Influence of Cholesterol on Equilibrium and Dynamic Bilayer Structure of Unsaturated Acyl Chain Phosphatidylcholine Vesicles As Determined from Higher Order Analysis of Fluorescence Anisotropy Decay†

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ABSTRACT: The influence of cholesterol on equilibrium and dynamic bilayer structure in minimally to highly unsaturated phosphatidylcholine (PC) vesicles has been examined by characterization of the dynamic fluorescence properties of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-[4-(trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH). Large, unilamellar egg PC, palmitoyloleoyl-PC (POPC), dioleoyl-PC (DOPC), palmitoylarachidonoyl-PC (PAPC), and palmitoyldocosahexaenoyl-PC (P-22:6-PC) vesicles containing no cholesterol or approximately 15 or 30 mol % cholesterol have been examined. Equilibrium and dynamic DPH orientational properties were analyzed according to an orthogonal, bimodal orientational distribution function [Straume, M., & Litman, B. J. (1987) *Biochemistry* (preceding paper in this issue)]. The same mathematical formalism was applied to TMA-DPH except that probe orientational probability was permitted only in the distribution peak aligned parallel to the bilayer normal. TMA-DPH fluorescence lifetimes were consistently increased by incorporation of cholesterol into these vesicles. Greater acyl chain unsaturation and increasing temperature each promoted reduction of lifetimes in the presence or absence of cholesterol. DPH lifetimes were much less sensitive than those of TMA-DPH to changes in composition or temperature. This behavior is consistent with reduced water penetrability into liquid-crystalline bilayers as cholesterol content is increased and as acyl chain unsaturation and temperature are reduced. Cholesterol also induces substantial equilibrium ordering of the bilayer both at the hydrophobic core and at the bilayer–water interface. DPH orientational distributions were shifted in favor of alignment parallel to the acyl side chains. The distributions of both probes were narrowed in response to incorporation of cholesterol. The acyl chain terminal region at the bilayer median therefore permitted less freedom for molecular motion, and the lateral bilayer molecular packing in the plane of the membrane surface became more dense when cholesterol was included in the vesicles. Reducing either acyl chain unsaturation or sample temperature induced bilayer ordering; however, the presence of cholesterol reduced the magnitude of these effects on bilayer order. The order of the bilayer core was generally more sensitive to changes in composition and temperature than was that of the bilayer–water interfacial region. Probe rotational dynamics were accelerated by cholesterol. Molecular motions were slowed as temperature was reduced, but cholesterol continued to promote more rapid bilayer dynamics, relative to samples without cholesterol, independent of sample temperature.

Cholesterol is a common constituent of biological membranes. Its relative content is variable and dependent on the source of the membrane (Benga & Holmes, 1984; Thompson & Huang, 1986). Examination of the effects of cholesterol on unsaturated acyl chain bilayer dynamics is of interest due to the substantial proportions of unsaturated acyl chains present in biological membranes (Akino & Tsuda, 1979; Benga & Holmes, 1984; Thompson & Huang, 1986). In general, acyl chain unsaturation promotes liquid-crystalline bilayer structure (Stubbs et al., 1981). However, equilibrium and dynamic lipid physical properties do vary, depending on the level of acyl chain unsaturation and on the cholesterol and protein contents of the membrane (Benga & Holmes, 1984; Brown & Williams, 1985; Simon et al., 1982; Stubbs et al., 1981; Thompson & Huang, 1986). Controlled studies to characterize the differential effects of varying acyl chain composition and cholesterol content on bilayer physical properties will permit correlation of this information with membrane protein structural and functional properties observed in equivalent bilayer environments. Structural and functional properties of membrane proteins are sensitive to changes in the properties of their bilayer environment (Applebury et al., 1974; Baldwin & Hubbell, 1985a,b; Devaux & Seigneuret, 1985; Morton et al., 1986; O'Brien et al., 1977; Salesse & Garnier, 1984; Stubbs & Litman, 1978). Lipid matrix composition, and associated physical properties, thus may be involved in mediating membrane protein structure and function.

The effects of varying levels of acyl chain unsaturation on the structure and dynamics of large, unilamellar PC[1] vesicles have been reported (Straume & Litman, 1987). Dynamic

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[1] Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-[4-(trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene; POPOP, 1,4-bis(5-phenyloxazol-2-yl)benzene; PC, phosphatidylcholine; egg PC, egg phosphatidylcholine; POPC, palmitoyloleoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; PAPC, palmitoylarachidonoylphosphatidylcholine; P-22:6-PC, palmitoyldocosahexaenoylphosphatidylcholine; Δ4, Δ5, and Δ9, positions of the first acyl chain carbon–carbon double bonds relative to the covalent ester linkage to the glycerol moiety of phospholipids; NMR, nuclear magnetic resonance.

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characterization of the fluorescence lifetime and rotational depolarization properties of DPH and TMA-DPH is performed by way of limited-frequency phase-modulation fluorometry. Anisotropy decay kinetics are analyzed by a higher order model capable of deriving orthogonal, bimodal equilibrium orientational probe distributions (Straume & Litman, 1987). In these studies, cholesterol has been incorporated into PC vesicles at approximately 15 and 30 mol % so as to characterize its influence on lipid structural properties. PC samples of varying acyl chain unsaturation have been examined ranging from the minimally unsaturated POPC to the highly unsaturated P-22:6-PC.

Experimental Procedures

Lipid Vesicle Preparation. Large, unilamellar phosphatidylcholine vesicles containing no cholesterol or approximately 15 or 30 mol % cholesterol were prepared by an octyl glucoside dialysis method described by Jackson et al. (1982) with the modifications of Straume and Litman (1987). Egg PC, POPC, DOPC, PAPC, and P-22:6-PC vesicles were prepared. In the preparation of PC–cholesterol vesicles, dry cholesterol was added to lyophilization tubes prior to the addition of phospholipid in chloroform. Cholesterol and PC were thus co-lyophilized prior to solubilization and dialysis.

Sucrose density step gradients from 0 to 8% (w/w) sucrose in buffer were layered with dialyzed vesicle preparations and centrifuged at 25 000 rpm in an SW-27 rotor for 6 h at 4 °C. Single bands were detected, and the positions of the bands indicated higher equilibrium vesicle density with increasing cholesterol. Cholesterol-free vesicles prepared in this manner displayed vesicle diameters of up to approximately 1000 Å (Jackson et al., 1982). Negative-stain electron micrographs indicated that cholesterol-containing vesicles were primarily unilamellar and that mean vesicle diameter increased 2–3-fold with 15 mol % cholesterol and approximately 5-fold with 30 mol % cholesterol.

Total phospholipid was determined by phosphate analysis according to the method of Bartlett (1959).

Lipid peroxidation of PAPC and P-22:6-PC lipids was determined by both 233-nm absorbance and thiobarbituric acid assay (Baker & Wilson, 1969; Slater, 1984; Sunamoto et al., 1985). No more than 0.5% PAPC peroxidation and 2–3% P-22:6-PC peroxidation was detected.

Cholesterol oxidase assays were employed to quantitate total cholesterol (Moore et al., 1977). Cholesterol content of the vesicles was within a few mole percent of the desired 15 and 30 mol % levels.

Fluorescence Measurements. All fluorescence data were obtained on an SLM 4800 phase-modulation spectofluorometer as described by Straume and Litman (1987).

Methods of Analysis. DPH and TMA-DPH fluorescence lifetimes were analyzed by a constrained biexponential decay model, and their rotational depolarization properties were determined according to a bimodal equilibrium orientational distribution model as described by Straume and Litman (1987).

Results

Fluorescence Lifetimes. The fractional amplitude of short lifetime fluorophores α_1 derived from the constrained two-population decay model for DPH and TMA-DPH in large, unilamellar, unsaturated acyl chain phosphatidylcholine vesicles varying in cholesterol content is usually less than 0.5 [see the Appendix, available as supplementary material (see paragraph at end of paper regarding supplementary material)]. The long-lifetime fluorophore population therefore contributes

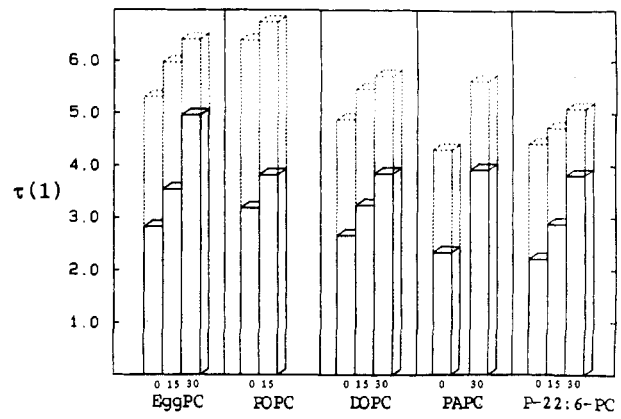

FIGURE 1: Intensity-weighted mean fluorescence lifetimes, $\tau(1)$ (in nanoseconds), of TMA-DPH-labeled vesicles containing no cholesterol or approximately 15 or 30 mol % cholesterol. Solid lines, T = 37 °C; dotted lines, T = 5 °C.

approximately 80% or more of the total fluorescence intensity (Straume & Litman, 1987). The intensity-weighted mean fluorescence lifetimes $\tau(1)$ characterize average probe excited state lifetimes and permit comparison of average lifetime behavior as a function of vesicle composition and temperatue (Straume & Litman, 1987). DPH lifetimes are much less sensitive than are those of TMA-DPH to differences in composition and temperature. The trends detected by TMA-DPH, however, are generally qualitatively present in the parameters derived from DPH-labeled vesicles (see the Appendix). Figure 1 presents the intensity-weighted mean fluorescence lifetimes of TMA-DPH at 37 and 5 °C as a function of acyl chain composition and cholesterol content. TMA-DPH lifetimes increase with increasing cholesterol content in each of these liquid-crystalline vesicle systems. This trend is observed at all temperatures examined. Greater degrees of acyl chain unsaturation continue to yield shorter TMA-DPH lifetimes, even in the presence of approximately 15 or 30 mol % cholesterol. All systems also exhibit longer lifetimes in response to cooling.

Equilibrium Order. The distribution widths θ_g derived for TMA-DPH are greater than those derived for DPH (see the Appendix). This may occur because of the availability of additional modes of reorientational relaxation to DPH (bimodal distributions) than to TMA-DPH (unimodal distributions) (Straume & Litman, 1987). Both probes experience narrowing of distribution widths in response to incorporation of cholesterol into these vesicles. This cholesterol-induced ordering is more substantial at higher sample temperatures. At all temperatures, however, greater acyl chain unsaturation generally permits broader probe distributions for both DPH and TMA-DPH in the presence or absence of cholesterol. Distributions for both probes are narrowed in response to cooling independent of acyl chain composition or cholesterol content, but greater temperature sensitivity is apparent in the absence of cholesterol than in its presence.

The fraction of DPH molecules occupying the orientational maximum aligned parallel to the bilayer normal, i.e., parallel to the phospholipid acyl chains, is quite sensitive to the cholesterol content of these vesicles. Figure 2 shows that cholesterol induces a redistribution of DPH orientational probability in favor of alignment with the bilayer normal (also, see the Appendix). This cholesterol-dependent increase in $f_\parallel$ is observed independent of acyl chain composition or sample temperature, but the effect is more substantial at higher temperatures. Cooling the vesicles induces an increase in $f_\parallel$ in each of these systems, but the temperature-dependent redistribution

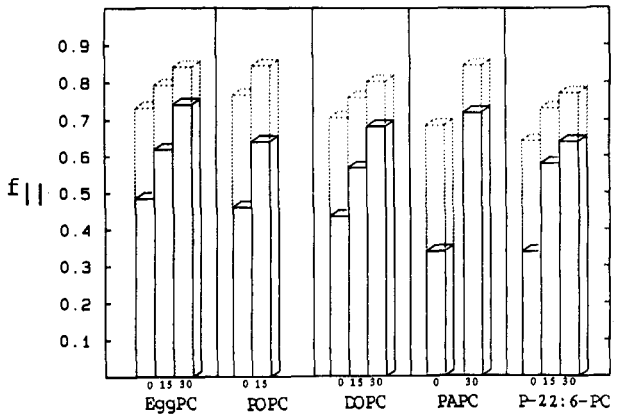

FIGURE 2: Fractions of DPH equilibrium orientational distributions aligned parallel to the bilayer normal, $f_{||}$, in vesicles containing no cholesterol or approximately 15 or 30 mol % cholesterol. Solid lines, T = 37 °C; dotted lines, T = 5 °C.

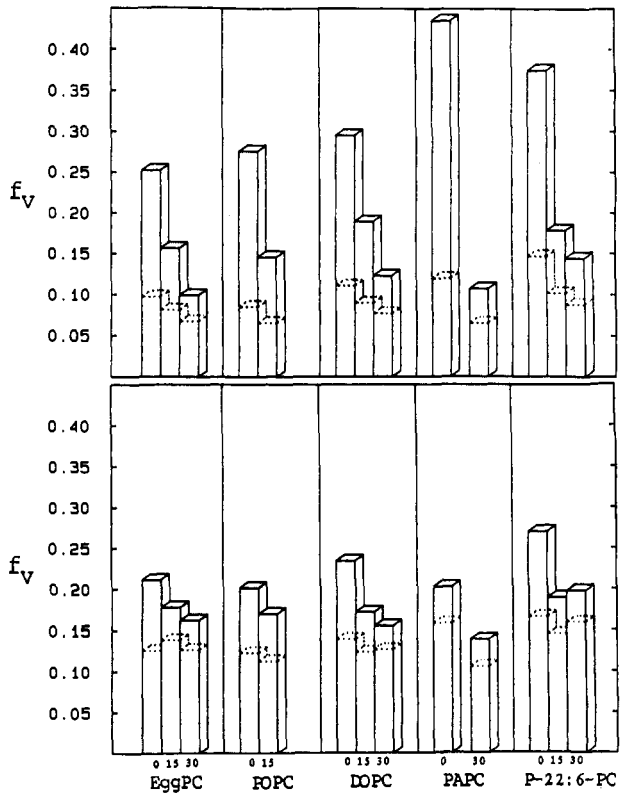

FIGURE 3: Fractional volumes, f_v, derived for DPH- (top) and TMA-DPH- (bottom) labeled vesicles containing no cholesterol or approximately 15 or 30 mol % cholesterol. Solid lines, T = 37 °C; dotted lines, T = 5 °C.

of DPH is greater in cholesterol-free vesicles than in those containing cholesterol.

Characterization of the overall equilibrium ordering experienced by DPH or TMA-DPH by the fractional volume parameter f_v indicates a substantial ordering effect of cholesterol in these vesicles (see Figure 3 and the Appendix). The extent of cholesterol-induced ordering is much greater at 37 °C than at 5 °C, and DPH fractional volumes are seen to be more sensitive to cholesterol content than are those of TMA-DPH. All samples permit less freedom for probe reorientational motion as temperatures are reduced. Cholesterol, however, reduces the magnitude of this temperature-dependent response by inducing substantially more order at 37 °C than at lower temperatures. The temperature-dependence of f_v is therefore greatly reduced for both probes when cholesterol is present, an effect also observed in the behavior of θ_g and $f_{||}$. At

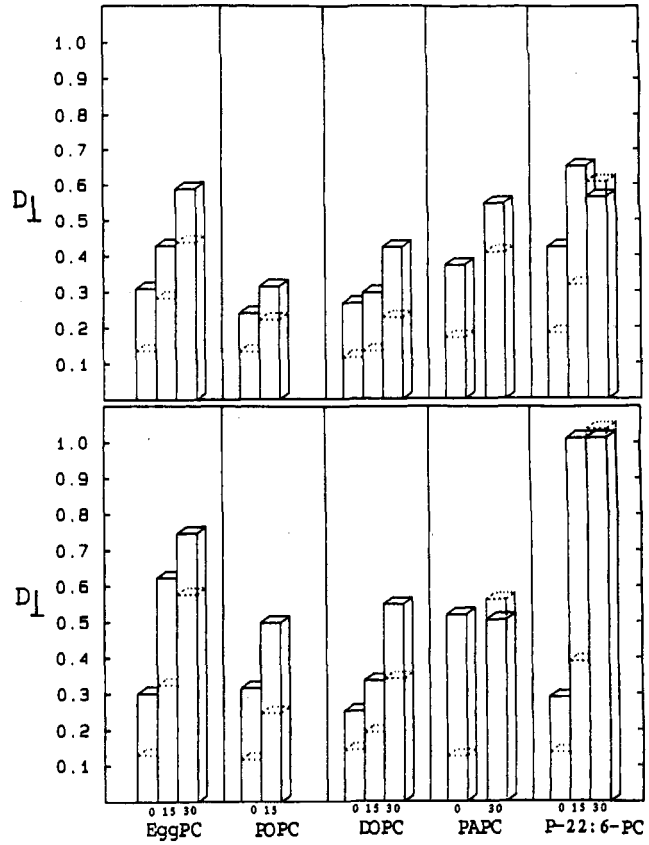

FIGURE 4: Perpendicular rotational diffusion coefficients, $D_\perp$ (in ns^{-1}), derived for DPH- (top) and TMA-DPH- (bottom) labeled vesicles containing no cholesterol or approximately 15 or 30 mol % cholesterol. Solid lines, T = 37 °C; dotted lines, T = 5 °C.

physiological temperatures and in the absence of cholesterol, the volume available for DPH reorientational motion is greater than that available to TMA-DPH. Incorporation of approximately 30 mol % cholesterol, however, reduces the fractional volume of DPH to values less than those of TMA-DPH. at 5 °C, DPH fractional volumes are less than those of TMA-DPH independent of cholesterol content. Cholesterol-free vesicles permit greater f_v values for both DPH and TMA-DPH as the level of acyl chain unsaturation increases independent of sample temperature. Cholesterol at 30 mol %, however, largely eliminates these acyl chain dependent differences in the fractional volumes derived for DPH and TMA-DPH.

Rotational Dynamics. Derived perpendicular rotational diffusion coefficient values for DPH and TMA-DPH are presented in Figure 4 (also, see the Apprendix). Cholesterol consistently induces accelerated rates of probe depolarizing motions in each of the vesicle systems examined. $D_\perp$ values for DPH are increased approximately 1.5–3-fold by 30 mol % cholesterol whereas values for TMA-DPH are generally accelerated to a greater extent under the same conditions. In almost all cases, however, dynamic fluctuations responsible for probe depolarization are more rapid at higher sample temperatures.

Discussion

The total fluorescence intensity decay properties derived from the constrained, two-population model employed in our analysis indicate that only approximately 20% or less of the fluorescence intensity is contributed by the derived short lifetime population. (This is the result of transforming fractional amplitudes, α_i, to fractional intensities f_i.) Previously derived DPH lifetime distributions obtained from biexponential decay models support this result (Ameloot et al., 1984; Chen

et al., 1977; Hildenbrand & Nicolau, 1979; Kawato et al., 1977; Kinosita et al., 1981a,b; Klausner et al., 1980; Lakowicz et al., 1985; Parasassi et al., 1984; Stubbs et al., 1984, 1981). Recent analysis of DPH in multilamellar liposomes by multifrequency phase-modulation measurements has been capable of deriving continuous lifetime distributions (Glaser et al., 1986), an alternative way of accounting for nonmonoexponential decay behavior which may more accurately represent the true situation. A precise interpretation of the model-dependent derived α_i and τ_i parameters is thus not offered since these are only the result of an analysis permitting better characterization of total intensity decay than does a monoexponential function. The effects of temperature, acyl chain composition, and cholesterol content on average probe decay behavior may, however, by quantified by the intensity-weighted mean fluorescence lifetime, $\tau(1)$.

Mean DPH lifetimes are much less sensitive than are those of TMA-DPH to changes in sample temperature or vesicle composition. TMA-DPH, partitioned into bilayers at the bilayer–water interface (Engel & Prendergast, 1981; Prendergast et al., 1981), is more likely to encounter water molecules than is DPH, localized within the hydrophobic core of the membrane (Lakowicz, 1983; Shinitzky & Barenholz, 1978). Migration of β-coupled gauche-trans-gauche conformations along the carbonyl-terminal acyl chain region may facilitate water transport into phospholipid bilayers (Edholm, 1981; Thompson & Huang, 1986). Probe–water interactions may then result in dynamic quenching of fluorescence, causing a decrease in fluorophore lifetimes. TMA-DPH fluorescence may therefore be quenched by increased water permeability into bilayers leading to reduced lifetimes whereas DPH lifetimes are expected to be much less sensitive. The presence of cholesterol in unsaturated acyl chain, liquid-crystalline PC vesicles results in longer TMA-DPH lifetimes and in only minimally affected DPH lifetimes. This suggests that cholesterol inhibits water penetrability into these bilayers independent of acyl chain composition and that DPH rarely encounters water even in the absence of cholesterol.

Cholesterol has been observed by X-ray diffraction and capacitance measurements to inhibit water penetration into bacterial phosphatidylethanolamine bilayers (Simon et al., 1982). The results of Simon et al. (1982) also indicate that water penetrates only a few angstroms into these bilayers, consistent with less observed variability of DPH lifetimes than of TMA-DPH lifetimes in the present experiments. The presence of different head groups in these experiments does not allow direct comparison; however, the observed lifetime behavior is qualitatively consistent with this phenomenon. A condensing effect of cholesterol on liquid-crystalline phospholipid bilayers has been observed that reduces the mean molecular area of phospholipid molecules and correlates with decreased bilayer permeability to hydrophilic molecules (Chapman, 1973; Demel & de Kruijff, 1976; Jain, 1975; Lecuyer & Derivichian, 1969; Phillips, 1972). In addition, cholesterol reduces the content of gauche conformations in liquid-crystalline PC bilayers as determined by Raman spectroscopy (Levin, 1984; Mendelsohn, 1972). If migration of β-coupled gauch-trans-gauche conformations is indeed responsible for water transport into bilayers (Edholm, 1981; Thomspon & Huang, 1986) and if the observed reduction of gauche conformations induced by cholesterol is responsible for reducing the probability of such β-coupled conformations, then this may be a mechanism whereby cholesterol induces (1) tighter lateral molecular packing density (the condensing effect) by allowing fewer gauche conformations and (2) less penetrability of water into bilayers by reducing the probability of β-coupled gauche-trans-gauche conformations. Increasing acyl chain unsaturation or sample temperature each cause shorter fluorescence lifetimes, even when cholesterol is present. Increased acyl chain unsaturation is known to promote head group and acyl chain mobility (Barton & Gunstone, 1975; Kohler et al., 1972; Levine et al., 1972), and elevated temperatures are also expected to enhance molecular dynamics due to increased energy content of bilayer structures.

The derived equilibrium orientational distributions for DPH and TMA-DPH indicate that cholesterol restricts the freedom available for probe reorientation both in the hydrophobic core and at the bilayer–water interface. Equilibrium probe orientational distribution widths are narrowed and DPH orientational probability is redistributed parallel to the phospholipid acyl chains in response to increasing cholesterol. This cholesterol-dependent ordering is more substantial at physiological temperatures than at lower temperatures. However, the more highly unsaturated PAPC and P-22:6-PC vesicles show more low-temperature, cholesterol-dependent ordering than do the less highly unsaturated egg PC, POPC, and DOPC systems. This behavior is consistent with greater observed residual, low-temperature disorder present in the more highly unsaturated, cholesterol-free vesicles.

The derived fractional volumes available for probe reorientation show the order of the hydrophobic bilayer core to be more sensitive to acyl chain composition and cholesterol content than is the bilayer–water interface, particularly at physiological temperatures. Cholesterol-free vesicles at 37 °C permit more freedom for DPH reorientation in the bilayer interior than for TMA-DPH motion at the interfacial and head group regions. Cholesterol up to approximately 30 mol %, however, increases the molecular order of the hydrophobic bilayer core more substantially than it does the bilayer–water interface region such that more freedom for molecular motions now exists at the interfacial and head group regions than in the bilayer interior. At low temperature (5 °C), the bilayer core is more ordered than the bilayer–water interface independent of cholesterol content.

This cholesterol-induced ordering of liquid-crystalline bilayers is consistent with condensing effects of cholesterol that reduce the mean area per phospholipid (Chapman, 1973; Demel & de Kruijff, 1976; Jain, 1975; Lecuyer & Derivichian, 1969; Phillips, 1972). Such condensing of bilayers by cholesterol, as well as the reported reduction in gauche acyl chain conformations (Levin, 1984; Mendelsohn, 1972), is expected to increase the molecular order of the membrane. Cholesterol-free vesicles permit as much as approximately 65% of DPH orientational probability in a distribution at the bilayer median parallel to the plane of the bilayer. In the absence of cholesterol, the acyl chain terminal region is therefore quite disordered. Cholesterol substantially reduces the proportion of DPH distributed parallel to the bilayer plane indicative of considerable ordering of the acyl chain terminal region. Cholesterol-induced ordering of the bilayer median has been detected by X-ray diffraction studies (Levine & Wilkins, 1971) consistent with the observed redistribution of DPH.

Reducing temperature or acyl chain unsaturation each results in more equilibrium molecular ordering independent of cholesterol content. Acyl chain dependent differences in bilayer order are reduced by the presence of cholesterol, although the qualitative trend of more disorder with more unsaturation appears to be maintained. Because the ordering effects of cholesterol are more significant at higher temperatures, the equilibrium order of cholesterol-containing vesicles is much

less temperature dependent than is that of vesicles free of cholesterol.

Rates of probe depolarizing motions are greatly accelerated by cholesterol both in the bilayer interior and at the bilayer–water interface. Cholesterol therefore orders these bilayers but also appears to induce more rapid lipid molecular fluctuations throughout the bilayer. NMR results support the existence of more rapid motion of acyl chain methyl termini in the presence of cholesterol but slower and unaffected rates of molecular motion at the middle of acyl chains and at the bilayer–water interface, respectively (Thompson & Huang, 1986). ^{13}C NMR results have been interpreted to suggest direct contact between cholesterol and C-9 and C-10 of egg PC acyl chains in bilayer structures, thus restricting the motion of the middle of the acyl chains relative to the methyl termini (Brainard & Cordes, 1981; Godici & Landsberger, 1975). The disagreement between the fluorescence results derived in this paper and the NMR results reported above may be attributed to a number of potential factors: (1) the time scales detected by NMR techniques are frequently slower than those detected by fluorescence measurements; (2) the reporter groups observed in NMR experiments are smaller and more localized than the fluorophores used in this paper; (3) the NMR reporter groups are nonperturbing, intrinsic lipid molecular structures whereas DPH and TMA-DPH are potentially perturbing molecules extrinsically incorporated into bilayers. These differences make it necessary to consider the phenomena actually being observed by these respective biophysical techniques in regard to interpretation and comparison of derived equilibrium and dynamic parameters.

Cholesterol is known to disrupt cooperativity of thermal phase transitions in saturated acyl chain PC bilayers (Estep et al., 1978; Kinosita & Ikegami, 1984; Lentz et al., 1980). The observed condensing effect and decreased gauche conformational content of acyl chains in cholesterol-containing phospholipid structures is consistent with the observed increase in molecular order induced by cholesterol. More rapid molecular fluctuations may accompany a more ordered environment that is less capable of laterally dissipating lipid matrix molecular kinetic energy. Enhanced rates of probe depolarization may therfore be expected, although the extent of depolarization is more limited. ^{1}H–^{31}P nuclear Overhauser measurements of phospholipid bilayers detect less tightly associated head group structure when cholesterol is present (Yeagle et al., 1977). Fewer and/or less stable head group–head group interactions may also contribute to more rapid bilayer dynamics.

The behavior described above is consistent with more condensed lateral packing in Δ9 acyl chain systems (containing oleic acyl chains, 18:1) than in the more highly unsaturated Δ4 or Δ5 systems (containing arachidonic or docosahexaenoic acyl chains, 20:4 or 22:6) (Demel et al., 1972). The different positioning of carbon–carbon double bonds may affect the stability of acyl chain–cholesterol interactions, based on consideration of steric molecular packing models (Huang, 1977; Huang & Mason, 1982). The present results suggest that the acyl chain terminal region of cholesterol-containing bilayers is also substantially restricted. Molecular dynamics, however, are enhanced throughout the bilayer by inclusion of cholesterol into these vesicles.

These studies demonstrate that the probe environment can be dramatically affected by both temperature and cholesterol content as well as by degree of acyl chain unsaturation. The temperature variation of the derived fluorescence parameters is largest for the more highly unsaturated systems. Since most biological systems exist in a relatively narrow temperature range, one would expect that a method would have evolved to adjust bilayer properties under essentially isothermal conditions. This can potentially be done through compositional variation. Here, the effect of approximately 30 mol % cholesterol is shown to shift the f_v parameter at 37 °C, in DPH-containing systems, to values very close to those observed at 5 °C in cholesterol-free vesicles. Thus, variation in cholesterol content is capable of providing the same dynamic range in f_v under isothermal conditions as is achieved in the absence of cholesterol by varying temperature from 37 to 5 °C. The largest range of variation for both temperature- and cholesterol-induced changes is in the more highly unsaturated systems. The presence of high levels of both acyl chain unsaturation and cholesterol in the same membranes, as is the case in the retinal rod outer segment disk membrane, may be explained in terms of the wide latitude of lipid matrix properties afforded the organism, in a very narrow temperature range, by variation of cholesterol content in the membrane.

Ongoing studies involving reconsistution of rhodopsin into synthetic vesicles of defined molecular composition will permit an examination of the effect of this protein on lipid physical properties. In addition, studies related to the functional characterization of the protein are being carried out [see, for example, Applebury et al. (1974), Baldwin and Hubbell (1985a,b), Litman et al. (1981), Morton et al. (1986), O'Brien et al. (1977), and Stubbs and Litman (1978)]. Knowledge of the effects of each membrane component on bilayer structure and dynamics in conjunction with functional information about integral proteins will permit an interpretation of lipid–protein interactions in terms of structural and functional parameters.

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Supplementary Material Available

Appendix containing a table showing derived fluorescence intensity decay and dynamic depolarization parameters for DPH and TMA-DPH as a function of temperature, acyl chain composition, and cholesterol content for large, unilamellar octyl glucoside dialysis vesicles (11 pages). Ordering information is given on any current masthead page.

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