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Effect of Lysolecithin on the Structure and Permeability of Lecithin Bilayer Vesicles†

Yunko Lee and Sunney I. Chan*

ABSTRACT: In order to elucidate the role of lysolecithin in membranes, we have examined the effect of lysolecithin on the structure and permeability of lecithin bilayer membranes. Small L-α-dimyristoyllecithin (DML) vesicles with myristoyllysolecithin (MLL) incorporated as well as small L-α-dipalmitoyllecithin (DPL) vesicles with palmitoyllysolecithin (PLL) were studied by nuclear magnetic resonance (NMR) methods at temperatures both above and below the α-gel ⇌ liquid crystalline phase transition temperature (T_c) and as a function of the concentration of the incorporated lysolecithin. Europium(III) ion was used as a probe to measure the permeability of the vesicular bilayer membrane. At temperatures below T_c, these vesicles were found to be extremely permeable to europium(III) ions. The ion translocation was found to be too fast to be measured by the NMR method under these conditions. However, above the phase transition temperature the ionic permeability decreases to a rate which could be conveniently monitored, and the permeability was shown to increase with temperature and lysolecithin concentration. Analysis of the lysolecithin concentration dependence suggests the formation of ion channels within the lipid bilayer involving four lysolecithin molecules. The data below T_c suggest a phase separation below the phase transition temperature of the host lipid, leading to the formation of patches of lysolecithin molecules within the lecithin matrix. These lysolecithin clusters are presumably long-lived under these conditions and are sufficiently structurally perturbed or disordered to serve as channels for rapid ion permeation.

Lysolecithin is an important metabolite, produced by many cells and widely distributed in a variety of tissues. For example, chromaffin granules from the adrenal medulla contain a fairly high concentration of lysolecithin in their membranes (Blaschko et al., 1967; Kirshner, 1974). The functional role of lysolecithin in these membranes is not fully understood. Since it has been proposed that lysolecithin is capable of promoting fusion between cells (Guttler and Clausen, 1969; Poole et al., 1970; Croce et al., 1971), the function of lysolecithin in these membranes may be related to its fusion inducing property. It has been shown that chromaffin granules from the adrenal medulla undergo fusion among themselves (Edwards et al., 1974), and it has been proposed that these granules fuse with the chromaffin plasma membrane during the process of catecholamine release (Douglas, 1966; Diner, 1967; Winkler, 1971; Smith and Van Orden, 1973).

In order to elucidate the role of lysolecithin in these membranes, we have investigated the effect of lysolecithin on the structure and ionic permeability of lecithin bilayer membranes.

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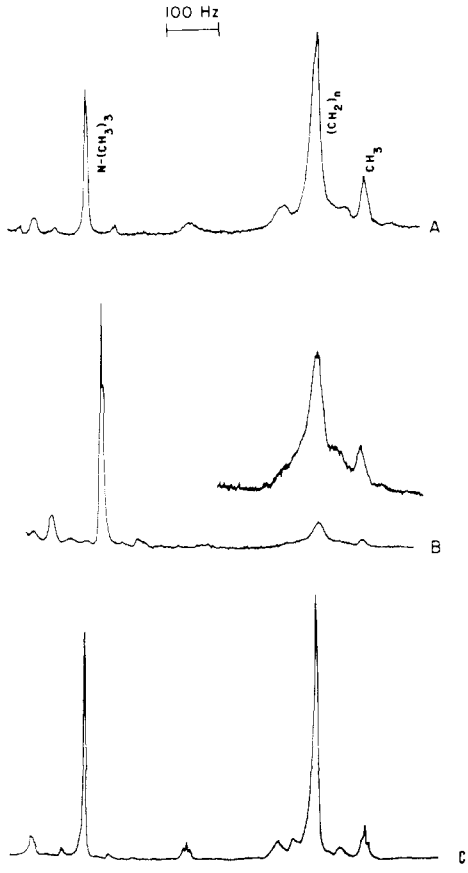

FIGURE 1: Typical ^{1}H NMR spectra (220 MHz) of lysolecithin containing lecithin bilayer vesicles: (A) PLL/DPL vesicles containing 18.0 mol % of PLL; (B) PLL/dideuteriopalmitoyllecithin vesicles containing 14.0 mol % of PLL; (C) 2.5% (w/v) PLL micelles in D_2O; temperature, 65 °C.

The proton magnetic resonance (^{1}H NMR) method has been used in this work.

Experimental Section

Materials. L-α-Dipalmitoyllecithin (DPL)[1] and L-α-dimyristoyllecithin (DML) were purchased from Calbiochem. Palmitoyllysolecithin (PLL) and myristoyllysolecithin (MLL) were obtained from Serdary Research Laboratories, Inc. The lipids did not show detectable impurities by thin-layer chromatography and were used without further purification. Europium and lanthanum nitrates were from Research Inorganic/Organic Chemical Corporation. Deuterium oxide (99.8% D) was from Stohler Isotope Chemicals.

Sample Preparation. Preparation of the vesicle suspension was carried out as follows. Weighed amounts of lecithin and lysolecithin were mixed and dissolved in a 3:1 (v/v) chloroform/methanol solution. Most of the solvent was removed by evaporating at room temperature in the fume hood. The remaining viscous solution was completely dried under high vacuum. The homogeneously mixed lipids (50 mg) were suspended in 1 mL of 10 mM lanthanum nitrate solution in deuterium oxide. The suspension was shaken by a vortex mixer and sonicated with a 150-W MSE sonicator with Ti-microtip at power level Hi-4 for 15 min. During the sonication, the sample was partially immersed in a glycerol cooling bath to avoid overheating. After sonication the sample was centrifuged to remove any metallic particles present. Asymmetric bilayer vesicles were prepared by adding equal volumes of 10 mM europium nitrate solution in deuterium oxide to the vesicle suspensions, thus maintaining isotonic conditions. The pD of the final vesicle suspension was typically 5.3.

Nuclear Magnetic Resonance Measurements. Proton magnetic resonance (^{1}H NMR) spectra were taken on a modified Varian HR-220 superconducting NMR spectrometer equipped with frequency sweep and multinuclear capabilities. Probe temperature was maintained with a Varian V4540 temperature control unit and calibrated with a standard ethylene glycol sample. Although the permeability of the vesicle toward europium(III) ions was ascertained at various temperatures, the spectrum recording temperature was always 55 °C for the MLL/DML vesicle samples and 65 °C for the PLL/DPL samples.

Results and Discussion

The Structure of Lysolecithin-Containing Lecithin Vesicles. At temperatures above the thermal phase transition temperature (T_c) of the lipid, the ^{1}H NMR spectra of the sonicated bilayer vesicles of lecithin are characterized by sharp resonance signals of the choline methyl and fatty acid protons and the intensities of these proton signals approach 100% of those expected. The dependence of the spectral line width and intensity of the fatty acid proton signals on vesicle size has previously been established and attributed to the subtle variations in the molecular packing of the phospholipid molecules with surface curvature of the vesicles (Sheetz and Chan, 1972; Lichtenberg et al., 1975).

A typical ^{1}H NMR spectrum of lysolecithin containing lecithin bilayer vesicles is shown in Figure 1A. The spectrum is essentially identical with that of sonicated vesicles of 250–300 Å in diameter. In order to ensure that lysolecithin is incorporated into the lecithin bilayer, we have also recorded the ^{1}H NMR spectrum of sonicated vesicles containing palmitoyllysolecithin incorporated into diperdeuteriopalmitoyllecithin at a temperature above the T_c of the DPL. This spectrum is shown in Figure 1B and is seen to show the same spectral characteristic features as small DPL vesicles, aside from the expected reduction in intensity of the hydrocarbon chain signals. In this spectrum, the half-width of the acyl chain methylene signal was about 30 Hz. This line width may be compared with the line width of 8 Hz for the acyl chain methylene proton signal of palmitoyllysolecithin or myristoyllysolecithin micelles obtained simply by dispersing the lysolecithin in deuterium oxide (Figure 1C). These results indicate that when vesicles containing lecithin and lysolecithin are prepared by cosonication, the lysolecithin is favorably partitioned into the bilayer. There is no evidence of sharper resonances in the ^{1}H NMR spectrum of these vesicles characteristic of lysolecithin molecules coexisting as micelles in the solution.

The thermal stability of the sonicated lysolecithin/lecithin vesicles was ascertained by keeping the vesicle solutions at the probe temperature for up to 5 h. No significant change was observed either in the ^{1}H NMR spectra or in the turbidity of the sample.

The Effect of Lysolecithin on the Permeability of Lysolecithin-Containing Lecithin Vesicles. Small sonicated vesicles containing lysolecithin were found to be permeable to ions under certain conditions of temperature and concentration of lysolecithin. At a given temperature, the permeability was observed to increase with increasing lysolecithin concentration. At a given concentration of lysolecithin in the bilayer, the

[1] Abbreviations used are: DPL, L-α-dipalmitoyllecithin; DML, L-α-dimyristoyllecithin; PLL, palmitoyllysolecithin; MLL, myristoyllysolecithin.

vesicles were more leaky to ions at temperatures below than above the T_c of the host lipid.

In the present work, the permeability of lysolecithin-containing lecithin bilayer vesicles to ions was determined through the use of vesicles containing an asymmetrical distribution of cations across the bilayer membrane. This method is based on the use of europium(III) ions, which serve both as a spectroscopic shift reagent and an ionic permeability indicator (Fernández et al., 1973; Barsukov et al., 1973, 1975; Hunt, 1975; Lawaczeck et al., 1975, 1976; Lau and Chan, 1976). In the ^{1}H NMR spectrum of sonicated lecithin bilayer vesicles, the choline methyl protons give rise to two partially resolved signals due to different surface curvature of the inner and outer halves of the bilayer. Levine et al. (1973) and Kostelnik and Castellano (1972) showed that by using appropriate amount of lanthanum shift reagent such as europium(III) ions, it is possible to resolve the two choline proton signals completely without incurring severe spectral broadening, provided the shift reagent is added to either the inner or the outer compartment of the vesicle, but not to both, and the bilayer is impermeable to the shift reagent. However, should the bilayer membrane become permeable to these cations, there would be a net passage of the europium(III) ion from one compartment to the other. The rate of europium(III) ion transport across the bilayer can be monitored by the change in the chemical-shift difference between the two choline methyl proton signals.

A typical time course of the ^{1}H NMR spectra of lysolecithin-containing lecithin bilayer vesicles at 55 °C is illustrated in Figure 2. After the addition of europium(III) ions to the extravesicular medium the inside choline methyl signal was observed to gradually approach that of the outside choline signal in the spectra. These observations have been obtained for DML vesicles containing 7.01, 9.96, and 13.7 mol % of MLL. The rate of collapse of the choline methyl signals increased with increasing lysolecithin concentration. In fact, for a sample containing 26.4 mol % of MLL, only one upfield-shifted choline signal was observed, suggesting that the rate of europium(III) ion transport became too fast to be monitored by the present technique under this condition of concentration. Note that all the above measurements have been made at a temperature of 55 °C, which is above the T_c of the host lipid.

Similar observations were obtained for PLL-containing DPL vesicles at 65 °C, except that the rate of europium(III) ion translocation was much slower than in the cases of MLL/DML bilayer vesicles. We have studied the ionic permeability at PLL concentrations of 7.15, 14.0, 18.0, 21.8, and 26.8 mol %. Again, the rate of ion translocation was found to increase with PLL concentration, and at the highest PLL concentration examined (26.8 mol %) the rate was too fast to measure.

It has been shown that sonicated bilayer vesicles of pure lecithin which have been annealed above the thermal transition temperature are impermeable to europium(III) ions (Lawaczeck et al., 1975, 1976). Accordingly, we have attributed our observations here to the formation of ion-conducting entities by the lysolecithin molecules in the lecithin host matrix.

The observed transport of europium(III) ions across the bilayer membrane represents the net flow resulting from the influx and outflux of the ions:

$$Eu^{3+}\ (\text{outside}) \underset{k_o}{\overset{k_i}{\rightleftharpoons}} Eu^{3+}\ (\text{inside})$$

where k_o and k_i are the rate constants for the outward and the inward fluxes per unit cross-section area of membrane. For the

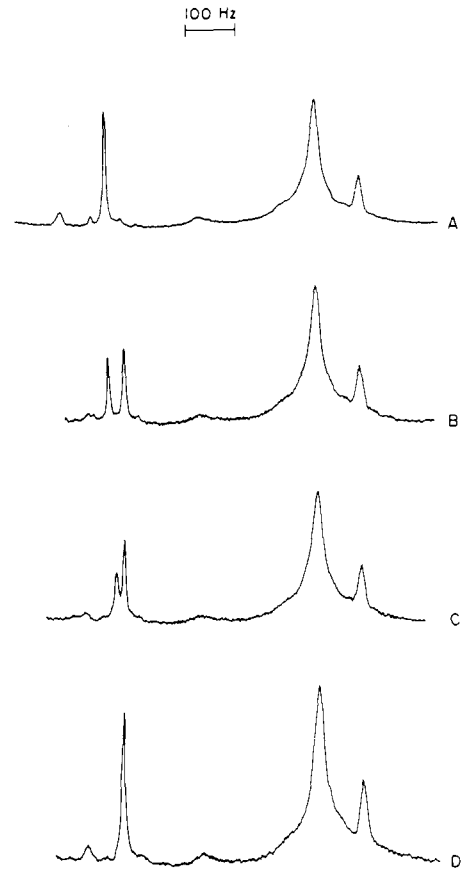

FIGURE 2: ^{1}H NMR spectra (220 MHz) of MLL/DML vesicles containing 9.96 mol % of MLL: (A) before adding europium(III) ions; (B) 5 min; (C) 100 min; and (D) 270 min after the addition of europium(III) ions; temperature, 55 °C.

vesicle systems under investigation here, in addition to the concentration gradient, there are a number of other driving forces which can influence the ion fluxes. For a vesicle suspension which has been prepared essentially under isoosmotic and isotonic conditions, any effects which arise from a small osmotic pressure difference or a small steady-state electrical potential[2] should be negligible. However, Sheetz and Chan (1972) have pointed out that for small vesicles of 250–300 Å in diameter, there exists a hydrostatic pressure difference of several atmospheres across the bilayer membrane. While such a hydrostatic pressure difference is probably not high enough to influence the bilayer structure, it leads to a streaming potential which is capable of producing a streaming current (Lakshminarayanaiah, 1969). If we take this streaming potential into consideration, then the inward and the outward ion fluxes can be expressed as follows:

$$\text{influx} = \left(\kappa_i A_o + \frac{P_o A_o}{\delta\rho}\right) N_o \qquad (1)$$

$$\text{outflux} = \left(\kappa_o A_i + \frac{P_i A_i}{\delta\rho}\right) N_i \qquad (2)$$

Here N_o and N_i denote the concentration of europium(III) ions in the extra- and intravesicular compartments, respectively;

[2] Based on the Goldman equation (Goldman, 1943), the steady-state electrical potential across the bilayer membrane in our isotonic systems should be zero if the ionic diffusivities of the lanthanium(III) and europium(III) ions may be assumed to be the same. We believe that this is a reasonable assumption since the lanthanium and europium ions bear the same charge and have essentially the same ionic radii.

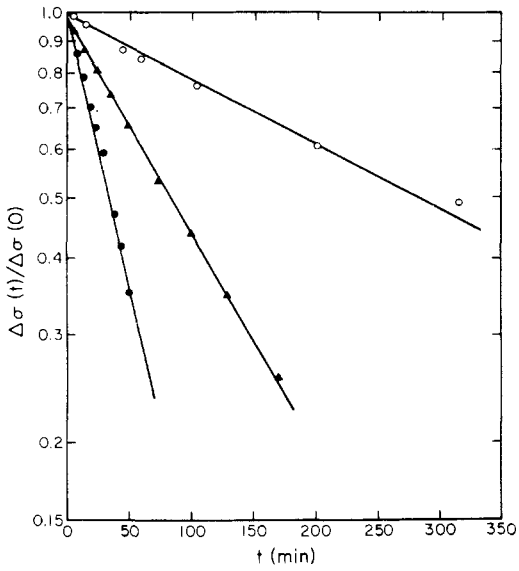

FIGURE 3: Time dependence of the chemical-shift difference of the choline methyl proton signals of the sonicated MLL/DML bilayer vesicles containing 7.01% (○), 9.96% (▲), and 13.7% (●) by mole of MLL; temperature, 55 °C.

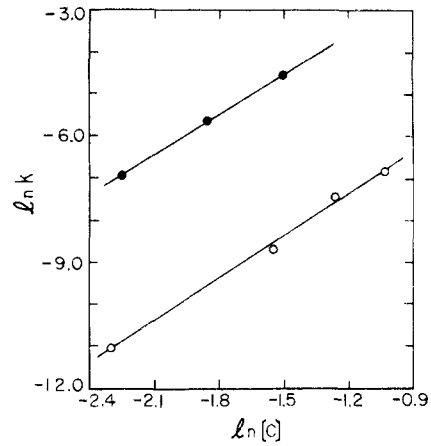

FIGURE 4: Lysolecithin concentration dependence of the rate constant of europium(III) ion translocation across the lecithin bilayer membranes: (●) MLL/DML vesicles at 55 °C; (○) PLL/DPL vesicles at 65 °C.

A_o and A_i are the outer and inner surface areas of the bilayer vesicle; and P_o and P_i are the hydrostatic pressures outside and inside the vesicle. δ is the thickness of the bilayer membrane; ρ is the europium(III) ion flow resistance across the bilayer; and κ_o and κ_i denote the intrinsic rate constants for the outward and inward ion translocation. Although, here, we have assumed that these intrinsic rate constants are different because of the asymmetrical nature of the bilayer, the structural difference between the two monolayers may not be sufficiently large for these constants to be significantly different.

When the system is displaced from equilibrium, the rate of ion translocation is given by:

$$\frac{dN_i}{dt} = -\left(\kappa_o A_i + \frac{P_i A_i}{\delta\rho}\right) N_i + \left(\kappa_i A_o + \frac{P_o A_o}{\delta\rho}\right) N_o \quad (3)$$

and

$$\frac{dN_o}{dt} = -\left(\kappa_i A_o + \frac{P_o A_o}{\delta\rho}\right) N_o + \left(\kappa_o A_i + \frac{P_i A_i}{\delta\rho}\right) N_i \quad (4)$$

At equilibrium, the total influx must equal the total outflux, and $N_i \simeq N_o$, so that:

$$\kappa_i A_o + \frac{P_o A_o}{\delta\rho} = \kappa_o A_i + \frac{P_i A_i}{\delta\rho} \equiv k \quad (5)$$

Equations 3 and 4 can then be combined to yield the first-order rate equation:

$$\frac{d(N_o - N_i)}{dt} = -2k(N_o - N_i) \quad (6)$$

In this study, we have chosen to work in the region (N_0 and N_i both less than 10 mM) where the chemical-shift difference between the two choline methyl proton peaks ($\Delta\sigma$) is linearly proportional to the concentration difference of europium(III) ions between the two compartments of the vesicle (Levine et al., 1973). Under this condition:

$$\frac{d(\Delta\sigma)}{dt} = -2k(\Delta\sigma) \quad (7)$$

Thus, a plot of ln ($\Delta\sigma$) vs. time should give a straight line with the slope equal to twice the overall rate constant k, and the intercept equal to $\Delta\sigma(0)$, the chemical-shift difference between the two choline signals at $t = 0$. The data presented in Figure 3 for MLL containing DML vesicles follow this predicted behavior. From the log $(\Delta\sigma(t)/\Delta\sigma(0))$ vs. time plots, we obtained the europium(III) ion translocation rate constants k values of 1.0×10^{-3}, 3.8×10^{-3}, and 1.1×10^{-2} min^{-1} for the DML vesicles containing 7.01, 9.96, and 13.7 mol % of MLL, respectively.

The dependence of the rate constants on the concentration of lysolecithin may be inferred by assuming that it is proportional to the mth power of the molar concentration of lysolecithin in the mixed lipid bilayer, [C], i.e.,

$$k \propto [C]^m \quad (8)$$

In Figure 4, a ln-ln plot of k vs. [C] is presented. A straight line is obtained with a slope of $m = 3.2$. m should be related to the number of lysolecithin molecules involved in the ion transporting mediator. We shall return to discuss the implication of this result further on in this paper.

Our data for the PLL/DPL vesicles shown in Figure 5 revealed nonlinear behavior of the log $(\Delta\sigma(t)/\Delta\sigma(0))$ vs. time plot at high lysolecithin concentrations. The origin of this observed nonlinearity is not fully understood at this time. However, it is possible that for this lipid system the ionic diffusivities of the ions involved in the transmembrane exchange (i.e., Eu(III) and La(III) ions) are sufficiently different that a transmembrane potential develops with the accumulation of Eu(III) ions in the inner compartment which slows down the influx of these ions with time. In view of this possibility, we have chosen to interpret only the rate data depicted by the linear region at the shorter times. From the initial slopes of the log $(\Delta\sigma(t)/\Delta\sigma(0))$ vs. time plots, we obtained the following rate constants: 1.6×10^{-5}, 1.6×10^{-4}, 6.0×10^{-4}, and 1.1×10^{-3} min^{-1} for the PLL/DPL vesicles containing 7.15, 14.0, 18.0, and 21.8 mol % of PLL, respectively. Here again, the dependence of k on the concentration of PLL may be deduced from a ln-ln plot of k vs. [C]. As shown in Figure 4, a linear plot with a slope of m equal to 3.3 was obtained.

Effect of Temperature on the Ionic Permeability of Lysolecithin/Lecithin Bilayer Vesicles. We have studied the effect of temperature on the ionic permeability of lysolecithin containing lecithin bilayer vesicles. These studies were carried out as follows. A stock solution of PLL/DPL bilayer vesicles containing 14.0 mol % of PLL was first prepared by sonicating a PLL/DPL mixture in a 10 mM lanthanum nitrate-D_2O solution. Six samples were prepared from the stock solution

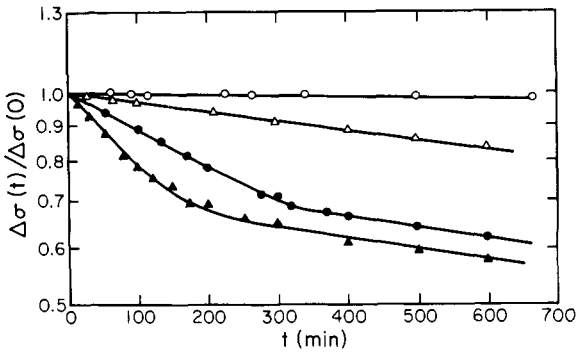

FIGURE 5: Time dependence of the chemical-shift difference between the two choline methyl proton signals of the PLL/DPL bilayer vesicles containing 7.15% (○), 14.0% (△), 18.0% (●), and 21.8% (▲) by mole of PLL; temperature, 65 °C.

and were stored separately in temperature baths at 2, 25, 37, 44.6, 57, and 74 °C. After equilibration at the bath temperature, an equal volume of 10 mM europium nitrate–D_2O solution (at the same temperature as the bath temperature) was added. The sample was then shaken for complete mixing and its ^1H NMR spectrum was recorded immediately (within 5 min). Only one upfield-shifted choline methyl proton resonance signal was observed for the samples when europium nitrate was added at 2, 25, 37, and 44.6 °C. This result suggested that at these temperatures, the PLL/DPL vesicles were highly permeable to europium(III) ions. When europium ions were added at 57 and 74 °C, two separated choline methyl resonance signals were observed. The PLL/DPL vesicles were only slowly permeable to europium(III) ions at these higher temperatures.

Lecithin bilayer vesicles which have been prepared by sonication below the thermal phase transition temperature of the lipid have recently been shown to contain structural defects which permit rapid ion permeation across the bilayer membrane (Lawaczeck et al., 1976). These structural defects can be eliminated by annealing the vesicle suspension above the phase transition temperature of the lipid. The annealing effect is irreversible and the vesicles are impermeable to europium(III) ions at temperatures both above and below the phase transition temperature once the vesicles have been annealed. This irreversibility of ionic permeability was not observed in the lysolecithin/lecithin vesicles under consideration here.

When vesicle suspensions from the stock solution were purposely annealed at 65 °C for 1.5 h prior to equilibration at various bath temperatures and the addition of europium nitrate solution at the same bath temperature, identical results were obtained as described earlier for vesicles which have not been previously annealed. Even when europium nitrate was added at the annealing temperature (65 °C), where the membrane is presumably impermeable to the shift reagent, the ^1H NMR spectrum showed only one upfield-shifted choline methyl resonance after the samples have been brought back and incubated at temperatures below the thermal phase transition temperature, e.g., 2, 25, 37, and 44.6 °C, for 15 min.

Similar observations were obtained for MLL/DML vesicles containing 7.4 mol % of MLL. At low temperatures, e.g., 2, 15, and 25 °C, the MLL/DML vesicles were extremely permeable to europium(III) ions, while at high temperatures, e.g., 30, 50, and 55 °C, they became quite impermeable.

N. O. Petersen from this laboratory has shown from differential thermal analysis studies that PLL/DPL mixtures with PLL concentrations of up to 30 mol % in the multilayer phase exhibit an α-gel–liquid crystalline phase transition

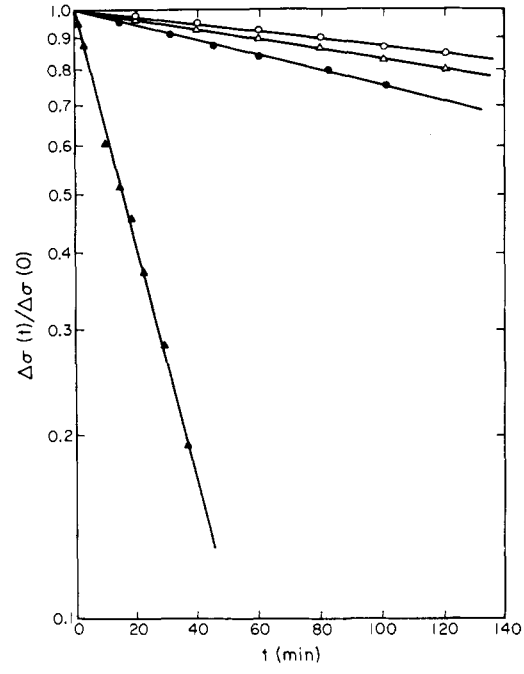

FIGURE 6: Time dependence of the chemical-shift difference between the two choline methyl proton signals of MLL/DML bilayer vesicles containing 7.4 mol % of MLL at temperatures of 39 °C (○), 50 °C (△), 55 °C (●), and 65 °C (▲).

temperature of 41 ± 1 °C, which is close to that for pure DPL. This result suggests immiscibility of the lysolecithin and lecithin at temperatures below the T_c of the host lipid. The fairly sharp transition observed from the thermogram indicates that the exclusion of lysolecithin from the host lipid is fairly complete. These results, together with the extremely high ionic permeability of lysolecithin/lecithin vesicles below the phase transition temperature of the host lipid, indicate the formation of lysolecithin patches below the T_c of the host lipid.

Above the phase transition temperature of the host lipid, we expect no species separation in the bilayer membrane. Under these conditions, the rate of europium(III) ion transport across the bilayer membrane was found to increase with increasing temperature. We have examined the temperature dependence of MLL/DML vesicles containing 7.4 mol % of MLL in some detail. Four samples were prepared from a stock solution and were stored separately in constant temperature baths of 39, 50, 55, and 65 °C. After allowing to equilibrate to the bath temperature, an equal volume of europium nitrate solution was added and the sample was thoroughly mixed. Each sample was kept in its temperature, except that, at various times, it was taken out for a short period of time to record the ^1H NMR spectrum.

The results of this temperature study are summarized in Figure 6. The rate constants at the various temperatures were found to be: 39 °C, 6.6×10^{-4}; 50 °C, 8.8×10^{-4}; 55 °C, 1.2×10^{-3}; and 65 °C, 2.2×10^{-2} min^{-1}. The ionic permeability was found to be abnormally high at 65 °C. Other studies on sonicated bilayer vesicles from our laboratory also showed evidence for abnormal properties and behavior of such vesicles at temperatures much higher than the T_c for this lipid (R. Lawaczeck, R. Blackman, and M. Kainosho, personal communication). If the data point at this temperature (65 °C) could be ignored, then the rate constants can be shown to satisfy Arrhenius behavior with the activation energy equal to 5.4 kcal/mol.

A similar study on PLL/DPL vesicles containing 14.0 mol

% of PLL gave the following europium(III) ion translocation rate constants: 57 °C, 9.6×10^{-5}; 65 °C, 1.6×10^{-4}; and 74 °C, 2.5×10^{-4} min^{-1}. An Arrhenius plot of the rate data yielded an activation energy of 13.0 kcal/mol for this system.

Some Thoughts on the Nature of the Ion-Transport Mediator. The observations reported here indicate that lipid bilayer membranes can be permeable to ions in the presence of incorporated lysolecithin. This ionic permeability can be the result of the formation of ion channels or pores involving a number of lysolecithin molecules. Alternatively, a few lysolecithin molecules may act as an ion carrier and, in fact, the possibility of flip-flop carrier immediately comes to mind. However, the relatively low activation energies which we have observed here for the lysolecithin mediated ion translocation process would seem to argue against the carrier mechanism. In systems for which a flip-flop mechanism is presumably involved, activation energies of 20 kcal/mol or higher have typically been reported (Dalmark et al., 1971; Toyoshima and Thompson, 1975). Unfortunately, this point of pore vs. carrier cannot be unambiguously settled at this time.

If, indeed, an ion channel or an activated pore is involved, then the size of the ion channel can be inferred from the dependence of the ion transport rate constant on the concentration of lysolecithin. The value of m determined (3.2–3.3) would suggest that the ion channel involves at least four lysolecithin molecules. Since a lysolecithin molecule itself does not span the membrane, we envision the formation of an ion channel when two pairs of lysolecithin molcules, one within each monolayer, meet coincidentally at some point in the bilayer and the resultant tetramer somehow becomes activated. It is conceivable that activation of the ion channel occurs when a cation binds to the phosphate moiety of the polar headgroup of the lysolecithin molecule. This ion binding can modify the headgroup configuration which in turn can cause a transient disruption of the packing of the hydrocarbon chains of the lysolecithin molecules with those of the contiguous lecithin molecules in the host matrix.[3]

The formation of the tetrameric lysolecithin ion channel can be described in terms of the following equilibria:

$$L + L \overset{K_1}{\rightleftharpoons} L_2; \quad L^* + L^* \overset{K_1}{\rightleftharpoons} L_2^*$$

and

$$L_2 + L_2^* \overset{K_2}{\rightleftharpoons} L_4$$

where L and L_2 denote the lysolecithin monomer and dimer, respectively, and we have differentiated between species in the two monolayers by designating those on the outer layer by an asterisk. K_1 is the dimer formation constant and K_2 is the formation constant for the tetramer, L_4. If $[C_o]$ and $[C_i]$ denote the stoichiometric concentrations of lysolecithin molecules on the outer and inner monolayers, respectively, then:

$$[C_o] = [L^*] + 2[L_2^*] = [L^*] + 2K_1[L^*]^2 \quad (9)$$

$$[C_i] = [L] + 2[L_2] = [L] + 2K_1[L]^2 \quad (10)$$

As a first approximation, we may take $[C_o]$ to be equal to $[C_i]$. However, this need not be the case, as the lysolecithin molecules may be unequally distributed between the two halves of the bilayer due to differences in their surface curvatures. In general we may write:

$$[C_o] = f_o[C]; \quad [C_i] = f_i[C]$$

where f_o and f_i are factors introduced to account for possible unequal partitioning of the lysolecithin molecules between the monolayers. Within the framework of this model, the value of m may be ascertained for various ranges of K_1f values. As an illustration, we first consider the limit where the concentration of dimers is small compared with that of the monomer, i.e., $K_1f[C] \ll 1$. Then:

$$[L^*] \rightarrow f_o[C]; \quad [L] \rightarrow f_i[C]$$

and it follows that:

$$[L_4] \simeq K_1^2K_2f_o^2f_i^2[C]^4 \quad (11)$$

The rate of ion channel formation and hence the concentration dependence of the europium(III) ion transport rate would then be proportional to the fourth power of the lysolecithin concentration. However, if the dimer concentration within each monolayer is not negligibly small compared with that of the monomer, the effective power dependence, i.e., m, can be shown to be less than 4. In fact, in the limit $K_1f[C] \gg 1$:

$$[L_2^*] \rightarrow \tfrac{1}{2}f_o[C]; \quad [L_2] \rightarrow \tfrac{1}{2}f_i[C]$$

and

$$[L_4] \simeq \tfrac{1}{4}K_2f_of_i[C]^2 \quad (12)$$

so that the effective value of m is 2. Our experimentally determined value of $m \simeq 3.2$ would imply that $K_1f \simeq 1\ M^{-1}$.

If the lysolecithin ion channel formation can be represented by the above picture, simple model building suggests that the average size of the ion channel should be approximately 7.5 Å in diameter. An ion channel of this size is clearly large enough to accommodate a hydrated europium(III) or lanthanum(III) ion, the size of which is estimated to be approximately 7 Å. A schematic illustration of such a channel is de-

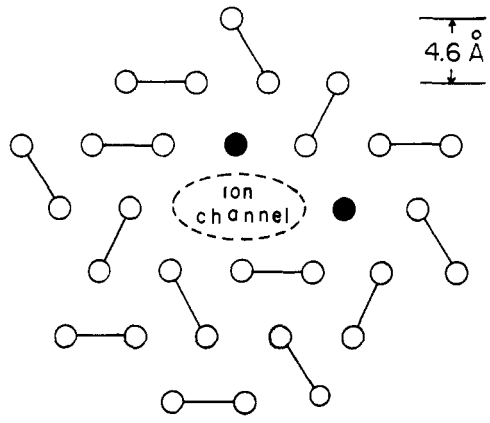

FIGURE 7: Schematic illustration of the cross-section of a possible lysolecithin ion channel in a lecithin host matrix: (●) lysolecithin; (○) lecithin.

[3] Much evidence has been accumulated to indicate that the average headgroup orientation of lecithin molecules in lipid bilayer is tilted with respect to the bilayer normal (Gally et al., 1975; Yeagle et al., 1976). This tilted headgroup orientation is, however, not expected for lysolecithin molecules which are imbedded within a lecithin bilayer matrix. Reference to the Corey-Pauling-Koltun (CPK) molecular model shows that for optimum packing of the lysolecithin chain with the hydrocarbon chains of the surrounding lecithin molecules, it is necessary for the headgroup of the lysolecithin molecule to be oriented perpendicular to the bilayer surface. Should, however, the headgroup conformation of a lysolecithin molecule be modified upon cation binding, the hydrocarbon chain packing must necessarily be disrupted in the vicinity of the lysolecithin molecules affected. Presumably a lysolecithin ion channel could be activated in this manner.

picted in Figure 7. Here we have assumed hexagonal packing of the acyl chains with a chain–chain distance of 5.3 Å (4.6 Å/sin 60°), but with the lecithin molecules oriented at random, as expected at temperatures above the T_c of the host lipid.

We believe that our findings here can have important biological implications, as lysolecithin is known to cause lysis of cells. Therefore, in membranes which contain a relatively high concentration of lysolecithin such as in the membranes of chromaffin granules, it is reasonable to expect that there are processes which regulate the manifestation of these ion channels.

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