was found: the rotational diffusion of the CSL long axis (wobbling diffusion) in unsaturated PC membranes was greatly decreased in the presence of cholesterol. When the dynamics of the CSL long axis was modeled as Brownian rotational diffusion within the confines of a cone, both the wobbling diffusion constant (D_w) and the semi cone angle (θ_C) are decreased with an increase of cholesterol mole fraction in DOPC membranes. Very recently, Shin and Freed (1989) have studied lateral and rotational diffusion of CSL and 16-doxyl-PC spin-label in 1-palmitoyl-2-oleoyl-PC (POPC)–cholesterol oriented multilamellar membranes and also found that cholesterol affects CSL motion strongly and PC motion weakly in these membranes.

On the basis of these findings, in the present study, we have further investigated the PC–cholesterol interaction with special attention paid to differences between saturated and unsaturated PC membranes. In spite of intensive studies of PC–cholesterol membranes in many laboratories, we were unable to find in the literature any indication of studies that focused on differences in cholesterol effects between saturated and unsaturated PC membranes. Thus, we have undertaken systematic research on the effects of alkyl chain unsaturation and cholesterol mole fraction on cholesterol motion in PC–cholesterol membranes.

We selected androstane spin-label (ASL) as a spin-labeled cholesterol analogue. Previously, we used CSL as a cholesterol analogue because its molecular structure and phase behavior in PC–cholesterol membranes are similar to those of cholesterol (Müller-Landau & Cadenhead, 1979a,b; Cadenhead & Müller-Landau, 1979; Presti & Chan, 1982). However, there are two major drawbacks to the use of CSL compared with ASL: (1) labeling of biological membranes with CSL is extremely difficult (Schreier et al., 1978); (2) the magnetic parameters of CSL are very sensitive to the environment such as host PC species, and they have to be determined for each membrane separately before motional parameters of CSL are obtained (Kusumi et al., 1986; Kusumi & Pasenkiewicz-Gierula, 1988).

In spin-label studies of membrane lipids, two classes of labels have been used: phospholipid spin-labels and SASL to study alkyl chain motion and CSL and ASL to study cholesterol motion. It must be remembered that the experiments carried out with the probe molecules necessitate due caution in interpretation of the results and that the labeled molecules cannot be expected to mimic all properties of phospholipid alkyl chains and cholesterol. Nevertheless, the interaction of ASL (or CSL or SASL) with phospholipid and cholesterol should, to a certain degree, approximate cholesterol–PC and cholesterol–cholesterol interactions in the membrane because of the overall similarity of the molecular structure (Müller-Landau & Cadenhead, 1979a,b; Cadenhead & Müller-Landau, 1979; Presti & Chan, 1982; Kusumi et al., 1986).

In the present work, ASL dynamics was studied in the liquid-crystalline phase of L-α-dilauroylphosphatidylcholine (DLPC, 12 carbons in a saturated alkyl chain), DMPC (14 carbons, saturated), L-α-dipalmitoylphosphatidylcholine (DPPC, 16 carbons, saturated), and DOPC (18 carbons, a cis double bond between C9 and C10) membranes as a function of the mole fraction of cholesterol and temperature. In the analysis of ESR spectra by computer simulation, ASL was treated as a rigid rod undergoing Brownian rotational diffusion within the confines of a cone (Israelachvili et al., 1975; Wang & Pecora, 1980; Kinosita et al., 1984; Kusumi & Pasenkiewicz-Gierula, 1988). By comparing the data obtained in the present work with our previous results (Kusumi et al., 1986; Kusumi & Pasenkiewicz-Gierula, 1988; Merkle et al., 1987; Subczynski et al., 1989, 1990), it is concluded that more extensive phase separation in unsaturated PC–cholesterol membranes in the liquid-crystalline phase takes place than in saturated PC–cholesterol membranes at physiological temperatures partially due to the conformational mismatch of cholesterol and unsaturated PC.

Experimental Procedures

Sample Preparation. ASL was obtained from Syva (Palo Alto, CA). All phospholipids were purchased from Sigma (St. Louis, MO), and cholesterol (crystallized) was from Boehringer (Indianapolis, IN). All reagents were used without further purification. Lipids and ASL were mixed at a molar ratio of 400:1 in chloroform, and the solvent was evaporated with a stream of nitrogen gas. Residual lipids were placed under vacuum ($\approx$0.06 mmHg) for at least 12 h. Liposomes (multilamellar dispersion of lipids) were formed by addition of 65 mM NaCl buffered with 10 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (pH 7.6) to dried lipid at temperatures 25 °C above the phase transition temperature of each phospholipid and vortexing. Liposomes were centrifuged at 12800*g* for 10 min at 4 °C, and the loose pellet was used for ESR measurements.

ESR Measurements of ASL—Fast Motional Case. All samples were deoxygenated by placement in gas-permeable capillary (i.d. 0.7 mm) made from a methylpentene polymer called TPX (Subczynski & Hyde, 1989). This plastic is permeable to oxygen, nitrogen, and carbon dioxide and is substantially impermeable to water. Samples were equilibrated with nitrogen gas that was used for temperature control.

ESR spectra of ASL undergoing fast motion were obtained with a Varian E-109 X-band spectrometer equipped with a Varian variable-temperature accessory and an E-231 Varian multipurpose cavity (rectangular TE_{102} mode). The same dewar insert was used to maintain a constant effective microwave field on the sample (Kusumi et al., 1978, 1980). A modulation width (peak to peak) of 0.5 G and an incident microwave power of 0.5 mW were used. Although this power is much less than the power level employed conventionally, we observed very slight saturation of the ESR signal. Nev-

ertheless, 0.5 mW was utilized to improve the signal-to-noise ratio.

ESR Measurements of Rigid-Limit Spectra at Q-band. Q-band spectra were recorded at −120 °C with a Varian E-110 microwave bridge connected to a Varian E-9 spectrometer with a Varian variable-temperature accessory and a Varian cylindrical cavity (TE_{011} mode). For the second-derivative display, field modulation of 50 kHz with in-phase detection at 100 kHz was used.

Simulation of ESR Spectra. Determination of magnetic parameters (**g** and **A** tensors) was carried out by simulating Q-band second-derivative ESR spectra at −120 °C (Pasenkiewicz-Gierula et al., 1983; Kusumi & Pasenkiewicz-Gierula, 1988). Since, at the rigid limit, the second-derivative Q-band spectrum is much richer in spectral features compared with the conventional first-derivative X-band spectrum, a more reliable simulation is possible in this display (Pasenkiewicz-Gierula et al., 1983). Simulation of rigid-limit Q-band spectra was carried out on a CDC Cyber 72 computer by an automated nonlinear least-squares minimization using the program MINUIT from the CERN program library. The central processing unit time for a simulation was about 60 s. Obtaining the best-fit parameters required at least 300 iterations.

Fast-motional spectra at X-band were simulated as described previously (Kusumi & Pasenkiewicz-Gierula, 1988). Simulation was carried out on an MC5500 Masscomp computer. The central processing unit time for a simulation was less than 10 s.

Results

Determination of Magnetic Parameters of ASL in Various PC–Cholesterol Membranes. Previously, we used CSL to study rotational diffusion of cholesterol in PC–cholesterol membranes. However, working with CSL can be very cumbersome because the magnetic parameters (**g** and **A** tensors) of CSL have to be determined for each membrane system due to their strong dependences on the polarity of the environment. In addition, labeling the biological membranes with CSL is difficult because of its much lower dispersibility in water (or in detergent micelles) than ASL, fatty acid spin probes, and phospholipid spin probes. To circumvent these problems, ASL has been employed in the present study. Since the nitroxide group of ASL is located inside the membrane and not in the membrane surface region as is the case with CSL, the magnetic parameters of ASL are expected to be quite insensitive to environmental variations such as the host phospholipid alkyl chain length and unsaturation and the cholesterol mole fraction.

ASL has been extensively utilized to study artificial and biological membranes (Hubbell & McConnell, 1971; Kusumi et al., 1983). Nevertheless, the magnetic parameters of ASL have never been determined in the membrane. In quantitative ESR studies of the molecular motion in the membrane by simulation of ESR spectra of spin-labels, their magnetic parameters have to be determined in situ (in the membrane) rather than in single crystals, as previously shown by us (Kusumi & Pasenkiewicz-Gierula, 1988).

To examine the effect of PC species and cholesterol content on the magnetic parameters of ASL, A_z's of ASL in various PC–cholesterol membranes were measured at the rigid limit by lowering the temperature to −120 °C (Griffith & Jost, 1976; Kusumi & Pasenkiewicz-Gierula, 1988). A_z is very sensitive to environmental changes and easy to obtain experimentally (Kusumi et al., 1986; Kusumi & Pasenkiewicz-Gierula, 1988). In Table I are listed A_z values for ASL in DLPC, DPPC, and DOPC membranes in the presence and absence of 50 mol % cholesterol. The variations in A_z are small.

Table I: A_z Values (G) of ASL and CSL in Various PC–Cholesterol Membranes

host lipid	ASL	CSL[a]
DLPC	32.4	33.8
DLPC/50% chol[b]	32.4	34.8
DPPC	32.7	34.0
DPPC/50% chol	32.3	35.05
DOPC	32.6	33.05
DOPC/50% chol	32.4	35.0

[a] From Kusumi et al. (1986). [b] 50% chol = 50 mol % cholesterol.

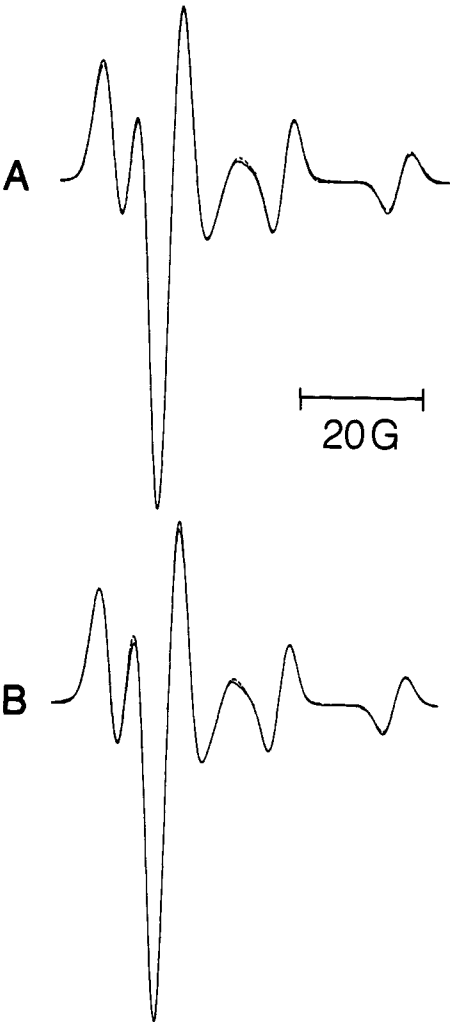

FIGURE 1: Experimental (solid line) and simulated (broken line) second-derivative Q-band ESR spectra of ASL in DMPC (A) and egg yolk PC (B) membranes at −120 °C.

In the course of obtaining six magnetic parameters, first g_z and A_z were determined experimentally from the X-band ESR spectrum recorded at −120 °C. The other magnetic parameters were determined as best-fit parameters to the Q-band second-derivative ESR spectrum recorded at −120 °C. Pasenkiewicz-Gierula et al. (1983) showed that a Q-band second-derivative spectrum is a favorable display for the determination of all six magnetic parameters. They werr able to determine **g** and **A** values of nitroxide spin-labels more accurately by using frozen solutions than by using single crystals as performed before their study. The χ^2 value increased for changes of ±0.00002 in **g** values and ±0.02 G in **A** values.

Figure 1 shows the superimposed experimental and simulated second-derivative spectra for ASL in DMPC and egg yolk PC membranes at Q-band at −120 °C. Further lowering

Table II: Magnetic Parameters of ASL in DMPC and Egg Yolk PC Membranes Determined by Computer Simulation[a]

host lipid	g_x	g_y	g_z	A_x	A_y	A_z
DMPC	2.008 74	2.006 04	2.002 34	5.45	5.04	32.96
egg yolk PC	2.008 74	2.006 01	2.002 33	5.45	5.07	32.72

[a] g_z's and A_z's were obtained experimentally. A's are expressed in gauss.

of temperature did not change the peak positions, and the mobility of ASL was regarded as almost frozen in the time scale of the anisotropy of the nitroxide radical at this temperature. We consider the quality of the fits exceptional. The magnetic parameters of ASL in various PC–cholesterol membranes that give the best fits are listed in Table II and are shown to be very similar to each other.

These results indicate again that the magnetic parameters of ASL are rather insensitive to changes in PC species and cholesterol content in the membrane. Thus, we used the same magnetic parameters (i.e., those in the DMPC membranes) throughout this study.

Fast Rotational Diffusion of ASL in the Membrane. ASL motion in the membrane is treated as Brownian rotational diffusion of a rigid rod within the confines of a cone of a semi cone angle of θ_C (Kusumi & Pasenkiewicz-Gierula, 1988). These spatial confines imposed by the environment (restoring potential) can be described as a square well potential with infinitely high barriers at θ_C (which may approximate a Gaussian shape of the potential). In this model, one considers two *independent* modes of ASL motion: (1) axial rotation of ASL about its long axis (i.e., ASL rotates rapidly about its long axis with a rotational diffusion coefficient $D_{\parallel}$, irrespective of the orientation of the long axis) and (2) wobbling of the long axis itself within a cone [i.e., ASL (rigid rod) reorients rapidly with a rotational diffusion coefficient D_w within the confines of a cone of a semi cone angle of θ_C].

To simulate ESR spectra of ASL in the membrane, the theory by Israelachvili et al. (1975) was employed as was done previously (Pasenkiewicz-Gierula & Kusumi, 1988). The line width can be expressed as

$$\Gamma_m = \Gamma_r + A^2(\langle\cos^4\theta\rangle - \langle\cos^2\theta\rangle^2)\gamma_e\tau_w + (1/8)B^2\langle\sin^4\theta\rangle\gamma_e\tau_R \quad (1)$$

$$A = (h\nu/\beta_e g_0^2)[g_y - (1/2)(g_z + g_x)] + m[T_y - (1/2)(T_z + T_x)] \quad (2)$$

$$B = (h\nu/\beta_e g_0^2)(g_x - g_z) + m(T_x - T_z) \quad (3)$$

where Γ_r is the residual line width, β_e is the Bohr magneton, γ_e is the magnetogyric ratio of the free electron, τ_w and τ_R are rotational correlation times, θ is the instantaneous orientation of the nitroxide z axis with respect to the magnetic field direction, g_0 is the trace of the **g** tensor, and m is the magnetic quantum number. $\langle\ \rangle$ indicates averaging over the semi cone angle θ_C and depends on θ_C and the angle between the cone axis and the magnetic field.

The correlation time τ_w appearing in the second term of eq 1 arises from pure wobbling within the cone (Israelachvili et al., 1975), and D_W can be calculated from τ_w and θ_C (which are obtained by simulating the ESR spectrum) according to the theory of Wang and Pecora (1980):

$$D_W = 1/[\nu_2^0(\nu_2^0 + 1)\tau_w] \quad (4)$$

where ν_2^0 only depends on the semi cone angle and is calculated according to the method of Pal (1918).

The other correlation time τ_R appearing in the third term of eq 1 arises from both axial rotation and wobbling. Therefore, τ_R represents a mixed correlation time of rotation and wobbling (Israelachivili et al., 1975) and is a function of $D_{\parallel}$, D_W, and θ_C. Since we have not succeeded in calculating $D_{\parallel}$ from τ_R, D_W, and θ_C, we will not discuss $D_{\parallel}$ (and τ_R) any further in the present paper. For more details of the theory and ESR spectral simulation, readers are referred to Kusumi and Pasenkiewicz-Gierula (1988).

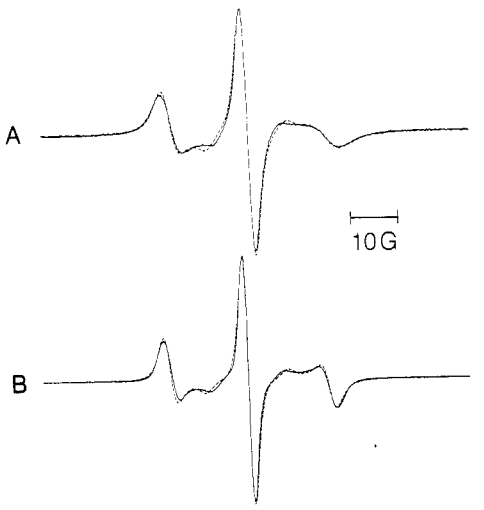

FIGURE 2: Experimental (solid line) and simulated (broken line) X-band ESR spectra of ASL in 15 mol % cholesterol–DMPC membranes at 27 °C (A) and in 30 mol % cholesterol–DMPC membranes at 65 °C (B).

Figure 2 shows experimental and simulated X-band spectra of rapidly moving ASL in DMPC–cholesterol membranes. The fit is good for spectra taken in the liquid-crystalline phase, where the assumption of rapid motion is satisfied. The fit between simulated and experimental spectra is poor when the motion becomes slow, e.g., in DMPC–50 mol % cholesterol membranes or at lower temperatures. This shows a limitation of the theory employed in this work. An attempt was made to use computer programs based on the stochastic Liouville theory developed by Freed's group (Freed et al., 1971; Goldman et al., 1972; Polnaszek et al., 1973, 1981; Meirovitch & Freed, 1984; Shin & Freed, 1989). However, when restoring potential was included in the program, computation time became inhibitively long even for ASL undergoing rapid motion. When the restoring potential was removed from the program, it was impossible to satisfactorily simulate ASL spectra in membranes. The motion of spin-labels in oriented multilamellar membranes has been analyzed by the Freed's theory (Meirovitch & Freed, 1984; Shin & Freed, 1989). However, these membrane samples contain only 15–20% (w/w) water, which is known to considerably affect the lipid motion and phase transition (Kuo & Wade, 1979). Thus, the oriented membranes are not suitable for the purpose of the present study.

Estimation of the accuracy of motional parameters is difficult due to complex interplay of these parameters. However, (1) changes in the cone angle by more than 2° or (2) changes in τ_R by 15% lead to an obviously worse fit; (3) simulation is quite sensitive to wobbling rotational correlation time τ_w (a function of both D_w and θ_C) when τ_w is smaller than 0.9 ns.

The Lorentzian residual line widths (half-width at half-height) of ASL in DMPC–cholesterol and DOPC–cholesterol membranes are plotted against temperature in Figure 3. The residual line widths in other membranes are similar to those shown in Figure 3 (data not shown). The range of spin–spin relaxation time calculated from the residual line width (notice that all samples were deoxygenated) is between 6.9×10^{-8} s

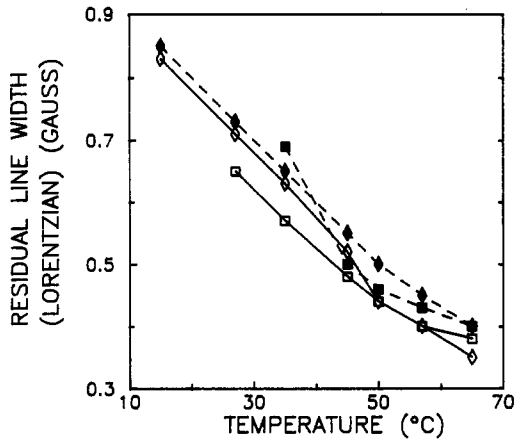

FIGURE 3: Lorentzian residual line width of ASL in various PC–cholesterol membranes plotted against temperature: (□) DMPC; (■) DMPC–30 mol % cholesterol; (◇) DOPC; (◆) DOPC–30 mol % cholesterol.

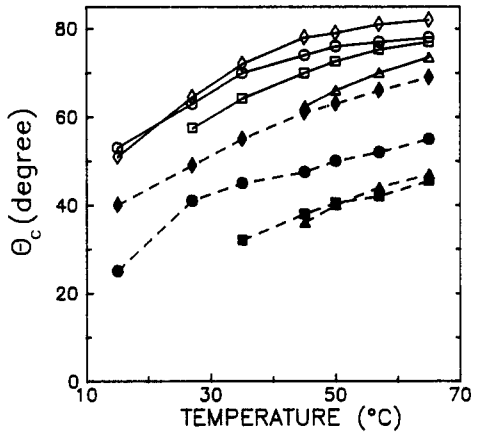

FIGURE 4: Semi cone angle (θ_C) for ASL plotted against temperature in various PC membranes containing either 0 mol % cholesterol (open keys) or 30 mol % cholesterol (closed keys): (○) DLPC; (□) DMPC; (△) DPPC; (◇) DOPC.

(at 15 °C) and 1.6×10^{-7} s (at 65 °C), which is in general agreement with the transverse relaxation times for CSL in egg yolk PC membranes obtained by the electron spin-echo method by Madden et al. (1980).

Figure 4 displays the temperature dependence of the semi cone angle of ASL in DLPC, DMPC, DPPC, and DOPC membranes in the presence and absence of 30 mol % cholesterol. It is rather surprising that θ_C is always larger than 50° in the absence of cholesterol and can be as large as 80° in DOPC membranes.

The semi cone angle for ASL decreases with increase in the alkyl chain length in saturated PC membranes as was previously found for CSL. This result is apparently at variance with our previous observation in which order parameters for 5-SASL were shown to be about the same in DMPC, DPPC, and DSPC membranes when they were compared at the same temperature (Kusumi et al., 1986). Because the intramolecular mobility of ASL is much smaller than that of 5-SASL alkyl chains due to the rigid planar steroid ring structure of ASL, ASL must be sensitive to changes at various "depths" in the membrane. It follows then that the alkyl chain order is larger for longer alkyl chains toward the center of the lipid bilayer, while it is similar near the surface of PC membranes for various alkyl chain lengths. These results are indicative of the dependence of the "alkyl chain flexibility gradient" (Hubbell & McConnell, 1971; McConnell, 1976; Stockton et al., 1976; Rice et al., 1978) on the length of phospholipid alkyl chains.

Incorporation of cholesterol decreases θ_C in all membranes. The effect is particularly large in DMPC and DPPC membranes. As was found previously, the 5-SASL and 16-SASL motion in saturated PC membranes is also affected by the presence of cholesterol. The cholesterol effect on the motional freedom of SASL in unsaturated PC membranes is much smaller than that in saturated PC membranes (Kusumi et al., 1986).

The presence of 30 mol % cholesterol substantially decreases the cone angle of ASL in DOPC membranes. The cholesterol effect is smaller in DOPC membranes than in saturated PC membranes, but it is much larger than one might expect from the cholesterol effect on 5-SASL and 16-SASL in DOPC membranes [see Table I and II in Kusumi et al. (1986)]. These results for ASL agree with our previous data for CSL. These data are summarized in Table III. The differences in cholesterol effect between steroid type spin-labels and SASL's and between saturated and unsaturated PC membranes will be discussed in the next section.

The rotational diffusion constant D_w is plotted against inverse temperature in Figure 5. D_w's for DMPC and DPPC membranes are about the same, and D_w's for DLPC and DOPC membranes are considerably larger. D_w is decreased as the mole fraction of cholesterol is increased in all membranes. Incorporation of 30 mol % cholesterol decreases D_w by a factor of 3–10. The cholesterol effect is much larger in saturated PC membranes. Due to the slow motion of ASL, we were unable to simulate ESR spectra for ASL in the 50 mol % cholesterol–DMPC membrane.

The straight lines shown in Figure 5 are drawn on the basis of a leasts-square fitting of the points in the indicated range,

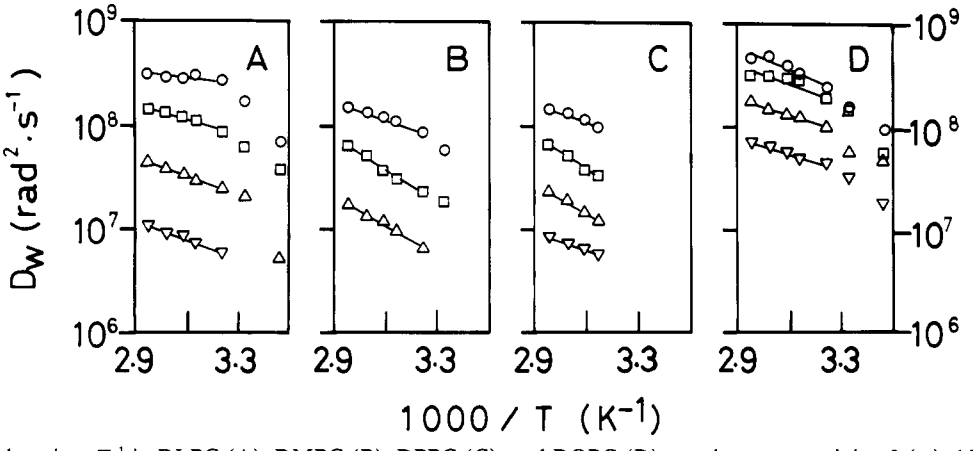

FIGURE 5: D_w plotted against T^{-1} in DLPC (A), DMPC (B), DPPC (C), and DOPC (D) membranes containing 0 (○), 15 (□), 30 (△), and 50 (▽) mol % cholesterol. The lines are the best linear fits in the indicated temperature range.

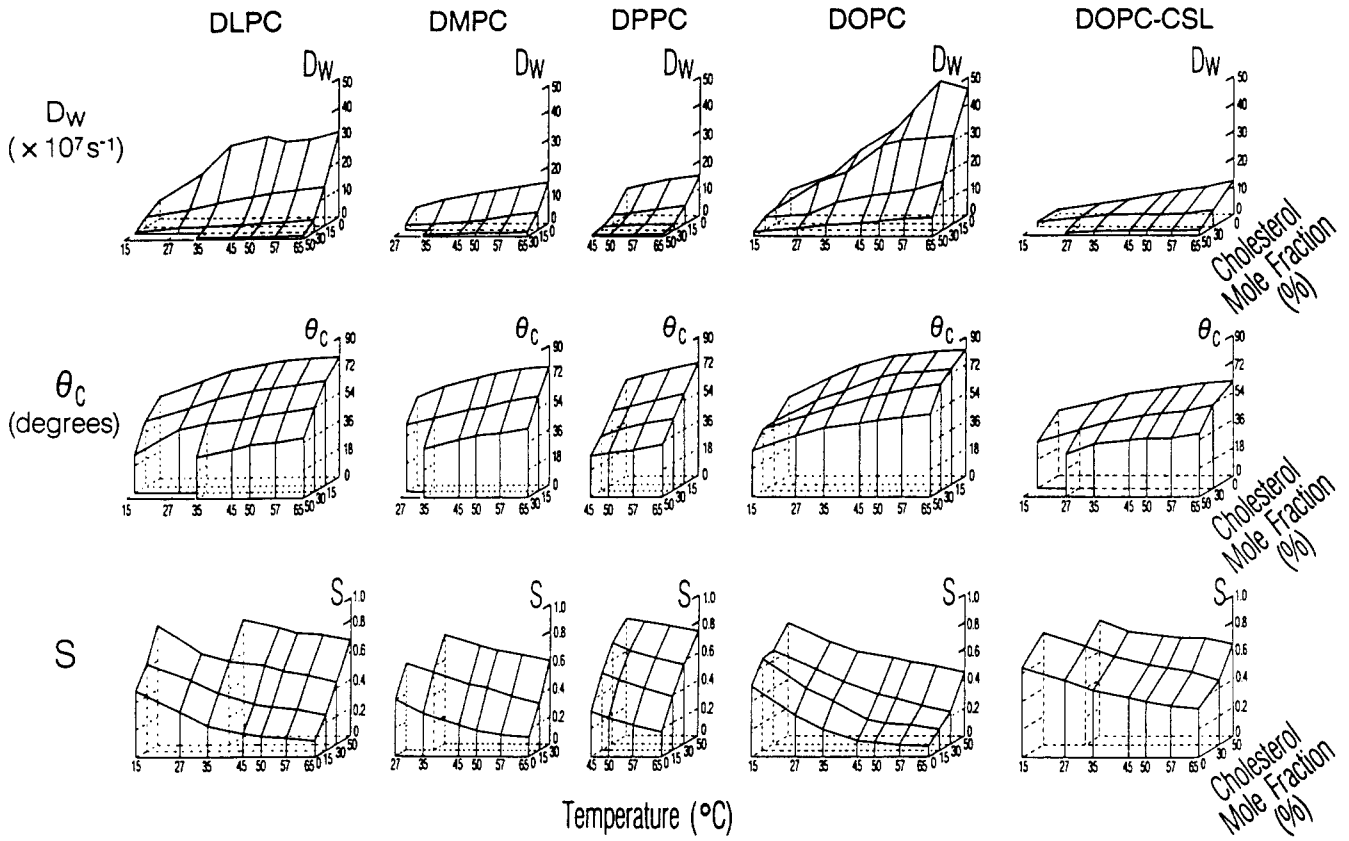

FIGURE 6: D_w, θ_C, and S of ASL in various PC membranes plotted as a function of temperature (x axis) and cholesterol mole fraction (y axis). CSL data on DOPC membranes (Kusumi & Pasenkiewicz-Gerula, 1988) are also shown.

Table III: Effect of Inclusion of 30 mol % Cholesterol in DMPC and DOPC Membranes on the Cone Angle (deg) of 5-SASL, ASL, and CSL

temp (°C)	host lipid	5-SASL[a]	ASL	CSL[b]
27	DMPC	46	57	ND[c]
27	DMPC + chol[d]	34	ND	ND
27	DOPC	46	64	50
27	DOPC + chol	45	49	39
45	DMPC	50	70	ND
45	DMPC + chol	41	38	ND
45	DOPC	51	78	57
45	DOPC + chol	48	61	46
60	DMPC	52	75[e]	ND
60	DMPC + chol	45	41[e]	ND
60	DOPC	52	81[e]	61
60	DOPC + chol	51	66[e]	49

[a] From Kusumi et al. (1986). [b] From Kusumi and Pasenkiewicz-Gierula (1988). [c] ND = not determined. [d] chol = 30 mol % cholesterol. [e] Measured at 57 °C.

which is well above the phase transition. The fit is better in the presence of cholesterol. The slope, which may be related to the activation energy, shows a complex and interesting behavior. However, we refrain from further discussion on this point in this paper because the quality of fit varies from sample to sample.

Figure 6 shows the summary of our results on D_w, θ_C, and the order parameter (S) as a function of cholesterol mole fraction and temperature. In our model, the order parameter can be related to θ_C as

$$S = (1/2)(\cos \theta_C)(1 + \cos \theta_C) \quad (5)$$

(Kusumi & Pasenkiewicz-Gierula, 1988). Figure 6 also shows D_w, θ_C, and S for CSL in DOPC–cholesterol membranes (Kusumi & Pasenkiewicz-Gierula, 1988). Both D_w and θ_C are smaller for CSL than for ASL. This difference may be explained by the presence of the anchoring alkyl chain tail in CSL.

Discussion

We have systematically studied rotational diffusion of ASL, a cholesterol analogue, with special attention paid to the differences in the cholesterol effects between saturated and unsaturated PC membranes. We focused our investigation on the liquid-crystalline phase because it is related to biological membranes under physiological conditions. Viewing PC–cholesterol membranes from the side of cholesterol as well as from the side of PC is important because the phase separation of the cholesterol-rich domains is proposed to take place even in the liquid-crystalline phase of PC–cholesterol membranes (Lentz et al., 1980; Recktenwald & McConnell, 1981; Presti & Chan, 1982; Kusumi et al., 1986). Taylor et al. (1982), Dufourc et al. (1984), and Dufourc and Smith (1986) have also emphasized studies of PC–cholesterol membranes from the viewpoints of both constituents. Although the cholesterol dynamics and molecular ordering in PC–cholesterol membranes have been studied extensively [in addition to the references already cited, see Hemminga (1974, 1977), Oldfield et al. (1978), Rogers et al. (1979), Polnaszek et al. (1981), Kelusky et al. (1983), and Smutzer and Yeagle (1985)], no systematic comparison of the cholesterol effects between saturated and unsaturated PC membranes has been carried out.

Recktenwald and McConnell (1981) proposed a phase diagram for DMPC–cholesterol membranes such as that displayed by the solid lines in Figure 7. The area in which our experiments were carried out is shown by stippling. According to Lentz et al. (1980), Recktenwald and McConnell (1981), and Presti et al. (1982), fluid-phase–fluid-phase separation occurs in region I. Region II also consists of two phases: the

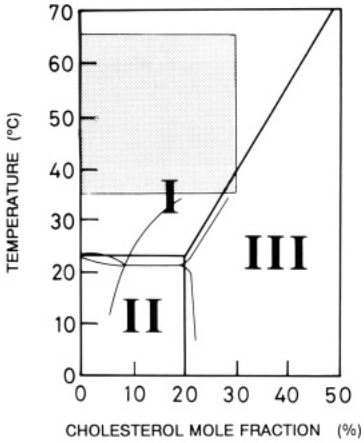

FIGURE 7: Phase diagram for DMPC–cholesterol membranes (Recktenwald & McConnell, 1981). The phase boundaries presented by Ipsen et al. (1987) are approximately shown by thin lines. The stippled area indicates the regions in which activation energy for rotational diffusion of ASL was estimated in the present work. The dotted line in region I indicates a phase boundary proposed by Shimshick and McConnell (1973) (see text). Lentz et al. (1980) published a very detailed phase diagram for DPPC–cholesterol membranes. However, the major features of the diagram, i.e., the phase boundaries between regions I and II and between regions II and III, are similar if the temperature is shifted by ≈18 °C (based on the phase transition temperatures for DMPC and DPPC membranes; Ladbrooke and Chapman, 1969).

solid phase of pure DMPC and the liquid-crystalline phase (in terms of translational mobilities of the constituents) that contains 20 mol % cholesterol and 80 mol % DMPC. Region III consists of one fluid phase. Most methods agree with the location of the boundaries between these regions (Shimshick & McConnell, 1973; Haberkorn et al., 1977; Recktenwald & McConnell, 1981; Kusumi et al., 1986). The phase diagram presented by Ipsen et al. (1987) is slightly more complex (schematically shown by the thin lines in Figure 7). Knoll et al. (1985), however, indicated that no boundary exists between regions I and III on the basis of their small-angle neutron scattering study. In the following discussion, we assume the phase diagram presented in Figure 7 for sake of clearer presentation. Our major conclusions are not dependent on the details of the phase diagram. (Discussion of the phase boundary will be made when necessary.)

The molecular structure of the membrane in region II has been extensively studied and largely elucidated (Copeland & McConnell, 1980; Owicki & McConnell, 1980; Hui & He, 1983; Imaizumi & Hatta, 1984). However, the molecular details in regions I and III have not been known although they are physiologically more important than those in region II. We have been addressing this problem in this series of work (Kusumi et al., 1986, 1989; Merkle et al., 1987; Kusumi & Pasenkiewicz-Gierula, 1988; Subczynski et al., 1989a,b). One of the most general rules in regions I and III in terms of *saturated* PC–cholesterol interactions is that addition of cholesterol leads to a more conformationally ordered state of the alkyl chains of the PC molecules; i.e., cholesterol interacts more favorably with PC alkyl chains in an extended conformation [Presti & Chan, 1982; Presti et al., 1982; Kusumi et al., 1986; Ipsen et al., 1987; also see Table I in Subczynski et al. (1989a)].

Saturated PC–Cholesterol Membranes. Strong effects of cholesterol on both the semi cone angle and the wobbling rotational diffusion constant for ASL were found in saturated PC membranes (Figures 4–6). These results are in general agreement with the previous NMR observation with deuterated cholesterol (Taylor et al., 1982; Dufourc et al., 1984; Dufourc & Smith, 1986). The cholesterol effects are the largest in DMPC membranes. An increase of cholesterol mole fraction from 0 to 30% decreases D_w by a factor of 9–15 (depending on temperature) and θ_C by a factor of about 2. The good match in hydrophobic lengths of DMPC and cholesterol may be related to this observation. It is noted that, in the absence of cholesterol, ASL motion (both θ_C and D_w) is also most restricted in DMPC membranes when the membranes used here are compared at the same temperature in the liquid-crystalline phase.

Table IV: Comparison of Motional Parameters of Various Rigid-Body Probes in PC–Cholesterol Membranes

host lipid	temp °C	probe	D_w ($\times 10^7$ s^{-1})	θ_C (deg)	S	ref
DMPC	35	ASL	8.7	64	0.31	*a*
DMPC	35	CSL	7.0	48	0.57	*b*
DMPC (sonicated)	35	DPH[h]	15.6	62	0.34	*c*
DMPC/30% chol[i]	35	ASL	0.63	32	0.78	*a*
DMPC (sonicated)	35	DPH	10.6	30	0.81	*c*
DPPC	50	ASL	11.4	66	0.28	*a*
DPPC	50	CSL	7.7	48	0.55	*b*
DPPC	50	DPH	29	61	0.36	*c*
DPPC (sonicated)	50	DPH	25	64	0.31	*d*
DPPC/30% chol	50	ASL	1.4	40	0.68	*a*
DPPC/50% chol	50	ASL	0.65	29	0.82	*a*
DPPC/50% chol	49	CSL	0.34	25	0.86	*e*
POPC	35	CSL	2.6	ND	0.45	*f*
POPC/30% chol	35	CSL	0.31	ND	0.81	*f*
DOPC	35	ASL	25	72	0.20	*a*
DOPC	35	CSL	5.8	53	0.48	*h*
DOPC	37	DPH	22	78	0.13	*d*
DOPC/30% chol	35	ASL	9.8	55	0.45	*a*
DOPC/25% chol	35	CSL	4.7	44	0.62	*b*
DOPC/50% chol	35	ASL	4.1	45	0.60	*a*
DOPC/50% chol	35	CSL	1.7	33	0.77	*b*
DOPC	30	CSL	4.8	50	0.53	*b*
DOPC/50% chol	30	CSL	1.4	31	0.80	*b*
EYPC[j]/30% chol	30	[^{2}H]chol	ND[k]	ND	0.75	*g*
EYPC/50% chol	30	[^{2}H]chol	ND	ND	0.80	*g*
DOPC	30	ASL	16	68	0.26	*a*
DOPC/30% chol	30	ASL	6.3	51	0.51	*a*
DOPC/50% chol	30	ASL	3.2	43	0.63	*a*

[a] This study. [b] Kusumi and Pasenkiewicz-Gierula (1988). [c] Kinosita and Ikegami (1984). [d] Stubbs et al. (1981). [e] Hemminga (1974). [f] Shin and Freed (1989). [g] Taylor et al. (1982). [h] DPH = 1,6-diphenyl-1,3,5-hexatriene. [i] Chol = cholesterol in mol percent [j] EYPC = egg yolk PC. [k] ND = not determined.

When D_w's are compared at the same temperature and at the same cholesterol content, D_w is larger in the order DOPC > DLPC > DPPC > DMPC. The difference between DOPC and saturated PC membranes becomes larger as the cholesterol mole fraction is increased.

The cone angle is larger in the order DOPC > DLPC > DMPC ≥ DPPC when θ_C's are compared at the same temperature and at the same cholesterol content.

D_w, θ_C, and S determined for four rigid-body probes, i.e., ASL, CSL, [^{2}H]cholesterol, and 1,6-diphenyl-1,3,5-hexatriene (DPH), in various PC–cholesterol membranes are summarized in Table IV. When comparing ASL data with CSL data, one notices larger D_w and θ_C for ASL probably due to the lack of the isooctyl side chain. DPH motion in the membrane can also be analyzed as a rigid rod undergoing Brownian rotational diffusion in a cone (Kawato et al., 1977; Kinosita et al., 1977). θ_C values for DPH are quite comparable to those for ASL and larger than those for CSL. D_w values for DPH are larger than those for ASL and CSL. Since DPH is expected to localize more in the inner part of the membrane than ASL and CSL and since the molecule is less bulky than ASL and CSL, greater mobility of DPH seems to be reasonable. The S value determined for cholesterol in egg yolk PC membranes by the NMR method (Taylor et al., 1982) agrees with that for CSL in DOPC–cholesterol (1/1) membranes, while it is larger than

S for CSL in DOPC–cholesterol (3/1) membranes. The lack of a 3-β-OH group in CSL and/or a difference in the definition of orienting potential may explain this difference. The mobility of ASL in DOPC–cholesterol membranes is much larger than that of [^{2}H]cholesterol in egg yolk PC–cholesterol membranes, indicating that due caution must be exercised in the interpretation of ASL results (ASL motion is not the same as cholesterol motion). The difference in the PC–cholesterol interaction between egg yolk PC and DOPC membranes can also contribute to these results.

DOPC–Cholesterol Membranes. Since biological membranes contain many unsaturated alkyl chains, an understanding of unsaturated PC–cholesterol interaction is important. The prevalent idea on unsaturated PC–cholesterol membranes appears to be a better miscibility of cholesterol with unsaturated PC than with saturated PC due to the "fluidizing effect" of unsaturated PC in the membrane (Jain & Wagner, 1980). The present data together with the results by Kusumi et al. (1986), Kusumi and Pasenkiewicz-Gierula (1988), and Shin and Freed (1989) strongly suggest just the opposite: the fluid-phase microimmiscibility is prevalent at physiological temperatures in unsaturated PC–cholesterol membranes.

Three characteristics of the cholesterol effects in PC–cholesterol membranes have emerged from the comparison of ASL data with SASL data and from the comparison of results of saturated PC membranes with those of unsaturated PC membranes. The following are key observations:

(I) In DOPC–cholesterol membranes, the cholesterol effect is much larger on ASL than on SASL, as summarized in Table III (this study; Kusumi et al., 1986; Kusumi & Pasenkiewicz-Gierula, 1988).

(II) The cholesterol effects on ASL motion are smaller in DOPC membranes than in saturated PC membranes. An increase in cholesterol mole fraction from 0 to 30% decreases D_w by a factor of about 2.5 and θ_C by a factor of about 1.3 in DOPC membranes as compared with decreases of a factor of 9–15 for D_w and 2 for θ_C in DMPC membranes.

(III) In saturated PC membranes, both ASL and SASL are strongly influenced by the presence of cholesterol.

We propose that the key feature of the DOPC–cholesterol interaction to explain these results is the considerable nonconformability of the molecular shapes of DOPC and cholesterol in the membrane. The cholesterol backbone is the rigid planar transfused tetracyclic ring structure of a steroid which reaches up to 9 or 10 carbons of extended alkyl chains and to a somewhat deeper level in the hydrophobic region of the membrane in the liquid-crystalline phase (Stockton & Smith, 1976; Huang, 1977; Presti & Chan, 1982; Presti, 1985). The cis configuration of the double bonds between C-9 and C-10 in DOPC molecules produces a bend of about 30° in the aliphatic chain (Franks, 1976; Worcester & Franks, 1976; Huang, 1977; Presti & Chan, 1982). As shown in Figure 8 (see the figure legend for details), the rigid skeleton of cholesterol (and ASL and CSL) and the bent structure of DOPC may not conform with each other when they are in direct contact in the membrane. Although the effect of this sharp bend is somewhat reduced by the simultaneous occurrence of kinds in the oleoyl chain (Huang, 1977; Pace & Chan, 1982a,b), the cis double bond would certainly create serious problems in the packing of cholesterol and oleoyl chains in the membrane.

This nonconformability would lead to two major differences in the PC–cholesterol interaction between saturated and unsaturated PC–cholesterol membranes:

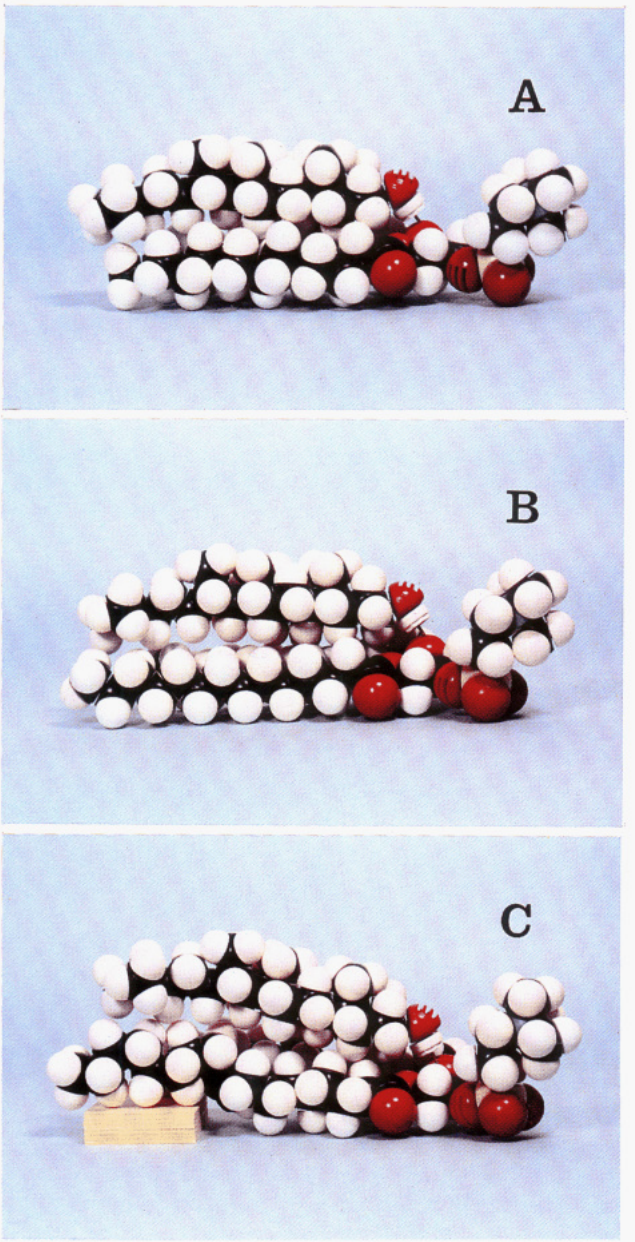

FIGURE 8: Space-filling models showing the interaction of cholesterol with DMPC in a crankshaft conformation (A), DMPC in an all-trans conformation (B), and DOPC with a cis double bond in a crankshaft structure (C). The alkyl chain conformation, the crankshaft structure in particular, is based on Figures 1 and 6 in Pace and Chan (1982a). The conformational mismatch between cholesterol and DOPC (C) is larger than that between cholesterol and DMPC (A and B), although the effect of the bend at the cis double bond is reduced by kink conformations (crankshaft structure) in the unsaturated chains.

(A) Cholesterol molecules tend to be excluded from DOPC domains and segregated out as shown in Figure 9. Thus, it is likely that the cholesterol mole fraction in the cholesterol-rich phase is higher in DOPC membranes than in saturated PC membranes.[2]

[2] The formation of cholesterol-rich domains (or cholesterol oligomers) in DOPC–cholesterol membranes is supposedly driven by the differences in enthalpy of PC–cholesterol, PC–PC, and cholesterol–cholesterol interactions. The PC–cholesterol interaction is the weakest. The balance between these interaction enthalpies and mixing entropy determines the equilibrium between the two phases. A similar delicate balance between interaction enthalpies and mixing entropy in membranes was found in the molecular association (transient dimer formation in the liquid-crystalline phase) of rhodopsin molecules in rod outer segment membranes and in reconstituted membranes (Kusumi et al., 1980; Kusumi & Hyde, 1982).

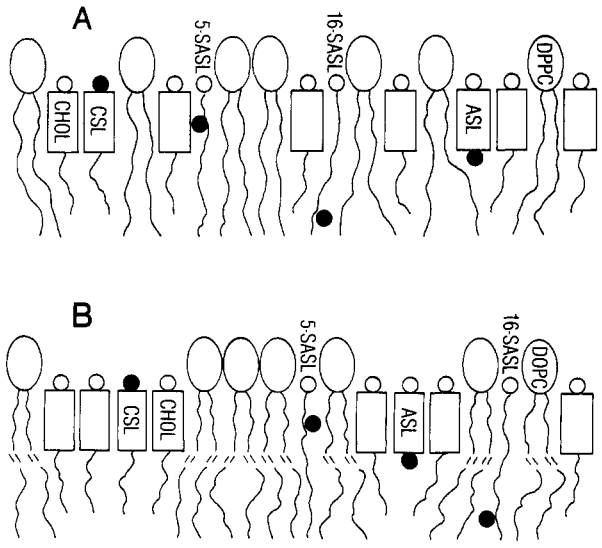

FIGURE 9: Schematic drawings of PC–cholesterol membranes (with spin probes), showing fluid-phase separation into cholesterol-rich domains and cholesterol-poor domains in membranes. (A) Saturated PC–cholesterol membranes. (B) Unsaturated PC–cholesterol membranes, in which microimmiscibility is enhanced by structural nonconformability of unsaturated PC and cholesterol.

(B) The ordering effect of cholesterol [a contact interaction of cholesterol with saturated alkyl chains which promotes the extended PC alkyl chains (more trans bonds) next to cholesterol] is much weaker in DOPC membranes compared with that in saturated PC membranes: although addition of cholesterol leads to a more conformationally ordered state of the saturated alkyl chains (Stockton & Smith, 1976; Oldfield et al., 1978; Recktenwald & McConnell, 1981; Presti et al., 1982; Kusumi et al., 1986; Ipsen et al., 1987; Yeagle, 1988), this ordering effect of cholesterol would not work well in DOPC membranes due to the conformational mismatch between DOPC and cholesterol.

A strong effect of cholesterol on cholesterol analogue spin-labels and a moderate effect on PC analogue spin-labels in DOPC membranes (observation I above) can be explained by the first consequence (A) of structural nonconformability between DOPC and cholesterol; i.e., cholesterol molecules and ASL are mostly located in the cholesterol-rich domains, and the PC mole fraction (thus the content of SASL) in the cholesterol-rich domains is low. As a result, cholesterol affects ASL motion strongly and SASL motion weakly.

A larger effect of cholesterol on ASL motion in saturated PC membranes than in DOPC membranes (observation II above) can be explained mostly by the second consequence of the nonconformability (B), a less effective ordering effect of cholesterol on unsaturated alkyl chains. Even though the direct interaction between cholesterol and ASL is the same in DOPC and saturated PC membranes, since PC molecules surrounding cholesterol cannot be effectively ordered by the cholesterol molecule in DOPC membranes, ASL motion in DOPC membranes is less affected by the presence of cholesterol.[3]

Strong effects of cholesterol on both SASL and ASL motion in saturated PC membranes (observation III above) can be explained by the fact that the mole fraction of PC is high even in the cholesterol-rich domains. As can be seen from the phase diagram in Figure 7, the cholesterol-rich domains in saturated PC membranes contain both ASL and SASL, and the ordering effect of cholesterol on saturated alkyl chains is strong.

A condensing effect of cholesterol on a variety of monolayer PC membranes, which may be related to the interactions discussed above, has been studied by Demel et al. (1972). The effect was evident for 1-stearoyl-2-oleoyl-PC and POPC membranes, but was not found for dilinoleoyl-PC membranes. Although no measurement was made for DOPC membranes, a small condensing effect of cholesterol on DOPC membranes is expected since the most significant influence of unsaturation generally appears after the introduction of the first double bond (Demel et al., 1972; Kinosita & Ikegami, 1984; Kinosita et al., 1984).

Lateral and rotational diffusion data for CSL and 16-doxyl-PC spin-label in POPC–cholesterol oriented multilamellar membranes obtained by Shin and Freed (1989) are consistent with our results[4] (Kusumi et al., 1986; Kusumi & Pasenkiewicz-Gierula, 1988; this work). They indicated that POPC–cholesterol membranes can be viewed as a nonideal solution containing cholesterol-rich clusters by the self-association of cholesterol in POPC membranes and emphasized the tendency of cholesterol self-association. Since cholesterol effects are quite different between saturated PC and unsaturated PC membranes, we place our emphasis on the balance among PC–cholesterol, cholesterol–cholesterol, and PC–PC interactions.

It would be of great interest to gain some knowledge on the stability of each cholesterol-rich domain, or more precisely on the size and the lifetime of each cholesterol-rich (or cholesterol oligomeric) domain. Our observation II and its explanation above suggest that the size and/or the lifetime of cholesterol-rich domains in DOPC–cholesterol membranes can be quite small. Since the effects of cholesterol (at the same bulk mole fraction) on ASL motion are smaller in DOPC membranes than in saturated PC membranes in spite of the higher mole fraction of cholesterol in cholesterol-rich domains in DOPC membranes than in saturated PC membranes, ASL in the cholesterol-rich domain is probably in contact with DOPC molecules in the bulk phase on the time scale of the magnetic anisotropy of the spin probe, 10^{-8} s.

Deuterium NMR of deuterated lipids can provide information on events undergoing on the time scale of 10^{-5} s. Stockton and Smith (1976) observed an ordering effect of cholesterol on perdeuterated stearic acids in egg yolk PC membranes using this method. Direct comparison of their results with our data is difficult because the high concentration of perdeuterated stearic acids used (16 mol %) may disturb the phase equilibrium in the membrane, and the pH of the medium (pure water) was not controlled in these NMR experiments (pK of stearic acid in the membrane is as high as pH 6.9 and is lowered in the presence of cholesterol; Sanson et al., 1976; Egret-Charlier et al., 1978; Kusumi et al., 1982). Nevertheless, these results suggest that cholesterol can affect

[3] In these arguments, only a short-range effect of cholesterol is considered; i.e., cholesterol affects the motion of lipids in direct contact, and it minimally influences the lipid in the second annular ring around the cholesterol molecule in the liquid-crystalline phase. In contrast, the long-range effect of cholesterol was observed below the phase transition temperature (Subczynski & Kusumi, 1986).

[4] In the study by Shin and Freed (1989), 16-doxyl-PC was used to monitor the motion of PC alkyl chains. They observed a slight *increase* in D_w of 16-doxyl-PC in the presence of cholesterol. This result is consistent with the cholesterol-induced increase of oxygen transport in the middle of DOPC membranes (Subczynski et al., 1989), which can be explained by creation of free space in the middle of the DOPC bilayer due to the shorter length of cholesterol than of DOPC alkyl chains. Due to this cholesterol effect in the central part of the bilayer, coupled with the low water content in their sample, it is somewhat difficult to directly compare their POPC data with our results of saturated and unsaturated PC membranes.

alkyl chain order in unsaturated PC membranes on the time scale of 10^{-5} s, suggesting that cholesterol-rich domains may be forming and dispersing continually at time scales faster than 10^{-5} s (and slower than 10^{-8} s).[5]

More experimental work is obviously needed to elucidate the stability of the cholesterol-rich domains. We are in the process of studying the size and lifetime of cholesterol-rich domains by using the spin–lattice relaxation time of the nitroxide spin-label as a basic clock (10^{-6}–10^{-5} s) by employing the saturation–recovery pulse ESR technique (Kusumi et al., 1982; Subczynski et al., 1987, 1989, 1990). Our preliminary results using a planar-square copper complex, 3-ethoxy-2-oxabutyraldehyde–bis(N^4,N^4-dimethylthiosemicarbazonato)-copper(II), as a relaxing agent suggest that the cholesterol-rich domains are in fact quite small and unstable (Subczynski et al., 1989, 1990).

In the case of saturated PC membranes, Hui (1988) reported that cholesterol-rich domains show a ribbon-like structure (20–30 nm × several micrometers) as observed by diffraction contrast electron microscopy (40 mol % cholesterol in DPPC at 35 °C). Several models have been proposed for the cholesterol-rich phase in PC–cholesterol membranes (Engelman & Rothman, 1972; Müller-Landau & Cadenhead, 1979b; Rogers et al., 1979; Presti et al., 1982).

In recent years, the domain structure of cellular plasma membranes has been extensively studied (Karnovsky et al., 1982). The molecular mechanisms for formation of various specialized domains in the plasma membranes are of particular interest because of their functional significance (Palade, 1985). Since the plasma membranes of eukaryotic cells contain various amounts of cholesterol and unsaturated phospholipid molecules, fluid-phase separation of the cholesterol-rich domains may play important roles in formation of these specialized domains and structures in the plasma membranes (Bridgman & Nakajima, 1981; Castuma & Brenner, 1986).

Conclusions

(1) Cholesterol effects on saturated and unsaturated PC membranes in the fluid phase are different. In unsaturated PC–cholesterol membranes, fluid-phase microimmiscibility (cholesterol-rich domains) takes place. Cholesterol-rich (poor) domains in unsaturated PC membranes contain a much higher (lower) fraction of cholesterol than those proposed for saturated PC membranes, as evidenced in the measurements of the dynamics of SASL, CSL, and ASL.

(2) Cholesterol molecules tend to mix with saturated PC, inducing a more ordered state of the saturated alkyl chains. Cholesterol decreases the mobilities of both cholesterol-type and phospholipid-type spin probes in saturated PC membranes.

(3) Cholesterol molecules tend to be segregated out in unsaturated PC membranes, probably due to the structural noncomformability of the bend at the cis double bond in unsaturated alkyl chains and the rigid tetracyclic fused ring of cholesterol. Cholesterol decreases the mobilities of cholesterol-type spin-labels but shows only small effects on phospholipid-type spin probes in unsaturated PC membranes.

(4) When discussing cholesterol effects on membranes, one must specify the degree of unsaturation in the membrane.

Registry No. DMPC, 18194-24-6; DOPC, 4235-95-4; DPPC, 63-89-8; DLPC, 18194-25-7; cholesterol, 57-88-5.

[5] Another explanation for these NMR results is that the collective motion of alkyl chains such as time-dependent changes of collective tilt angles is suppressed in the presence of cholesterol (Gaffney & McConnell, 1974; Godici & Landsberger, 1975; McConnell, 1976; Seelig & Seelig, 1977; Petersen & Chan, 1977).

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