Asymmetry of Lysophosphatidylcholine/Cholesterol Vesicles Is Sensitive to Cholesterol Modulation†

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ABSTRACT: Sonication of lysophosphatidylcholine (lysoPC; 20 μmol/mL) and cholesterol (chol) in aqueous medium produces lamellar structures over a wide range of concentrations. From 25 to 47 mol % cholesterol, electron microscopy (EM) after negative staining showed extended stacklike lamellae about 40 Å thick. From 50 to 60 mol % chol, freeze–fracture EM showed homogeneous populations of small unilamellar vesicles averaging 260–310 Å in diameter. Phosphorus-31 nuclear magnetic resonance was used to characterize the stacklike lamellae and to measure the distribution of the lysophospholipid between the outer and inner leaflet of the vesicles as a function of sterol concentration. We found that in lysoPC/chol dispersions containing less than equimolar amounts of cholesterol (25–47 mol %), the entire phosphorus signal (40.5 ppm) was shifted downfield by 10.5 ppm upon addition of Pr^{3+} (2.4 mM), consistent with the stacklike lamellar structures in which all lysoPC head groups are accessible to the ions. By contrast, addition of Pr^{3+} to lysoPC/chol vesicles containing equimolar or higher amounts of cholesterol (up to 60 mol %) gave rise to two phosphorus peaks. The more intense downfield signal (51.0 ppm) responsive to paramagnetic ions was assigned to lysoPC located in the outer vesicle leaflet. The upfield signal (40.5 ppm), which was not affected by the ions, was assigned to inside lysoPC. For lysoPC/chol (1:1) vesicles, an outside to inside lysophospholipid ratio ($R_{o/i}$) of 6.5 was determined. Essentially the same $R_{o/i}$ value (6.7) was obtained on lysoPC/chol (1:1) vesicles which after dialysis contained only entrapped Pr^{3+}. Ion leakage did not occur. The data show that vesicles containing equimolar amounts of lysoPC and cholesterol are drastically asymmetric and that lysoPC has a distinct preference for the outer bilayer shell. Furthermore, an increase in cholesterol levels from 50 to 60 mol % not only decreased vesicle size (from 310 to 260 Å) but also caused a drastic decrease in the lysoPC $R_{o/i}$ ratio from 6.5 to 2.2. The high sensitivity of the lysoPC outside/inside distribution to small incremental changes in cholesterol content may have a regulatory function in lysoPC-mediated membrane processes in highly curved bilayer domains.

Lysophosphatidylcholine (lysoPC)[1] forms micelles in aqueous medium (Saunders & Thomas, 1958; Saunders, 1966). This arrangement is quite consistent with the wedge or inverted cone shape of the lysoPC molecule (Haydon & Taylor, 1963; Cullis & de Kruijff, 1979). When lysoPC is incorporated into phosphatidylcholine (PC) bilayers, it induces structural changes. These usually result in increased membrane permeability (Lee & Chan, 1977; Morris et al., 1980), can cause membrane fusion (Elamrani & Blume, 1982), and eventually lead to lamellar disruption (Bangham & Horne, 1964). By contrast, cholesterol (chol) in phospholipid lamellae rather behaves as if it were a truncated cone (Carnie et al., 1979). Cholesterol incorporation into PC bilayers reduces membrane permeability, broadens the gel to liquid-crystalline phase transition, and decreases average molecular surface areas (de Bernard, 1958; Demel et al., 1967; Bittman et al., 1981; Murari et al., 1986). It is not only that lysoPC and cholesterol affect phospholipid bilayers quite differently, but when both are present in similar proportions, they seem to counteract each other's effect (Inoue & Kitagawa, 1974; Klopfenstein et al., 1974; Kitagawa et al., 1976; van Echteld et al., 1981). Hence, much current interest focuses on lysoPC/cholesterol interactions and on how lysoPC and cholesterol affect membrane properties.

In the present study, we have examined the lamellar structures that are produced by prolonged sonication of lysoPC and cholesterol. Morphological changes were followed by electron microscopy. Phosphorus-31 nuclear magnetic resonance (NMR) and lanthanide ions were used to characterize the lamellar structures and to measure changes in lysophospholipid distribution between the outer and inner bilayer leaflet of lysoPC/chol small unilamellar vesicles (SUV) as they are affected by changes in sterol concentration.

Experimental Procedures

Materials. Lysophosphatidylcholine (1-*O*-hexadecanoyl-*sn*-glycero-3-phosphocholine, lysoPC) was purchased from Avanti Polar Lipids (Birmingham, AL) and was shown to be pure by thin-layer chromatography (TLC); developing solvent A, $CHCl_3/CH_3OH/H_2O$ (65:35:8, by volume). LysoPC was characterized by ^{13}C NMR (Murari et al., 1982) using $CDCl_3/CD_3OD/D_2O$ (50:50:15, by volume) as solvent (Murari & Baumann, 1981). Cholesterol was purchased from Nu-Chek Prep (Elysian, MN) and was shown to be pure by

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[1] Abbreviations: lysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; chol, cholesterol; EM, electron microscopy; NMR, nuclear magnetic resonance; SUV, small unilamellar vesicle(s); TLC, thin-layer chromatography; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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TLC; developing solvent B, hexane/diethyl ether/acetic acid (70:30:1, by volume). Deuteriated solvents ($CDCl_3$, 99.8% D; CD_3OD, 99.5% D; D_2O, 99.8% D) were from KOR Isotopes (Cambridge, MA). Praseodymium chloride (99.9%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Small aliquots (20 μL) of a stock solution of the shift reagent in D_2O were directly added into the NMR tubes containing the lipid dispersions.

Preparation of Lipid Dispersions. LysoPC (50 μmol) and cholesterol in appropriate proportions were dissolved in $CHCl_3/CH_3OH$ (1:1) in a round-bottom flask, the solvent was removed at room temperature on a rotary evaporator, and the sample was dried in vacuo for at least 6 h. The lipid film was dispersed by vortexing in 2.5 mL of 10 mM Tris-HCl buffer in D_2O containing 0.15 M KCl and 0.001% NaN_3, and the dispersion was sonicated under nitrogen for 3 h in an ice bath (Branson tip sonifier, Model 350; output setting 4). Debris was removed at 105000*g* for 1 h using a Beckman Model L5-75 ultracentrifuge. Aliquots of the sonicated dispersions, before and after NMR, were routinely tested to assure the integrity of the lipid components. For this purpose, lipids were extracted (Bligh & Dyer, 1959) and then analyzed by TLC using developing solvents A and B (see above).

Electron Microscopy. The lipid dispersions prepared by sonication were characterized by transmission electron microscopy (EM) after negative staining or freeze–fracture.

For negative staining, a drop of lipid dispersion was placed on an inverted 200-mesh carbon-coated copper grid (E. F. Fullam, Inc., New York, NY) which had been preconditioned by brief exposure (45 s) to long-wave UV light, and excess sample was drawn off with a filter paper point after 30 s. A drop of 2% aqueous phosphotungstic acid was placed onto the grid, excess stain was picked up after 30 s, and the grid was air-dried for 20 min. Samples were immediately examined under a JEOL 100S electron microscope at 60 kV. Micrographs were taken at magnifications of up to 40000× and then enlarged to up to 100000×.

For freeze–fracture, a drop of sample was placed on a 3-mm cup-type specimen holder, rapidly frozen by immersion in liquid nitrogen cooled Freon, and transferred to a Balzer BAF 300/301 apparatus. Immediately following fracture, the sample was coated from a carbon/platinum electrode at a 45° angle, and a layer of carbon was deposited from a vertical electrode. Replicas were removed by floating in distilled water, cleaned with chloroform/methanol, placed on a 200-mesh copper grid, and then examined under the JEOL 100S electron microscope at 60 kV. Vesicle size distributions were determined from freeze–fracture electron micrographs by measuring the diameter of concave hemispheres that were 50% shadowed (van Venetië et al., 1980).

Phosphorus-31 NMR. Spectra were recorded at 32.20 MHz on a Varian FT-80A pulse Fourier-transform instrument equipped with a broad-band probe. Spectra were measured at 37 ± 1 °C using 10-mm o.d. sample tubes (90° flip angle, 10-s recycle time, 5000 transients, 4-kHz sweep width, 8K data points).

All spectra were recorded at 20 mM lysoPC concentration in the presence of increasing amounts of cholesterol (0–60 mol %). Identical instrument settings were used throughout. This made it possible to directly compare integrated peak areas for quantitation. Spectra recorded with or without proton noise decoupling gave identical results. All measurements were done at least in duplicate.

Dialysis. To remove external Pr^{3+} ions, lysoPC/chol (1:1) vesicles prepared in the presence of $PrCl_3$ were dialyzed by using Spectrapor membrane tubing (15.9-mm diameter, up to 14000 daltons; Spectrum Medical Industries, Los Angeles, CA). Approximately 2.5 mL of the vesicle preparation was placed into the tubing, the tubing was clamped at both ends, and the sample was dialyzed twice for 12 h by immersing into 200 mL of D_2O buffer while stirring.

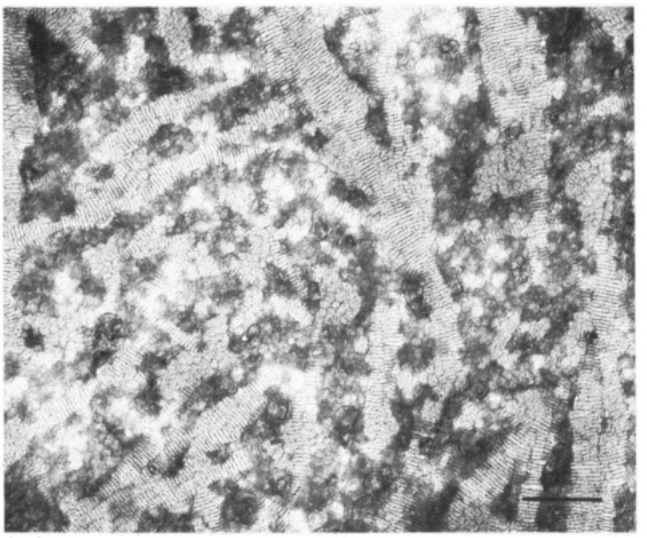

FIGURE 1: Electron micrograph, after negative staining, of a lysophosphatidylcholine/cholesterol sonicated dispersion (3 h) containing 25 mol % chol. Average distance of the stacklike lamellae, 40 ± 5 Å. The bar (insert) equals 1000 Å.

RESULTS

Prolonged sonication of lysophosphatidylcholine (lysoPC) and cholesterol (chol) in aqueous medium (20 mM lysoPC/10 mM Tris-HCl, 3 h) produces lamellar structures. The type of lamellae formed is very much dependent upon the cholesterol concentration. LysoPC/chol dispersions containing less than equimolar amounts of cholesterol (25–47 mol % chol) preferentially form extended stacklike lamellae with the occasional appearance of vesicular structures at the higher cholesterol levels. A representative electron micrograph of a lysoPC/chol dispersion containing 25 mol % chol is shown in Figure 1. The micrograph clearly depicts the characteristic rouleaux of disks produced. While the diameter of the discoid stacks can vary over a considerable range, the periodicity of the lamellae remains quite constant, averaging 40 ± 5 Å in thickness, similar to that of most phospholipid bilayers.

Phosphorus-31 NMR was used to further characterize the stacklike discoid structures. We have previously shown that lysoPC micelles produce a narrow, isotropic peak near 40.5 ppm with a half-height line width ($\nu_{1/2}$) of 1.8 Hz (Kumar & Baumann, 1986). The lysoPC/chol stacks containing 25–44 mol % chol gave rise to a slightly broader resonance with a line width of 2.6–4.8 Hz, respectively. Figure 2 displays the ^{31}P NMR spectra of lysoPC/chol sonicated dispersions containing up to 50 mol % cholesterol. The insert (top) shows the spectrum of equimolar lysoPC/chol ($\nu_{1/2}$ 6.3 Hz) in the absence of the lanthanide ions. It can be seen that upon addition of 2.4 mM Pr^{3+} to lysoPC/chol dispersions containing less than equimolar amounts of cholesterol, the phosphorus signal, which normally appears near 40.5 ppm, is shifted downfield to 51.0 ppm (Figure 2). A shift of the entire phosphorus signal (by 10.5 ppm) indicates that all lysoPC head groups in these structures are accessible to the paramagnetic ions. This, of course, is quite consistent with the discoid rouleaux produced (Figure 1) by these sonicated lysoPC/chol dispersions.

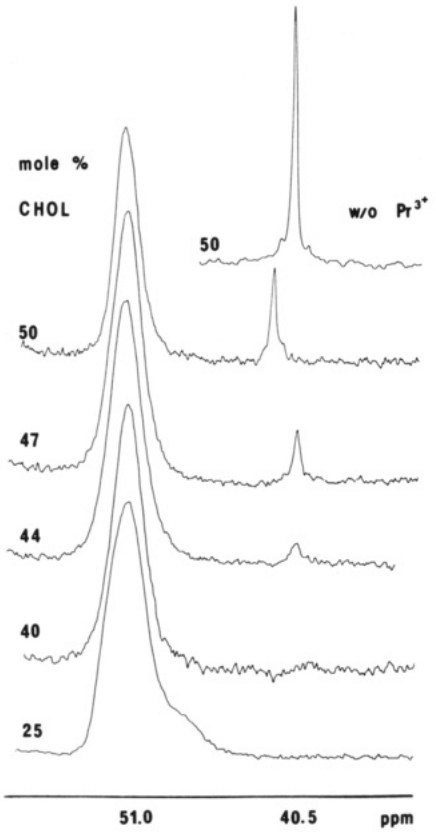

FIGURE 2: Phosphorus-31 NMR spectra of lysoPC/chol sonicated dispersions containing 25–50 mol % cholesterol in the presence of 2.4 mM Pr^{3+}. The signals near 51.0 ppm represent lysoPC phosphorus shifted by Pr^{3+}. The small upfield signals and the reference signal (top insert; $\nu_{1/2}$ 6.3 Hz) near 40.5 ppm represent lysoPC not exposed to the paramagnetic ions.

The gradual appearance of vesicular structures in electron micrographs of lysoPC/chol dispersions approaching equimolar proportions, that was mentioned above, was accompanied by the emergence of a weak phosphorus signal near 40.5 ppm characteristic of lysoPC phosphorus not shifted by lanthanide ions (Figure 2, at 44 mol % and higher chol). This signal became quite prominent at 50 mol % cholesterol (Figure 2).

Three-hour sonication of equimolar lysoPC/chol (1:1) dispersions produced a homogeneous population of small unilamellar vesicles (SUV; ~300-Å diameter). On the basis of electron microscopy after negative staining, the presence of lysoPC micelles[2] (or "stacks") was ruled out. A representative freeze–fracture electron micrograph of the spherical lysoPC/chol (1:1) SUV is shown in Figure 3 (panel A; vesicle diameter, 310 ± 50 Å).

The lysoPC/chol (1:1) small unilamellar vesicles were further characterized by phosphorus-31 NMR, and the distribution of lysoPC ($R_{o/i}$ ratio) between the outer and inner bilayer leaflets was determined. As is shown in Figure 4 (panel A, bottom), lysoPC/chol (1:1) SUV give rise to a single peak near 40.5 ppm with a half-height line width ($\nu_{1/2}$) of 6.3 Hz (T_2^* 0.05 s; T_1 0.65 s). This line width is distinctly greater than that of lysoPC micelles (1.8 Hz) and compares well with the half-height line width which we measured for 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine small unilamellar vesicles (8.0 Hz; V. V. Kumar and W. J. Baumann, unpublished data) or with data published for similar PC vesicles ($\nu_{1/2}$ 9–12 Hz at 36.4 MHz; de Kruijff et al., 1975). Upon addition of Pr^{3+} (2.4 mM) to lysoPC/chol (1:1), an additional, more intense signal appears downfield near 51.0 ppm (Figure 4, panel A, top). The more intense signal responsive to paramagnetic ions can be assigned to lysoPC molecules located in the outer leaflet of the vesicles. The less intense signal not affected by the ions can be assigned to lysoPC located in the inner vesicle shell. On the basis of integrated peak intensities, which were measured under proton noise-decoupled and proton-coupled conditions to rule out a contribution from NOE,

[2] LysoPC micelles can readily be recognized and distinguished from lysoPC/chol small unilamellar vesicles (~300-Å diameter) by electron microscopy (at 100000× magnification) after negative staining. Our lysoPC micellar preparations showed a diameter of less than 90 Å which is in good agreement with literature values (77 Å; Hauser, 1976).

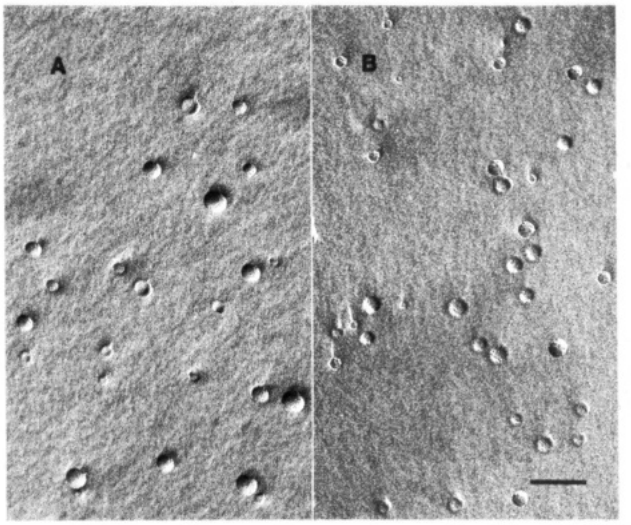

FIGURE 3: Freeze–fracture electron micrographs of lysoPC/chol unilamellar vesicles. (Panel A) LysoPC/chol vesicles containing 50 mol % chol; vesicle diameter, 310 ± 50 Å. (Panel B) LysoPC/chol vesicles containing 60 mol % chol; vesicle diameter, 260 ± 20 Å. The bar (insert) equals 1000 Å (panels A and B).

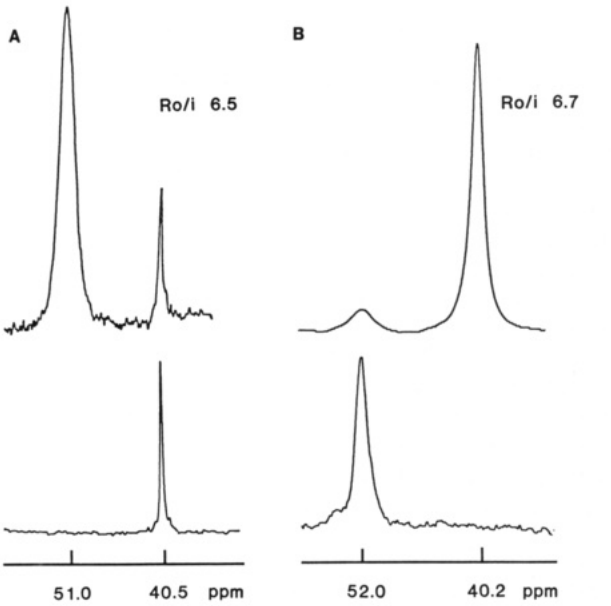

FIGURE 4: Phosphorus-31 NMR spectra of lysoPC/chol unilamellar vesicles containing 50 mol % cholesterol (see also electron micrograph, Figure 3, panel A). (A) LysoPC/chol (1:1) vesicles before (bottom) and after addition of 2.4 mM Pr^{3+} (top). The signals near 40.5 ppm are due to lysoPC phosphorus in the absence of Pr^{3+} (inside lysoPC in top spectrum); the signal near 51.0 ppm represents lysoPC phosphorus shifted by Pr^{3+} (outside lysoPC). On the basis of integrated peak areas, an outside to inside lysoPC ratio ($R_{o/i}$) of 6.5 was determined. (B) LysoPC/chol (1:1) vesicles prepared in the presence of 2.4 mM Pr^{3+} before (bottom) and after removal of outside ions by dialysis (top). The signals near 52.0 ppm represent lysoPC phosphorus shifted by Pr^{3+}; the signal near 40.2 ppm (top) is due to lysoPC in the absence of Pr^{3+} (outside lysoPC; $R_{o/i}$ 6.7).

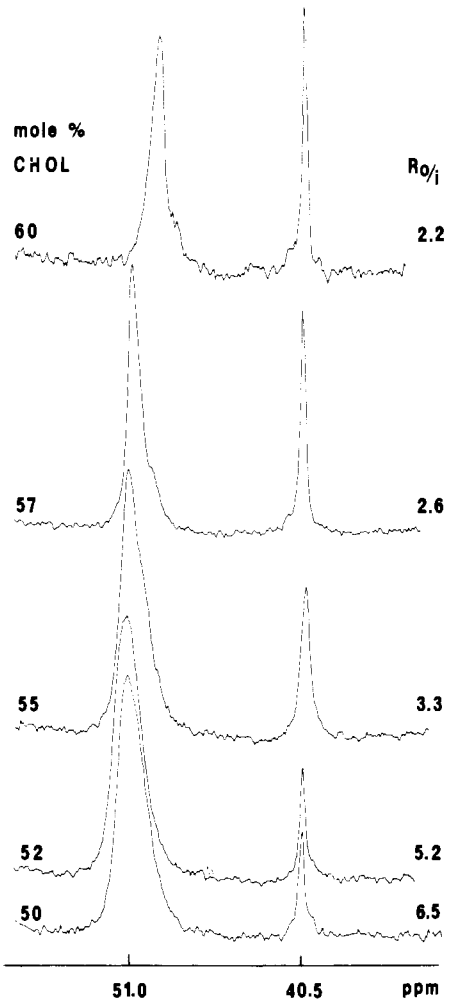

FIGURE 5: Phosphorus-31 NMR spectra of lysoPC/chol unilamellar vesicles containing 50–60 mol % cholesterol (see also electron micrographs, Figure 3, panels A and B) in the presence of 2.4 mM Pr^{3+}. The signals near 40.5 ppm represent lysoPC phosphorus that is not affected by Pr^{3+}, i.e., lysoPC located in the inner vesicle leaflet. The more intense signals near 51.0 ppm responsive to Pr^{3+} represent lysoPC located in the outer vesicle leaflet. The distribution of lysoPC between the outer and the inner vesicle leaflets ($R_{o/i}$ ratio) was determined by integration of respective peak areas.[4]

an outside to inside lysoPC ratio of 6.5 was determined. This distinct preference of lysoPC for the outer bilayer leaflet was confirmed on lysoPC/chol (1:1) SUV with lanthanide ions entrapped in their interior space. For this purpose, lysoPC and cholesterol in equimolar proportions were sonicated in the presence of Pr^{3+}, and external ions were removed by dialysis. As is shown in Figure 4 (panel B, bottom), lysoPC/chol (1:1) vesicles having Pr^{3+} at the inside and the outside give rise to a single peak near 52.0 ppm. When external ions were removed by dialysis, an additional, more intense signal appeared upfield near 40.2 ppm which is characteristic of lysoPC in the absence of paramagnetic ions (Figure 4, panel B, top). In this case, an $R_{o/i}$ value of 6.7 was determined. Spectral changes were not observed for more than 2 months, indicating that the vesicles remained impermeable to lanthanide ions. The results of these complementary experiments support the conclusion that small unilamellar lysoPC/chol (1:1) vesicles are highly asymmetric in respect to the distribution of the lysophospholipid between the outer and inner vesicle shells ($R_{o/i}$ 6.6 ± 0.2).

When the proportion of cholesterol in lysoPC/chol dispersions was increased from 50 to 60 mol %,[3] the vesicle diameter decreased from 310 ± 50 to 260 ± 20 Å. Representative freeze–fracture electron micrographs of vesicles containing 50 mol % chol (panel A) and 60 mol % chol (panel B) are shown in Figure 3. Multilamellar or stacklike structures were not apparent.

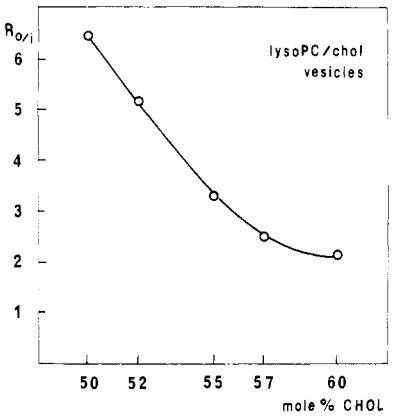

FIGURE 6: Outside/inside ratio ($R_{o/i}$) of lysoPC in lysoPC/chol small unilamellar vesicles as a function of cholesterol concentration.

In the absence of praseodymium, the lysoPC/chol small unilamellar vesicles containing 50–60 mol % cholesterol gave rise to a rather narrow and symmetrical phosphorus signal near 40.5 ppm. The half-height line width increased only little (from 6.3 to 7.8 Hz), and there was no indication of a "powder pattern" for any of the preparations.

In the presence of praseodymium (2.4 mM), a prominent outside lysoPC signal was observed near 51.0 ppm in addition to the unshifted signal near 40.5 ppm characteristic for inside lysoPC (Figure 5). As all spectra were recorded at identical lysoPC concentrations (20 mM) using identical instrument settings, peak integrals could directly be compared in a quantitative fashion.[4] A comparison of the spectra shows that with small incremental increases in the cholesterol levels of the small unilamellar lysoPC/chol vesicles, the intensity of the inside lysoPC signal (40.5 ppm) increases markedly at the expense of the outside signal (51.0 ppm). Throughout the range of concentrations measured (50–60 mol % chol), lysoPC exhibits a distinct preference for the outside leaflet. In equimolar lysoPC/chol SUV, almost 87% of the total lysoPC is found on the outside, whereas in lysoPC/chol vesicles containing 60 mol % chol about 69% of the total lysophospholipid can be accommodated in the outside shell. The percentage of total lysoPC located on the inside increases almost 2.5-fold (from 13% to 31%) by a change in cholesterol levels by 10 mol %.

The change in the lysoPC outside to inside ratio as a function of cholesterol concentration is depicted in Figure 6. It is apparent that an initial increase in the cholesterol mole percentage by 5% (from 50% to 55% chol) causes a 50% decrease in the lysoPC outside to inside ratio (from 6.5 to 3.3). After this drastic and essentially linear decline, the $R_{o/i}$ value for lysoPC asymptotically approaches 2.2 (at 60 mol % chol). This translates into a 3-fold change in the lysoPC $R_{o/i}$ value for a 10% change in the cholesterol mole percentage.

Discussion

Lysophosphatidylcholine, being a micellar lipid, acts as a natural modulator of biological membranes (Nicolson, 1976; Weltzien, 1979). LysoPC increases cell membrane permeability (Miller et al., 1979), facilitates cell fusion (Lucy, 1970; Poole et al., 1970; Croce et al., 1971), regulates the activity

[3] At cholesterol concentrations higher than 60 mol %, the dispersions remained turbid even after extended sonication.

[4] "Truncation" was excluded by comparing total peak integrals and finding them conserved (±2%) for each of the spectra.

of various membrane-bound enzymes (Mookerjea & Yung, 1974; Parthasarathy & Baumann, 1979; Parthasarathy et al., 1981), appears to be involved in endocytotic processes (Malewicz et al., 1981), and ultimately causes cell lysis (Reman et al., 1969; Weltzien, 1979).

In the present context, it is of particular interest that the modulation of biological membranes by lysoPC is very much affected by membrane cholesterol. Collier (1947) observed some 40 years ago that cholesterol feeding to rabbits increased the antihemolytic value of erythrocytes, as measured by lysoPC-induced red cell lysis, and that the increase paralleled the cell cholesterol concentration (Collier & Chen, 1950). Cholesterol was also shown to inhibit lysoPC-induced changes in membrane potential and potassium release from erythrocytes (Shinozawa et al., 1978). More recent studies have indicated that lysoPC and cholesterol affect red cell shape in an interdependent fashion (Lange & Slayton, 1982) and that the rate of lysoPC translocation across the erythrocyte membrane is cholesterol dependent (Mohandas et al., 1982).

Thus, while there is considerable evidence that lysoPC and cholesterol affect phospholipid bilayers as well as biological membranes in an opposing manner, there is also an indication that lysoPC and cholesterol, even in the absence of other phospholipids, interact in aqueous medium and form a lamellar phase. Dervichian (1946) first observed that "lysolecithin associated with cholesterol in equimolar proportions swells and gives myelinic figures as does lecithin alone". The formation of lysoPC/chol (1:1) lamellar structures in water was later confirmed by differential scanning calorimetry (Klopfenstein et al., 1974), X-ray diffraction (Rand et al., 1975), electron spin resonance (Purdon et al., 1975), and barrier property measurements (Kitagawa et al., 1976; van Echteld et al., 1981). It was also shown by NMR spectroscopy that, in aqueous phase, lysoPC and cholesterol can combine in equimolar proportion and, upon sonication, form stable vesicles (Ramsammy & Brockerhoff, 1982; Kumar & Baumann, 1986) as would double-stranded phospholipids such as phosphatidylcholine.

In the present study, we have examined the lysoPC/chol system over a wide range of concentrations. Extended sonication (3 h) of lysoPC/chol dispersions containing less than equimolar amounts of cholesterol produced characteristic discoid rouleaux (Figure 1) that varied in diameter but showed constant periodicity of the bilayer-like lamellae (40 Å). The structures are similar to those described by Purdon et al. (1975) and are reminiscent of the discoid stacks produced by sonication of lysoPC/PC (1:1) mixtures (Inoue et al., 1977). The morphological similarity between lysoPC/PC (1:1) and lysoPC/chol dispersions containing excess lysoPC could suggest that lysoPC/chol (1:1) behaves as if it were a lamellar lipid mimicking phosphatidylcholine. The phenomenon has been explained by the formation of lysoPC/chol (1:1) molecular complexes of approximately cylindrical shape (Rand et al., 1975).

It is well-known that equimolar lysoPC/chol dispersions in water form lamellar structures (Dervichian, 1946; Purdon et al., 1975; Ramsammy et al., 1984). However, our study has shown that extensive 3-h sonication is required to produce a homogeneous population of lysoPC/chol (1:1) small unilamellar vesicles of uniform size (~300 Å; Figure 3A).

Prolonged sonication of lysoPC/chol dispersions containing higher than equimolar amounts of cholesterol (up to 60 mol %)[3] also produced vesicles (Figures 3B). However, their average diameters were consistently smaller (260 Å) than those of lysoPC/chol (1:1) SUV (310 Å). A decrease in diameter (700 to 600 Å) with increasing cholesterol content (50 to 60 mol %) has also been observed on the larger spherical particles formed in lysoPC/chol dispersions without sonication (Purdon et al., 1975). It is tempting to hypothesize that excess cholesterol may cause a tighter packing of the lysoPC/chol (1:1) lamellar phase in a similar fashion as it affects phosphatidylcholine bilayers ("condensing effect").

The main objective of the present study was to measure the lysophosphatidylcholine distribution between the outer and the inner bilayer leaflets ($R_{o/i}$) of lysoPC/chol small unilamellar vesicles and to determine how lysoPC distribution is modulated by changes in membrane cholesterol. A more detailed knowledge of the underlying physical principles of lysoPC/cholesterol interactions and lysoPC sidedness, as it is affected by cholesterol, would be an important first step toward a better understanding of various biological membrane processes that are regulated by lysoPC and cholesterol. Phosphorus-31 NMR in the presence of praseodymium ions as shift reagents proved to be a most suitable tool to gain such insights.

Lysophospholipid asymmetry in lysoPC/chol (1:1) SUV was measured by two complementary techniques with Pr^{3+} present either only in the exterior volume or only entrapped in the vesicle interior. By either technique, integration of respective peak areas gave essentially identical results. We found that lysoPC/chol (1:1) small unilamellar vesicles are drastically asymmetric with respect to lysoPC which shows a distinct preference for the outer bilayer leaflet. An outside to inside lysophospholipid ratio of 6.6 was determined.[5] No changes in chemical shifts or signal intensities were observed for at least 2 months, indicating that, for all practical purposes, lysoPC/chol (1:1) vesicles are impermeable to lanthanide ions.

The phospholipid asymmetry that we observed for lysoPC/chol (1:1) vesicles is much greater ($R_{o/i}$ 6.6 ± 0.2) than that ($R_{o/i}$ 2.2 ± 0.6) reported for phosphatidylcholine vesicles (Bystrov et al., 1972; de Kruijff et al., 1975, 1977) or phosphatidylcholine/cholesterol vesicles (de Kruijff et al., 1976;, 1977). On the other hand, PC vesicles containing a small percentage of lysoPC (5–15%) had also been shown to preferentially accomodate the lysophospholipid in the outer vesicle shell (de Oliveira Filgueiras et al., 1977; de Kruyff et al., 1977; van den Besselaar et al., 1977). This distinct preference of lysoPC for the outer bilayer leaflet of small unilamellar vesicles is likely to reflect the geometric requirements of lysoPC and is consistent with the inverted cone shape of the lysoPC molecule (Haydon & Taylor, 1963; Cullis & de Kruijff, 1979; Carnie et al., 1979).

The most important result of the present study is the observation that small incremental increases in cholesterol content between 50 and 60 mol % cholesterol not only caused a decrease in vesicle size (from 310 to 260 Å) but also resulted in a sharp decrease in the lysoPC $R_{o/i}$ ratio. While lysoPC/chol (1:1) SUV were found to be highly asymmetric ($R_{o/i}$ 6.6), lysoPC/chol vesicles containing 60 mol % cholesterol approached a lysoPC $R_{o/i}$ of only 2.2. This degree of phospholipid asymmetry is similar to that of vesicles containing phosphatidylcholine with or without cholesterol. The drastic effect of small changes in cholesterol levels on the lysoPC outside/inside distribution in small unilamellar vesicles appears to be noteworthy not only as an intriguing physical phenomenon but may well have a regulatory function in various lysoPC-mediated biological processes taking place in highly curved membrane regions.

[5] The $R_{o/i}$ value of 2.0 reported for lysoPC/chol (1:1) sonicated dispersions (Ramsammy & Brockerhoff, 1982) was apparently determined on a population of larger liposomes (Ramsammy et al., 1984; Figure 3a).

Registry No. CHOL, 57-88-5; lysoPC, 17364-16-8.

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