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Kinetic Studies of the Micellar to Lamellar Phase Transition of 1-Stearoyllysophosphatidylcholine Dispersions†

Wen-guey Wu and Ching-hsien Huang*

ABSTRACT: Steady-state 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence depolarization anisotropy, ^{31}P nuclear magnetic resonance (^{31}P NMR), and high-sensitivity differential isothermal calorimetry were performed to investigate the kinetics of the micellar to lamellar phase transition of 1-stearoyllysophosphatidylcholine dispersions at temperatures below the lamellar → micellar transition temperature (T_m). Around the supercooling temperature of 25 °C below T_m, the kinetic process of this phase transition was found to consist of an initial lag time followed by a cooperative step of lamellar formation. The cooperativity of this lamellar formation was strongly negatively temperature dependent. Within experimental error, no positive concentration dependence was found for the micellar → lamellar transition with lysophospholipid concentrations in the 0.01-100 mM range. Immediately following the completion of lamellar formation, the lipid aggregates were shown, by ^{31}P NMR, to be large extended lamellar structures. The chaotropic salt NaSCN was shown to slow down the rate of formation of the large extended lamellar structures. However, the lamellar formation detected by either calorimetry or DPH anisotropy was not affected by chaotropic salts. The mechanism of the transition of the lysophospholipid micelles to lamellae at a constant supercooling temperature is discussed in terms of two-dimensional nucleation and growth processes. The heat of formation for the lamellar phase was found to be much lower than that expected from consideration of the melting enthalpy of the lamellar to micellar transition. We suggest that the packing of the lysophospholipid acyl chain in micelles at the supercooling temperature is probably tighter than that of normal micelles. This different micellar structure in conjunction with the driving force associated with supercooling is believed to play a major role in the enhancement of the rate of lamellar formation.

In a recent paper (Wu et al., 1982), it was shown by quasi-elastic light scattering, Raman spectroscopy, and high-sensitivity differential scanning calorimetry that 1-stearoyllysophosphatidylcholine in excess water can undergo a sharp, cooperative lamellar → micellar transition. This transition has a phase transition temperature, T_m, centered around 26.2 °C and a transition enthalpy, ΔH, of 7 kcal/mol. It was further observed that on immediate cooling of the micellar solution to a temperature slightly below T_m the lysophospholipids did not return to the lamellar structure. This indicated that the lamellar → micellar transition is irreversible at temperatures near the T_m. However, the lamellar structure of 1-stearoyllysophosphatidylcholine could be formed by prolonged annealing of the lysophospholipid micellar solution at 0 °C. This phenomenon raises an interesting question as to how lysophospholipid micelles are converted into lamellae at the supercooling temperature, a temperature which is far below T_m. The lamellar structure of 1-stearoyllysophosphatidyl-

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choline is highly ordered as demonstrated by the order/disorder parameter obtained from Raman spectra (Wu et al., 1982), and the highly ordered structure is consistent with the view that the single acyl chains of lysophospholipids are fully interdigitated in the lamella (Wu et al., 1982). Hence, the conversion of lysophospholipid molecules from a dynamic micellar structure to a two-dimensional lamella must involve the ordering and interdigitation of the long acyl chain. Although the molecular mechanism by which the lysophospholipid molecules present in the micelles change into the more ordered conformations found in the lamellae is not known, contrasting models can be proposed. For instance, one can argue that the micellar to lamellar phase transition mimics the sonicated unilamellar vesicle fusion in the gel state; therefore, the collision model proposed for the gel-state vesicle fusion may be feasible (Wong & Thompson, 1982). On the other hand, the kinetics of metastable phase transitions have often been described in terms of a nucleation and growth process (Zettlemoyer, 1969). Since the configuration of the acyl chain in micelles can be treated as systems of long linear hydrocarbons under certain geometric constraint (Dill & Flory, 1981), the direct application of the nucleation mechanism of polymer phase transformation to the micellar → lamellar transition is possible. The understanding of the mechanism as represented in this work will, we hope, provide a rationale for the behavior of other amphiphilic molecules used for model membrane studies. In fact, various nonbilayer micellar structures have been proposed as an obligatory intermediate during membrane–membrane fusion (Lucy, 1970; Cullis & Hope, 1978; Mantsch et al., 1981); our kinetic study of the micellar → lamellar transition may therefore provide useful information which will aid in elucidating the mechanism of membrane fusion.

In the present study, we used ^{31}P nuclear magnetic resonance (NMR), differential isothermal calorimetry, and steady-state 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence depolarization anisotropic spectroscopy to investigate the kinetics of the micellar to lamellar phase transition at various temperatures and at different concentrations. The spectroscopic techniques of ^{31}P NMR and DPH fluorescence anisotropy detect the dramatic structural rearrangements that occur in the head-group and hydrocarbon region of the lysophospholipid aggregate, respectively. High-sensitivity calorimetry, however, not only allows us to correlate results obtained from both spectroscopic techniques with thermodynamic data but also enables us to study the lysophospholipid–H_2O system over a lipid concentration range which is not experimentally feasible by either of the above-mentioned spectroscopic techniques. Our structural and thermodynamic results indicate that bimolecular collision does not play a primary role in the micellar → lamellar phase transition. Furthermore, this process can be described by the language of classic polymer nucleation.

Materials and Methods

1-Stearoyllysophosphatidylcholine was obtained from Avanti Polar Lipids, Inc., Birmingham, AL. Dipalmitoylphosphatidylcholine was from Calibochem-Behring Corp., La Jolla, CA. The purity of the lipids was checked by following the procedure described elsewhere (Wu et al., 1982). DPH, a gift of Dr. J. Mason, was zone purified and stored as a 1.2 mM stock solution in tetrahydrofuran at 0 °C. Sodium thiocyanate (certified, lot 775634) was obtained from Fisher Scientific Co. Deuterium oxide (99.8%, D-4501) was purchased from Sigma Chemical Co., St. Louis, MO. A standard buffer solution [50 mM KCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM *N*-[tris(hydroxymethyl)-methyl]-2-aminoethanesulfonic acid (Tes), and 10 mM histidine, pH 6.8] was used through all the experiments except where noted otherwise.

Sample Preparation. The lysophospholipid was dispersed in the desired buffer by stirring several times for a total of about 10 min at temperatures 10–15 °C higher than the T_m until clear micellar aggregates formed. The samples were annealed at 0 °C and then heated through the T_m up to 40 °C. The samples were then cooled to 0 °C and heated to 40 °C again. After two or three cycles of annealing and melting, the samples were then stored at 0 °C. Prior to use, all the samples were heated to 40 °C for at least 5 min to re-form the micellar structure. Since some samples exhibited anomalous kinetic behavior after they were incubated at 0 °C for more than 1 week, the reported experiments were conducted within 3 days after the dispersions were prepared. For the fluorescence studies, a calculated amount of DPH solution was dispersed in the lipid sample to give a DPH to lysophospholipid molar ratio of 1/1000. The lipid samples were then kept at 35 °C for 1 h to allow evaporation of any traces of organic solvent remaining and then stored at 0 °C for 24 h prior to use.

Steady-State Fluorescence Measurements. DPH is currently one of the most widely used fluorescent probes to monitor the lipid fluidity by using the steady-state fluorescence technique. The motion of the rigid DPH molecule in the hydrophobic region of the lipid assembly is extremely sensitive to changes in the environment adjacent to the chromophore probe. The motion of DPH is characterized by the fluorescence anisotropy, r, a parameter which can be directly obtained from the fluorescence experiment according to the relationship (Shinitzky & Barenholz, 1978; Jähnig, 1979)

$$r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$$

where $I_{\parallel}$ and $I_{\perp}$ represent the emission intensity parallel and perpendicular, respectively, to the plane of polarization of the excitation beam.

The fluorescence intensities, $I_{\parallel}$ and $I_{\perp}$, were obtained by using a SLM 4800 spectrofluorometer. Since we were only interested in observing a change in the fluorescence anisotropy, not in the absolute value of either r or Δr, and since the lipid concentration used was so low (<0.1 mM) that the scattering would not affect appreciably the fluorescence anisotropy, the parameter r was thus calculated directly from the fluorescence intensities without correcting for light scattering. The temperature of the sample (±0.2 °C) within the cuvette holder was controlled by a refrigerated water-bath circulator (Neslab RTE-4) equipped with a digital temperature controller (Neslab DCR-1). The DPH-containing sample was excited at 360 nm, and the emission intensity was recorded at 431 nm by using a filter to cutoff wavelengths below 389 nm.

Differential Isothermal Calorimetry. The high-sensitivity differential scanning calorimeter and the detailed procedure for determining the melting behavior of lipid dispersions have been described elsewhere (Suurkuusk et al., 1976; Mason et al., 1981). In this study, the scanning rate was set to zero; hence, the calorimetry was conducted isothermally. Before each experiment, the sample holder was warmed up to between 35 and 40 °C and then quenched in the calorimetric cell to the supercooling temperature. Although substantial equilibrium of the sample can be reached in 5–10 min, true equilibrium of the sample can only be approached in about 20 min as judged by the asymptote to the heat capacity curve. The kinetic zero time was defined as the time at which the micellar sample was placed into the sample holder which had just been quenched to the desired supercooling temperature within the

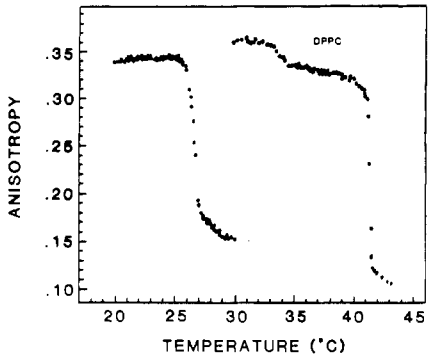

FIGURE 1: Fluorescence anisotropy of DPH vs. temperature for 1-stearoyllysophosphatidylcholine and DPPC (dipalmitoylphosphatidylcholine) dispersions. The concentrations of lipids in both plots were the same (0.1 mM), and the amount of DPH in the lipid–water system was 0.1% (DPH:lipid). The scans were performed at the rate of about 20 °C/h in the ascending temperature direction.

calorimeter. During the experiments, the temperature of the sample holder was electronically controlled to within ±0.001 °C, and the differential capacity was then recorded as a function of time.

^{31}P NMR Spectroscopy. All ^{31}P nuclear magnetic resonance spectra of lysophospholipid dispersions were obtained at 24.15 MHz with a Joel-FX60Q Fourier-transform spectrometer operating under continuous broad-band proton decoupling. Accumulated free induction decays were obtained, in 5 min, from 500 scans on a nonspinning 1.3-mL sample (15% D_2O, 500 mM lipid) in a 10-mm NMR tube. A 4-kHz sweep width and a 10-μs (45°) pulse with a 2.5-ms pulse delay were used. In general, 1–5-Hz line broadenings were introduced during signal enhancement, and 4K data points were collected.

Results

DPH Fluorescence Anisotropy Studies. Figure 1 shows the temperature scanning profiles of the DPH fluorescence anisotropy for both 1-stearoyllysophosphatidylcholine and dipalmitoylphosphatidylcholine (DPPC) dispersions. The observed temperatures at the center of the order–disorder transition for both dispersions (26.3 °C for 1-stearoyllysophosphatidylcholine and 41.4 °C for DPPC) are in excellent agreement with the T_m values obtained by calorimetric measurements (Wu et al., 1982; Ladbrooke & Chapman, 1969). However, the DPH anisotropy of 1-stearoyllysophosphatidylcholine micellar aggregates is significantly higher than that of the multilamellar dispersions of DPPC at equivalent temperatures above their respective T_ms. This could be due to the perturbation of the relatively small micellar aggregates by the rigid DPH molecule.

The large difference in the DPH anisotropy between the micellar and lamellar states can be used to monitor the rate of the micellar → lamellar phase transition. Figure 2 shows the effect of temperature on the change of DPH anisotropy in 1-stearoyllysophosphatidylcholine micelles as a function of time. The lysophospholipid concentration used in this series of experiments was 0.05 mM. This study demonstrates several important features of the micellar → lamellar phase transition of 1-stearoyllysophosphatidylcholine. First, a relative fast rate for the micellar to lamellar phase transition can be observed only at temperatures considerably lower than the T_m. For instance, the rate observed at 0 °C is appreciably faster than that at 8 °C. Second, the rate of lamellar formation has a strong negative temperature dependence. It took only 2 min to complete the transition process at 2 °C, but at least 30 min was needed at 6 °C. Third, the micellar → lamellar transition

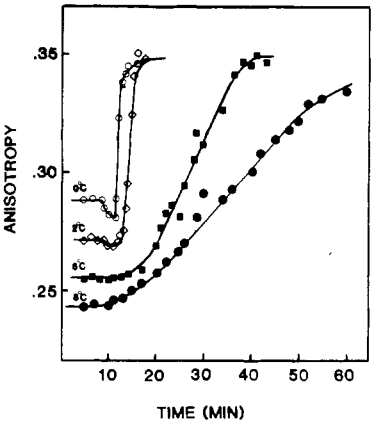

FIGURE 2: Effect of temperature on the kinetics of lamellar formation as detected by the fluorescence anisotropy of DPH. Lipid concentration was 0.05 mM.

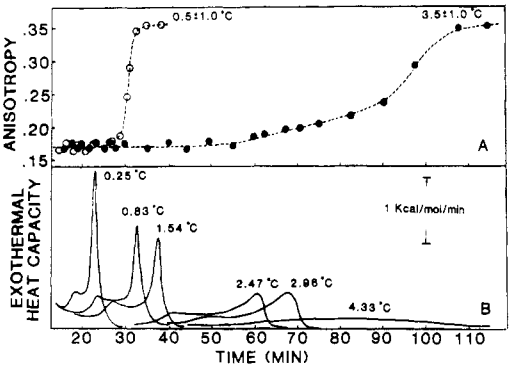

FIGURE 3: Comparison of the kinetics of the phase transition of 1-stearoyllysophosphatidylcholine by (A) the fluorescence anisotropy of DPH. The lysophospholipid samples (10 mM) were incubated in a water bath at the two indicated temperatures; then at different incubation times, an aliquot was transferred and diluted into the fluorescent cuvette to a final concentration of 0.1 mM, and the fluorescence measurement was immediately carried out at 20 °C. (B) Differential isothermal calorimetry. The lysophospholipid concentration was 8.3 mM. Both studies were performed in the presence of DPH, and the molar ratio of DPH to lysophospholipid was 1 to 1000.

profile shows a lag time which is then followed by the lamellar formation stage. At 6 °C, for example, the anisotropy was constant up to 10 min followed by an increase in the anisotropy which reached a plateau value at a time of 35 min. At temperatures near the freezing temperature of water, there is always a small but reproducible anisotropy decrease before the lamellar formation starts. The cause of this phenomenon is not clear, but it is not concentration dependent.

Our efforts have failed to work out the exact concentration dependence of the micellar → lamellar phase transition. The problem is that fluctuations of the lag time were observed for concentrations below the submillimolar range. Although more data are required for a rigorous statistical analysis, there are clear trends which indicate that at lower concentrations both the lag time and the lamellar formation time are shorter. As an example, Figure 3A shows data for lysophospholipid dispersions at a 10 mM lysophospholipid concentration. Despite the longer lag time and slower rate, the character of the negative temperature dependence is clearly demonstrated at this high concentration of lysophospholipid.

Differential Isothermal Calorimetric Studies. Figure 3B shows the relative heat capacity as a function of incubation time for a DPH-containing 1-stearoyllysophosphatidylcholine micellar solution (8.3 mM) determined at various supercooling temperatures. Here, the supercooling temperature is a preset

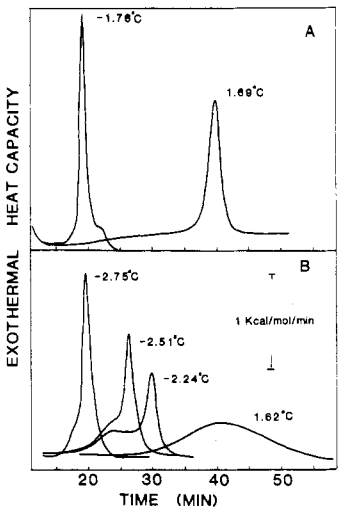

FIGURE 4: Effect of temperature on the kinetics of lamellar formation as detected by differential isothermal calorimetry: (a) 500 mM lysophospholipid with 300 mM NaSCN; (B) 15 mM lysophospholipid in water.

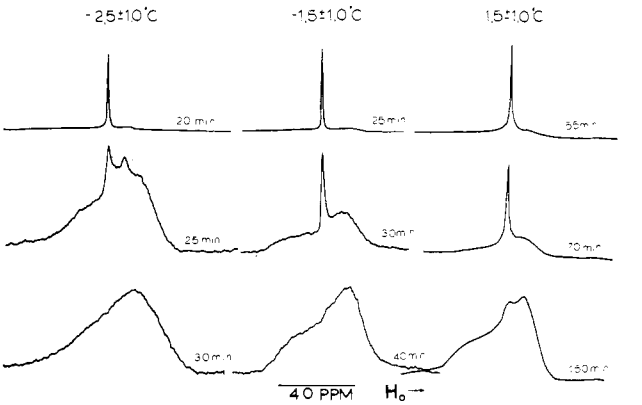

FIGURE 5: ^{31}P NMR spectra at 24.1 MHz of 1-stearoyllysophosphatidylcholine (500 mM) in 15% D_2O as a function of time at the indicated temperatures.

temperature which is considerably lower than the T_m for the lysophospholipid lamellar → micellar transition. In fact, the calorimetric cell is maintained at the supercooling temperature, that is, the temperature at which the lysophospholipid micellar solution is slowly converted into lysophospholipid lamellae in excess water. The area under the calorimetric scan shown in Figure 3B represents the total heat released from the lysophospholipid system, or the formation enthalpy, as the lipid undergoes the micellar → lamellar transition. It is clearly demonstrated by the various calorimetric scans that the rate of conversion of micelles to lamellae depends on the supercooling temperature; the lower the supercooling temperature, the faster the rate of conversion. This result is in excellent agreement with the anisotropy results shown in Figure 3A.

Figure 4B shows the relative heat capacity determined by differential isothermal calorimetry as a function of incubation time at various supercooling temperatures for 1-stearoyllysophosphatidylcholine (15 mM) dispersions. Unlike the exothermal curves shown in Figure 3B, these curves were determined for the lysophospholipid–water system in the absence of the fluorescent probe, DPH. Lysophospholipid concentrations of 5, 10, 35, 40, and 50 mM were examined, and the results were found to be similar to those given in Figure 4B. Clearly, Figure 4B also shows that the lower the supercooling temperature, the faster the rate of lamellar formation from the micelle. However, in comparing Figure 4B with Figure 3B, it was observed that in order to achieve an equivalent rate of conversion of lysophospholipid micelles to lamellae, the system without doping with DPH has to be incubated at a supercooling temperature which is at least 2 °C lower than that of the same system doped with DPH. Also, the relative heat capacity curves were more asymmetric for the lysophospholipid–H_2O system doped with DPH; this indicates that the kinetics of the micellar → lamellar transition are more complex when DPH is added to the system.

The effect of D_2O (20%, 40%, 60%, and 80%) on the rate of conversion of lysophospholipid micelles to lamellae was also studied by using differential isothermal calorimetry. Results show that there is no significant change in the kinetics of the micellar → lamellar transition (data not shown).

The value of the formation enthalpy, or the heat released from the 1-stearoyllysophosphatidylcholine–water (or D_2O–H_2O) system accompanying the lipid micellar → lamellar phase transition, was found to depend on the supercooling temperature. Within the temperature range of −2.5 to 2 °C, the formation enthalpy varies from 3 to 6.2 kcal/mol; moreover, the formation enthalpy decreases with decreasing supercooling temperature. All values, however, are smaller than the value of the melting enthalpy (7 kcal/mol) associated with the reversed process, i.e., the lamellar → micellar transition, detected at the T_m (Wu et al., 1982). If we assume that the final interdigitated lamellar structures for 1-stearoyllysophosphatidylcholines are virtually identical for the lysophospholipid–water system incubated at various supercooling temperatures, the observed change in the formation enthalpy can then be attributed to the difference in the ordering of the long acyl chains in micelles at various supercooling temperatures. The smaller value of the formation enthalpy is probably due to the fact that the acyl chains of micelles at lower temperatures have a tighter molecular packing.

^{31}P NMR Studies. Figure 5 shows three series of 5-min accumulations of proton-decoupled ^{31}P NMR spectra of a 1-stearoyllysophosphatidylcholine (500 mM)–water system. The micellar solution shows a single sharp isotropic ^{31}P NMR peak after it has been incubated at −2.5 °C for 20 min. This sharp isotropic ^{31}P NMR peak can be attributed to the fast rate of micellar tumbling and rapid lateral diffusion of lysophospholipids in micelles. A complete anisotropic spectrum, however, is detected for the lysophospholipid–water system after the sample has been incubated in the NMR tube at −2.5 °C for 30 min; this anisotropic spectrum has the resonance shape of a "bilayer" type spectrum which is characterized by a high-field peak with a broad low-field shoulder. Furthermore, as shown in Figure 5, the incubation time required before anisotropic spectra were detected is dependent upon the incubation temperature at which the NMR tube containing the micellar solution of 1-stearoyllysophosphatidylcholine is kept. For instance, 30, 40, and 150 min are required for the lysophospholipid sample when incubated at −2.5, −1.5, and 1.5 °C, respectively, before anisotropic ^{31}P NMR spectra can be detected. As seen previously, a negative temperature coefficient is observed for the lag time as detected by the ^{31}P NMR technique.

The effect of NaSCN, a chaotropic salt, on the kinetics of the micellar → lamellar transition of 1-stearoyllysophosphatidylcholine was examined by ^{31}P NMR. Preweighed NaSCN was added to a 10-mm NMR tube containing 1-stearoyllysophosphatidylcholine to form a final 0.5 M lysophospholipid dispersion containing 0.3 M NaSCN. It can be seen in Figure 6 that at the supercooling temperature of −2.5 °C, NaSCN decreases the rate of conversion of the isotropic micellar spectrum to the anisotropic bilayer spectrum. An

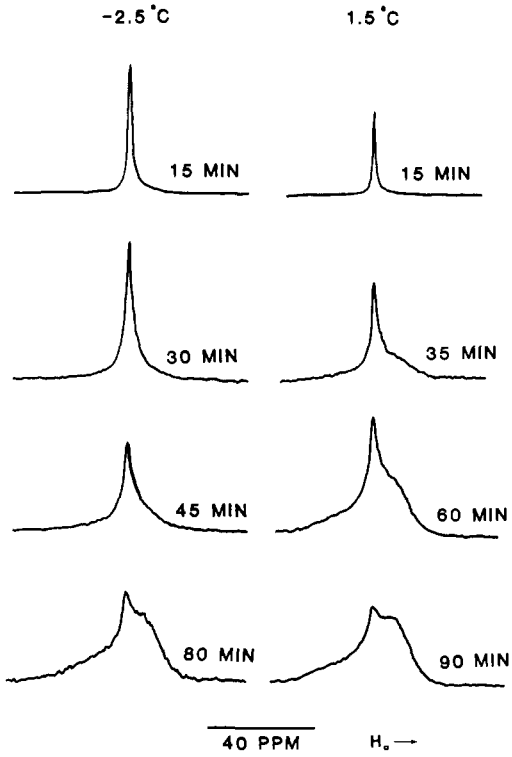

FIGURE 6: Effect of NaSCN (0.3 M) on the kinetics of ^{31}P NMR spectra of lysophospholipid dispersions (0.5 M) at the indicated temperatures.

aliquot (50 μL) of the same sample was used for differential isothermal calorimetric studies. Results show that the rate of lamellar formation as judged from the scanning curve in the relative heat capacity vs. time plot is not at all decreased by NaSCN, even though the shape of the scanning curve is altered (Figure 4A). In the presence of NaSCN, it seems that the results obtained from ^{31}P NMR are incompatible with the calorimetric observations. However, it is most likely that the rate of lamellar formation is not affected by NaSCN, but the subsequent aggregation of the small lamellar structure to form large extended assemblies is inhibited by NaSCN. It is well-known that NaSCN, a chaotropic agent, disrupts structural water and prevents protein aggregation at relatively low concentrations (Sawyer & Puckridge, 1973; Trotta et al., 1974). The aggregation of lysophospholipid lamellae to form large assemblies which give the characteristic anisotropic ^{31}P NMR spectrum is thus most likely inhibited by NaSCN. As a result, there is a slower rate of conversion of the isotropic spectrum to the anisotropic spectrum of the ^{31}P NMR experiment.

Discussion

Based on our fluorescent, calorimetric, and NMR studies (Figures 2–5), we have shown that within the wide concentration range of 1–100 mM 1-stearoyllysophosphatidylcholine, the transition of the lysophospholipid micelles, in excess water, to lamellae requires a considerable degree of supercooling and that the micellar → lamellar transition exhibits the following characteristics. First, a lag time is always observed at various supercooling temperatures before the transition of the micelles to lamellae actually takes place, and the lag time is inversely dependent on the supercooling temperature. Second, the relative rate of lamellar formation from micelles has an extremely large negative temperature coefficient. Finally, the rate of the micellar → lamellar transition appears to be independent of lysophospholipid concentration. However, in the presence of trace amounts of impurities, such as DPH, the phase transition occurs at a higher temperature of supercooling.

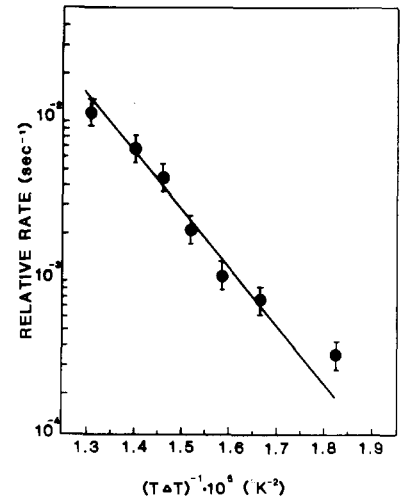

FIGURE 7: Semilogarithmic plot of the relative rate vs. $1/[T(T_m - T)]$. T is the supercooling temperature, and T_m (26.2 °C) is the transition temperature of the lamellar → micellar transition (Wu et al., 1982). It is assumed that the anisotropy increases linearly in the lamellar formation stage. The relative rate is defined as the inverse of the time span between the initial and final anisotropic plateaus (see Figure 2). The lysophospholipid concentration was 0.05 mM.

The negative temperature coefficient of the rate of the micellar → lamellar transition immediately rules out the collision mechanism as a plausible model to explain the micellar → lamellar transition. At the very least, the bimolecular collision of micelles can be ruled out as the rate-limiting step in the complex process of the micellar → lamellar transition.

Nucleation and growth have been widely discussed for the homogeneous crystallization of molten polymers (Zettlemoyer, 1969). The models used in the homogeneous nucleation of long linear hydrocarbons from melt cannot, strictly speaking, be applied to our system, since we are studying a two-component system, namely, the lysophospholipid–H_2O system. Nevertheless, the models used in polymer systems can provide the starting point for our understanding of lysophospholipid micellar → lamellar transitions.

For a two-dimensional nucleus with a cyclindrical shape, the rate of homogeneous nucleation at a given supercooling temperature T is given by (Zettlemoyer, 1969)

$$R = A \exp\{-[\pi\sigma^2 d T_m/(k\Delta H_m)]/[T(T_m - T)]\}$$

where σ is the interfacial free energy of the crystalline long-chain hydrocarbon at the nucleus surface, d is the length of the cylindrical nucleus, ΔH_m is the endothermic transition enthalpy of the crystal → melt transition at the chain melting temperature, T_m, of the macroscopic crystal, k is the Boltzmann constant, and A is a preexponential rate factor. This two-dimensional nucleation process can be reduced to $R = A \exp[-B/[T(T_m - T)]] = A \exp[-B/(T\Delta T)]$, where T is the absolute magnitude of the difference between the supercooling temperature and the crystal → melt transition temperature. This equation describes the negative temperature coefficient of the nucleation process. Taking the logarithm of the equation gives $\ln R = \ln A - B/(T\Delta T)$. A plot of the $\ln R$ against $(T\Delta T)^{-1}$ should be a straight line.

The relative rate of the lamellar formation from 1-stearoyllysophosphatidylcholine micelles can be estimated from Figure 2 by taking the inverse of the time elapsed between the lower and upper plateau regions. A plot of the logarithm of the relative rate against $(T\Delta T)^{-1}$ is given in Figure 7. Clearly,

the data are best fit by a least-squares line. The micellar → lamellar transition observed at supercooling temperatures can, therefore, be described by the two-dimensional nucleation process of polymer crystallization.

We should point out that the spontaneous initiation of crystal growth from a polymer melt at supercooling temperatures typically does not show a lag time (Turnbull & Cormia, 1961). In the case of 1-stearoyllysophosphatidylcholine lamellar formation from micelles, however, a lag time is always detected. Recently, Mazer and Carey reported that a lag time is observed for the formation of cholesterol microprecipitates from cholesterol containing mixed micelles at low temperatures (Mazer & Carey, 1983). It is possible that for a two-component system such as the 1-stearoyllysophosphatidylcholine–H_2O system, the formation of an embryonic nucleus, which allows two-dimensional nucleation and growth to proceed, requires an induction time at the supercooling temperature.

It is reasonable to assume that the lysophospholipid acyl chains pack tighter in micelles at supercooling temperatures than at temperatures above the T_m; this raises the possibility that the kinetics of the micellar → lamellar phase transformation are sensitive to the micellar structure; i.e., the tighter the micellar packing, the more easily they will form the lamellar phase. In fact, this interpretation is also consistent with the experimental observation of the negative temperature coefficient of the lag time for the lamellar formation detected at various supercooling temperatures. Although the exact nature of the much tighter packing within the micelles at the studied supercooling temperature is unknown, we speculate that the hydration state of the lysophosphatidylcholine head group may be involved. Recently, several studies have shown that the molecular packing of dipalmitoylphosphatidylcholines in bilayers at the crystalline state (L_c) below the subtransition temperature is tighter than that in the gel state ($L_{\beta'}$) above the subtransition temperature and that the degrees of hydration for the same lipid in the two states are also different (Füldner, 1981; Ruocco & Shipley, 1982). It is possible that a decrease in the hydration layer of the lysophospholipid head group can also induce a more ordered acyl chain packing in micelles and that the degree of hydration is markedly decreased at supercooling temperatures.

Finally, the macroscopic picture of the lamellar formation can be envisioned from the ^{31}P NMR studies. A complete anisotropic ^{31}P NMR spectrum can be detected for the lysophosphatidylcholine assemblies only when the lysophospholipid assembly is large enough so that the chemical shift anisotropy will not be averaged out by its tumbling motion. It seems that once the interdigitated lamellar structures are formed from the embryonic nucleus, they immediately grow to fully extended large structures with sizes up to a few thousand angstroms. This observation is supported by our unpublished freeze–fracture studies. However, the formation of the lamellar structure with interdigitated hydrocarbon packing and the growth of the lamella to fully extended structures are not physically the same process. From previous studies on the crystallization processes, it is known that the postnucleation event is very complex. A good example is the epitaxial growth in the solid state of thin-film crystallization (Venables & Price, 1975). In our studies, the experiments using a chaotropic salt show that it is possible for lysophospholipids to decouple the process of hydrocarbon packing rearrangement from the growing process and that interdigitation of the hydrocarbon chain of the lysophospholipid can proceed to completion even if the growing process is inhibited.

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