Inhibition of Membrane Fusion by Lysophosphatidylcholine†

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ABSTRACT: The ability of lysophosphatidylcholine to inhibit membrane fusion at subsolubilizing concentrations (between 1 and 9 mol % with respect to the membrane lipids) was examined. Fusion between *N*-methyldioleoylphosphatidylethanolamine (DOPE) large unilamellar vesicles (LUV) and fusion between Sendai virus and *N*-methyl-DOPE LUV were measured. A contents mixing fusion assay was used for LUV fusion (ANTS/DPX), and a lipid mixing assay (octadecylrhodamine B) was used for the virus fusion experiments. Lysophosphatidylcholine was effective at inhibiting both LUV fusion and Sendai virus/LUV fusion. Lysophosphatidylcholine also inhibited leakage from *N*-methyl-DOPE LUV. ^{31}P nuclear magnetic resonance data were obtained of *N*-methyl-DOPE in the presence of lysophosphatidylcholine. Lysophosphatidylcholine stabilized the lamellar phase and reduced the incidence of nonlamellar structures at all temperatures. The destabilization of nonlamellar structures with a negative radius of curvature may be a mechanism for inhibition of fusion by lysophosphatidylcholine in these systems.

Lysophospholipids were recently reported to inhibit several membrane fusion reactions, including planar bilayer fusions (Chernomordik et al., 1985), Ca^{2+}, GTP-γ-S, GTP, and H^+-stimulated fusions (Chernomordik et al., 1993), and phospholipase C-induced fusion (Nieva et al., 1993). This is in contrast to early reports that lysophospholipids stimulated cell/cell fusion. However, these latter experiments were performed at concentrations at which the lysolipids exhibited their detergent properties, and consequently the lysolipids were lytic to the cells (Poole et al., 1970).

Recent studies have revealed a correspondence between the inhibition of membrane fusion by small hydrophobic peptides in vesicle fusion and in viral fusion (Kelsey et al., 1990) and the ability of such peptides to inhibit viral infection (Richardson et al., 1980; Richardson & Choppin, 1983). One of the most simple of fusion systems inhibited by these antiviral peptides was fusion of large unilamellar vesicles (LUV) of *N*-methyldioleoylphosphatidylethanolamine (*N*-methyl-DOPE). It was concluded that these inhibitory peptides acted by inhibiting the membrane fusion step of the viral infection pathway (Kelsey et al., 1990).

The above two sets of data suggested that lysophosphatidylcholine (lysoPC) may also inhibit the fusion of LUV of *N*-methyl-DOPE. Observation of inhibition of membrane fusion in the more simple lipid vesicle system would offer the opportunity to explore further the mechanism of inhibition in a system that fuses through nonlamellar intermediates. Because of the packing properties of lysoPC in a membrane, it would be expected to inhibit the formation of some of the nonlamellar structures formed by *N*-methyl-DOPE (Gagne et al., 1985). If such structures were involved in the membrane fusion pathway as was previously suggested (Ellens et al., 1989), then lysoPC might inhibit fusion in the *N*-methyl-DOPE system. We have therefore examined the effects of lysoPC on the fusion of LUV of *N*-methyl-DOPE. Fusion between Sendai virus and *N*-methyl-DOPE LUV was also tested because this fusion system was inhibited by the hydrophobic, antiviral peptides referred to above and may utilize a pathway for fusion involving nonlamellar intermediates. LysoPC was found to inhibit *N*-methyl-DOPE LUV fusion and the fusion between Sendai virus and *N*-methyl-DOPE LUV. LysoPC was found to destabilize highly-curved nonlamellar structures in the *N*-methyl-DOPE LUV and favor the bilayer structure at subsolubilizing concentrations. A preliminary report on these results appeared previously (Yeagle & Flanagan, 1991).

MATERIALS AND METHODS

N-Methyldioleoylphosphatidylethanolamine (*N*-methyl-DOPE) and lysophosphatidylcholine (from egg phosphatidylcholine) were obtained from Avanti Polar Lipids, Birmingham, AL. Octadecylrhodamine B chloride (R_{18}), 1-aminonaphthalene-3,6,8-trisulfonic acid (disodium salt) (ANTS), and *p*-xylylenebis(pyridinium bromide) (DPX) were from Molecular Probes, Inc., Junction City, OR.

Vesicle Preparation. Large unilamellar vesicles encapsulating ANTS or DPX were prepared according to methods described previously (Szoka et al., 1980) with some modifications (Ellens et al., 1989). *N*-Methyl-DOPE was hydrated for 3 h on ice, under N_2, either in 25 mM ANTS, 45 mM NaCl, and 10 mM glycine, pH 9.5, or in 90 mM DPX/10 mM glycine, pH 9.5. The lipid suspension was next subjected to 5 freeze–thaw cycles followed by 10 extrusions through a polycarbonate membrane with 0.1-μm pores (Nucleopore Corp., Pleasanton, CA). Encapsulated material was separated from unencapsulated material on a Sephadex G-50 column (Pharmacia) with 100 mM NaCl, 10 mM glycine, and 0.1 mM EDTA, pH 9.5, used as the elution buffer. Vesicles were stored on ice, under N_2, and were used within 1 day. Vesicles were characterized by negative-stain transmission electron microscopy and by gel chromatography as a function of the number of extrusions. After 10 extrusions, no further improvement in homogeneity of vesicle size was seen. Also, no evidence of multilamellar vesicles was observed. According to measurements of the electron micrographs, the LUV ranged in size from 200 to 900 nm, with most LUV near 400 nm.

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Sendai Virus Preparation and Labeling. Sendai virus was grown in the chorioallantoic membrane of embryonated chicken eggs. The allantoic fluid was harvested 72-h postinfection, and the virus was partially purified by centrifugation through a 30–60% sucrose density gradient. The amount of virus used in the fusion assays was quantified by the number of milligrams of viral protein determined by the dye binding assay (Bradford 1976). Virus was labeled with octadecylrhodamine B chloride (R_{18}) as described (Hoekstra et al., 1984). Briefly, 20 nmol of R_{18} in 10 mL of ethanol was added for each milligram of viral protein in a total volume of 1 mL. The mixture was vortexed and allowed to incubate at room temperature for 1 h. Labeled virus was separated from unincorporated R_{18} by passing the incubation mixture over a Sephadex G-75 column and eluting with 100 mM NaCl, 10 mM glycine, and 0.1 mM EDTA, pH 7.4.

Preparation of Erythrocyte Ghosts. Human erythrocyte ghosts were prepared from freshly drawn human blood according to established procedures (Dodge et al., 1963).

Fusion and Leakage Assays. Fluorescence measurements were made on an SLM 8000D spectrofluorometer or on a Perkin Elmer LS-50B spectrofluorometer. The ANTS/DPX fusion assays were carried out as described (Ellens et al., 1985). Vesicles either contained 25 mM ANTS and 45 mM NaCl or contained 90 mM DPX. Fluorescence intensity was monitored with an excitation wavelength of 380 nm and an emission wavelength of 510 nm. Where appropriate, 25 μL of methanol with or without lysophosphatidylcholine was injected into the suspension of LUV. The LUV were allowed to equilibrate to the appropriate temperature with or without inhibitor for 5 min prior to initiation of the assay. All assays were carried out in a total volume of 1 mL. Fusion of LUV was initiated by lowering the pH from 9.5 to 4.5 with 25 μL of 2 M sodium acetate/acetic acid buffer. Addition of inhibitor did not change that final pH. For fusion assays, a 9:1 molar ratio of DPX-containing LUV to ANTS-containing LUV was used. Fluorescence quenching due to contents mixing (resulting in an ANTS–DPX complex with a reduced quantum yield) reflected the rate of LUV fusion. For vesicle/vesicle fusion assays, the base-line fluorescence was taken to be the level obtained with the shutters of the fluorometer closed, and 100% fluorescence was taken to be the initial fluorescence intensity before lowering the pH. The initial rate of fusion was then calculated from the slope of the fluorescence decay curve during the period (20 s) immediately following the initiation of fusion.

The R_{18} fusion assay for virus/vesicle fusion was carried out as described (Hoekstra et al., 1984). A stock suspension of *N*-methyl-DOPE LUV in 100 mM NaCl/10 mM glycine, pH 9.5, was used; 1 μmol (approximately 50 μL) of these LUV was added such that the combined volume was 975 μL. Where appropriate, 25 μL of methanol with or without lysophosphatidylcholine was injected into the suspension of LUV. A total volume of 1 mL was used for each assay. The LUV were allowed to equilibrate with or without inhibitor at the appropriate temperature for 5 min. Fifty microliters of R_{18}-labeled virus (0.05 mg/mL) was added to the vesicles to drop the pH of the LUV and to initiate the fusion of Sendai virus with *N*-methyl-DOPE LUV near pH 7. Fluorescence was monitored with an excitation wavelength of 560 nm and an emission wavelength of 586 nm. The initial rate of fusion was then calculated from the slope of the fluorescence decay curve during the period (1 min, or less) immediately following the initiation of fusion, normalized to 100% fluorescence. The fluorescence intensity obtained at zero time was taken as the base line; 100% fluorescence was determined by adding 100 mL of 10% Triton X-100 to the vesicle/virus mixture. The final pH of the assay mixture was found to be between 7.6 and 7.8.

Leakage assays were carried out as described previously using LUV containing both ANTS and DPX (Ellens et al., 1984).

Other Assays. Phosphate was determined by the method of Bartlett (1959).

Nuclear Magnetic Resonance. ^{31}P nuclear magnetic resonance (NMR) spectra were obtained with a JEOL FX270 Fourier transform spectrometer on a broad-band probe in 10-mm tubes at the indicated temperatures. A fully phase cycled (32 pulse) chemical shift anisotropy (CSA) echo was used with a 40-μs echo (Rance & Byrd, 1983). Gated proton decoupling (on only during acquisition) at a decoupling field of 9 kHz was employed to eliminate sample heating. A 50-kHz spectral width was used, with 50-Hz of line broadening in the Fourier transformation. A delay time of 1 s was used between pulses. The only ^{31}P nuclei in the preparation were in the phospholipid component of these membranes. For the NMR experiments where the amphipathic compounds under study were present, the appropriate amount of compound was added to the dissolved phospholipid in chloroform prior to drying the phospholipid. The lipid mixture was then hydrated by the addition of buffer.

RESULTS

Previous studies had revealed that LUV of *N*-methyl-DOPE would spontaneously fuse at low pH and that the rate of membrane fusion (as measured by contents mixing) increased monotonically with an increase in temperature (Ellens et al., 1989). Furthermore, it was reported that Sendai virus could fuse with these LUV in the absence of a receptor in the LUV (Kelsey et al., 1990). This study examined the effect of lysoPC on *N*-methyl-DOPE LUV fusion and the fusion of Sendai virus with these LUV.

The first set of experiments described here tested the effects of lysoPC on the fusion of Sendai virus with *N*-methyl-DOPE LUV. These experiments were carried out using the lipid mixing assay (R_{18}) as described under Materials and Methods, as a function of temperature. The concentrations of inhibitor were expressed as a mole percent with respect to the phospholipid in the vesicles. The concentrations of these inhibitors were well below the level at which solubilization of the membrane would occur. Figure 1 shows representative traces of the change in fluorescence intensity with time when R_{18}-labeled Sendai virus was introduced to a suspension of *N*-methyl-DOPE LUV. Curve A was observed in the absence of lysoPC, and curve B was observed in the presence of lysoPC. LysoPC showed inhibition of virus/vesicle fusion in a dose-dependent manner and at all temperatures measured. Figure 2 summarizes these data. At 2 mol % (with respect to the phospholipids), the initial rate of virus/LUV fusion was 50% inhibited by lysoPC, averaged over the data from all the temperatures at which the fusion was studied.

Whether lysoPC could inhibit *N*-methyl-DOPE LUV fusion, using the contents mixing assay, was then examined. Figure 3 shows representative traces of the change in fluorescence intensity with time when *N*-methyl-DOPE LUV containing ANTS were mixed with *N*-methyl-DOPE LUV containing DPX, following the protocols described under Materials and Methods. Curve A was observed in the absence of lysoPC. The behavior of the fluorescence intensity at short time was consistent with fluorescence quenching due to contents mixing

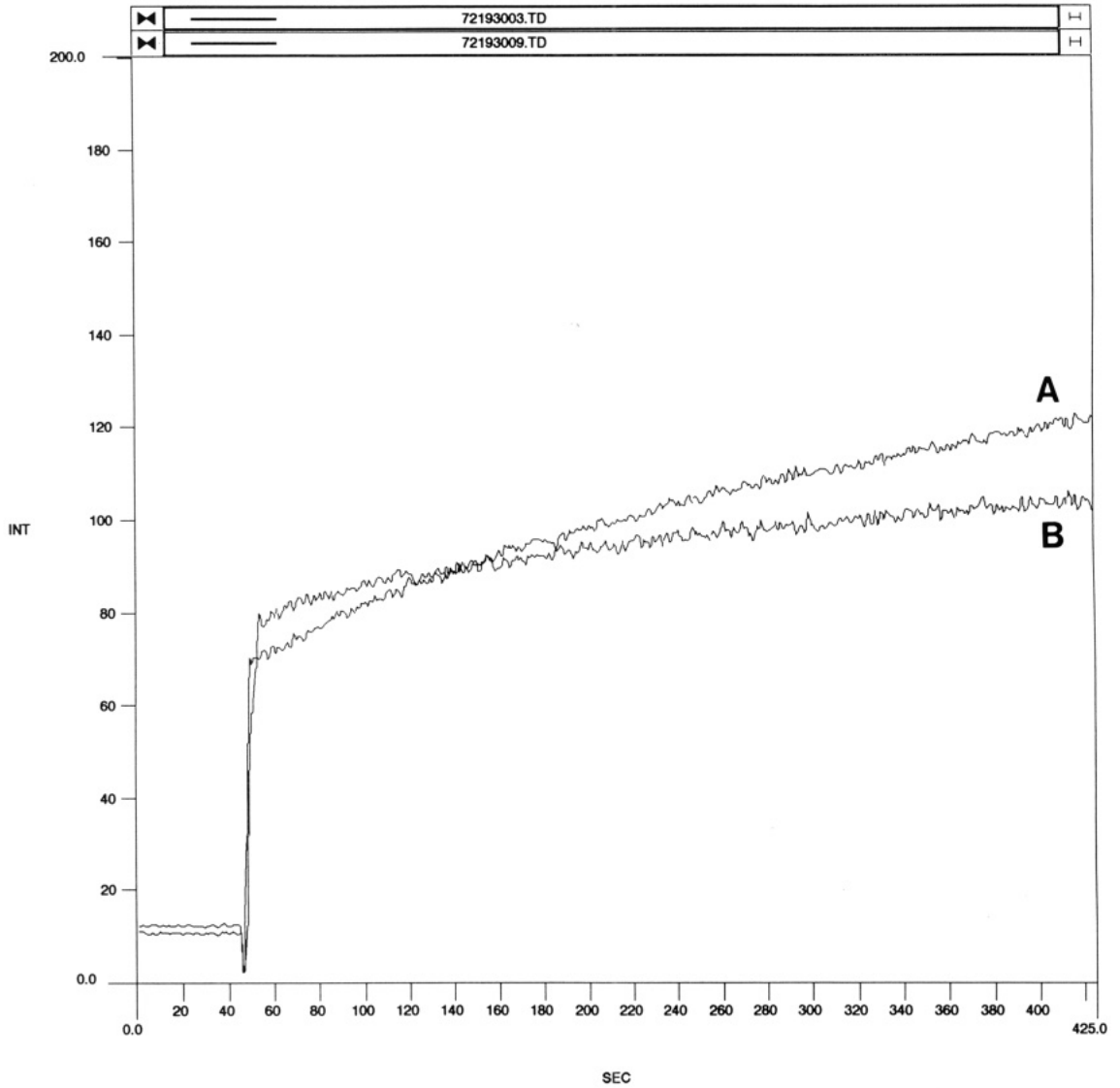

FIGURE 1: Fusion of Sendai virus with *N*-methyl-DOPE LUV measured by the R_{18} lipid mixing assay. Fluorescence was measured as a function of time; the large increase in fluorescence at short time reflects the addition of the R_{18}-labeled Sendai. Measurements were made at 35 °C. 2.5 μg of R_{18}-labeled Sendai and 1 μM *N*-methyl-DOPE LUV was used in 975 μL. Curve A was in the absence of lysoPC, and curve B was in the presence of lysoPC.

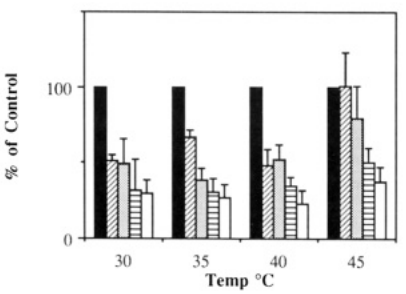

FIGURE 2: Effects of lysophosphatidylcholine on the fusion of Sendai virus with *N*-methyl-DOPE LUV measured by a lipid mixing assay (R_{18}) as described in the text, as a function of temperature. The results represent the average of at least three independent experiments in each case. The black bars represent the control (normalized to 100%). The diagonal hatched bars represent inhibitor present at 1.2 mol %; the stippled bar is 2.4 mol %, the horizontal hatched bar is 4.8 mol %, and the white bar is 9 mol %.

of the LUV, as described previously (Ellens et al., 1989). At longer times (about 2 min after initiation of the fusion reaction), the fluorescence intensity increased, consistent with leakage from the vesicles. Within the time period of these measurements, the fluorescence trace did not return to the maximum value at time = 0. The initial rate of fusion was measured from the slope in the first 20 s of this curve, when membrane fusion was the dominant process. Measurement of the kinetics at short times after initiation of fusion was essential to avoid artifacts at longer times introduced by leakage and possible precipitation. Curve B was observed in the presence of lysoPC. In the presence of lysoPC, only a steady decrease in fluorescence was observed over the whole time period recorded. The initial rate of this process was considerably less than in the absence of lysoPC. As shown in Figure 4, lysophosphatidylcholine inhibited vesicle fusion in a dose-dependent manner at all temperatures between 25 and 45 °C, at subsolubilizing concentrations. These experiments were performed at 50 μM phospholipid. At 1 mol % added lysoPC (with respect to the membrane phospholipids), the initial rate of *N*-methyl-DOPE LUV fusion was 50% inhibited, averaged over all the data from all the temperatures at which fusion was studied. As a control, *N*-methyl-DOPE LUV containing only ANTS were subjected to the same protocol as in Figure 3. As shown in Figure 5, no change was observed in fluorescence intensity with time, after initiation of the fusion conditions by a drop in pH. Therefore, loss of fluorescence intensity due to precipitation of membranes likely

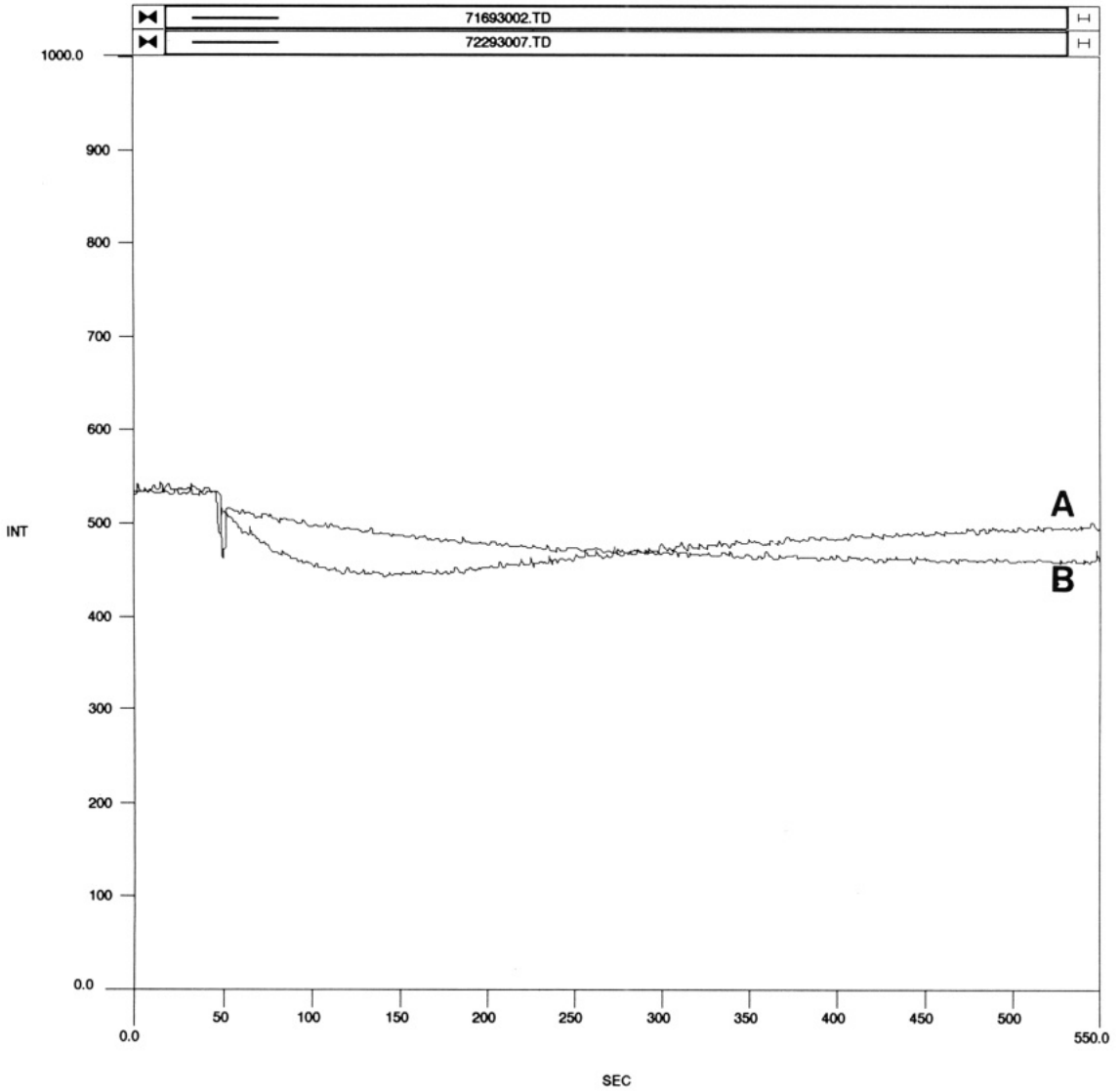

FIGURE 3: Effect of lysophosphatidylcholine on the fusion of *N*-methyl-DOPE LUV measured with a contents mixing assay as described in the text. Fluorescence was measured as a function of time at 35 °C. Curve A was in the absence of lysoPC, and curve B was in the presence of 5 mol % lysoPC. *N*-Methyl-DOPE LUV concentration was 100 μM, and lysoPC was mixed with the lipid prior tot he formation of LUV.

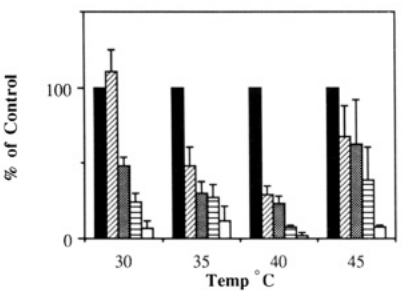

FIGURE 4: Effect of lysophosphatidylcholine on the fusion of *N*-methyl-DOPE LUV measured with a contents mixing assay as described in the text, as a function of temperature. LysoPC was added to preformed LUV. Other details are in the text. The results represent the average of at least three independent experiments in each case. The black bars represent the control (normalized to 100%). The diagonal hatched bars represent inhibitor present at 1.2 mol %; the stippled bar is 2.4 mol %, the horizontal hatched bar is 4.8 mol %, and the white bar is 9 mol %.

did not contribute to the observations in Figure 3. Inhibition of membrane fusion of *N*-methyl-DOPE LUV by lysoPC was also observed at 100 μM phospholipid.

The addition of lysoPC to preformed LUV leads predominantly to incorporation of lysoPC into the outer monolayer of the vesicle. The local concentration of lysoPC would be expected to be higher than the composition averaged over all the phospholipid in the sample. Therefore, experiments were performed in which lysoPC was mixed with the *N*-methyl-DOPE in organic solvent prior to the formation of the LUV. In this case, the lysoPC also showed inhibition, but appeared to be somewhat less effective than when added to preformed LUV at the same overall concentration (see Figure 3 for an example). This result may support the concept that the localized concentration of lysoPC in the outer monolayer of the LUV was somewhat higher than reflected in the overall composition due to transmembrane movement rates that were less than the fusion rate.

In Figure 3 an absence of the leakage phase was noted in the measurement of fusion of *N*-methyl-DOPE LUV in the presence of lysoPC. Therefore, the effects of lysoPC on the leakage of *N*-methyl-DOPE LUV were determined. Figure 6 shows representative traces of fluorescence intensity as a function of time for LUV loaded with ANTS/DPX. The increase in fluorescence intensity in curve A corresponds to a dequenching of the probe fluorescence due to a leakage of the vesicle contents and a loss of close approach between fluorophore and quencher. Under these conditions, leakage is a slower process than is membrane fusion, which is consistent with the observations in Figure 3 that membrane fusion

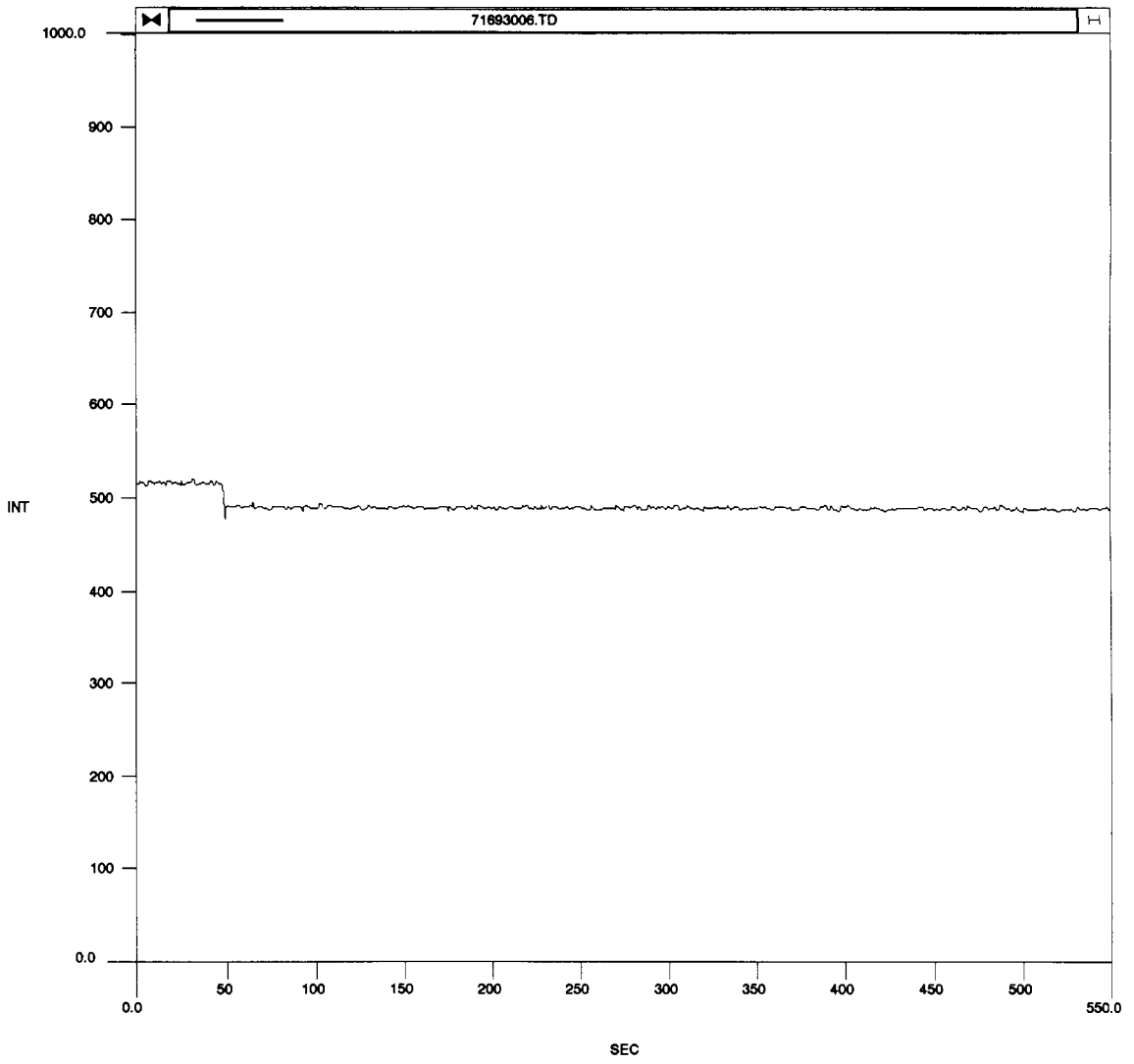

FIGURE 5: *N*-Methyl-DOPE LUV containing only ANTS were subjected to the same protocol as in Figure 3. The pH was dropped at the point on the curve where there is a sudden decrease in fluorescence intensity.

dominated the early phase of that assay and leakage dominated a later phase of that same assay. The effect of lysoPC on this process can be seen in curve B. The inhibition of the initial rate of leakage of *N*-methyl-DOPE LUV by lysoPC as a function of lysoPC concentration is presented in Figure 7. Inhibition of 50% of the initial rate of leakage occurred between 1 and 2 mol % added lysoPC. This is similar to the effectiveness of lysoPC at inhibiting the initiation rate of fusion of *N*-methyl-DOPE LUV.

It was previously suggested that a nonlamellar intermediate was involved in the fusion of the *N*-methyl-DOPE LUV. This putative intermediate was proposed to be the source of the isotropic resonance in the ^{31}P NMR spectra of these LUV as well as lipidic particles in freeze–fracture electron microscopy of the preparations (Ellens et al., 1989). It may also be the target of another class of fusion inhibitors which are hydrophobic peptides (Kelsey et al., 1991). Therefore, the effects of lysophosphatidylcholine on the isotropic resonance in the ^{31}P NMR spectra of *N*-methyl-DOPE were determined. The ^{31}P NMR spectra as a function of temperature for the pure phospholipid were published previously (Gagne et al., 1985; Ellens et al., 1989). They consisted of a pure lamellar powder pattern at low temperatures. At temperatures 35 °C and above, the ^{31}P NMR spectra contained the lamellar powder pattern and, superimposed, an isotropic resonance. The sample temperature in these experiments was not increased above T_h, the temperature at which the hexagonal II phase appeared. Data were only taken from samples on their first heating after hydration at 5 °C. The percent of the total phospholipid in structures represented by this isotropic resonances increased with increased temperature from 35 °C. The percent of the total phospholipid in the isotropic resonance was calculated from spectral simulations of spectra of *N*-methyl-DOPE pure and also containing lysoPC, as a function of temperature. The results are summarized in Figure 8 for 5 mol % lysoPC in the bilayer (with respect to the phospholipid). Each set of data represents the average of two independent measurements on separate preparations. As in the pure phospholipid, the isotropic resonance increases in intensity as a function of temperature. LysoPC stabilized the bilayer so that the isotropic structures formed only at higher temperatures.

DISCUSSION

LysoPC was found to inhibit two different membrane fusion systems in this study: one, a simple (one-lipid component) phospholipid vesicle fusion, two, a receptor-independent fusion between Sendai virus and phospholipid vesicles. These results are consistent with the inhibition of Ca^{2+}-, GTP-, and pH-dependent biological fusions by lysoPC reported recently (Chernomordik et al., 1993). These results also may explain the inhibition by lysoPC of syncytia formation by Newcastle disease virus (Kohn, 1965) and of fusion of myoblasts

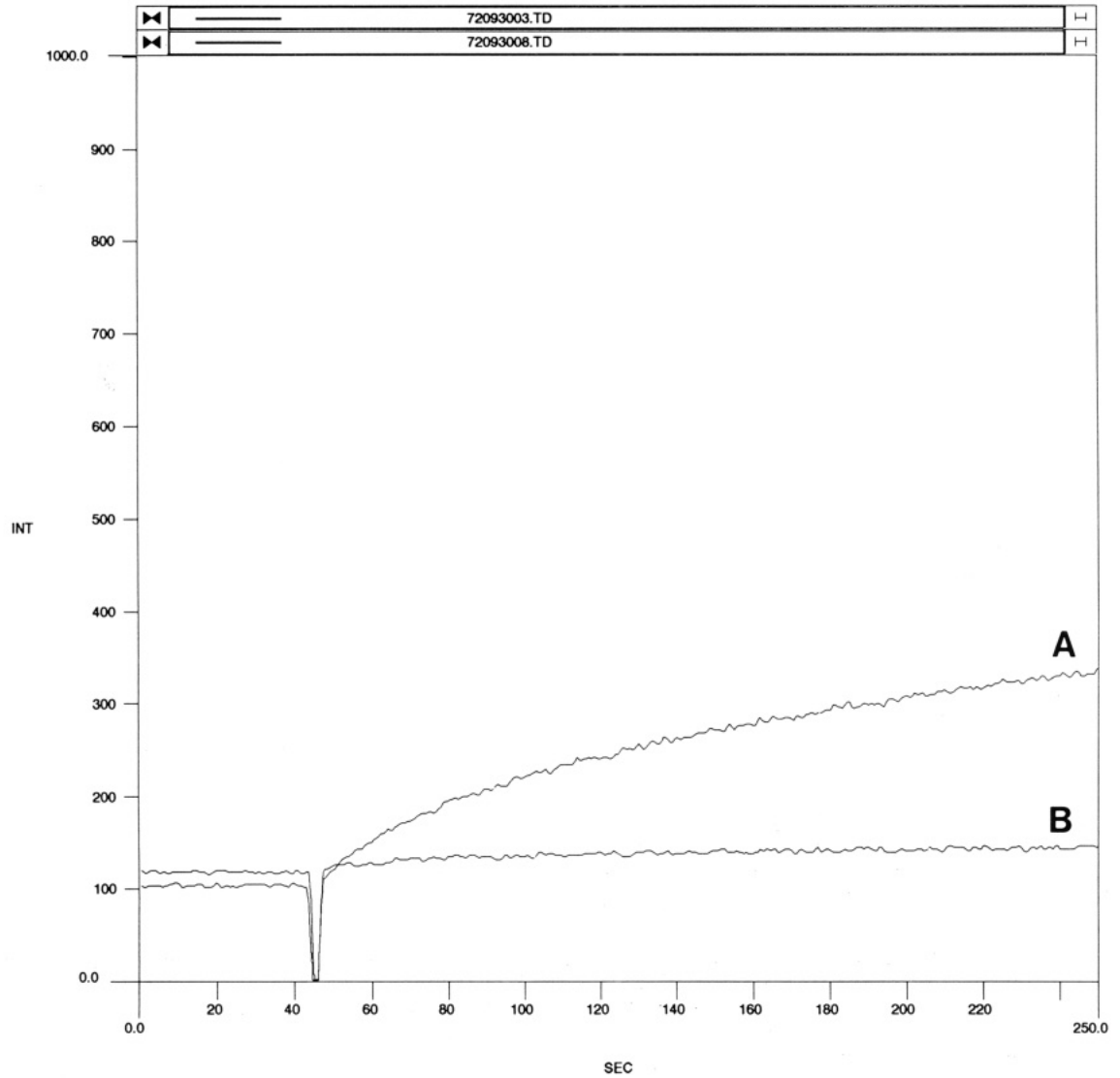

FIGURE 6: Leakage of contents of *N*-methyl-DOPE LUV as a function of time using vesicles loaded with ANTS and DPX, as described in the text. Fluorescence was measured as a function of time at 35 °C. *N*-Methyl-DOPE LUV were at 100 μM. Curve A was in the absence of lysoPC, and curve B was in the presence of lysoPC. Leakage was initiated at about 45 s.

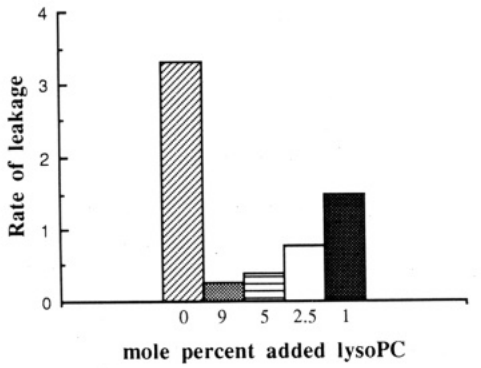

FIGURE 7: Inhibition of the initial rate of leakage of *N*-methyl-DOPE LUV by lysoPC as a function of lysoPC concentration at 35 °C.

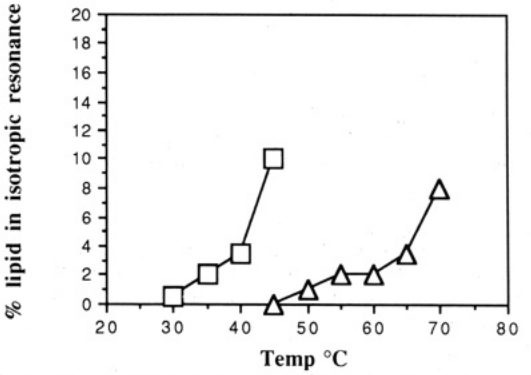

FIGURE 8: Percent of total phospholipid in the isotropic resonance of ^{31}P NMR spectra of *N*-methyl-DOPE LUV as a function of temperature: pure (□) and in the presence of 5 mol percent (△) lysophosphatidylcholine.

(Reporter & Raveed, 1973) as an inhibition of membrane fusion by this phospholipid.

LysoPC inhibited *N*-methyl-DOPE LUV fusion. The ^{31}P NMR studies revealed that the lysophosphatidylcholine also stabilized the lamellar phase of *N*-methyl-DOPE. Previous studies had noted a direct correspondence between the percent of the lipid in nonlamellar isotropic structures and the rate of fusion. It was suggested in these reports that the isotropic ^{31}P NMR resonance either was directly reporting on a fusion intermediate or was sensitive to a structure which reflected the presence of a fusion intermediate (Ellens et al., 1989). The most simple explanation of the data obtained in the present study was that lysophosphatidylcholine, by stabilizing the lamellar phase, inhibited the formation of membrane fusion intermediates for *N*-methyl-DOPE LUV fusion.

LysoPC also inhibited fusion of the virus with LUV. There is no traditional receptor for the Sendai virus in the target membrane in this fusion system. As shown previously, the initial rates of Sendai fusion with *N*-methyl-DOPE LUV were directly related to the incidence of the isotropic structures with highly curved surfaces in the target membrane (Kelsey et al., 1990). Elimination of this isotropic structure, and others with similar structural characteristics, by lysophosphatidylcholine might then be expected to reproduce the environment for fusion offered by a purely lamellar phospholipid vesicle system, like phosphatidylcholine. LUV of phosphatidylcholine do not rapidly fuse with Sendai virus. Therefore, the lack of fusion in *N*-methyl-DOPE LUV in the presence of lysophosphatidylcholine can be simply understood in terms of the lack of structure in the target membranes that allows Sendai virus to fuse with lipid vesicles in the absence of a receptor.

However, it is possible that these data suggest more about the membrane fusion process. The inhibition by the antiviral peptides has been suggested to be due to the ability of this peptide to destabilize phospholipid structures with small radii of curvature (Kelsey et al., 1991). From such results, it was suggested that intermediates with small radii of curvature are common to all these fusion events. In the case of *N*-methyl-DOPE LUV fusion, considerable independent evidence (freeze–fracture electron microscopy, cryo-transmission electron microscopy, and ^{31}P NMR) supports such a conclusion (Ellens et al., 1989; Siegel et al., 1989). Furthermore, Sendai virus fusion with *N*-methyl-DOPE LUV also seems to require similar structures, as discussed above. Enveloped virus fusion with cell plasma membrane is mediated by the fusion protein of the virus, F in the case of Sendai. The "fusion peptide" from the fusion protein of measles virus has been shown to induce structures of small radii of curvature in phospholipid bilayers (Yeagle et al., 1991). Thus, a compound with the ability to inhibit the formation by phospholipids of structures with small radii of curvature might be expected to inhibit the membrane fusion reactions studied here, as was observed.

More specifically, lysoPC has been suggested to inhibit the formation of a hypothesized fusion intermediate, the "stalk" (Chernomordik et al., 1993). Such stalk structures were suggested previously to act as intermediates in membrane fusion (Markin et al., 1984; Chernomordik et al., 1987). More recently this model has been refined and applied to the fusion pathway of influenza virus (Siegel, 1993). The intermediate in the stalk model is constructed in part of lipid surfaces with a short radius of curvature. Therefore, the data reported here of inhibition of fusion by lysoPC are consistent with the stalk model to the extent that model relies on surfaces with a short radius of curvature.

The distribution of lysoPC across the bilayer of small unilamellar vesicles has been measured by accessibility to phospholipase and by accessibility to shift reagents using ^{13}C NMR. In pure PC vesicles, about 90% of the lysoPC was located in the outer leaflet of the vesicle bilayer (Besselaar et al., 1977; Kruijff et al., 1977). On the other hand, in a bilayer without significant differences in curvature in the two sides, lysoPC is nearly symmetrically distributed (Besselaar et al., 1979). This indicates a strong preference of lysoPC for the outer vesicle surface, over the inner surface with its negative radius of curvature. It is possible, therefore, that the inhibition of fusion by lysoPC is the result of a destabilization of surfaces with a short, negative radius curvature, and that intermediates in the fusion pathway are formed with a negative radius of curvature. However, it should be noted that the stalk intermediate has surfaces of both negative and positive radius of curvature. Therefore, these data, while consistent with the stalk model, cannot be used to unambiguously support that model for fusion intermediates.

The interpretation of these data in terms of inhibition of membrane fusion is complicated by one ambiguity. The rate-limiting step of this fusion reaction has been previously stated to be aggregation [e.g., see Siegel et al. (1989)]. However, reference to the quantitative aspects of the existing study makes it unlikely that the action of the lysoPC is exclusively at the level of inhibiting vesicle aggregation. LysoPC inhibits LUV fusion and leakage to the level of about 50% at 1 mol % lysoPC. This corresponds to about a 2% increase in density of methyl groups in the membrane surface. According to the data of Rand and Parsegian (1989), this is too small a change in surface composition to be detected in the measurements of hydration repulsion. It seems more likely that at 50 μM phospholipid in the presence of 1 mol % or higher lysoPC, the rate of fusion has been slowed sufficiently by lysoPC to become the rate-limiting step. However, there necessarily remains an ambiguity in this interpretation.

These data on the inhibition by lysoPC are consistent with the conclusions reached earlier on the mechanism for inhibition of membrane fusion by certain specific hydrophobic antiviral peptides (Kelsey et al., 1990, 1991; Yeagle et al., 1992). The data from the latter studies led to the conclusion that at least some intermediates in the membrane fusion pathway for the *N*-methyl-DOPE LUV fusion and in the pathway of Sendai viral fusion contained lipid surfaces with a short radius of curvature. Some of these experiments produced data that were consistent with the involvement of membrane fusion intermediates with a short, negative radius of curvature.

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