PRESSURE EFFECT ON THE RATE OF CRYSTALLINE PHASE FORMATION OF L-α-DIPALMITOYLPHOSPHATIDYLCHOLINES IN MULTILAMELLAR DISPERSIONS

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ABSTRACT Fully hydrated dipalmitoylphosphatidylcholine (DPPC) in multilamellar dispersions undergoes a subtransition between the crystalline L_c phase and the gel $L_{\beta'}$ phase. This so-called subtransition occurs only if the DPPC-H_2O system has been incubated at temperatures near 0°C for an extended period. We have examined the effect of pressure, up to 336 atm, on the rate of crystalline L_c phase formation of multilamellar DPPC dispersions at 0°C. The hydrostatic pressure is generated by a centrifugal field; the formation of the lamellar L_c phase in the multicomponent DPPC dispersions is monitored calorimetrically at ambient pressure. Results indicate that the rate of formation of the hydrated crystalline L_c phase decreases with increasing pressure. Based on transition state theory, the retardation of the formation of the hydrated crystalline L_c phase by pressure is due to an increase in the volume of the lipid-water system when the activated state is formed. We interpret that the positive value of activation volume is attributed primarily to the dehydration of the lipid polar head group. Although the acyl chain ordering and the head group dehydration are both associated with the $L_{\beta'} \rightarrow L_c$ phase transition, the observation of the pressure effects on the rate of crystalline L_c phase formation is used to show that the head group dehydration plays a predominant role in controlling the kinetics of the $L_{\beta'} \rightarrow L_c$ phase transition.

INTRODUCTION

L-α-dipalmitoyl phosphatidylcholine (DPPC) in excess water has been extensively studied as a model for saturated diacyl phospholipids in the lipid bilayer. Multiple endothermic phase transitions of fully hydrated DPPC molecules in multilamellar dispersions have been detected at ambient pressure by various physical techniques (Chapman, et al., 1967; Tardieu et al., 1973; Chen et al., 1980; Wong and Mantsch, 1983). The endothermic phase transition at ~18°C, the so-called subtransition, is attributable to the lamellar crystalline (L_c)-to-gel ($L_{\beta'}$) phase transition, which can be observed only if the DPPC-H_2O system has been incubated at temperatures near 0°C for an extended period (Chen et al., 1980; Füldner, 1981; Ruocco and Shipley, 1982; Cameron and Mantsch, 1982). Accompanying the lamellar gel to crystalline phase transition, the acyl chains of DPPC molecules in the bilayer are converted from a distorted quasi-orthorhombic packing to a more ordered two-dimensional orthorhombic subcell structure (Ruocco and Shipley, 1982). Moreover, a decrease in hydration of the lipid polar head group is discernible in the formation of the crystalline phase (Füldner, 1981; Ruocco and Shipley, 1982; Cameron and Mantsch, 1982). The slow kinetics of the $L_{\beta'} \rightarrow L_c$ phase transformation has been studied by incubation temperature jump measurements, and the results are interpreted phenomenologically in terms of nucleation and growth processes (Nagle and Wilkinson, 1982).

The formation of a highly ordered crystalline phase is by no means confined to fully hydrated DPPC molecules in lamellae. Other phospholipids or glycolipids, in excess water, have also been demonstrated to undergo the lamellar gel → crystalline phase transition with the same characteristic slow rate of conversion (Chang and Epand, 1983; Mantsch et al., 1983; Seddon et al., 1983; Sen et al., 1983; Stümpel et al., 1983; Serrallach et al., 1984). Thermodynamic parameters such as temperature and pressure are expectd to affect the slow rate of conversion of the $L_{\beta'}$ phase to the L_c phase. In fact, it has been postulated

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that the conversion occurs only within a narrow temperature range of −8°C to +6°C (Cameron and Mantsch, 1982). However, little is known about the possible effect of pressure. In this communication, we examined the effect of hydrostatic pressure generated by ultracentrifugation on the rate of crystalline phase formation in multilamellar DPPC dispersions.

METHODS

L-α-Dipalmitoylphosphatidylcholine (DPPC), claimed to be 99% pure, was obtained from Avanti Polar Lipids Inc. (Birmingham, AL). The purity of the commercial lipid was ascertained by thin-layer chromatography (TLC) with the solvent mixture chloroform/methanol/48% ammonium hydroxide (65:35:5). The commercial lipid was judged to be chromatographically pure by TLC, since only a single spot on the thin-layer plate was detected with iodine after migration in the developing solvent at 1–2 μmol loading of the lipid sample. Multilamellar DPPC dispersions were prepared by suspending 20 mg of DPPC in 9 ml of deionized, distilled water. The sample was first heated to 50°C and then was immediately vortexed for 2 min at the elevated temperature. The sample was then subjected to ultracentrifugation at 0°C (±1°C) at the desired fixed rotational speed. In the centrifuge tube, the DPPC liposomes were packed at the bottom of the centrifuge tube by high speed centrifugation. The hydrostatic pressure generated by the centrifugal field ($\omega^2 r$) can be calculated from the relationship: $(dP/dr) = \rho\omega^2 r$, where r is the distance from the center of rotation to the bottom of the tube, ω is the angular velocity of the spinning rotor, and ρ the density of the solvent. Upon integration, the pressure, P, can be estimated as $P = P_o + (1/2) \cdot \rho \cdot \omega^2(r^2 - r_o^2)$, where P_o is the pressure, 1 atm, at the air-solution meniscus in the centrifuge tube, r_o is the distance from the center of rotation to the solution-air meniscus. For instance, if a Ty65 rotor is spinning at 45,000 rpm in a Beckman L5-50B ultracentrifuge, and if the solution is 6 cm in height in the centrifuge tube, the pelleted DPPC sample can be calculated to be under a hydrostatic pressure of 336 atm. The advantage of using the ultracentrifuge is that the temperature and the speed can be strictly controlled. Consequently, various hydrostatic pressures can be generated on the fully hydrated DPPC sample at 0°C by adjusting the rotational speed of the rotor. After centrifugation, the clear supernatant was discarded and the pellet was resuspended in H_2O, at 0°C, to give a final lipid concentration of ~16.7 mg/ml. This resuspended DPPC sample was subsequently used for calorimetric measurements. All the above experimental procedures, after the step of centrifugation, were carried out in the cold room at 0°C.

The observation of the crystalline L_c phase after incubation of DPPC samples under various pressures at 0°C for a defined time was monitored calorimetrically at ambient pressure. All differential scanning calorimetric (DSC) measurements were performed with a high-sensitivity DSC instrument of the heat conduction type based on the design of Ross and Goldberg (1974). The construction, sensitivity, and the procedure for determining the phase transition behavior of multilamellar DPPC dispersions have been described in detail elsewhere (Suurkuusk et al., 1976; Mason et al., 1981). DPPC samples, transferred immediately from the centrifuge tube into the calorimeter, which had been pre-equilibrated at 0°C, were scanned from 4 to 45°C in the ascending temperature mode at a scanning rate of 20°C/h. Since the $L_{\beta'}$ to L_c phase transition is extremely slow, and since the fully hydrated DPPC samples were incubated under various pressures at 0°C for long periods (≥13 h), the values of ΔH and T_m obtained from the various DSC endotherms, taken at ambient pressure, can in practice be taken as the values for the fully hydrated DPPC sample incubated at 0°C, under various pressures, for a defined time.

RESULTS

Scanning Calorimetry of Multilamellar DPPC Dispersions Following Prolonged Storage at 0°C and at Ambient Pressure

Fig. 1 shows a series of calorimetric scans for multilamellar DPPC dispersions that have been stored at 0°C and at ambient pressure for various long incubation times. In all these experiments, multilamellar DPPC dispersions exhibit three distinct phase transitions: the sub-, the pre-, and the main transition, although the pre- and main transitions are presented only in one of the endothermic transition profiles shown in Fig. 1. The main phase transition, attributed to the $P_{\beta'} \rightarrow L_\alpha$ phase transition, is

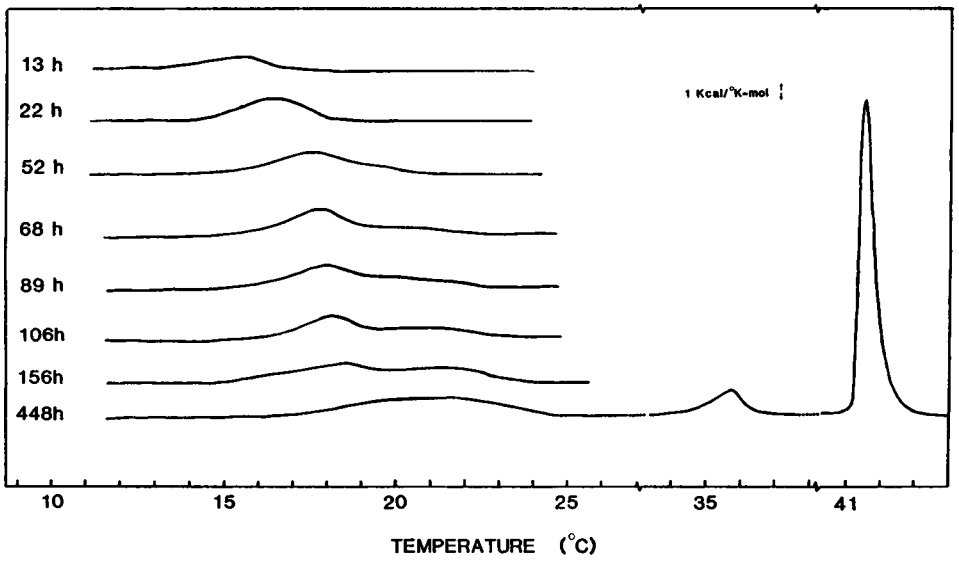

FIGURE 1 DSC traces of aqueous multilamellar dispersions of L-α-DPPC incubated at 0°C and at ambient pressure for various incubation times as indicated. Heating rate: 20°C/h.

characterized by a phase transition temperature (T_m) of 41.4 ± 0.1°C, an enthalpy change (ΔH) of 8.5 ± 0.5 kcal/mol, and a half-height width ($\Delta T_{1/2}$) of 0.5°C. The pre-transition, ascribed to the $L_{\beta'} \rightarrow P_{\beta'}$ phase transition, is centered at 35.7 ± 0.1°C with a half-height width of 1.0°C and ΔH of 1.5 kcal/mol. Both the pre- and the main phase transition characteristics are independent of the long incubation time.

In contrast, the calorimetric behavior of the subtransition for the DPPC-H_2O system depends significantly upon the thermal history of the sample. The subtransition can be detected calorimetrically only in the first heating scan obtained after the multilamellar DPPC dispersion has been incubated at 1 atm and at 0°C for 9 h or longer. This observation can be attributed to the extremely slow rate of conversion of the phospholipids from the $L_{\beta'}$ phase to the L_c phase in multilamellar DPPC dispersions. As shown in Fig. 1, the area under the broad endothermic subtransition peak, which is proportional to the calorimetric enthalpy (ΔH), and the subtransition temperature (T_m) of DPPC dispersions depend on the incubation time. Moreover, a slowly emerging broad feature, centered at 21°C, is discernible in the calorimetric scan of DPPC dispersions that have been incubated at 0°C and at ambient pressure for 3 d or longer. The values of ΔH and T_m are plotted in Fig. 2 as a function of incubation time. The results show that the calorimetric parameters increase hyperbolically with increasing incubation time. For example, the subtransition enthalpy increases rapidly during an incubation period of 9–40 h; however, it increases at a much slower rate at longer incubation times. A similar hyperbolic curve for the increase in the subtransition enthalpy was observed for DPPC dispersions as a function of prolonged incubation times at −2°C (Ruocco and Shipley, 1982). In Fig. 2 *A*, the value of ΔH is calculated, after correcting the temperature factor in the calorimeter, from the total area including the shoulder, if any, under the broad endothermic subtransition peak. The value of T_m, shown in Fig. 2 *B*, is calculated from the temperature corresponding to the center of the broad endothermic peak when the incubation time is <3 d. After 3 d of incubation at 0°C, a smaller broad high-temperature shoulder appears and the estimated T'_m of this shoulder is also included in Fig. 2 *B* (dashed lines). As yet, we have no explanation for the appearance of this discernible shoulder of this subtransition when the sample is stored at 0°C for more than 3 d. The *y*-intercept of the plot of T_m against the reciprocal of the incubation time at 1 atm is 18.5°C (plot not shown). This extrapolated T_m at infinite incubation time, $T_m(\infty)$, is in excellent agreement with that reported by Chen et al. (1980).

Pressure Dependence of the Subtransition

The multilamellar DPPC dispersion was first subjected to a fixed hydrostatic pressure, at 0°C, by spinning the lipid sample in a centrifuge tube at the desired angular velocity, at 0°C, for a preset time interval. Following the centrifugation, the pelleted sample was first resuspended in H_2O at 0°C, and was then immediately scanned in the calorimeter at ambient pressure. The thermal behavior of the sample was then plotted as a function of the preset centrifugation time. Results indicate that both the main and the pretransition characteristics are independent of the 0°C incubation time under various pressures. It should be pointed out, however, that the time interval between the rotor deceleration and the onset of the subsequent DSC experiment is on the order of 30 min. Any pressure-induced changes of the lipid-H_2O system associated with the pre- and main phase transitions resulting from the centrifugation process are expected to have relaxed back to the original state by the time of the DSC experiment, since the relaxation times related to the pre- and main phase transition kinetics are much shorter than 30 min (Gruenewald et al., 1980; Lentz et al., 1978; Akiyama et al., 1982; Yager and Peticolas, 1982).

In contrast, the rate of appearance of the subtransition at 0°C for the multilamellar DPPC dispersion depends on

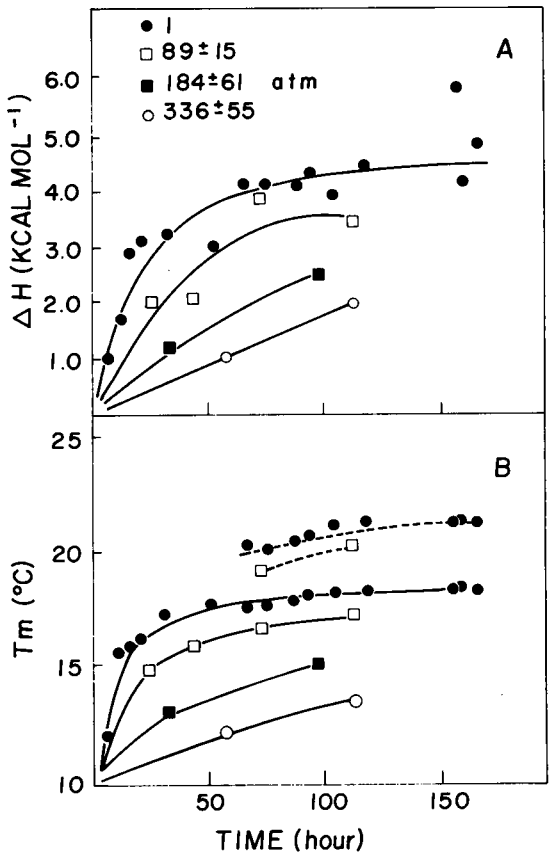

FIGURE 2 Pressure-dependent kinetics of (*A*) the subtransition enthalpy (ΔH) and (*B*) the subtransition temperature (T_m). The errors for the hydrostatic pressure are estimated from the possible variation in the height of the lipid dispersion in the centrifuge tube (± 2 mm). The dashed lines connect the data points corresponding to the transition temperature of the high-temperature shoulder of the broad endothermic subtransition peak for the DPPC dispersion obtained at various incubation times, at 0°C, and at various pressures.

the pressure. Fig 2 *A* and *B* illustrates the effect of hydrostatic pressure generated by ultracentrifugation on the subtransition ΔH and T_m plotted as a function of the incubation time at 0°C. As an example, a value of 2 kcal/mol is obtained as the subtransition enthalpy for multilamellar DPPC dispersions after incubating the lipid-H_2O system for 110 h at 0°C, under 336 atmospheric pressure, whereas this value of ΔH can be achieved by incubating the same sample, at 0°C, for only 15 h under 1 atmospheric pressure. A similar conclusion can be drawn for the subtransition T_m as shown in Fig. 2 *B*; the slow upward shift in the subtransition T_m as a function of the incubation time has been reported earlier at ambient pressure by Chen et al. (1980).

From the limited experimental data, the kinetics of the L_c phase formation at 89 ± 15 atmospheric pressure appears to be hyperbolic, whereas kinetics of the initial phase with an apparent linear form, probably due to the limited number of data points, are observed for the L_c phase formation at higher pressures (Fig. 2). Despite the different shapes of the various kinetic curves, one can conclude that the rate of formation of the crystalline L_c phase in multilamellar DPPC dispersions at 0°C decreases appreciably with increasing pressure within the centrifugation period of 4.6 d.

DISCUSSION

It is now well established that as multilamellar DPPC dispersions undergo a transition from the gel $L_{\beta'}$ phase to the crystalline L_c phase, the fully extended acyl chains of DPPC molecules are changed from a slightly less rigid quasi-orthorhombic subcell to a highly ordered two-dimensional orthorhombic packing (Ruocco and Shipley, 1982). Moreover, ^{31}P NMR experiments indicate that the ^{31}P resonance changes from an axially symmetric pattern to an axially asymmetric one in going from the $L_{\beta'}$ to the L_c phase, suggesting that the rotational diffusion of the DPPC molecule as a whole about the long molecular axis in the lamellar gel $L_{\beta'}$ phase is considerably higher than that in the L_c crystalline phase (Füldner, 1981). Besides the structural and motional changes, infrared results show that the $L_{\beta'} \rightarrow L_c$ phase transition is an extremely slow process that occurs only within a narrow temperature range below 6°C and is accompanied by a slowly progressive dehydration of the carbonyl group and by inference of the polar head group (Cameron and Mantsch, 1982). X-ray diffraction results indicate that fully hydrated DPPC loses 8 mol H_2O/mol DPPC as the hydrated $L_{\beta'}$ bilayer phase slowly converts to the L_c phase (Ruocco and Shipley, 1982). However, it is not at all clear whether the acyl chain ordering or the head group dehydration plays the more dominant role in controlling the kinetics of the $L_{\beta'} \rightarrow L_c$ phase transition in hydrated DPPC bilayers.

In this communication, we demonstrate that the slow conversion of the fully hydrated $L_{\beta'}$ bilayer phase in multilamellar DPPC dispersions to the L_c phase can be monitored calorimetrically. At ambient pressure, the subtransition enthalpy and transition temperature of the fully hydrated DPPC sample incubated at 0°C increase with increasing incubation time, and the increase, which reflects the rate of crystalline L_c phase formation, proceeds in a hyperbolic manner. An important result is the observation that the slow rate of the L_c phase formation is further retarded over a range of hydrostatic pressures generated in the centrifugation tube by adjusting the angular velocity of the centrifuge rotor.

Using the transition state theory, the effect of pressure at a constant temperature on the rate of transformation of fully hydrated DPPC from the gel $L_{\beta'}$ phase to the crystalline L_c phase at the nucleation site in the bilayer can be expressed as $(\mathrm{d} \ln k)/\mathrm{d}P = -(\Delta V\dagger)/RT$, where k is the rate constant, P is the hydrostatic pressure, R is the universal gas constant (82 cm^3 atm/°K mol), and $\Delta V\dagger$ is the change in volume of the DPPC-H_2O system, or (DPPC), in passing from the initial prenucleated state, (DPPC) $L_{\beta'}$, to the activated transition state (DPPC)† following the reaction pathway: (DPPC) $L_{\beta'} \rightleftharpoons$ (DPPC)† $\rightarrow$ (DPPC)L_c (Morild, 1981). According to this expression, the rate constant, k, decreases with increasing pressure if $\Delta V\dagger$, the volume of activation, is positive. In principle, $\Delta V\dagger$ can be calculated if both k and P are known. Because of our limited data at high pressure (Fig. 2 *A*), calculation of $\Delta V\dagger$ is unwarranted.

The fact that the rate of formation of the L_c phase at 0°C in multilamellar DPPC dispersions is retarded by pressure (Fig. 2 *A*) and the fact that the concentrations of DPPC used in these experiments are identical (16.7 mg/ml) indicate unambiguously that the $\Delta V\dagger$ value is positive. Interpretation in molecular and hence in mechanistic terms of the increase in activation volume is fraught with difficulty. However, there are two major factors that contribute to the increase in $\Delta V\dagger$ (Laidler and Bunting 1973; Low and Somero, 1975; Morild, 1981). To a first approximation, the magnitude of $\Delta V\dagger$ can be considered as the sum of contributions from the intrinsic structural change ($\Delta V_1\dagger$) and the volume change resulting from reorganization of solvent molecules ($\Delta V_2\dagger$) by the expression of $\Delta V\dagger \cong a_1 \Delta V_1\dagger + a_2 \Delta V_2\dagger$, where a_1 and a_2 are the relative (%) contribution of $\Delta V_1\dagger$ and $\Delta V_2\dagger$, respectively, to the overall activation volume. Unfortunately, both the coefficients a_1 and a_2 are quantitatively uncertain. We can, however, discuss the other terms qualitatively. First, the intrinsic structural factor, $\Delta V_1\dagger$, can be regarded as the volume increase in DPPC molecules themselves as they pass from the $L_{\beta'}$ phase into the activated transition state on the pathway to the crystalline L_c phase formation. It is known, however, that the acyl chains of DPPC molecules are packed in a more disordered manner in the lamellar $L_{\beta'}$ phase than in the crystalline L_c phase (Ruocco and Shipley, 1982); the apparent specific volume of DPPC molecules in the $L_{\beta'}$ phase is also known to be larger than

that in the L_c phase by 9 μL/g at the subtransition temperature (Nagle and Wilkinson, 1982). In fact, the phospholipid molecules with a more disordered acyl chain packing in the lamella have a larger value of specific volume. Since the subtransition temperature of DPPC bilayers is ~18°C, it is thus inconceivable that at 0°C the DPPC molecules in the less ordered $L_{\beta'}$ phase would undergo additional acyl chain disordering and hence would give rise to an increase in $\Delta V_1\dagger$ at the transition state along the kinetic pathway from the $L_{\beta'}$ phase to the highly ordered crystalline L_c phase. Instead, it is more reasonable to assume that some DPPC molecules in the lamellar $L_{\beta'}$ phase, at a temperature 18°C below its subtransition temperature, may pack more orderly to form nucleation sites and to constitute the rate-limiting step on the kinetic pathway of the formation of the highly ordered crystalline L_c phase. This concept of ordered intermediates has been widely discussed in interpreting the kinetic folding pathway of proteins (Jaenicke, 1980). The putative ordered nucleation sites in DPPC lamellae at 0°C would certainly lead to a decrease in the volume of DPPC molecules at the transition state, giving a negative $\Delta V_1\dagger$. In any case, there is as yet no theoretical or experimental evidence suggesting that the observed positive $\Delta V\dagger$ value can possibly arise from the contribution from $\Delta V_1\dagger$, the intrinsic structural factor. Second, the change in electrostriction of water molecules around the carbonyl group and polar head group of DPPC molecules can have a marked effect on the volume change ($\Delta V_2\dagger$). It should be noted that the head-group bound H_2O molecules are not icelike immobilized molecules (Borle and Seelig, 1983). Although they are more compactly packed around the lipid dipolar head group due to electrostriction, these bound water molecules are in constant exchange with the interstitial water located between the lipid lamellae. As the fully hydrated DPPC bilayer is converted from the gel $L_{\beta'}$ phase to the crystalline L_c phase, it is known that 8 mol of tightly bound water molecules per mole of DPPC at the polar lipid interface are released into the bulk water (Ruocco and Shipley, 1982). The DPPC in the activated transition state along the pathway from the gel $L_{\beta'}$ phase to the crystalline L_c phase can thus be reasonably considered to have less tightly bound water at the polar lipid interface than that in the original $L_{\beta'}$ state. When the activated (DPPC)$\dagger$ is attained, there will be some release of tightly bound water molecules, resulting in a positive $\Delta V_2\dagger$. Consequently, the volume of activation of the DPPC-H_2O system will be increased.

The fact that $\Delta V\dagger$ is positive argues strongly that the dehydration process, not the acyl chain ordering, plays a dominant role in the rate-limiting step controlling the slow kinetics of the $L_{\beta'} \rightarrow L_c$ phase transition in multilamellar DPPC dispersions. In fact, the kinetic-structural data of DPPC, in excess H_2O, at -2°C, carried out at ambient pressure by Ruocco and Shipley (1982), clearly show that the change in the bilayer periodicity from 63 Å to 59.5 Å occurs at a considerably slower rate than that of the initial change in x-ray diffraction patterns in the wide-angle region (1/4.18 and 1/4.08 $Å^{-1}$), showing that the alteration in interbilayer hydration is indeed slower than changes in acyl chain packings of DPPC molecules in bilayers accompanying the slow $L_{\beta'} \rightarrow L_c$ phase change at -2°C. It needs to be stressed, however, that the intrinsic structural changes of lipid molecules themselves ($\Delta V_1\dagger$) and the reorganization of water molecules around the polar head group of lamellar lipids ($\Delta V_2\dagger$) at the transition state generated during the prolonged incubation period at 0°C will necessarily offset each other in their overall net contribution to the total activation volume. It is the fine balance of these two factors that holds a particular lipid species, such as any of those discussed in the Introduction section, in a transition state with a distinctive magnitude of $\Delta V\dagger$ and with either a positive or negative sign in the aqueous surrounding, thus determining the specific rate of formation of the crystalline L_c phase for the particular lipid species including DPPC.

When a pressure is applied to the DPPC-H_2O system, the $P\Delta V\dagger$ term and hence the activation energy ($\Delta G\dagger$) for the transition state are increased. The effect of hydrostatic pressures is therefore to make the transition state for the $L_{\beta'} \rightarrow L_c$ phase transition reaction more difficult to reach. This is reflected in a decreased rate of conversion of hydrated DPPC from the $L_{\beta'}$ phase to the L_c phase.

Recently, the subtransition temperatures of fully hydrated lamellar DPPC with one or two deuterated acyl chains have been reported (Casal et al., 1983). Results indicate that the subtransition temperature is virtually independent of the degree of deuteration, suggesting that the head group hydration is the main driving force for the $L_c \rightarrow L_{\beta'}$ phase transition. Our pressure-dependent studies further indicate that the rate of the reversed $L_{\beta'} \rightarrow L_c$ transition in multilamellar DPPC dispersions depends primarily on the head group dehydration. Future pressure-dependent studies on other lamellar lipid species, in excess water, should be useful in elucidating the general mechanism by which the slow kinetic pathway of the crystalline L_c phase formation in the lamella may be proceeded.

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REFERENCES

Akiyama, M., Y. Terayama, and N. Matsushima. 1982. Kinetics of pretransition in multilamellar dimyristoylphosphatidylcholine vesicle. *Biochim. Biophys. Acta.* 687:337–339.

Borle, F., and J. Seelig. 1983. Hydration of *Escherichia coli* lipids.

Deuterium T. relaxation time studies of phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. *Biochim. Biophys. Acta.* 735:131–136.

Cameron, D. G., and H. H. Mantsch. 1982. Metastability and polymorphism in the gel phase of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine: A Fourier transform infrared study of the subtransition. *Biophys. J.* 38:175–184.

Casal, H. L., H. H. Mantsch, D. G. Cameron, and B. P. Gaber. 1983. On the subtransitions of deuterated derivatives of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. *Chem. Phys. Lipids.* 33:109–112.

Chang, H., and R. M. Epand. 1983. The existence of a highly ordered phase in fully hydrated dilauroylphosphatidylethanolamine. *Biochim. Biophys. Acta.* 728:319–324.

Chapman, D., R. M. Williams, and B. D. Ladbrooke. 1967. Physical studies of phospholipids. VI. Thermotropic and lyotropic mesomorphism of some 1,2-diacylphosphatidylcholines. *Chem. Phys. Lipids.* 1:445–475.

Chen, S. C., J. M. Sturtevant, and B. J. Gaffney. 1980. Scanning calorimetric evidence for a third phase transition in phosphatidylcholine bilayers. *Proc. Natl. Acad. Sci. USA.* 77:5060–5063.

Füldner, H. H. 1981. Characterization of a third phase transition in multilamellar dipalmitoyllecithin liposomes. *Biochemistry.* 20:5707–5710.

Gruenewald, B., A. Blume, and F. Watanabe. 1980. Kinetic investigations on the phase transition of phospholipid bilayers. *Biochim. Biophys. Acta.* 597:41–52.

Jaenicke, R. 1980. Protein Folding. Elsevier Publishing Company, Amsterdam, Holland. 585 pp.

Laidler, K. J., and P. S. Bunting. 1973. The Chemical Kinetics of Enzyme Action. Oxford University Press (Clarendon), London. 220–232.

Lentz, B. R., E. Freire, and R. L. Biltonen. 1978. Fluorescence and calorimetric study of phase transitions in phosphatidylcholine multilayers: kinetics of the pretransition. *Biochemistry.* 17:4475–4480.

Low, P. S., and G. N. Somero. 1975. Activation volume in enzymic catalysis: their sources and modification by low-molecular weight solutes. *Proc. Natl. Acad. Sci. USA.* 72:3014–3018.

Mantsch, H. H., S. C. Hsi, K. W. Butler, and D. G. Cameron. 1983. Studies on the thermotropic behavior of aqueous phosphatidylethanolamine. *Biochim. Biophys. Acta.* 728:325–330.

Mason, J. T., C. Huang, and R. L. Biltonen. 1981. Calorimetric Investigations of saturated mixed-chain phosphatidylcholine bilayer dispersion. *Biochemistry.* 20:6086–6092.

Morild, E. 1981. The theory of pressure effects on enzymes. *Adv. Protein Chem.* 34:93–166.

Nagle, J. F., and D. A. Wilkinson. 1982. Dilatometric studies of the subtransition in dipalmitoylphosphocholine. *Biochemistry.* 21:3817–3821.

Ross, P. P., and R. Goldberg. 1974. A scanning microcalorimeter for thermally induced transitions in solution. *Thermochim. Acta.* 10:143–151.

Ruocco, M. J., and G. G. Shipley. 1982. Characterization of the subtransition of hydrated dipalmitoyl phosphocholine bilayers. Kinetic, hydration and structural study. *Biochim. Biophys. Acta.* 691:309–320.

Seddon, J. M., K. Harlos, and D. Marsh. 1983. Matastability and polymorphism in the gel and fluid bilayer phases of dilauroyl phosphoethanolamine. *J. Biol. Chem.* 258:3850–3854.

Sen, A., D. A. Manock, D. J. Collins, P. J. Quinn, and W. P. Williams. 1983. Thermotropic phase properties and structure of 1,2-distearoylgalactoglycerols in aqueous systems. *Proc. R. Soc. Lond. B. Biol. Sci.* 218:349–364.

Serrallach, E. N., R. Dijkman, G. H. de Haas, and G. G. Shipley. 1983. Structure and thermotropic properties of 1,3-dipalmitoyl-glycero-2-phosphocholine. *J. Mol. Biol.* 170:155–174.

Stümpel, J., E. Hansjörg, and A. Nicksch. 1983. X-ray analysis and calorimetry on phosphatidylcholine model membranes. *Biochim. Biophys. Acta.* 727:246–254.

Suurkuusk, J., B. R. Lentz, Y. Barenholz, R. L. Biltonen, and T. E. Thompson. 1976. A calorimetric and fluorescent probe study of the gel-liquid crystalline phase transition in small single lamellar dipalmitoyl phosphatidylcholine vesicles. *Biochemistry.* 15:1393–1401.

Tardieu, A., V. Luzzatti, and F. C. Reman. 1973. Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases. *J. Mol. Biol.* 75:711–733.

Wong, P. T. T., and H. H. Mantsch. 1983. A low-temperature structural phase transition of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine bilayers in the gel phase. *Biochim. Biophys. Acta.* 732:92–98.

Yager, P., and W. L. Peticolas. 1982. The kinetics of the main phase transition of aqueous dispersions of phospholipids induced by pressure jump and monitored by Raman spectroscopy. *Biochim. Biophys. Acta.* 688:775–785.