Thermal History Alters Cholesterol Effect on Transition of 1-palmitoyl-2-linoleoyl Phosphatidylcholine

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ABSTRACT The effect of cholesterol on the bilayer phase behavior of heteroacid phosphatidylcholines with one unsaturated fatty acid depends on the nature of the unsaturated chain. Previous differential scanning calorimetry (DSC) studies showed that 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (16:0–18:2 PC) had a broad, weak transition at about −18°C, which was effectively eliminated by less than 15 mol % cholesterol. Phospholipids with greater and lesser degrees of unsaturation displayed stronger phase transitions and less sensitivity to cholesterol. In this work, deuterium nuclear magnetic resonance has been used to examine the phase behavior of 1-perdeuteriopalmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (16:0–18:2 PC-d_{31}) alone, and with 15 mol % cholesterol. The behavior is found to be sensitive to sample thermal history. Moderately fast cooling (1°/h) results in a continuous phase change from a fluid to an ordered phase in the pure lipid. Under similar cooling conditions, the sample containing cholesterol displays increased chain order and a continuous phase change with no apparent isothermal transition. However, when these systems are cooled at a reduced rate (0.3°/h), the continuous phase change is pre-empted by a sharp transition into a more ordered phase that gives a deuterium spectrum having intensity at a value of the quadrupole-splitting characteristic of a rigid lattice system. In the pure lipid, this transition effectively coincides with the center of the continuous phase change. Addition of 15 mol % cholesterol lowers the temperature of this sharp transition by about 3°C. These observations provide some insights into the behavior of this system seen using differential scanning calorimetry. Results of deuteron transverse relaxation measurements under these conditions are also reported.

INTRODUCTION

Because of the importance of cholesterol in a variety of cellular membranes, its influence on the structure, dynamics, and phase behavior of lipid bilayers has been investigated in a wide variety of model systems. A number of reviews have dealt with the results of such investigations (Yeagle, 1985, 1991, 1993; Bayerl and Sackmann, 1993; Finegold and Singer, 1993; O'Leary, 1993). Cholesterol has been found to have an ordering effect on chains of unsaturated phospholipids in many bilayer and monolayer systems. The result is a condensation of chain packing and a consequent increase in fluid bilayer thickness. The effect of cholesterol on phospholipid order and dynamic behavior, as observed by ^{2}H NMR, has been reviewed by Davis (1993).

There is evidence that there exists some variation, among lipid species, in the nature or magnitude of the interaction with cholesterol (de Kruyff et al., 1973; Davis and Keough, 1983). Differential scanning calorimetric (DSC) studies of the effect of acyl chain unsaturation on the phospholipid-cholesterol interaction (Keough et al., 1989; Hernandez-Borrell and Keough, 1993) have found that the degree of lipid chain unsaturation affects sensitivity of the phase behavior to cholesterol. In particular, it was found that the phase transition in 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (16:0–18:2 PC) could be effectively removed by the addition of less than 17 mol % cholesterol (Keough et al., 1989; Hernandez-Borrell and Keough, 1993). For phospholipids with both greater and lesser degrees of unsaturation, the concentration of cholesterol required to cause similar effects was greater than for 16:0–18:2 PC. It was also found, however, that the transition enthalpy of pure 16:0–18:2 PC was smaller than that of pure phospholipids with greater and lesser degrees of unsaturation.

Polyunsaturated phospholipids generally display chain-melting transitions well below 0°C. At these temperatures, kinetic effects can be important and metastability of the ordered phases may significantly complicate the observed behavior of single-component bilayers and phospholipid-cholesterol mixtures. To draw meaningful conclusions regarding the effect of cholesterol on the phase behavior of 16:0–18:2 PC, it is thus essential that the nature of the low temperature phases be established and the effect of metastability be carefully characterized. We have used ^{2}H NMR to examine the phase behavior of 16:0–18:2 PC perdeuterated on the saturated chain (16:0–18:2 PC-d_{31}) and mixtures of this phospholipid with cholesterol. Metastability and sensitivity to thermal history, in these systems, have been investigated by varying cooling rates and equilibration times. These observations have been compared and contrasted with ones made by DSC using typical protocols. It is found, for dispersions of 16:0–18:2 PC, that the calorimetrically observed peak in the pure lipid excess heat capacity may not correspond to a normal gel-liquid crystal transition and that the behavior of the system is sensitive to cooling

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history. If the sample is cooled sufficiently slowly, a sharp transition to a highly ordered phase can be observed. This highly ordered phase is similar to the lamellar crystalline or subgel (Lc) phase observed in saturated diacyl phospholipids at low temperature. For sufficiently slow cooling, the transition to this phase is not eliminated by 15 mol % cholesterol.

MATERIALS AND METHODS

The phospholipid 16:0–18:2 PC-d_{31}, perdeuterated on the saturated chain, was purchased from Avanti Polar Lipids (Pelham, AL). A single band, corresponding to PC, was observed on thin layer chromatography (Keough and Davis, 1979) of this lipid and the lipid was used without further purification. Acyl chain migration as measured by phospholipase A_2 hydrolysis (Davis and Keough, 1979) was found to be less than 18% in these samples.

Phospholipid and cholesterol were co-dissolved in chloroform, which was then removed under nitrogen. The samples were then evacuated in the presence of P_2O_5 in the dark for at least 8 h. Lipid dispersions were prepared by vortexing the dried film at room temperature in deionized, doubly distilled water from which oxygen had been purged by bubbling with argon. The resulting dispersions were transferred to 8-mm NMR tubes, which were then sealed under an argon atmosphere. Each sample contained about 25 mg of lipid in 200 μl water.

^{2}H NMR spectra were obtained at 23.215 MHz using the quadrupole echo pulse sequence (Davis et al., 1976) on a locally constructed spectrometer with a superconducting solenoid (Nalorac Cryogenics, Martinez, CA). The sample coil was mounted on the NMR probe within a copper oven, the temperature of which was controlled by a microcomputer. Transients were digitized using a Nicolet 2090A digital oscilloscope interfaced to a second microcomputer. Oversampling by a factor of two (Prosser et al., 1991) was used to give an effective digitizer dwell time of 2 s for most spectra collected. For the majority of spectra, 4000 transients were collected with a repetition time of 0.5 s. After the NMR experiments, the samples used were found to migrate as single spots by thin layer chromatography.

The quadrupole echo pulse sequence consists of two $\pi/2$ pulses, with a phase difference of 90°, separated by an interval, τ, which was 40 μs for spectral studies and between 40 μs and 400 μs for measurements of chain deuteron average transverse relaxation rates. The $\pi/2$ pulse lengths were between 2.1 μs and 2.5 μs. The amplitude of the quadrupole echo, at time 2τ after the initial pulse, decays according to

$$A(2\tau) = A(0)e^{-2\tau\langle R_{2e}\rangle} \quad (1)$$

where $\langle R_{2e}\rangle$ is the mean transverse relaxation rate for deuterons along the chain. Effective transverse relaxation times, $T_{2e} = \langle R_{2e}\rangle^{-1}$ were estimated from the initial slope of each decay curve.

Using a Perkin-Elmer DSC II instrument, DSC measurements were also performed on some of the samples used in the NMR experiments. Samples consisting of between 0.6 mg and 1.1 mg of the dispersions used to obtain the NMR spectra were loaded into aluminum sample pans and sealed under argon gas. These were cooled from 27°C to −2°C at 5°/min and subsequently cooled from −2°C to −33°C at 5°/min or 0.3°/h. Samples cooled at 5°/min were immediately scanned from −33°C to −0.3°C at 5°/min. Samples cooled at 0.3°/h were held at −33°C for 3 h and then scanned from −33°C to −0.3°C at 5°/min. The phospholipid content in the sample pans was determined by measurement of total phosphorus after extraction of the sample pan contents with chloroform-methanol (2:1, v/v).

RESULTS

Fig. 1, *A* and *B* show DSC traces for 16:0–18:2 PC-d_{31} in the absence of cholesterol and with 15 mol % cholesterol.

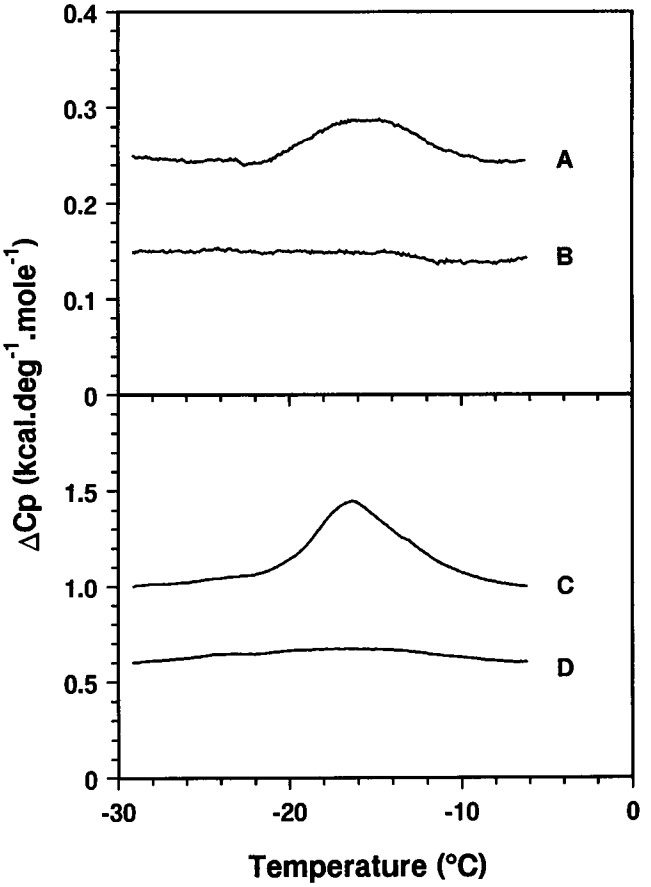

FIGURE 1 Differential scanning calorimetric traces for (*A*) 16:0–18:2 PC-d_{31} and (*B*) 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol obtained after cooling of the samples at 5°/min, and for (*C*) 16:0–18:2 PC-d_{31} and (*D*) 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol obtained after cooling of the samples at 0.3°/h.

These scans were obtained using a conventional DSC cooling protocol and are consistent with the behavior reported previously (Keough et al., 1989; Hernandez-Borrell and Keough, 1993). The samples were cooled relatively quickly (5°/min) to −33°C in the DSC II instrument and then scanned upward at a rate of 5°/min. The scans were repeated three times. The second and third scans were consistent. The traces shown were obtained from the third cycle. In the absence of cholesterol, the observed transition is centered slightly below −16°C and is of low enthalpy (ΔH = 0.9 kcal/mol) and several degrees wide ($\Delta T_{1/2}$ = 5.0°). With the addition of 15 mol % cholesterol, the transition is effectively unobservable. The scans shown in Fig. 1, *C* and *D* were obtained with slower cooling and are discussed below.

For polyunsaturated lipid samples with low transition temperatures, cooling rate can be an important determinant of the bilayer state at the beginning of the DSC scan. To investigate the effect of thermal history on the phase behavior of such bilayers, three series of ^{2}H NMR spectra, corresponding to different thermal histories, were collected. Fig. 2, *A*, *C*, and *E*, show ^{2}H NMR spectra for 16:0–18:2 PC-d_{31}. Fig. 2, *B*, *D*, and *F*, show ^{2}H NMR spectra for

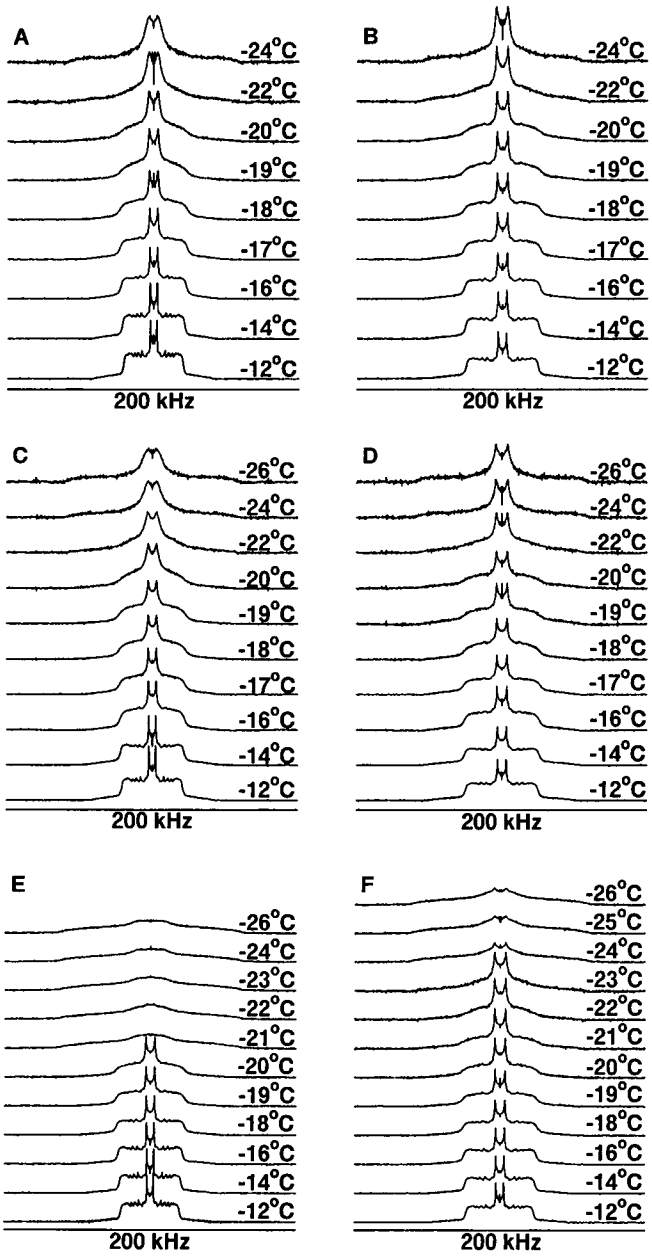

FIGURE 2 ^{2}H NMR spectra of 16:0–18:2 PC-d_{31} (*A*, *C*, and *E*) and 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol (*B*, *D*, and *F*). Spectra in *A* and *B* were obtained while cooling the samples with an effective rate of about 1°/h. Spectra in *C* and *D* were obtained while warming the samples from −26°C. Spectra in *E* and *F* were obtained while cooling the samples with an effective rate of about 0.3°C/h.

16:0–18:2 PC-d_{31} containing 15 mol % cholesterol. Spectra in Fig. 2, *A* and *B* were collected while cooling at an effective rate of about 1°/h. The pure lipid spectrum at −12°C is a superposition of Pake doublets indicating axially symmetric chain reorientation on a time scale short compared with the characteristic time for the deuterium NMR experiment. Liquid crystalline spectra from the cholesterol-containing sample display increased quadrupole splittings, which reflect cholesterol-induced chain ordering. These spectra also show some rounding of those doublets having the largest quadrupole splittings. The distortion of these doublets indicates that the correlation times for chain motion near the headgroup end of the chain are approaching the characteristic timescale of the ^{2}H NMR experiment ($\approx 10^{-5}$ s).

In Fig. 2, *A* and *B*, cooling causes a continuous broadening of the spectrum into a largely featureless distribution characteristic of axially asymmetric motion on a time scale comparable to or longer than the ^{2}H NMR timescale. Such spectra are generally characteristic of an ordered, but nonrigid, phase. The central doublet, however, is more prominent than is normally seen in the gel phase of diacyl phospholipids, suggesting that the chain methylene deuteron transverse relaxation rates rise more quickly with decreasing temperature than those for the methyl deuterons. Over this temperature range, the chain motion correlation times are becoming intermediate on the quadrupole echo time scale.

The liquid crystal spectrum of a chain deuteron displays characteristic axially symmetric powder pattern edges corresponding to molecules reorienting about axes perpendicular to the applied magnetic field. The splitting of these edges, $\delta\nu_Q$, is proportional to the orientational order parameter. The first spectral moment of the spectrum for a chain-perdeuterated sample is given by Davis (1983)

$$M_1 = \frac{4\pi}{3\sqrt{3}}\langle\delta\nu_Q\rangle \qquad (2)$$

where the average is over the chain deuterons. The temperature dependence of M_1 can provide insights into bilayer phase behavior. Fig. 3 *A* shows the temperature dependence of M_1 for spectra displayed in Fig. 2, *A* and *B*. The chain ordering effect of cholesterol increases M_1 values in the liquid crystalline phase of the cholesterol-containing sample. Neither sample shows a discontinuity in M_1 as might be expected for a phase transition, although there is a barely discernible change in slope near −18°C for the pure phospholipid sample. The continuous phase change seen in the pure lipid sample with ^{2}H NMR may be consistent with a very small excess heat capacity as observed by DSC.

At low temperature, equilibration in the ordered phase may be kinetically hindered. To determine whether the observations described above were sensitive to such effects, the ^{2}H NMR measurements were repeated while warming the sample after initially cooling to −26°C. ^{2}H NMR spectra and corresponding M_1 results are displayed in Fig. 2, *C* and *D*, and Fig. 3 *B*, again for 16:0–18:2 PC-d_{31} and for 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol. The spectra are qualitatively similar to those seen on cooling. First spectral moments corresponding to these spectra are shown in Fig. 3 *B*. The M_1 values seen in the ordered phase are slightly higher than those seen in the first series of experiments, and the change in slope of M_1 versus T near −20°C for the pure lipid is more apparent. The pure lipid

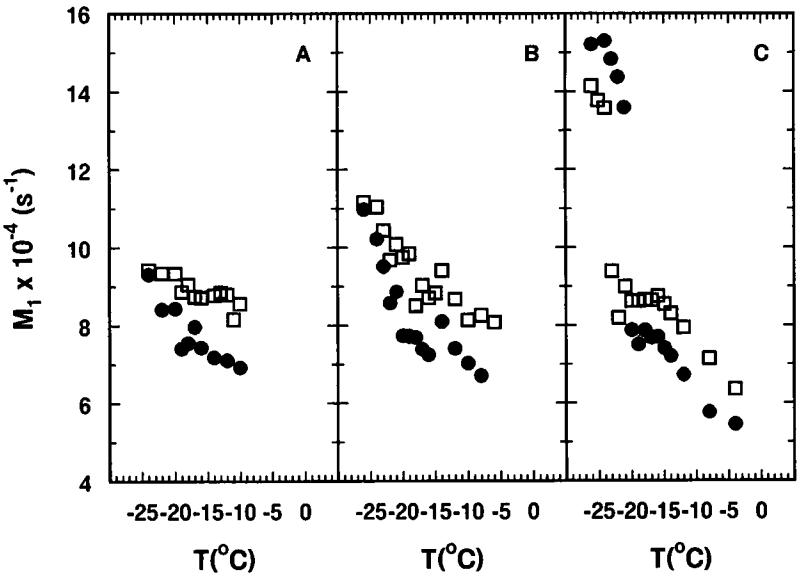

FIGURE 3 (*A*) Temperature dependence of first spectral moments (M_1) corresponding to the spectra in Fig. 2, *A* and *B*. (*B*) Temperature dependence of first spectral moments (M_1) corresponding to the spectra in Fig. 2, *C* and *D*. (*C*) Temperature dependence of first spectral moments (M_1) corresponding to the spectra in Fig. 2, *E* and *F*. Solid circles (●) correspond to 16:0–18:2 PC-d_{31}, and open squares (□) correspond to 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol.

phase change is still continuous and there is no clear indication of a transition in the presence of cholesterol.

The ^{2}H NMR measurements described above are consistent with the calorimetric evidence for a weak transition in the pure lipid and effectively no observable transition in the presence of cholesterol. Indeed, under the conditions of the experiments illustrated by Fig. 2, *A–D*, a continuous phase change rather than an isothermal phase transition is observed. A strikingly different picture emerges if the samples are cooled more slowly.

Fig. 2, *E* and *F* show ^{2}H NMR spectra for 16:0–18:2 PC-d_{31} and 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol collected as the samples were cooled more slowly than in the experiments described above. Each time the temperature was changed, the sample was allowed to equilibrate for about 30 min. Mean transverse relaxation rates were measured using transients collected with five different quadrupole echo pulse separations. The final pulse separation, 40 μs, was used to obtain the spectra displayed in Fig. 2, *E* and *F*. One consequence of this protocol was that the effective cooling rate in the neighborhood of −20°C, for these experiments, was about 0.3°/h. Fig. 3 *C* shows the temperature dependence of the first spectral moments corresponding to these spectra.

Spectra for the pure lipid sample in Fig. 2 *E* show the effect of chain ordering and slowing of chain reorientation as the sample is cooled toward −20°C. Between −20°C and −21°C, however, there is a sharp change from a distorted liquid crystalline spectrum to a spectrum with significant intensity out to ±63 kHz, which suggests the presence of a very ordered phase likely analogous to the L_C (or subgel) phase of saturated diacyl phospholipids in which motions of the chain methylene groups are slow on the ^{2}H NMR timescale (Davis, 1979; Davis, 1983; Morrow and Davis, 1987). The central feature, with a width of about a third of the rigid lattice spectrum, arises from deuterons on the methyl group, which still undergoes fast reorientation about its threefold symmetry axis. In 1-palmitoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (16:0–16:0 PC-d_{62}), gel-phase values of M_1 typically range from about 1.0×10^5 s^{-1} to 1.3×10^5 s^{-1}, while the appearance of the L_C phase raises M_1 toward 1.6×10^5 s^{-1} (Morrow et al., 1992). The values of M_1 for the pure 16:0–18:2-PC-d_{31} sample in the ordered phase fall in the range from 1.3×10^5 s^{-1} to 1.6×10^5 s^{-1}, and thus provide additional evidence that the transition observed for this lipid is into an L_C-like phase.

The spectra in Fig. 2 *F* for the sample containing cholesterol show a more complex behavior. From −12°C to −23°C, the spectra change continuously from being a superposition of axially symmetric powder patterns, characteristic of the liquid crystalline phase, to a broad spectrum, characteristic of asymmetric motion on a timescale close to the characteristic timescale for ^{2}H NMR. The prominence of the methyl feature in the spectrum for the sample containing cholesterol at −23°C reflects the divergence of methyl and methylene transverse relaxation times. There is relatively little intensity at ±63 kHz in the −23°C spectrum. It would thus appear that, above −23°C, the sample containing cholesterol undergoes a continuous change into a gel-like phase.

Between −23°C and −24°C, however, the cholesterol-containing sample displays a sharp transition into a much more ordered phase indicated by a broadening of the methyl feature, a drop in its relative intensity, and by the appearance of intensity at ±63 kHz. Fig. 3 *C* also shows that cholesterol increases chain order in the liquid crystalline phase and decreases the magnitude of the jump in order at the transition.

From the results presented above, it would appear that a complete description of the 16:0–18:2 PC-d_{31} phase behavior requires the consideration of two nearly coincident phase changes. Equilibration in the most ordered L_C-like phase

appears to be slow below the transition temperature. Rapid or even moderately fast cooling results in the sample being frozen into a less ordered gel-like phase. If the cooling rate is sufficiently low, chain order and motional correlation times increase continuously in the liquid crystalline state, but the transition that ultimately occurs is directly from the liquid crystalline to the highly ordered L_C-like phase. In the presence of cholesterol, the transition into the L_C-like phase is depressed by about 3°, and the sample changes continuously from liquid crystal-like to gel-like before the transition into the more highly ordered L_C-like phase occurs.

Transverse relaxation behavior is particularly sensitive to the continuous phase change seen in these samples. The flattening of M_1 versus T, just above the transitions in Fig. 3 *C*, is similar to behavior previously observed in phospholipid-gramicidin mixtures, which undergo a continuous phase change rather than a discontinuous phase transition (Morrow, 1990). It reflects an increasing dependence of transverse relaxation rate on position along the chain as the sample approaches the transition and the mean transverse relaxation rate increases. Because of this effect, some care must be taken when relating M_1 data, near the transition, to chain order.

Effects of transverse relaxation are illustrated in Figs. 4 through 6. Fig. 4 shows the dependence of spectral area on 2τ for both samples at a series of temperatures. Corresponding effective transverse relaxation times are shown in Fig. 5. If correlation times, τ_c, for those motions responsible for transverse relaxation satisfy $\Delta M_2 \tau_c^2 \ll 1$ where ΔM_2 is that part of the spectral second moment modulated by the motions concerned, then the quadrupole echo relaxation time is given by Pauls et al., (1985)

$$\frac{1}{T_{2e}} = \Delta M_2 \tau_c. \tag{3}$$

For both samples, the temperature dependence of T_{2e} in the liquid crystalline phase thus indicates a continuous increase in correlation time with decreasing temperature. The transverse relaxation time approaches a minimum as $\Delta M_2 \tau_c^2$ approaches unity.

Cooling a sample through a bilayer phase transition usually causes a discontinuous increase in the correlation times for those motions responsible for transverse relaxation. Below the transition, the condition $\Delta M_2 \tau_c^2 \gg 1$ is generally satisfied so that T_{2e} is proportional to τ_c, and thus increases with decreasing temperature. The observed behavior for 16:0–18:2 PC-d_{31} differs slightly from that seen in bilayers of saturated diacyl phospholipids, such as 1-myristoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (14:0–14:0 PC), where T_{2e} remains relatively constant in the fluid phase, drops sharply at the transition, and then rises more slowly than is seen for 16:0–18:2 PC. In the case of 1-myristoyl-2-myristoyl-*sn*-glycero-3-phosphocholine, the motions in the gel phase have correlation times closer to $(\Delta M_2)^{-1/2}$ than in the liquid crystalline case, and thus give rise to values of T_{2e} close to its minimum. For the samples of

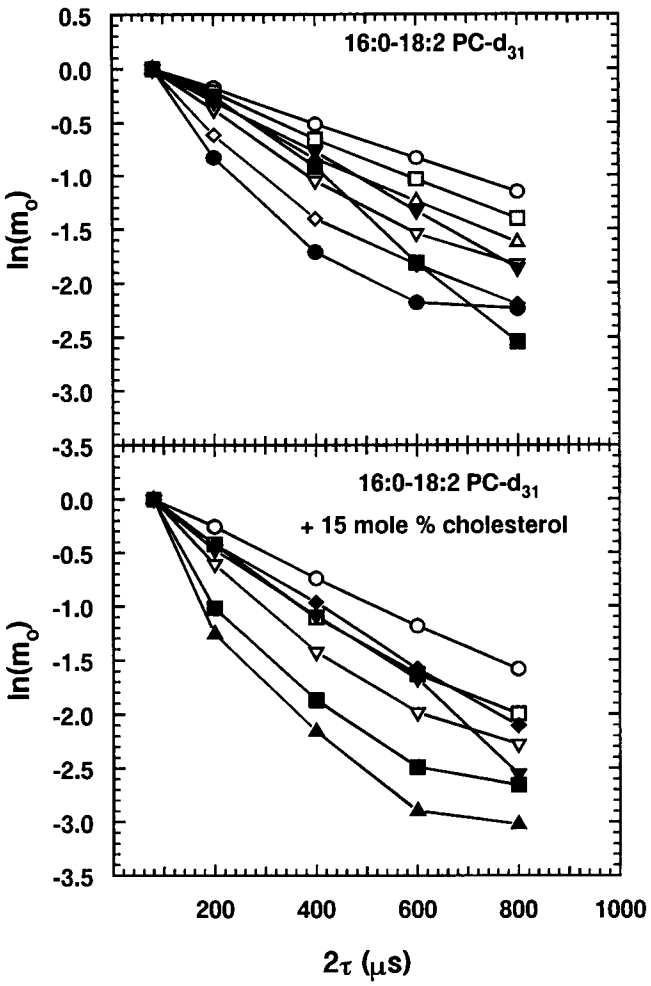

FIGURE 4 Dependence of the quadrupole echo amplitude on twice the quadrupole echo sequence pulse separation for a) 16:0–18:2 PC-d_{31} and b) 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol for a series of temperatures. ○, −12°C; □, −16°C; △, −17°C; ▽, −18°C; ◇, −19°C; ●, −20°C; ■, −21°C; ▲, −23°C; ▼, −24°C; ◆, −26°C.

16:0–18:2 PC-d_{31}, with and without cholesterol, it is the motions in the fluid phase for which correlation times approach $(\Delta M_2)^{-1/2}$ so that it is in the fluid phase that T_{2e} approaches a minimum.

The continuous slowing of chain motion with decreasing temperature just above the transition is accompanied by a strong variation in T_{2e} with position along the chain. Deuterons with the largest quadrupole splittings are the first to display a decrease in transverse relaxation time as the sample is cooled toward the transition. The result, in a quadrupole echo experiment, is a relative suppression of doublets with larger splittings and thus a strong dependence of M_1 on quadrupole echo pulse separation, τ, just above the transition. This sensitivity is illustrated in Fig. 6, which shows the dependence of M_1 on 2τ for both samples over a series of temperatures. In Fig. 3 *C*, this effect gives rise to a suppression of M_1 in the temperature range just above the transition where the dependence of M_1 on quadrupole echo pulse separation is strongest.

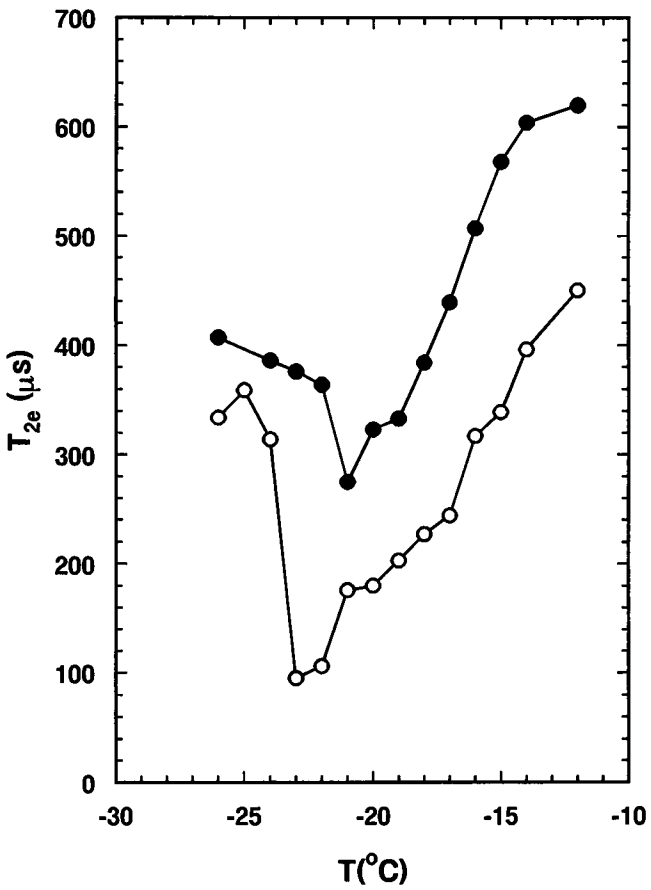

FIGURE 5 Temperature dependence of transverse relaxation times (T_{2e}) corresponding to the inverse mean transverse relaxation rate for chain deuterons obtained from the initial slope of quadrupole echo decays for 16:0–18:2 PC-d_{31} (●) and 16:0–18:2 PC-d_{31} (○) containing 15 mol % cholesterol.

These NMR results show that the low temperature states of the 16:0–18:2 PC-d_{31} bilayer, with and without cholesterol, are sensitive to the way in which the sample is cooled. DSC measurements should reflect this sensitivity to cooling protocol. To test this expectation, DSC experiments were repeated using a very slow cooling protocol before the start of the heating scan. Lipid dispersions were cooled stepwise at an effective cooling rate of 0.3°/h from −14°C to −33°C, and held at −33°C for 3 h. Samples were then scanned at 5°/min from −33°C to obtain the thermograms shown in Fig. 1, *C* and *D*. When cooled slowly, pure 16:0–18:2 PC-d_{31} dispersions yielded heating endotherms centered at −14.9°C that were nearly as broad as those observed following the quicker cooling protocol. However, the observed transition enthalpy, 1.7 kcal/mol, was greater in accord with the ^{2}H NMR results following slow cooling. The enthalpy associated with the calorimetrically-observed transition in the slowly cooled DSC sample may result from the transformation of a highly ordered phase, with some properties similar to L_C, directly into the liquid crystalline phase. If the more rapid cooling used in the earlier DSC experiments results in the bilayer being frozen into the gel phase, then the smaller transition at −16°C represents a change from gel to liquid crystal.

When 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol was cooled rapidly and scanned, no transition endotherm could be detected. When this dispersion was cooled more slowly, the resulting trace had a shape which, given the operating characteristics of the instrument in the range required, could not be unequivocally differentiated from some curvature in the operating baseline. Slow cooling before the DSC scan thus appears to have some effect on the pure 16:0–18:2 PC results, but no obvious effect on DSC observations with the cholesterol-containing system.

DISCUSSION

It is clear that the observed behavior is sensitive to the thermal history of the sample. For pure 16:0–18:2 PC, there appears to be a threshold cooling rate beyond which the sample undergoes a continuous ordering from liquid crystal into a gel-like phase. Once in the partially ordered state, low chain mobility may hinder complete rearrangement into a more ordered, crystalline state. The ability of the sample to enter the more ordered L_C-like phase appears to depend on the length of time during which it is held close to −20°C. At this temperature, the more ordered phase is stable and there is apparently sufficient mobility to allow the chains to rearrange into the more ordered state. In the presence of cholesterol, the continuous phase change appears to proceed further before the transition into the highly ordered L_C-like phase intervenes.

The low transition temperatures in these systems presumably reflect the influence of the double bonds in the 18:2 chain on the free energy of the ordered phase. Unsaturation may also influence the kinetics of this transition. Even at a temperature for which the ordered phase has the lowest free energy, and should thus be the equilibrium phase, the packing of the chains into the ordered structure is slow. This is due, in part, to the temperature at which the transition occurs but may also reflect the extent of molecular rearrangement required to accommodate double bonds in an ordered phase. The transition in 16:0–18:2 PC does, however, proceed rather quickly compared with the formation of the subgel phase in saturated diacyl phosphatidylcholines which, typically, is observable by DSC only after an incubation time on the order of days (Finegold and Singer, 1984; Finegold and Singer, 1986; Lewis and McElhaney, 1990). Tristram-Nagle et al. (1994) have studied the sensitivity of subgel formation kinetics to different cooling protocols. Based on their results, relatively little subgel formation would be expected for 1-palmitoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine after an incubation of only 3 h. The apparently quicker equilibration in 16:0–18:2 PC-d_{31} may reflect the fact that the transition on cooling is directly from a liquid crystalline phase in which the chains are still somewhat mobile, rather than from an intermediate gel phase in which some restriction of chain motion is already present.

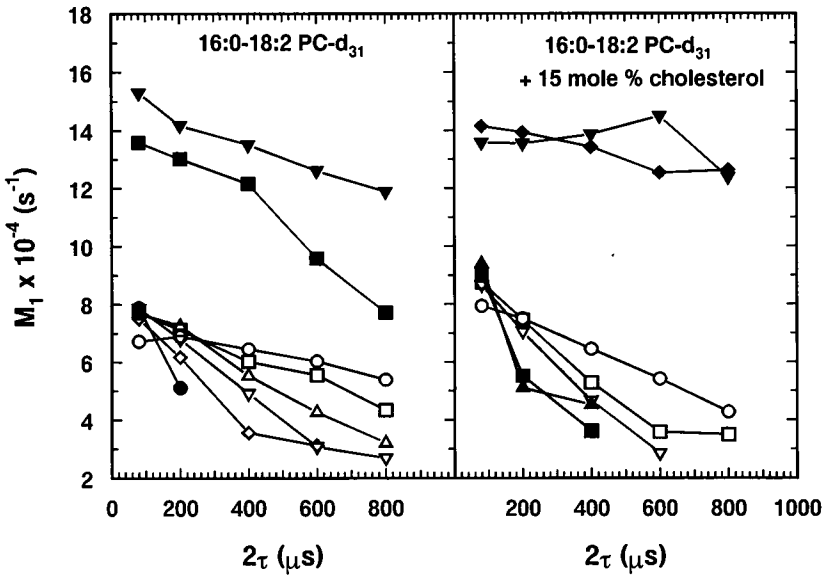

FIGURE 6 Dependence of spectral first moment (M_1) on twice the quadrupole echo sequence pulse separation for a) 16:0–18:2 PC-d_{31} and b) 16:0–18:2 PC-d_{31} containing 15 mol % cholesterol for a series of temperatures. ○, −12°C; □, −16°C; △, −17°C; ▽, −18°C; ◇, −19°C; ●, −20°C; ■, −21°C; ▲, −23°C; ▼, −24°C; ◆, −26°C.

The NMR spectra collected while slowly cooling the sample containing cholesterol show a continuous phase change to a partially ordered gel-like phase. This continuous change is effectively complete by −24°C, the temperature at which the transition to the more ordered crystalline phase occurs. The depression of the transition, by roughly 3°, by cholesterol may indicate an interesting difference between the highly ordered phase in 16:0–18:2 PC and the L_C phase in saturated diacyl phosphatidylcholines. In the L_C phase of saturated diacyl phosphatidylcholines, the chains are in an all-trans conformation. The unsaturated chain of 16:0–18:2 PC presumably influences the symmetry and complicates packing in the highly ordered phase. The presence of cholesterol, a roughly cylindrical and effectively rigid molecule, may further complicate packing of the ordered 18:2 chains and contribute to a lowering of the phase transition temperature.

Prior slow cooling did not have as strong an effect on DSC measurements as slow cooling during NMR measurements. Metastability, the effect of the finite DSC scan rate, and differences in the absolute calibrations of the NMR and DSC temperature controllers are some factors that may have contributed to the observed differences in the transition temperatures. However, in light of the stability of the liquid crystalline phase during relatively prolonged incubation of the sample at temperatures slightly above −20°C in the course of the NMR measurements, the pure 16:0–18:2 PC-d_{31} transition temperature is not likely to be far above this temperature.

While DSC scans following slow cooling showed increased transition enthalpy for 16:0–18:2 PC-d_{31}, the transition remained unobservable for the cholesterol-containing sample. The NMR results suggest that cholesterol hinders formation of the more highly ordered L_C-like phase and this may account for the apparent absence of a calorimetrically observable transition in the presence of cholesterol. Another possible explanation for the behavior of the cholesterol-containing sample is related to the apparent separation of the transition into a continuous change of chain order and a more abrupt change in packing. The latter transition has a strong effect on M_1. However, if the transition enthalpy is primarily associated with the change in chain order, the spreading of that part of the continuous part of the transition over a broad temperature range might render it effectively unobservable under the conditions of the DSC experiment.

CONCLUSIONS

The results obtained indicate that the behavior observed for 16:0–18:2 PC is dependent on sample history. Under conventional DSC cooling and heating regimes, the observed transition is fairly uncooperative and may reflect a change from some sort of intermediate gel-like state to the liquid crystal. Alternatively, it may reflect melting of material both in a highly ordered, low temperature (crystal) phase and in the gel state with the magnitude of the transition being determined by the thermal history.

If the gel state is more disordered than the gel states of saturated PC lipids, the apparent influence of cholesterol on the transition may appear to be relatively greater than for other PC systems with more ordered gel states. The distinction between the relatively disordered gel of the unsaturated lipid and the liquid crystalline phase in the presence of cholesterol-induced ordering might be small leading to the effective removal of the gel-liquid crystal transition at relatively lower cholesterol concentrations. Once a high degree of order is achieved in the solid phase, however, the influence of cholesterol may be of a magnitude that is similar to the influence seen in other systems studied previously.

This unsaturated PC appears to enter a state similar to the L_C phase of saturated PC in a substantially shorter time than

that required for saturated PC. This raises interesting questions about intrachain motions in these lipids which, while not yet understood, might have important implications for motions in these lipids at higher temperatures relevant to those in biological membranes.

In light of the observations presented here, some care must be taken, when comparing DSC results for this system and related unsaturated phospholipid systems, to ensure that the transitions being compared are, in fact, equivalent.

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