Lysophosphatidylcholine stabilizes small unilamellar phosphatidylcholine vesicles

Phosphorus-31 NMR evidence for the "wedge" effect

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ABSTRACT Sonication of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-*sn*-glycero-3-phosphocholine (lysoPC, up to ~30 mol %) produces small unilamellar vesicles (SUV, 250–265 Å diameter). Phosphorus-31 NMR of the POPC/lysoPC vesicles gives rise to four distinct peaks for POPC and lysoPC in the outer and in the inner bilayer leaflet which can be used to localize and quantify the phospholipids in both vesicle shells. Addition of paramagnetic ions (3 mM Pr^{3+}) enhances outside/inside chemical shift differences and allows monitoring of membrane integrity by the absence of Pr^{3+} in the vesicle interior. ^{31}P NMR shows that lysoPC in these highly curved POPC/lysoPC vesicles prefers the outer bilayer leaflet. LysoPC incorporation into POPC SUV furthermore causes a substantial and concentration-dependent decrease in spin–spin relaxations (T_2^*) of the outside POPC phosphorus signals from 55 ms for pure POPC vesicles ($\nu_{1/2}$, 5.8 Hz) to 29.5 ms ($\nu_{1/2}$, 10.8 Hz) for POPC/lysoPC vesicles containing 25 mol % lysoPC. Our findings are consistent with the idea of a cone-shaped lysoPC molecule which, for geometric reasons, is preferentially accommodated in the outer bilayer leaflet. LysoPC incorporation into POPC SUV restricts POPC headgroup motion and tightens phospholipid packing, but only in the outer bilayer shell.

INTRODUCTION

Molecular shape is an important consideration in membrane modeling. Based on the physical dimension of a membrane component, its location in the membrane and its aggregate effect on membrane architecture and properties can often be predicted or rationalized (see also Carnie et al., 1979, and references therein). Whereas hydrated phosphatidylcholine (PC) molecules with their approximately "cylindrical" shape readily form lamellar structures, lysophosphatidylcholine (lysoPC) forms spherical micelles when dispersed in water (Saunders and Thomas, 1958). The micellar arrangement of lysoPC is consistent with the much discussed "wedge" or "inverted cone" shape of the lysoPC molecule (Haydon and Taylor, 1963; Cullis and de Kruijff, 1979; Carnie et al., 1979). The "wedge" model can also illustrate why lysoPC when inserted into planar phospholipid bilayers induces structural changes which eventually lead to lamellar disruption (Bangham and Horne, 1964). Although the "wedge" model has become an appealing concept, direct experimental evidence in its support is lacking.

Studies in our laboratory and by others have shown that lysoPC in small unilamellar vesicles (SUV) prefers the outer bilayer leaflet (de Kruyff et al., 1977; van den Besselaar et al., 1977; de Oliveira Filgueiras et al., 1977; Kumar and Baumann, 1986; Kumar et al., 1988). We now found that in small unilamellar PC/lysoPC vesicles PC and lysoPC in both vesicle leaflets can individually be recognized by ^{31}P nuclear magnetic resonance (NMR), even in the absence of paramagnetic ions. This made it possible to measure phosphatidylcholine headgroup motion in terms of ^{31}P spin–spin relaxations as function of PC/lysoPC ratio in each vesicle shell.

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MATERIALS AND METHODS

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-*sn*-glycero-3-phosphocholine (lysoPC) were obtained from Avanti Polar Lipids (Birmingham, AL); both were pure as judged by thin-layer chromatography (developing solvent, $CHCl_3/CH_3OH/H_2O$, 65:35:8, by vol). POPC and lysoPC were characterized by ^{13}C NMR, using $CDCl_3/CD_3OD/D_2O$, 50:50:15 (by vol) as solvent to assure monomeric distribution (Murari and Baumann, 1981; Murari et al., 1982).

Vesicles were prepared by sonication after a modification of established procedures (Huang, 1969; Huang and Thompson, 1974). POPC and lysoPC (0.25 mmol total) in appropriate proportion were dissolved in 15 ml of $CHCl_3/CH_3OH$ (2:1), using a 50-ml roundbottom flask, and the solvent was slowly removed at room temperature on a rotary evaporator. The film was dried in high vacuum for at least 6 h and then hydrated in a total of 2.5 ml of buffer (20 mM Tris/HCl, pH 7.4, 150 mM NaCl, 0.001% NaN_3). For this purpose, the film was slowly suspended in 0.5-ml portions of buffer by gently swirling the stoppered flask under nitrogen for 10 min, and the milky dispersion was transferred into a sonication vial. The process was repeated until the lipid film was quantitatively suspended. The dispersion was sonicated for 20 min in an ice-bath under a gentle stream of nitrogen using a probe-type

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sonicator (model 350 tip sonicator; output setting, 4; Branson Sonic Power Co., Danbury, CT). Debris was removed at 105,000 *g* for 1 h (10°C; model L5-75 centrifuge; T50 rotor; Beckman Instruments Inc., Fullerton, CA). The vesicles were characterized by transmission electron microscopy after negative staining and after freeze-fracture as described previously (Kumar et al., 1988).

Phosphorus-31 NMR spectra were recorded at 32.2 MHz on a model FT-80A pulse Fourier transform NMR instrument (Varian Associates, Inc., Palo Alto, CA) equipped with a broadband probe. Spectra were measured at 37 ± 1°C in 10 mm (o.d.) sample tubes under proton noise decoupled conditions using identical instrument settings (90° flip angle, 4 KHz sweepwidth, 8 K data points). Linewidths at halfheight ($\nu_{1/2}$) were measured on spectra obtained in the absence of lanthanide ions by Fourier transforming the accumulated free induction decay (FID) without applying exponential line broadening. All measurements were done on at least two sample preparations.

RESULTS AND DISCUSSION

Upon hydration and sonication, mixtures of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-*sn*-glycero-3-phosphocholine (lysoPC) form small unilamellar vesicles over a wide range of concentrations (up to ~30 mol% lysoPC). The vesicles were uniform in size (265 ± 35Å), and vesicle size was independent of the lysoPC concentration. POPC/lysoPC vesicles showed four distinct peaks in phosphorus-31 NMR (e.g., Fig. 1 *A*). The signals at 40.1 ppm (PC_o) and 40.5 ppm (LPC_o), respectively, were assigned to POPC and lysoPC in the outer bilayer leaflet; the signals at 39.9 ppm (PC_i) and 40.4 ppm (LPC_i) were assigned to POPC and lysoPC in the inner vesicle shell. The chemical shift difference between the lysoPC signal and the respective POPC signal (0.4–0.5 ppm) in POPC/lysoPC SUV is similar to that reported for other PC/lysoPC systems (Brasure et al., 1978; Roberts et al., 1979). The separation which we now observed between the outside lysoPC signal and the inside lysoPC signal ($\Delta\nu$ 0.1 ppm) is similar in magnitude to that observed between outside POPC and inside POPC ($\Delta\nu$ 0.2 ppm; Berden et al., 1974). Chemical shift differences between outside phospholipid signals and inside phospholipid signals are likely to reflect differences in phospholipid headgroup conformations due to different packing requirements (Huang and Mason, 1978) in the outer and the inner leaflet of these highly curved bilayers.

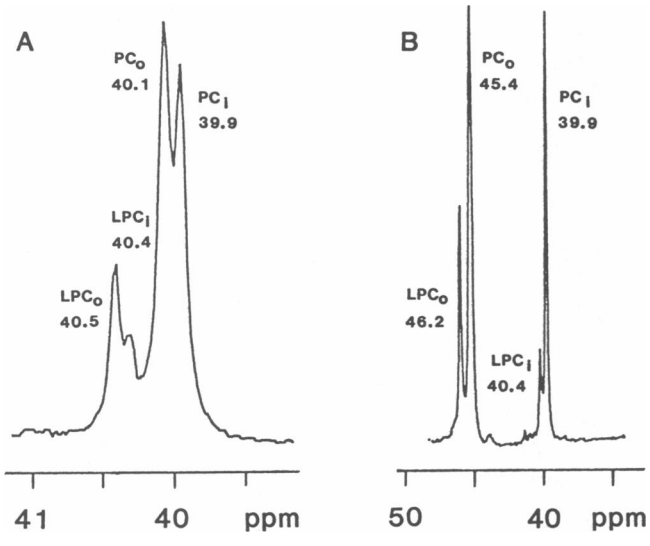

FIGURE 1. 32.2-MHz ^{31}P NMR spectra of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine/1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC/lysoPC, 75:25 mol%) small unilamellar vesicles in the (*A*) absence and (*B*) presence of 3 mM Pr^{3+}. LysoPC in the outer (LPC_o) and inner (LPC_i) bilayer leaflets and POPC in the outer (PC_o) and inner (PC_i) leaflets were assigned based on signal intensities and chemical shifts with and without Pr^{3+}. To optimize resolution, spectra were obtained by Fourier transforming the accumulated FID (15,000 transients for *A*, 25,000 for *B*) without applying exponential line broadening.

Upon addition of Pr^{3+} (3 mM) to the POPC/lysoPC vesicles, the outside signals were shifted downfield ($\Delta\nu$ 5.3–5.7 ppm; see Fig 1 *B*). This not only verified assignment of peaks but also facilitated quantitation of both phospholipids in each bilayer leaflet. In vesicles containing up to 25 mol% lysoPC, there was no change in chemical shift, signal intensity, or line shape for several weeks, indicating that membrane integrity and barrier properties were maintained.

We found that with increasing lysoPC mole percentage in POPC/lysoPC SUV, lysoPC in the outer leaflet increased sharply at the expense of outside POPC. Whereas the lysoPC outside-to-inside ratio ($R_{o/i}$) at 2.5 mol% lysoPC was 3.5, it rose to 6.5 at 25 mol% lysoPC. Our experimental data are in excellent agreement with the lysoPC outside/inside ratios predicted by Carnie et al. (1979) based on theoretical considerations.

The signal separation which we observed in the phosphorus spectra of the POPC/lysoPC vesicles, even in the absence of paramagnetic ions (Fig. 1 *A*), made it possible to measure half-height linewidths ($\nu_{1/2}$) and to calculate spin–spin relaxation times (T_2^*) of the outside and the inside POPC phosphorus. Fig. 2 illustrates that, throughout the entire range, T_2^* for the inside POPC signal remained constant ($\nu_{1/2}$, 7.2 Hz; T_2^*, 44 ms). However, the spin–spin relaxations of the outside POPC phosphorus showed a sharp decrease from 55 ms ($\nu_{1/2}$, 5.8 Hz) for pure POPC vesicles to 29.5 ms ($\nu_{1/2}$, 10.8 Hz) for POPC/lysoPC vesicles containing 25 mol% lysoPC. Because these unilamellar vesicles containing different mole percentages of lysoPC did not differ in size, line broadening due to differences in the rates of rotational diffusion can be ruled out. Hence, the decrease in T_2^* values must be explained in terms of increased motional constraints that are exerted on the POPC molecules in the outer shell at

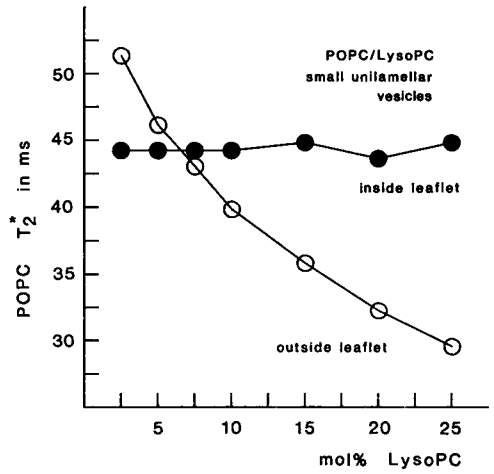

FIGURE 2 ^{31}P spin–spin relaxation times (T_2^*) of POPC in the outside leaflet (o) and in the inside leaflet leaflet (●) of small unilamellar phosphatidylcholine/lysophosphatidylcholine vesicles as function of total lysoPC mole percentage. T_2^* values were calculated from linewidths at half-height ($\nu_{1/2}$) of respective POPC phosphorus signals measured in the absence of Pr^{3+} (e.g., Fig. 1 *A*; two upfield signals).

higher lysoPC mole percentages. The fact that POPC in the outer leaflet and in the inner leaflet respond differently corroborates this interpretation.

It is furthermore of interest to note that the asymptotic decline in outside POPC T_2^* values, which we observed with increasing lysoPC mole percentage (Fig. 2), correlates well with the concomitant decline in measured

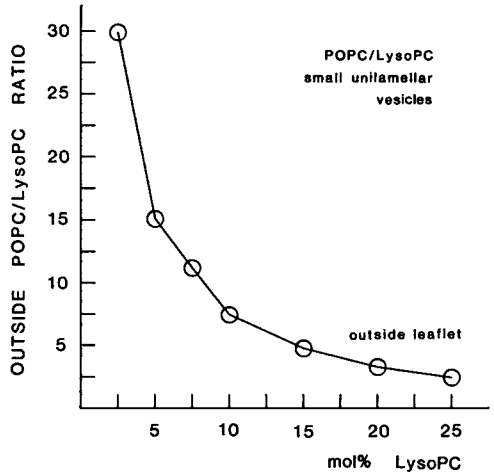

FIGURE 3 Ratio of POPC-to-lysoPC in the outer bilayer leaflet of small unilamellar phosphatidylcholine/lysophosphatidylcholine vesicles as function of total lysoPC mole percentage. Ratios were derived from ^{31}P NMR measurements on respective POPC/lysoPC SUV (265 ±35 Å diameter) in the presence of Pr^{3+} (e.g., Fig. 1 *B*, two downfield signals). Quantitation was by integration of respective peak areas, and also by cutting and weighing.

POPC/lysoPC ratios in the outer leaflet of these POPC/lysoPC vesicles (Fig. 3). This correlation is also consistent with our interpretation.

Our data show that incorporation of lysoPC into the outer bilayer leaflet of small unilamellar POPC vesicles significantly restricts headgroup motion of POPC. In this instance, the lysophospholipid "wedge" does not disrupt the lamellar phospholipid array, as it does in planar bilayers, but actually tightens it, apparently by filling the gaps between the tilted POPC "cylinders". Whether the lysoPC "wedge" disrupts or stabilizes a lamellar arrangement is therefore clearly a function of curvature. It is tempting to postulate that lysoPC may not only stabilize highly curved bilayer regions, but may actually induce formation of such regions. Various endocytotic and fusion phenomena may be favored by such a mechanism.

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