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Influence of Cholesterol on the Biophysical Properties of the Sphingomyelin/DOPC Binary System

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Abstract. The influence of cholesterol on the sphingomyelin (SM)/dioleoylphosphatidylcholine (DOPC) binary system was investigated in various respects. Electron spin resonance (ESR) measurements reveal that the order parameter of 5DS (5-doxyl stearic acid) in SM/DOPC bilayers increases notably when the concentration of cholesterol is over 30 mol%. Membrane potential measurements indicate that the K^+ permeability of the SM/DOPC bilayer decreases steeply at 40 mol% cholesterol concentration. Both these experiments suggest that cholesterol reduces the motion amplitude of hydrocarbon chains abruptly above 30 mol%. In contrast to the ordering effects on the hydrocarbon chains, ^{31}P-NMR results indicate that cholesterol slightly increases the motion of phosphate groups of the lipids. ^{31}P-NMR also raises the possibility of domain formation in the presence of cholesterol. Fluorescence-quenching experiments verified that solid domains appear in the binary system when cholesterol is present, and percolation threshold occurs at 50 mol% cholesterol concentration. The solid domains bear the properties of liquid ordered phase, which is the basic structure of caveolae and functional rafts. So this work provides an artificial model for the study of rafts and caveolae on biological membranes.

Key words: Electron spin resonance (ESR) — Fluorescence Measurements — ^{31}P-NMR — Percolation Threshold — Liquid Ordered Phase

Introduction

The significance of lipids and of the structural diversity of the self-assembled lipid bilayer membranes is still poorly comprehended, in contrast to the immense progress in the understanding of the functions of proteins and nucleic acids in living cells. The clearest function of membrane lipids is to form amphipathic bilayers that surround cells and organelles and block leakage of hydrophilic compounds while housing membrane proteins (Singer & Nicolson, 1972). Why, however, does a simple barrier require so many different types and species of lipids? Some recent advances indicate that lipids do play more multiple roles than what is at present commonly accepted (e.g., Dowhan, 1997; Brown, 1998). It has been known that lipids can serve as second messengers (Liscovitch & Cantley, 1994), regulators of membrane proteins (Newton, 1995), and perform other functions in biological membranes. Among these advances, the most outstanding discovery is that of the function of sphingolipids and cholesterol.

Sphingolipid, typified by sphingomyelin (SM), is one of the major classes of membrane phospholipids in eukaryotic organisms (Barenholz & Thompson, 1999). SMs and phosphatidylcholines (PCs) constitute more than 50% of the membranes' phospholipids and are strongly enriched in the external plasma membrane leaflet of cells (Barenholz & Thompson, 1999). Both phospholipid classes contain phosphoryl choline as the polar head group, but their backbones differ. SM has sphingosine as the hydrophobic backbone, together with an amide-linked acyl chain. PCs are glycerophospholipids with two acyl chains linked to the glycerol backbone with carbonyl ester linkages. Compared with natural glycerophospholipids, naturally occurring SMs are enriched in long saturated fatty-acyl chains, have a higher melting temperature, are more hydrophobic and can undergo a tight packing in cell membranes due to the absence of the kinked structure of unsaturated acyl chains (Barenholz & Thompson, 1999). For example, sphingomyelin from chicken egg yolk contains primarily palmit-

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ic acid and has a T_m of about 40°C (DSC results, *data not shown*).

Cholesterol is an alicyclic lipid molecule, consisting of four fused rings, a 3β-hydroxyl and a hydrophobic tail, all of which are significant in interacting with phospholipids (Yeagle, 1985). It is a major constituent of plasma membranes of eukaryotic cells, contributing to 30–40 mol% of the lipid fraction (Brown & London, 1998), so the understanding of the activity and function of cholesterol in biological and artificial membrane has been a challenging problem for many years (Yeagle, 1985; McMullen & McElhaney, 1996). Cholesterol has a wide variety of effects on the physical properties of membranes. It is generally accepted that cholesterol interacts with membrane phospholipids and fluidizes phospholipid hydrocarbon chains below their phase transition temperature (T_m), while rigidifying hydrocarbon chains above T_m (Davies et al., 1990). And when the concentration of cholesterol increases, at about 20 to 40 mol%, the phase transition "disappears" (Yeagle, 1985). Another biophysical effect attributed to cholesterol is its ability to condense the average cross-sectional area of fluid-phase PCs (Smaby et al., 1997) by decreasing the amount of *trans-gauche* isomerizations and increasing the fraction of *trans* dihedrals in lipid acyl chains (Robision et al., 1995). When interacting with nonlamellar lipids, cholesterol destabilizes the lamellar phase and, consequently, stabilizes the hexagonal II phase (Tilcock et al., 1984). Biological roles of cholesterol also involve reduction of passive permeability, increase of the mechanical strength of membrane, and modulation of the activity of many membrane-associated enzymes (Yeagle, 1985; McMullen & McElhaney, 1996).

The most exciting discovery about cholesterol in recent years is that it was found to play a key role in the formation of functional membrane rafts and caveolae, which are sphingolipid- and cholesterol-rich domains on plasma membranes and serve as platforms to support numerous cellular events in membrane traffic and signal transduction (Harder & Simons, 1997). Caveolae are nonclathrin-coated flask-like invaginations of the plasma membrane enriched in cholesterol, SM and glycosphingolipid (Harder & Simons, 1997). Though lacking the morphological properties, rafts have lipid composition and functions similar to caveolae (Simons & Ikonen, 1997). Recently, it has been suggested that the lipid structure of caveolae and rafts have the properties that are intermediate between those of the gel and l_d phases. In detail, the hydrocarbon chains of lipids in caveolae and rafts are extended and ordered as in the gel phase, but the lateral mobility in the bilayer is very high, as in the l_d phase (Harder & Simons, 1997). This lipid state is named liquid-ordered (l_o) phase. The l_o phase generates great interest because it is proposed to be more representative of plasma membrane organization. Although the l_o phase can be identified in binary mixtures of cholesterol and various neutral glycerolipids, such as PC (Sankaram & Thompson, 1990) and PE (Paré & Lafleur, 1998), the most attractive aspect is the interaction between sphingolipids and cholesterol, because caveolae and rafts are enriched in both these lipids.

In this paper, we study the effects of cholesterol on a model of plasma membranes. SM and DOPC were used as prototypes of sphingolipids and glycerolipids, because DOPC has a very low T_m, as do common biological glycerolipids.

Materials and Methods

Materials

Sphingomyelin from chicken egg yolk, dioleoylphosphatidylcholine (DOPC) and cholesterol were purchased from Sigma. 2-(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine (DPH-PC), 5-doxyl stearic acid (5DS) and 3,3′-dipropylthiacarbocyanine iodide ($DiSC_3(3)$) were purchased from Molecular Probes. All other reagents used were of analytical grade.

Preparation of Vesicles

A certain amount of SM and DOPC (1:1, molar ratio) was made into a thin film by dissolving in chloroform/methanol (v/v 5:1), evaporating under a stream of nitrogen and evacuating in a lyophilizer overnight. If some extrinsic probes were used, they were added during this step. The samples were hydrated with 20 mM Tris-HCl/150 mM KCl buffer solution (pH 7.0). The lipid vesicles were prepared by vigorously vortexing the sample for 5 min at 50°C.

Electron Spin Resonance Spectroscopy

ESR Spectroscopy was carried out on a BRUKER ER-200D spectrometer at 30, 40 and 50°C. The capillaries containing the 5DS-labeled lipid dispersions were introduced into a quartz tube and cavity temperature was controlled by using a calibrated Varian variable-temperature controller. Order parameters were calibrated from outer hyperfine splitting constants ($T_{//}$) and inner hyperfine splitting constants ($T_\perp$) by:

$$S = (T_{//}) - T_\perp)/[T_{zz} - (T_{xx} + T_{yy})/2] \cdot [a^o/a']$$

where $T_{zz} = 32.9$ gauss, $T_{xx} = 5.9$ gauss, $T_{yy} = 5.4$ gauss, $a^o = (T_{xx} + T_{yy} + T_{zz})/3$, and $a' = (T_{//} + 2T_\perp)/3$ (Mehlhorn et al., 1988).

Membrane Potential Measurements

The membrane potential of lipid bilayers was tested by monitoring shifts of the fluorescence maximum of the redistribution dye $diSC_3(3)$ caused by dye binding to the vesicles. The fluorescence measurements were performed on a HITACHI F-4010 Fluorescence Spectrophotometer at 30 and 50°C. Stock solution of $diSC_3(3)$ in ethanol was added to the suspension of vesicles with various K^+ gradients to reach a final concentration of 1.0 μM. Samples were gently mixed by a magnetic stirrer in a quartz cuvette for 1 min and then scanned from 560 to 650

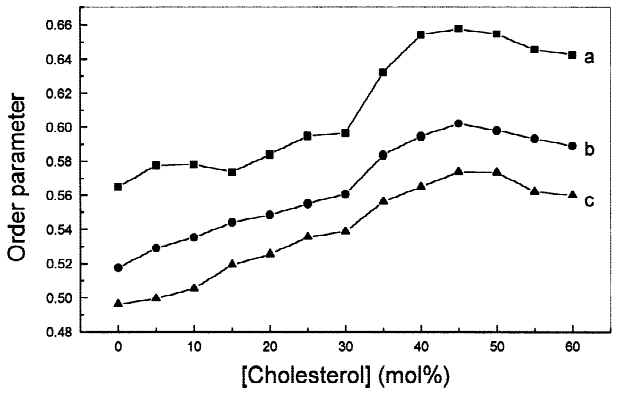

Fig. 1. Dependence of order parameter of 5DS-labled SM/DOPC (1:1) binary vesicles on the concentration of cholesterol. The temperature was 30°C (*a*), 40°C (*b*) and 50°C (*c*).

nm using an excitation wavelength of 550 nm. The concentration of total phospholipids was 20 μM.

^{31}P-NMR Spectroscopy

Samples for ^{31}P-NMR measurements were prepared in 20 mM Tris-HCl/150 mM KCl pH 7.0 containing 10% D_2O and the final concentration was 50 mg/ml. ^{31}P-NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer at 30°C and 45°C. A 5-mm broad band probe was used. The ^{1}H-^{31}P decoupling was carried out during excitation and acquisition. All chemical shifts are quoted in parts per million (ppm) with reference to H_3PO_4 in D_2O (0 ppm). All spectra were accumulated from up to 30,000 scans.

Fluorescence-Quenching Measurements

All samples for fluorescence-quenching study had the DPH-PC/(SM+DOPC) molar ratio of 1/500, and the molar ratio of quencher, 5DS, to total phospholipids was 0, 0.02, 0.04, 0.08, 0.12 and 0.16. All fluorescence spectra were recorded on a HITACHI F-4010 Fluorescence Spectrophotometer at 30 and 50°C. The excitation wavelength was 350 nm; emission spectra were recorded from 360 to 650 nm. The ratio of fluorescence maximum (F) and fluorescence of samples without quencher (F_0) was used to show the extent of quenching.

Results

Electron Spin Resonance Spectroscopy

An increase of order parameter corresponds to an increased motional restriction of the spin-labeled chains (Mehlhorn et al., 1988). So we used the order parameter to monitor the effects of cholesterol on the motions of SM/DOPC vesicles. Fig. 1 shows the change of order parameters of 5DS corresponding to the concentrations of cholesterol at various temperatures. At 30°C, the curve is complex in shape (Fig. 1, curve *a*). Order parameter increases slightly at 5 mol% but then decreases at 15 mol%. The small breaks may be the result of the change of cholesterol location in the bilayer. It was reported that, in DPPC vesicles, cholesterol is preferentially located into the hydrophobic core of the bilayer below 5 mol% (Videira, Antunes-Madeira & Madeira, 1999), which will increase the order of the acyl chains. At a critical concentration cholesterol diffuses to the region close to the lipid-water interface, packing with phospholipid chains in each leaflet of the membrane (Videira et al., 1999). This hypothesis may explain the small breaks between 5–15 mol% cholesterol. When the cholesterol concentration increases further to 30 mol%, the order parameter has only a slight increase, which indicates that cholesterol only has modest effects on the motion of hydrocarbon chains under 30 mol%.

A break in the positive slope occurs at 30 mol% cholesterol, and the slope reduces to nearly zero at 40 mol%. The order parameter reaches maximum at 45 mol%, then the slope becomes slightly negative at 45–50 mol%. The increase of order parameter indicates that cholesterol significantly decreases the amplitude of the hydrocarbon chain motion from 30 to 45 mol%, and the structure of the bilayer is ordered greatly. But the motion amplitude increases at 45–60 mol% cholesterol concentration. Some other studies with spin-labeled lipid (Sankaram & Thompson, 1990) and fatty acid (Kusumi et al., 1986) on the PC/cholesterol system obtained similar results. Sankaram and Thompson attribute this effect to a decrease in the packing density of the headgroups caused by a corresponding decrease in the density of the phospholipid molecules in the plane of the membrane. The decrease of order parameter from 45 mol% cholesterol seems to occur by a similar mechanism.

At 40 and 50°C, the curve is less complex than at 30°C, especially at low cholesterol concentration (Fig. 1, curves *b* and *c*). The small break at 5–15 mol% cholesterol gradually disappears because the effects of cholesterol dimer are mainly in the solid phase (Videira et al., 1999). The break at 30 mol% cholesterol becomes less abrupt, but the shape of the curves is still similar to that of 30°C. Cholesterol still has a marked ordering effect on the DOPC/SM system from 30–45 mol% cholesterol.

Membrane Potential Measurements

$DiSC_3(3)$ is a redistribution dye used for the assessment of membrane potential in living cells and artificial vesicles (Večeř, Heřman & Holoubek, 1997). When ion gradients exist across the lipid membrane, membrane potential makes the dye bind to the membrane, which results in a shift of fluorescence maximum. The fluorescence spectrum of free $diSC_3(3)$ in a buffer has its maximum at approx. 570 nm, while the bound form of the dye exhibits a red shift of 12 nm (Večeř et al., 1997). So the amplitude of red shifts can reflect the membrane poten-

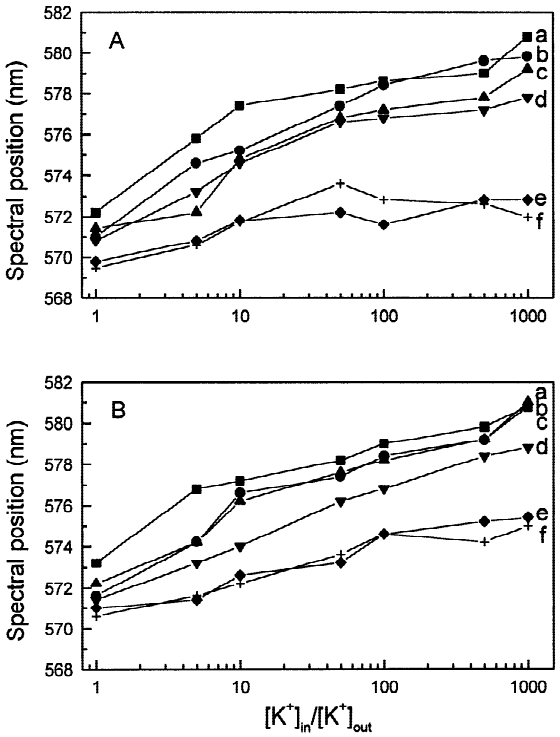

Fig. 2. Position of the fluorescence maximum of $diSC_3(3)$ in the SM/DOPC/CHL vesicles as a function of potassium gradient at 30°C (*A*) and 50°C (*B*). The concentrations of cholesterol correspond to 0 (*a*), 10 (*b*), 20 (*c*), 30 (*d*), 40 (*e*) and 50 (*f*) mol%.

tial and ion permeability of a certain membrane. Fig. 2 presents the shift of SM/DOPC vesicles in the presence of various concentrations of cholesterol. At 30°C, the spectral position red-shifts greatly when the K^+ gradient increases at cholesterol concentrations of 0 to 30 mol% (Fig. 2*A*, curves *a–d*). But at 40 and 50 mol%, the amplitude of the shift is much smaller (Fig. 2*A*, curves *e–f*). This indicates that the membrane potential of SM/DOPC decreases sharply when the cholesterol concentration increases to 40 mol%. The membrane potential exists as a consequence of the concentration gradient and the membrane permeability to ions. So, in the condition where the concentration gradient of K^+ remained the same, the decrease of membrane potential at 40 mol% cholesterol suggests that K^+ permeability decreased steeply at this concentration. At 50°C, the result is similar, only the shift amplitude at 40 and 50 mol% cholesterol is a little larger than at 30°C (Fig. 2*B*). These results indicate that cholesterol makes the hydrocarbon-chain packing more restricted at cholesterol concentrations above 30 mol% at both 30 and 50°C.

^{31}P-NMR Spectroscopy

^{31}P-NMR technique was employed to gain information on the local order and conformation in the phosphate group of the phospholipids' polar head group (Seelig, 1978). To check the effects of cholesterol on the head group motion of SM/DOPC vesicles, ^{31}P-NMR measurements at both 30 and 45°C were carried out. As shown in Fig. 3, the presence of up to 50 mol% cholesterol did not change the phospholipid phase organization, which remained in the lamellar phase. The chemical shift anisotropy (CSA) of the spectra in Fig. 3 are shown in the Table. CSA is a measure of the degree of order of the phosphate group, and a reduction in the value of CSA reflects an increase of amplitude of motion (Smith & Ekiel, 1984). At both temperatures, there was a slight decrease in the CSA of SM/DOPC vesicles on addition of as much as 50 mol% cholesterol, which indicates that cholesterol makes the phosphate group slightly less ordered in liquid crystalline phase, compared to the results of ESR measurements, which indicated that cholesterol makes the hydrocarbon chains more restricted. The reason may be that when cholesterol packs between lipid hydrocarbon chains and restricts their conformation choices, cholesterol separates phospholipid molecules, allowing perhaps more conformation freedom for the phosphodiester moiety. There is an interesting change in the lineshapes in the presence of cholesterol: the peak splits and the split expands as the concentration of cholesterol increases. This change may indicate phase separation in the system when cholesterol is present. We study this phase separation next by fluorescence-quenching measurements.

Fluorescence-Quenching Measurements

Phase percolation is an important physical property of the system of phases co-existing as microscopic domains. The critical point where the initially dispersed domains become connected with each other and the initially continuous phase becomes disconnected is called percolation threshold (Vaz & Almeida, 1993). Fluorescence quenching of a lipid-labeled fluorophore by a spin-labeled quencher has been used to study phase percolation (Piknova, Marsh & Thompson, 1996). Because the fluorophore (DPH-PC) preferentially partitions into fluid phase at a $K_{f/s}$ of 3.3 (Parente & Lentz, 1985) and the quencher (5DS) also prefers the fluid phase, the quenching drops abruptly at the percolation threshold (Piknova et al., 1996). Fig. 4 shows the sets of quenching data obtained at 30 and 50°C, which are similar at both temperatures. In the absence of cholesterol, the amplitude of quenching is large (Fig. 4 *A,a* and *B,a*), which shows that the quenching can occur freely and that the SM/DOPC binary system is in a homogeneous phase. This is in agreement with previous reports that SM and PC will mix ideally if the difference between their chain lengths is not too large (e.g., Calhoun & Shipley, 1979). While cholesterol is present (Fig. 4 *A,b* and *B,b*), the extent of quenching decreases, suggesting the formation of some

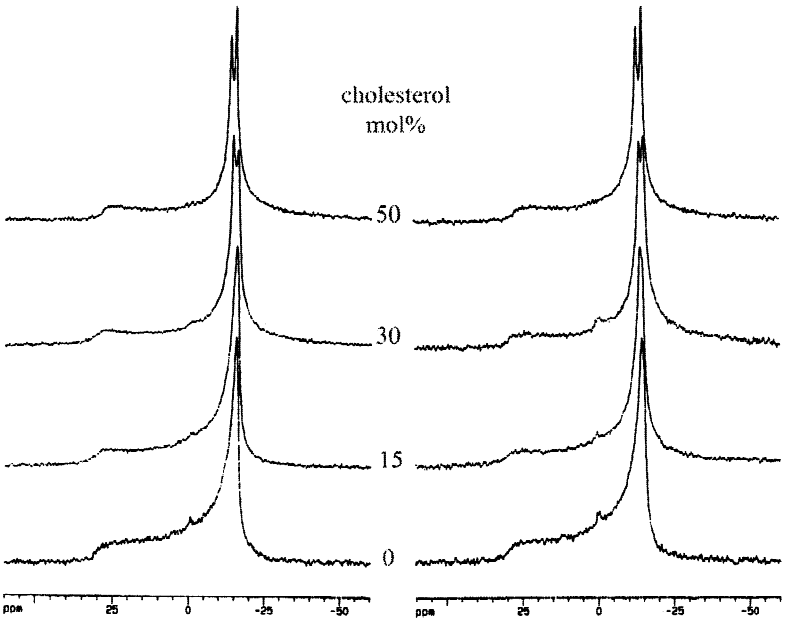

Fig. 3. ^{31}P-NMR lineshapes of SM/DOPC/CHL vesicles at 30°C (*left*) and 45°C (*right*). Cholesterol concentration is 0, 15, 30 and 50 mol% (from bottom to top).

Table. Effects of cholesterol on chemical shifts anisotropy of SM/DOPC vesicles

Temperature (°C)	Cholesterol concentration (mol%)			
	0	15	30	50
30	44	43	44	42 ppm
45	42	42	41	40 ppm

small, disconnected domains of solid phase. As the concentration of cholesterol increases, the quenching decreases further, but slowly (Fig. 4 *A,c–e* and *B,c–e*), which indicates that the percentage of the solid phase increases, but the solid phase domains are still disconnected. When the concentration of cholesterol reaches 50 mol% (Fig. 4 *A,f* and *B,f*), the quenching drops abruptly, which means the percolation threshold has been reached and the solid domains become continuous at this concentration. Further increasing the concentration of cholesterol has only slight effects on the domains; the system is still in the condition of disconnected domains of fluid phase floating in the continuous solid phase and does not transit to the solid phase after percolation threshold, even at 60 mol% cholesterol (Fig. 4 *A,g* and *B,g*), which is close to the maximum solubility of cholesterol in PCs (Huang, Buboltz & Feigenson, 1999).

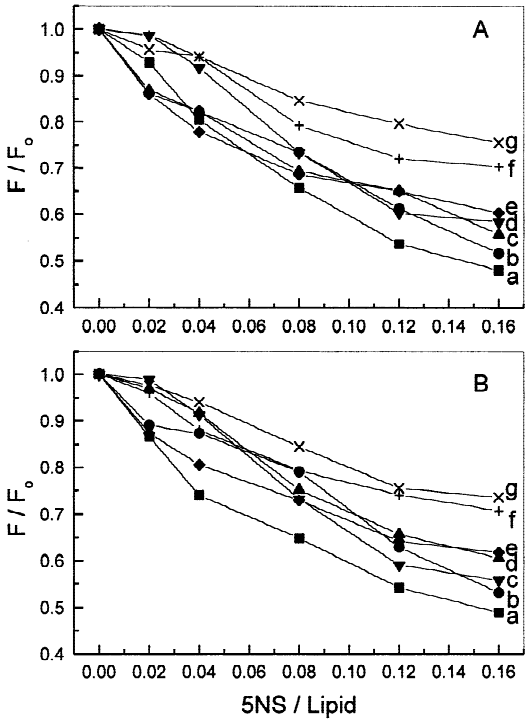

Fig. 4. Fluorescence quenching of DPH-PC by 5DS in SM/DOPC/CHL vesicles at 30°C (*A*) and 50°C (*B*). The concentrations of cholesterol correspond to 0 (*a*), 10 (*b*), 20 (*c*), 30 (*d*), 40 (*e*), 50 (*f*) and 60 (*g*) mol%.

Discussion

Both glycerolipids and sphingolipids are composed of a polar headgroup and one or two hydrocarbon chains linked covalently to the glycerol or sphingosine backbone. It is the variety in the hydrocarbon chain length, the degree of unsaturation of the chain, and the presence of branched chains that make membrane lipid an ex-

tremely complex mixture of different molecular species. At present, it is not known why membrane phospholipids have such a wide diversity of acyl chain lengths. The heterogeneity of the acyl chains allows the lipid bilayers to exhibit a wide range of physical properties. One of the many known physical parameters associated with the lipid bilayer, the main phase transition temperature, T_m, is perhaps the most important. T_m can be most accurately and reproducibly determined by high-resolution differential scanning calorimetry (DSC). Below T_m, the lipid bilayers will exist in an ordered gel phase, while above T_m, the bilayers will present in a liquid crystalline, or liquid disordered (l_d) phase.

In our system, DOPC, which has 18 carbon atoms and a C9–C10 cis double bond in each chain, has very low T_m, while SM from egg yolk has a T_m at about 40°C, which is close to physiological temperature. So we performed all the experiments at 30 and 50°C (or 45°C), either below or above the T_m of SM, and continually above the T_m of DOPC. But the order parameter of 5DS in SM/DOPC (1:1, molar ratio) vesicles without cholesterol at 30°C is about 0.565, which indicates a relatively high fluidity and suggests that this binary system is in liquid crystalline phase (Mehlhorn et al., 1988). Fluorescence polarization measurements also revealed that the phase transition of the SM/DOPC system occurs between 20 and 30°C (*data not shown*). We studied the effects of cholesterol on the hydrocarbon chain packing, head group motion, and phase behavior of the SM/DOPC binary system. Surprisingly, the results are similar at all temperatures between 30 and 50°C. So the discussion below is fitted to all temperatures in that range.

Cholesterol has different solubility in and affinity to various lipids. For instance, the maximum solubility of cholesterol in PC and PE bilayer is 66 and 51 mol%, respectively (Huang et al., 1999). It has been reported that cholesterol interacts preferentially with SM in the cell membrane (Slotte, 1999). In SM, acyl chains are amide-linked with the sphingoid base amino group, so hydrogen bonding of the 3β-hydroxyl to the amide group of SM appears to be more likely than to the oxygen atom at either the *sn*-1 or *sn*-2 position of phosphoglycerides (Brown, 1998). The affinity of cholesterol to lipids is also affected by the composition of acyl chains. The effect of cholesterol is much weaker in unsaturated PC (e.g., DOPC) membranes compared with that in saturated PC membranes. Because in DOPC-cholesterol membrane, steric nonconformability between the cis-unsaturated double bond in the alkyl chain and the rigid tetracyclic ring of cholesterol makes cholesterol molecules tend to be excluded from DOPC domains and segregated out (Subczynski et al., 1990). So the affinity of cholesterol to SM is much greater than to DOPC. We can conjecture that the formation of solid domains in the SM/DOPC/cholesterol system is driven by the interaction of SM and cholesterol, and that the solid domains are rich in SM and cholesterol when the cholesterol concentration is over 30 mol%. These domains have tight packing and relatively extended hydrocarbon chains, but the headgroups exhibit relatively rapid mobility. This is the most outstanding characteristic of liquid ordered phase (Brown & London, 1998), so we can deduce that the solid domains in the above system are in liquid-ordered (l_o) phase. Although microdomains of relatively solid phase also can form under 30 mol% cholesterol concentration, the fluidity of the system shows only a slight decrease, which indicates these microdomains are not in l_o phase, and may be in a state intermediate between the l_d and l_o phase (Brown & London, 1998). The l_o phase can form only when the concentration of cholesterol is over 30 mol%. When the concentration of cholesterol increases to 50 mol%, percolation threshold occurs and the domains of the l_o phase become continuous.

ESR is widely used in studying the physical properties of lipid membrane. Sankaram and Thompson studied simple systems of DPPC/cholesterol and SM/cholesterol using only ESR technology. They discovered a break in the positive slope of outer hyperfine splitting constants around 5 mol% cholesterol and the slope becomes negative near 25 mol%. The authors attribute the system between 5 and 25 mol% cholesterol to coexistence of the l_d and l_o phases. And above 25 mol%, the system is in l_o phase (Sankaram & Thompson, 1990). In our more complicated DOPC/SM/cholesterol system, we not only used ESR to study the order condition of hydrocarbon chains, but also studied the headgroup motion and phase behavior of the system. All experiments showed that in our system the l_o phase also appears, but at much higher cholesterol concentration, and even at 60 mol% the system still shows a coexistence of l_d and l_o phase. Ge et al. studied detergent-resistant membrane (DRM) from RBL-2H3 cells using ESR probes (Ge et al., 1999). They found that the order parameter of 5PC in DRM is comparable with that of the DPPC/cholesterol (1:1) system, which has been defined as l_o phase. So they concluded that DRM vesicles also have an l_o phase. Combining this work with our results, it can be concluded that our system has a similar structure of detergent-resistant membrane.

Caveolae and caveolae-like domains (or rafts) have recently been suggested to be liquid-ordered microdomains (Smart et al., 1999). Proteins may associate specifically with or may be excluded specifically from these domains. For example, GPI-anchored proteins, tyrosine kinases of the src family and some transmembrane proteins are bound to rafts and caveolae (Harder & Simons, 1997). In particular, an integral protein, caveolin, is used as the marker protein of caveolae (Anderson, 1998). Caveolin strongly binds cholesterol and may be responsible

for the morphological properties of caveolae (Harder & Simons, 1997). But are these proteins key factors in the formation of rafts and caveolae, or do they only prefer the lipid environments of rafts and caveolae? Our system is a simple model of plasma membrane, with DOPC as simulation of glycerolipids and SM as simulation of sphingolipids. At physiological temperature, microdomains of l_o phase form in this system at 30–50 mol% cholesterol, which is near the concentration of cholesterol in plasma membranes. The results on the simulation of plasma membrane of lipid composition indicate that, in physiological condition, microdomains of l_o phase can form independently of membrane proteins and other factors, just driven by the force of lipid-lipid interactions. The tight acyl chain packing of l_o phase lipids is probably responsible for their detergent insolubility. This also provides a rational explanation for the detergent insolubility of both rafts and caveolae (Harder & Simons, 1997). The proteins with a high affinity for the solid structures preferentially partition into them. In the cell types that can express caveolins, caveolae appear as a special form of raft when caveolins partition into the l_o phase domains. So we believe this system can give an insight to the mechanism of caveolae and raft formation, and serves as a good model to study the structure and function of the l_o phase.

References

Anderson, R.G.W. 1998. The caveolae membrane system. *Annu. Rev. Biochem.* **67:**199–225

Barenholz, Y., Thompson, T.E. 1999. Sphingomyelin: Biophysical aspects. *Chem. Phys. Lipids* **102:**29–34

Brown, D.A., London, E. 1998. Structure and origin of ordered lipid domains on biological membranes. *J. Membrane Biol.* **164:**103–114

Brown, R.E. 1998. Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. *J. Cell Sci.* **111:**1–9

Calhoun, W.I., Shipley, G.G. 1979. Sphingomyelin-lecithin bilayers and their interaction with cholesterol. *Biochemistry* **18:**1717–1722

Davies, M.A., Schuster, H.F., Brauner, J.W., Mendelsohn, R. 1990. Effects of cholesterol on conformational disorder in dipalmitoylphosphatidylcholine bilayers. A quantitative IR study of the depth dependence. *Biochemistry* **29:**4368–4373

Dowhan, W. 1997. Molecular basis for membrane phospholipid diversity: why are there so many lipids? *Annu. Rev. Biochem.* **66:**199–232

Ge, M., Field, K.A., Aneja, R., Holowka, D., Baird, B., Freed, J.H. 1999. Electron spin resonance characterization of liquid ordered phase of detergent-resistant membrane from RBL-2H3 cells. *Biophys. J.* **77:**925–933

Harder, T., Simons, K. 1997. Caveolae, DIGs, and the dynamics of sphingolipid-cholesterol microdomains. *Curr. Opin. Cell Biol.* **9:** 534–542

Huang, J., Buboltz, J.T., Feigenson, G.W. 1999. Maximum solubility of cholesterol in phosphatidylcholine and phosphatidylethanolamine bilayer. *Biochim. Biophys. Acta* **1417:**89–100

Kusumi, A., Subcznski, W.K., Pasenkiewicz-Gierula, M., Hyde, J.S., Merkle, H. 1986. Spin-label studies on phosphatidylcholine-cholesterol membrane: effects of alkyl chain length and unsaturation on the fluid phase. *Biochim. Biophys. Acta* **854:**307–317

Liscovitch, M., Cantley, L.C. 1994. Lipid second messengers. *Cell* **77:**329–334

Mehlhorn, I.E., Florio, E., Baker, K.R., Lordo, C., Grant, C.W.M. 1988. Evidence that trans-bilayer interdigitation of glycosphingolipid long fatty acid may be a general phenomenon. *Biochim. Biophys. Acta* **939:**151–159

McMullen, T.P.W., McElhaney, R.N. 1996. Physical studies of cholesterol-phospholipid interaction. *Curr. Opin. Colloid Interface Sci.* **1:**83–90

Needham, D., Nunn, R.S. 1990. Elastic deformation and failure of lipid bilayer membranes containing cholesterol. *Biophys. J.* **58:**997–1009

Newton, A.C. 1995. Protein kinase C: structure, function and regulation. *J. Biol. Chem.* **270:**28495–28498

Paré, C., Lafleur, M. 1998. Polymorphism of POPE/cholesterol system: a ^{2}H nuclear magnetic resonance and infrared spectroscopic investigation. *Biophys. J.* **74:**899–909

Parente, R., Lentz, B.R. 1985. Advantages and limitations of 1-palmitoyl-2-[[2-[4-(6-phenyl-*trans*-1,3,5-hexatrienyl)phenyl]ethyl]carbonyl]-3-*sn*-phosphatidylcholine as a fluorescence membrane probe. *Biochemistry* **24:**6178–6185

Piknova, B., Marsh, D., Thompson, T.E. 1996. Fluorescence-quenching study of percolation and compartmentalization in two-phase lipid bilayers. *Biophys. J.* **71:**892–897

Robision, A.J., Richards, W.G., Thomas, P.J., Hann, M.M. 1995. Behavior of cholesterol and its effects on head group and chain conformation in lipid bilayer: a molecular dynamics study. *Biophys. J.* **68:**164–170

Sankaram, M., Thompson, T.E. 1990. Interaction of cholesterol with various glycerophospholipids and sphingomyelin. *Biochemistry* **29:**10670–10675

Seelig, J. 1978. ^{31}P nuclear magnetic resonance and the head group structure of phospholipids in membrane. *Biochim. Biophys. Acta* **515:**105–140

Simons, K., Ikonen, E. 1997. Functional rafts in cell membrane. *Nature* **387:**569–572

Singer, S.J., Nicolson, G.L. 1972. The fluid mosaic model of the structure of cell membranes. *Science* **175:**720–731

Slott, J.P. 1999. Sphingomyelin-cholesterol interactions in biological and model membranes. *Chem. Phys. Lipids* **102:**13–27

Smaby, J.M., Mosen, M.M., Brockman, H.L., Brown, R.E. 1997. Phosphatidylcholine acyl unsaturation modulates the decrease in interfacial elasticity induces by cholesterol. *Biophys. J.* **73:**1492–1505

Smart, E.J., Graf, G.A., McNiven, M.A., Sessa, W.C., Engelman, J.A., Scherer, P.E., Okamoto, T., Lisanti, M.P. 1999. Caveolins, liquid-ordered domains, and signal transduction. *Mol. Cell. Biol.* **19:** 7289–7304

Smith, I.C.P., Ekiel, I.H. 1984. Phosphorus-31 NMR of phospholipids in membranes. *In* Phosphorus-31 NMR: Principles and applications. pp. 447–475. D.G. Gorenstein, editor. Academic Press, Orlando.

Subczynski, W.K., Antholine, W.E., Hyde, J.S., Kusumi, A. 1990. Microimmiscibility and three-dimensional dynamic structure of phosphatidylcholine-cholesterol membranes: translational diffusion of a copper complex in the membrane. *Biochemistry* **29:**7973–7945

Tilcock, C.P.S., Bally, M.B., Farren, S.B., Cullis, P.R., Grunner, S.M. 1984. Cation-dependent segregation phenomena and phase behav-

ior in model membrane system containing phosphatidylserine: influence of cholesterol and acyl chain composition. *Biochemistry* **23:**2696–2703

Vaz, W.L.C., Almeida, P.F.F. 1993. Phase topology and percolation in multi-phase lipid bilayers: is the biological membrane a domain mosaic? *Curr. Opion. Struct. Biol.* **3:**482–488

Večeř, J., Heřman, P., Holoubek, A. 1997. Diffusion membrane potential in liposomes: setting by ion gradients, absolute calibration and monitoring of fast changes by spectral shifts of $diSC_3(3)$ fluorescence maximum. *Biochim. Biophys. Acta* **1325:**155–164

Videira, P.A., Antunes-Madeira, M.C., Madeira, V.M.C. 1999. Perturbations induced by α- and β-endosulfan in lipid membranes: a DSC and fluorescence polarization study. *Biochim. Biophys. Acta* **1419:** 151–163

Yeagle, P.L. 1985. Cholesterol and the cell membrane. *Biochim. Biophys. Acta* **822:**267–287