Lamellar–Micellar Transition of 1-Stearoyllysophosphatidylcholine Assemblies in Excess Water†

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ABSTRACT: Although synthetic diacylphospholipid systems have been known for some time to undergo bilayer gel ↔ liquid crystalline or bilayer ↔ hexagonal phase transitions, lamellar to micellar transitions of lysophospholipid assemblies have only been briefly reported [Van Echteld, C. J. A., DeKruijff, B., Mandersloot, J. G., & DeGier, J. (1981) *Biochim. Biophys. Acta 649*, 211–220; Huang, C., Lapides, J. R., & Levin, I. W. (1982) *J. Am. Chem. Soc.* (in press)]. In the present study we apply dynamic light scattering, high-sensitivity differential scanning calorimetry, freeze–fracture electron microscopy, ^{31}P NMR, and Raman spectroscopic techniques toward investigating the lamellar to micellar transition of 1-stearoyllysophosphatidylcholine dispersions in excess water. At temperatures below the chain melting transition point, ^{31}P NMR data and freeze–fracture electron microscopic results together demonstrate the formation of extended multilamellar structures. Raman spectroscopic order/disorder indexes, characteristic of interchain interactions, suggest that acyl chains of the opposing leaflets are probably interdigitated in the lamellar structures. The various physical measurements indicate that in excess water the lamellar to micellar transition for 1-stearoyllysophosphatidylcholine dispersions occurs within a narrow temperature range between 24.5 and 26.5 °C with a transition enthalpy of 7.0 ± 0.5 kcal/mol. Calorimetric and light-scattering data demonstrate that the reverse micellar to lamellar transition occurs slowly. At temperatures slightly below the characteristic size transition temperature, the lamellar–micellar transition is essentially irreversible; prolonged incubation of micellar solutions at 0 °C, however, induces the micellar structures to re-form extended lamellar bilayers. The chain melting transition of 1-stearoyllysophosphatidylcholine is sharper than that for multilamellar dispersions of distearoylphosphatidylcholine. Since the transition enthalpy of the lysophosphatidylcholine system is less than the ΔH value for diacylphosphatidylcholine multibilayers, the sharp order/disorder transition observed for the lamellar to micellar change for lysophospholipid dispersions is interpreted in terms of a high lipid cooperativity resulting from tight, lateral chain packing arrangements within the extended, interdigitated bilayers.

Recently, Mason et al. (1981) reported the probable interdigitation across the bilayer center of multilamellar bilayers of asymmetric diacylphosphatidylcholine molecules such as 1-stearoyl-2-caproyl-*sn*-glycero-3-phosphorylcholine [C(18):C(10)PC] at temperatures below the gel ↔ liquid-crystalline phase transition temperature. Chain interdigitation presumably occurs across the bilayer center with the short acyl chain of a phospholipid in one monolayer packing end-to-end with the long acyl chain of another phospholipid from the opposing bilayer leaflet. Since lysophosphatidylcholine molecules may be regarded structurally as an extreme case of the asymmetric phosphophatidylcholine system in which the entire *sn*-2 acyl chain is substituted by a hydrogen atom, one expects the acyl chains of the lysophospholipids in the gel-state lamella to interdigitate fully, such that the thickness of the hydrocarbon region of the bilayer corresponds to the acyl chain length of a single lysophosphatidylcholine molecule. Indeed, Van Echteld and co-workers (1981), using ^{31}P NMR techniques, have recently shown that 1-palmitoyllysophosphatidylcholine forms extended bilayers in excess water at −10 °C. Further, a fully interdigitated lamellar packing of deoxylysophosphatidylcholine monohydrate, a lysophosphophatidylcholine analogue, has been demonstrated by Hauser et al. (1980) by an X-ray single-crystal analysis. In contrast to diacylphosphatidylcholine assemblies, lysophosphatidylcholine molecules are known to form micelles in excess water at temperatures above the chain melting point (Van Echteld et al., 1981; Reiss-Husson, 1967). The thermal transitions between the lamellar ↔ micellar states of lysophosphatidylcholines in excess water, however, have not been systematically studied, although Raman spectroscopic techniques indicate that a sharp order/disorder transition occurs for aqueous dispersions of 1-palmitoyllysophosphatidylcholine (Huang et al., 1982). In the present paper, we apply dynamic laser light scattering, high-sensitivity differential scanning calorimetry, freeze–fracture electron microscopy, ^{31}P NMR, and Raman spectroscopic techniques toward specifically investigating the lamellar ↔ micellar transition of aqueous 1-stearoyllysophosphatidylcholine dispersions. Our results indicate that the lamellar ↔ micellar transition for this system occurs within the very narrow temperature range from 24.5 to 26.5 °C with a transition enthalpy of 7.0 ± 0.5 kcal/mol. Further, the lamellar ↔ micellar transition is demonstrated to be virtually irreversible at temperatures slightly below the transition temperature; however, prolonged incubation of a micellar solution of 1-stearoyllysophosphatidylcholine at 0 °C reverses the lysophospholipid organization in excess water from the micellar structure back to the lamellar bilayer.

Materials and Methods

1-Stearoyllysophosphatidylcholine and distearoylphosphatidylcholine were obtained from Avanti Polar Lipids, Inc., Birmingham, AL. The purity of the lipid was ascertained by thin-layer chromatography with the solvent mixture chloroform/methanol/48% ammonium hydroxide (65:35:5). Only a single spot was detected with iodine or phosphate reagent following migration in a developing solvent of about 1 μmol of the lipid sample.

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Sample Preparation. In general, a known volume of 50 mM KCl aqueous solution was added to a preweighed dry lysophospholipid sample. The mixture was incubated at 30 °C for 3–5 min until the dispersion became transparent. If large aggregates were present, the heated sample was subject to centrifugation at 5000*g* for 5 min. The clear supernatant formed opaque dispersions at 0 °C in 2–5 h depending upon the lipid concentration. For a 10 mM lipid–50 mM KCl aqueous solution, the clear micellar solution formed lamellar aggregates after incubation at 0 °C for 1 h. The final lysophospholipid concentration was determined by the method of inorganic phosphate (Gomori, 1942).

Quasi-Elastic Light Scattering. Dynamic light-scattering measurements on lysophospholipid dispersions were carried out at a scattering angle of 90° with a He–Ne laser scattering spectrometer (6328 Å, Nicomp Model HN5-90) equipped with an autocorrelator (Model 6864). The apparent diffusion coefficients (D_a) of the lysophospholipid aggregates were determined directly in terms of the autocorrelation function of the scattered light. The plot of the logarithm of the autocorrelation function against time was fit by either a simple linear or higher polynormial expression by the cumulant method of Koppel (1972). The slope of the resultant curve yields $-2q^2D_a$, where q, the scattering vector, is a function of the refractive index of the solution (n), the wavelength of incident light (λ), and the scattering angle (θ), as given by $q = (4\pi n/\lambda) \sin(\theta/2)$. The Stokes radii ($R_s$) of the lysophospholipid aggregates were calculated from the apparent diffusion coefficient from the Einstein–Stokes relation $R_s = kT/(6\pi\eta D_a)$, where k is the Boltzmann constant, η the viscosity, and T the absolute temperature.

^{31}P NMR Spectroscopy. ^{31}P nuclear magnetic resonance (NMR) spectra of lysophospholipid dispersions were obtained at 145.7 MHz with a Nicolet NICFT-1180 spectrometer operating under full proton decoupling conditions. Accumulated free induction decays were obtained from 4000 transients on a 1.5-mL sample (150 mM) in a 10-mm NMR tube by using 20.48 ms as the acquisition time. A sweep width of 50 kHz and a 10-μs, 30° pulse, employing a 0.3-s interpulse time, were utilized. In order to enhance the signal/noise ratio, the ^{31}P FID signal was multiplied by an exponential function resulting in either a 50-Hz line broadening for the broad asymmetrical signal attributed to large lamellar structures or a 1-Hz line broadening for the narrow isotropic signal arising from micellar solutions.

Differential Scanning Calorimetry. All calorimetric measurements were determined on a high-sensitivity DSC instrument of the heat conduction type as described elsewhere (Suurkuusk et al., 1976). Prior to the calorimetric scans, the lysophospholipid samples were allowed to equilibrate with the calorimeter sink at 15–20 °C for 30 min. The scanning rate was set at 15.7 °C h^{-1} in an ascending mode; detailed experimental procedures were given previously (Mason et al., 1981).

Raman Measurements. Raman spectra of the lysophospholipid dispersions in the vibrational C–H stretching region (2800–3100 cm^{-1}) were recorded with a computer-controlled Spex Ramalog 6 spectrometer equipped with holographic gratings. The automated Raman system and the detailed procedures for determining the melting behavior of phospholipid dispersions were described elsewhere (Lavialle & Levin, 1980). In general, Raman spectra were recorded at a scanning rate of 1 cm^{-1}/s with a laser excitation power of 200 mW from an argon ion source. A total of three to four scans were signal averaged at each fixed temperature. Before the sample was scanned, the thermal equilibration time for the lysophospholipid dispersions (8–12 μL, 33%) was 12 min. The sample was heated in intervals of 2 °C before equilibrating at each step. The Raman intensity ratios, $I_{2936}/I_{2883(2892)}$, were used as indexes for monitoring the order/disorder changes in the lipid acyl chains as a function of temperature.

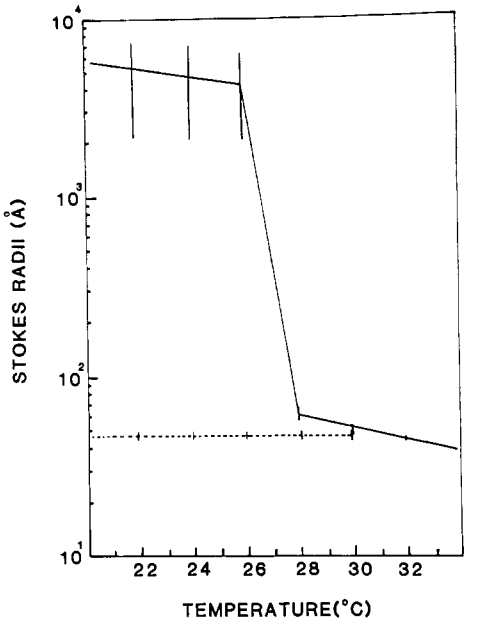

FIGURE 1: Stokes radii of 1-stearoyllysophosphatidylcholine aggregates as a function of temperature measured by dynamic laser light scattering. The solid curve represents the results acquired during an increasing temperature run for samples heated at a 2 °C interval. The dashed line displays the results of the sample, first being heated to 2 °C above the phase transition and then cooled to 22 °C within 30 min, while data were recorded at 2 °C intervals during a decreasing temperature run.

Results

Dynamic Laser Light-Scattering Studies. Prior to the light-scattering experiments, a 4 mM sample of the lysophospholipid dispersion was incubated at 0 °C for 48 h. An aliquot (0.5 mL) was transferred into the sample holder and allowed to thermally equilibrate within the light-scattering apparatus.

The Stokes radii of the aqueous lysophospholipid aggregates, calculated from D_a, are plotted in Figure 1 as a function of temperature. It is apparent in Figure 1 that the mean size of the lysophospholipid aggregate is relatively constant from 20 to 26 °C. In contrast, a marked decrease in the Stokes radii of about 2 orders of magnitude is clearly observed for the 1-stearoyllysophosphatidylcholine aqueous dispersion in the temperature interval from 26 to 28 °C. The turbidity of the 4 mM lysophosphatidylcholine dispersions at 28 °C is too low to give reliable values of D_a with our instrument; therefore, the Stokes radii of aqueous lysophosphatidylcholine aggregates in the 28–38 °C temperature range were calculated from the values of D_a obtained with a sample containing 95 mM 1-stearoyllysophosphatidylcholine in a 50 mM KCl aqueous solution. The Stokes radii of lysophosphatidylcholine aggregates for temperatures above 28 °C, as reflected by Figure 1, are typically the size of aqueous lipid micelles (Haberland & Reynolds, 1975).

Following the size transition from large aggregrates to small micelles, the resulting lysophospholipid micelles fail to revert reversibly to large aggregates should the sample be cooled within a 30-min interval, as shown in Figure 1. However, if the annealed sample is maintained at 0 °C for more than an

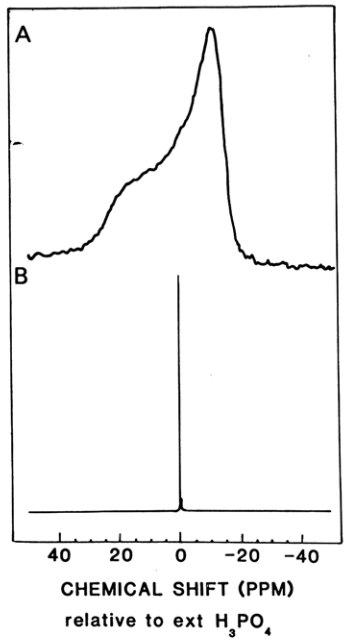

FIGURE 2: 145.7-MHz ^{31}P NMR spectra of aqueous dispersions of 1-stearoyllysophosphatidylcholine at 14 (A) and 30 °C (B).

hour, large aggregates are then detected by light-scattering measurements.

^{31}P NMR and Freeze–Fracture Electron Microscopy Studies. Changes in the characteristic ^{31}P NMR resonance patterns of aqueous dispersions of 1-stearoyllysophosphatidylcholine at two temperatures are displayed in Figure 2. Prior to the ^{31}P NMR experiment, the sample was maintained at 0 °C for 4 days. The aqueous lysophosphatidylcholine dispersion at 14 °C, shown in Figure 2A, exhibits a broad, asymmetric "solid-state" line shape with a characteristic low-field shoulder; this pattern is regarded as a typical bilayer or lamellar ^{31}P NMR resonance (Cullis & DeKruijff, 1978). Similar ^{31}P NMR spectra were also reported for 1-palmitoyllysophosphatidylcholine at −10 °C by Van Echteld et al. (1981). The ^{31}P NMR spectrum shown in Figure 2A is regarded as strong evidence for suggesting that 1-stearoyllysophosphatidylcholine molecules are packed in a lamellar array of bilayers at 14 °C, a temperature below the main size transition temperature detected by light scattering (Figure 1). In contrast, a very sharp isotropic ^{31}P NMR signal, shown in Figure 2B, is recorded for the same sample at a higher temperature (30 °C). This pattern is characteristic for lipid micelles (Cullis & DeKruijff, 1978); it is consistent with results obtained by light-scattering experiments and indicates that lysophospholipid molecules are packed in micelles rather than bilayers at a temperature above the sharp transition temperature. It should be noted that for an expanded spectroscopic trace there is a small side band at ~0.16 ppm upfield from the main ^{31}P NMR isotropic peak. This small peak is a result of acyl chain migration from the *sn*-1 to the *sn*-2 chain position (Plückthan & Dennis, 1982).

Our conclusion that 1-stearoyllysophosphatidylcholine molecules are packed in a lamellar structure at temperatures below the size transition temperature is further substantiated by freeze–fracture electron microscope experiments. Figure 3 displays a freeze–fracture replica of 1-stearoyllysophosphatidylcholine dispersions. The sample (10 mM) was incubated at 0 °C for 9 h prior to the rapid freezing in liquid Freon required by freeze–fracture techniques. Extended arrays of multilamellar structures are clearly observed in Figure 3. However, if the aqueous lysophospholipid sample was kept at

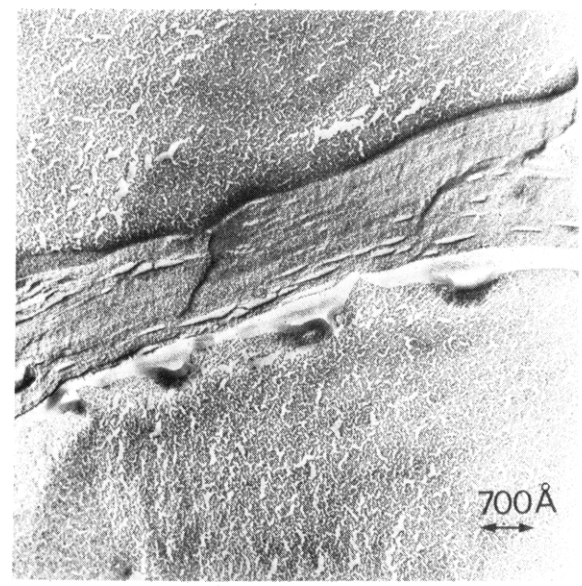

FIGURE 3: Freeze–fracture micrograph of 1-stearoyllysophosphatidylcholine dispersion (10 mM). Magnification is approximately 26800×.

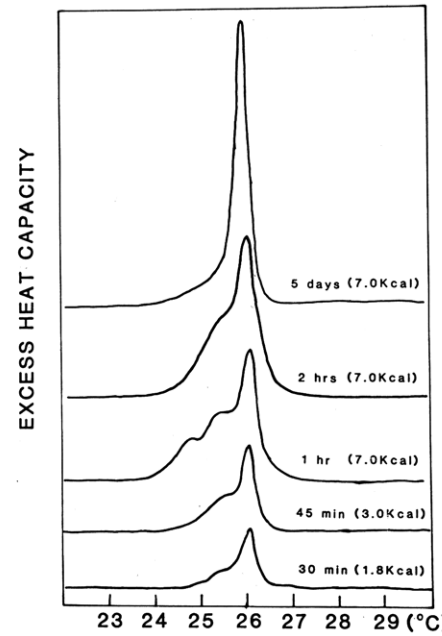

FIGURE 4: Effect of preincubation time at 0 °C for 1-stearoyllysophosphatidylcholine dispersions on the endothermic transition profile determined by high-sensitivity differential scanning calorimetry. Lysophospholipid concentrations were 11 mM in excess 50 mM KCl. A scan rate of 15.7 K h^{-1} was employed.

temperatures above the size transition temperature for 3–5 min prior to the freeze–fracture microscopic experiment, no multilamellar sheet structures were detected.

Calorimetric Studies. The thermotropic behavior of 1-stearoyllysophosphatidylcholine dispersions at various concentrations (6, 11, and 22 mM) were studied by high-sensitivity differential scanning calorimetry. Lysophospholipid dispersions were first heated above the size transition temperature and incubated at 30 °C for 3–5 min; the samples were then allowed to anneal at 0 °C for various time intervals. The endothermic transition profiles of the annealed samples as functions of the 0 °C preincubation time intervals are shown in Figure 4 for a sample with a lysophospholipid concentration of 11 mM in a 50 mM KCl aqueous solution.

After prolonged preincubation times (>24 h), 1-stearoyllysophosphatidylcholine dispersions display a sharp endo-

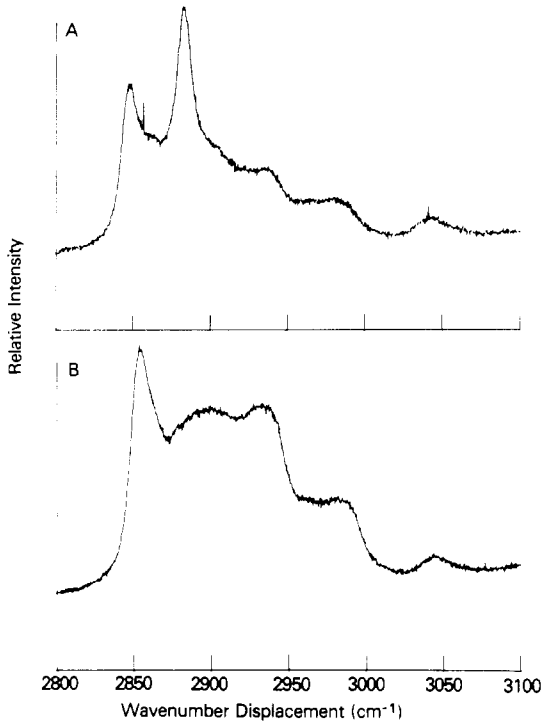

FIGURE 5: Raman spectra of the 2800–3100-cm^{-1} C–H stretching region for 1-stearoyllysophosphatidylcholine dispersions in (A) the gel state at 6.3 °C and (B) the aqueous micellar state at 41.2 °C.

thermic peak with a maximum peak height temperature of 26 °C, a half-width of 0.5 °C, and a transition enthalpy of 7.0 ± 0.5 kcal/mol. The endothermic peak occurs within a temperature range coinciding with the size transition temperature detected by the dynamic laser light-scattering experiments, an observation which strongly suggests that the endothermic transition corresponds to the reorganization of lysophospholipids from a multilamellar bilayer to a micellar structure.

As shown in Figure 4, samples which have been preincubated at 0 °C for less than 15 h display transition profiles with more than one peak. The main transition, however, is centered at 26 °C. Between a preincubation time of 1 h and 5 days, the transition enthalpy remains constant; however, the minor peaks on the ascending shoulder of the main peak gradually diminish with time, while the main peak grows in magnitude. The values of transition enthalpy corresponding to various endothermic profiles are given in Figure 4. The thermotropic behavior of 1-stearoyllysophosphatidylcholine at other concentrations (6 and 22 mM) is similar to the ones presented in Figure 4.

For a lysophospholipid sample heated to 30 °C, a temperature above the main endothermic transition temperature, and then immediately cooled to 10 °C, the calorimetric measurement performed within 30 min in a heating mode displays no thermal transition. This observation is consistent with the results obtained in the light-scattering experiments, indicating that the micellar → lamellar transition is a very slow process. On the basis of the observation that ΔH increases with increasing preincubation time, as shown in Figure 4, the regeneration time of a lamellar structure from 1-stearoyllysophosphatidylcholine micellar aggregates is estimated to be about an hour at 0 °C.

Raman Spectroscopic Studies. The lateral interactions involved in the order/disorder changes of the acyl chains in lysophospholipid assemblies as a function of temperature were investigated by monitoring the Raman spectral changes in the 2800–3100-cm^{-1} hydrocarbon chain C–H vibrational stretching

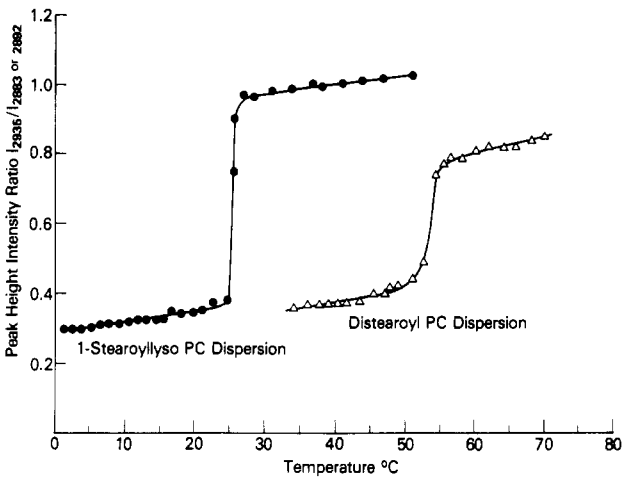

FIGURE 6: Comparison of the temperature profiles for 1-stearoyllysophosphatidylcholine and distearoylphosphatidylcholine dispersions in excess water. The $I_{2936}/I_{2883(or2892)}$ peak height intensity ratio was used as a spectral index for characterizing the structure and phase transitions of hydrocarbon chain region of the lipid assembly.

mode region of the system. Figure 5 shows Raman spectra of the C–H stretching mode interval for 1-stearoyllysophosphatidylcholine dispersions at two temperatures. Vibrational frequencies and assignments of Raman spectral features in the 2800–3100-cm^{-1} region are discussed elsewhere (Huang et al., 1982; Bush et al., 1980). It is worth mentioning here that on chain melting, the asymmetric methylene CH_2 stretching mode intensity at 2883 cm^{-1} is reduced and the peak position is shifted to 2892 cm^{-1} (Figure 5). In contrast, the peak intensity of the methyl symmetric stretching mode at 2936 cm^{-1} is significantly enhanced on chain melting, as compared to the gel-state bilayer spectrum (Figure 5). The peak height intensity ratio $I_{2936}/I_{2883(2892)}$ is taken as a spectral index to characterize the structural reorganizations and phase transitions occurring within the bilayer hydrocarbon chain region [see, for example, Levin et al., (1982)]. Figure 6 displays this spectral intensity ratio as a function of temperature. The temperature profile clearly indicates a marked increase in the lateral disorder of the lysophospholipid hydrocabon chain arrangements in the 25–26 °C interval. This observed chain melting temperature is in excellent agreement with the maximum peak height position (26 °C) of the endothermic peak determined by high-sensitivity differential calorimetry. For comparison, changes in the spectral ratios for distearoylphosphatidylcholine multibilayers as a function of temperature are also presented in Figure 6. It is clear from the values of the probe index, I_{2936}/I_{2883}, that below the chain melting temperature the lipid matrix forming the lamellar structure for 1-stearoyllysophosphatidylcholine molecules is more ordered than the gel-state bilayer organization characteristic of distearoylphosphatidylcholine molecules. At temperatures above the chain melting temperature, however, the relatively larger values for I_{2936}/I_{2892} indicate that the 1-stearoyllysophosphatidylcholine molecules in the micellar form exhibit greater lateral disorder than distearoylphosphatidylcholine in the liquid-crystalline bilayer state.

Discussion

In the presence of excess water, lipid assemblies of a variety of synthetic diacylphospholipids have been known for some time to undergo bilayer gel ↔ liquid-crystalline and/or bilayer ↔ hexagonal phase transition (Ladbrooke & Chapman, 1969; Cullis & DeKruijff, 1978). The lamellar ↔ micellar transition of 1-palmitoyllysophosphatidylcholine assemblies, however, has

only recently been briefly reported (Van Echteld et al., 1981; Ramsammy & Brockerhoff, 1982; Huang et al., 1982). In the present paper, we clearly demonstrate that 1-stearoyllysophosphatidylcholine molecules form extended multilamellar structures in excess water at temperatures below the chain melting transition point. These extended multibilayers undergo a highly cooperative endothermic phase transition from the lamellar to micellar form when heated above a temperature of 26 °C.

Our conclusion that 1-stearoyllysophosphatidylcholine molecules are organized in extended bilayer structures at low temperatures is drawn from the ^{31}P NMR and freeze–fracture electron microscopic results (Figures 2A and 3). These bilayer structures, however, are quite heterogeneous in their overall size, as shown by large error bars in laser light-scattering data (Figure 1). Within the lamellar structures, the acyl chains of the opposing leaflets are most likely interdigitated, since the Raman data indicate that the 1-stearoyllysophosphatidylchloline system is significantly more ordered than the gel-state distearoylphosphatidylcholine bilayer (Figure 6). Our earlier Raman investigations on bilayers of a homologous series of synthetic diacylphosphatidylcholines extending from dilauroylphosphatidylcholine (C_{12}) to dibehenoylphosphatidylcholine (C_{22}) indicated that for the same reduced temperatures the value of the I_{2936}/I_{2883} spectral intensity ratio decreases with increasing chain length for multilamellar bilayers in the gel state (Huang et al; 1982); that is, the longer chain systems display comparatively greater chain ordering properties. The analogous spectral index for 1-stearoyllysophosphatidylcholine lamellae reported in this paper is lower than that for dibehenoylphosphatidylcholine multibilayers, indicating a more ordered state for interchain arrangements.

The chain melting temperature detected by the Raman spectroscopic temperature profile agrees well with the main endothermic transition temperature determined by calorimetry. Furthermore, the temperature corresponding to the sharp size transformation observed by light-scattering measurements also coincides with the chain melting temperature. Since the same transition temperature (T_m) is detected for 1-stearoyllysophosphatidylcholine assemblies in excess water by three distinctly different measurements, the chain disordering induced by the endothermic process may be regarded as the major driving forces for the reorganization of the lipid assembly from a highly ordered lamellar structure to a disordered micellar solution.

The formation of micelles by lysophospholipids is well-known (Tanford, 1980). Light-scattering and ^{31}P NMR studies reported here confirm that at temperatures above T_m 1-stearoyllysophosphatidylcholine molecules aggregate into micellar structures. In addition, the Raman results indicate that this system displays greater intermolecular disorder than distearoylphosphatidylcholine bilayers in the liquid-crystalline state. That is, the spectral intensity parameter, I_{2936}/I_{2892}, for 1-stearoyllysophosphatidylcholine micelles at temperatures above T_m is considerably greater than that for distearoylphospholipid bilayers at the same reduced temperature.

Although the lamellar ↔ micellar transition described in this paper is analogous in many ways to the gel ↔ liquid-crystalline phase transition observed for synthetic diacylphospholipid bilayers, we emphasize that the conversion of micellar structures to lamellar assemblies is a very slow process for 1-stearoyllysophosphatidylcholine molecules in excess water in comparison to liquid-crystalline → gel phase changes. Currently, we are examining the effects of chaotropic and antichaotropic agents on the lamellar ↔ micellar transition; preliminary data suggest that the rate of conversion of lysophospholipid micelles to the lamellar assembly can be either promoted or inhibited by antichaotropic or chaotropic agents, respectively. Finally, we note that the chain melting transition shown in Figure 6 is sharper in the 1-stearoyllysophosphatidylcholine assembly than in the multilamellar dispersions of distearoylphosphatidylcholines. This sharper transition could arise from either an increase in the transition enthalpy or an increase in the cooperativity of the transition (Marsh et al., 1977). Since the transition enthalpy for 1-stearoyllysophosphatidylcholine (7.0 kcal/mol) system is smaller than the value (10.6 kcal/mol) obtained for distearoylphosphatidylcholine multibilayers (Mabrey & Sturtevant, 1976), the sharp transition observed for the lamellar ↔ micellar transition must be interpreted in terms of lipid cooperativity. The high cooperativity of the lamellar → micellar transition can, in turn, be attributed to the tighter, lateral packing of the hydrocarbon chains in the interdigitated bilayers.

Acknowledgments

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