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Calorimetric and nuclear magnetic resonance study of the phase behaviour of dilauroylphosphatidylcholine / water

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The phase behaviour of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) has been investigated by high sensitivity differential scanning calorimetry, ^{2}H nuclear magnetic resonance and X-ray diffraction. Depending on the thermal history of the sample, as many as three specific heat maxima are observed by DSC. Only two of these occur after prolonged incubation at temperatures below −6 °C or after repeated temperature cycling. The total enthalpy of these two peaks, 6.75 kcal / mol DLPC, and the changes in orientational order observed by ^{2}H-NMR, suggest that both of these peaks involve hydrocarbon chain melting and the structural reorganization that occurs in the sub-transition of longer chain diacylPC.

Introduction

Differential canning calorimetry and nuclear magnetic resonance are a powerful combination for the study of phase equilibria in simple two component mixtures [1–3]. Calorimetry provides important thermodynamic quantities, such as melting point temperatures and transition enthalpies, while NMR is sensitive to the detailed changes in molecular orientational order and dynamics which often occur when phase boundaries are crossed. These two techniques have been applied to a number of lipid/water mixtures, especially the diacylphosphatidylcholines (diacylPC) [4–6]. While some calorimetric studies on 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) have been reported [7,8], a comparison of the calorimetric behaviour with the observations of ^{2}H-NMR has not yet been published.

The hydrocarbon chains of DLPC, having only twelve carbons, are close to the minimum length required for bilayer formation by diacylPC. It has been suggested that the chain melting transition of DLPC may occur close to a critical point and that it may be possible to drive the transition into the critical region by changing the ionic environment [9]. Clearly, DLPC is one of the most interesting phospholipids and the description of its phase behaviour may provide important tests for the theories of lipid phase transitions and bilayer stability.

In an early DSC study of diacylPC [8], Mabrey and Sturtevant found that DLPC, in 15% ethylene glycol, exhibited a sharp endotherm at −1.8 °C, with an enthalpy of 1.7 kcal/mol, which agreed well with the linear relation between transition enthalpy and chain length observed for other di-

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acylPC. However, they also observed a broader peak near 4°C, and reported that the total enthalpy, integrated from −4 to 10°C, was 4.77 kcal/mol. Over 60% of the enthalpy was associated with this broad peak whose origin is still uncertain. Recently, Finegold and Singer [10] have attributed the broad higher temperature feature to a liquid-crystal phase metastability analogous to the gel phase metastability associated with the subtransition observed in longer chain PC [11].

In an effort to understand more clearly the phase behaviour of DLPC we have begun a series of experiments using DSC, ^{2}H-NMR and X-ray diffraction.

Materials and Methods

Proteated DLPC was obtained from Sigma (Sigma-Aldrich Corp., St. Louis, MO). Analysis by thin-layer chromatography showed it to run as a single spot, so it was used without further purification. Deuterium-labelled lauric acid was prepared either by the method of Hsiao et al. [12], for perdeuteration, or, for specific α-deuteration, was labelled by exchange with $KO^2H/^2H_2O$ at 180°C [13]. The phospholipid was then synthesized from deuterated lauric anhydride using the method of Gupta et al. [14]. The final purification was performed on a 1.5 m LH-20 column (Pharmacia, Uppsala, Sweden), eluting with 100% ethanol. For storage, part of the ethanol is removed by rotary evaporation, the remaining ethanol/lipid mixture is kept at −20°C until use. Thin-layer chromatography was again used to verify the purity of the lipid.

High sensitivity DSC experiments were performed on a modified MC-1 calorimeter (Microcal, Amherst, MA) interfaced to an 8085 based microcomputer used for data storage and analysis. Samples were prepared by weighing about 5 mg of dry lipid into the removable DSC sample cell and adding a quantity of 50 mM phosphate buffer at pH 7.0 to achieve the desired lipid/water molar ratio. In addition, two samples were prepared from buffer which had been mixed with 15% (by volume) ethylene glycol. Samples are then mixed by centrifugation and vortexing. This was found to be the most satisfactory method of maintaining the sample composition while ensuring sample reproducibility.

Unless otherwise noted, the sample was then incubated for at least 6 h at temperatures between −4 and −7°C. This incubation was performed in the calorimeter head which has been installed in a small refrigerator. After the incubation period, the DSC scans were performed at the slowest available scan rate, approx. 7 K/h, as measured by the microcomputer. At this scan rate, with incubation near −6°C, the calorimeter will have stabilized by the time the temperature reaches approx. −4.5°C.

NMR samples were prepared by mixing approx. 60 mg of dry lipid with the appropriate volume of buffer. These were mixed by gentle stirring with a fine glass rod. ^{2}H-NMR spectra were obtained with the quadrupolar echo technique [15], using two 4.0 μs, 90° pulses separated by 40 μs. The recycle delay was 0.9 s. Other aspects of the NMR experiments have been described previously [16]. The spectra were taken at a ^{2}H resonance frequency of 41.3 MHz. The sample and resonance coil were completely enclosed by a copper oven whose temperature can be readily set and controlled to within 0.1 K, although absolute temperature calibration is somewhat less accurate.

X-ray diffraction measurements were performed on samples of normal DLPC between −8°C and +8°C to insure that the samples remained in a bilayer phase as the temperature and water composition were varied. Samples were mixed as for the NMR samples, and gentle centrifugation was then used to force the mixture into the bottom of 2 mm diameter capillary tubes. The sample tubes were placed into an X-ray camera whose temperature was regulated by a circulating bath. The results verified that the samples were lamellar under all experimental conditions studied.

Results and Discussion

The two DSC traces shown in Fig. 1 were obtained with a sample containing 75 water molecules per lipid. The lower trace shows a sharp peak at approx. −2°C, labelled A in the figure, and a broad feature near 4°C, similar to the results reported earlier [8]. This sharp low-temper-

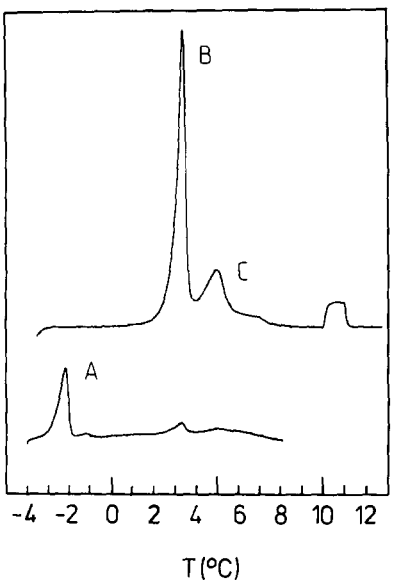

Fig. 1. High sensitivity DSC scans of DLPC/water at a molar ratio of 1 : 75. The lower trace is the first scan following sample preparation and incubation for about 6 h at temperatures below −6 °C. The upper trace is that obtained from subsequent scans of the same sample after similar incubation times. The scan rate is the same for both traces; approx. 7 K/h.

ature feature frequently occurs on the very first heating scan of a newly prepared sample. However, after incubating at temperatures below −6 °C for one week, the A feature was not observed even for the first heating scan. Subsequent scans, even after prolonged incubation (as long as one week) at temperatures between −4 and −7 °C, show only the higher temperature features, labelled B and C, in the upper trace of the figure. These two features occur reproducibly in all samples, whereas the feature labelled A never reappears once it has gone (occasionally a weak A peak is seen in the second heating scan). It is conceivable that cycling the sample through the range of temperatures from −7 to +10 °C results in the depression of the temperature of this peak. However, we can say that, in our samples, feature A never recurs at temperatures above about −4.5 °C. It is interesting to note that adding buffer to a few samples which no longer exhibited the A peak (to increase the water/lipid ratio), followed by the usual mixing procedure, caused the reappearance of peak A during the first heating scan of the newly mixed sample. Using 15% (by volume) ethylene glycol in the buffer used to prepare the samples does not significantly change the observed results. It does, however, permit incubation at lower temperatures. For the two samples prepared using ethylene glycol, we find that peak A does not reappear at temperatures above −7 °C.

A comparison of the areas under the calibration pulse with the total areas under each of the two traces in Fig. 1 indicates that there is a dramatic increase in the amount of heat required to warm the sample from −4 °C to +10 °C in the upper trace, i.e., in the situation where the A feature is absent. Whenever the A feature occurs, the area under the B and C features is relatively small, as shown in the figure. Thus, it appears that the three peaks, A, B and C, are somehow coupled. In addition, the low temperature phase seems to attain a lower enthalpy after cycling through this temperature region one or two times, but further cycling has no effect, and only the B and C features appear.

As the water (buffer) concentration is varied, there is a dramatic change in the relative areas of the B and C features. Fig. 2a shows DSC scans for samples at 10 different water concentrations, ranging from $W/L = 30$ to $W/L = 175$ (waters/lipid). These scans are typically the third or fourth heating scan at each concentration, so there is no A feature in any of them (here we display the scans only above 0 °C even though the scans all began at between −3 and −4.5 °C). At 30 waters/lipid, we observe only the B feature, but as the water content increases, the C feature grows at the expense of B. The ratio of the area of C to the total area increases monotonically with water concentration until we reach $W/L = 125$, after which the ratio remains approximately constant. The total area under both B and C is nearly independent of W/L with a total enthalpy of 6.75 (±0.25) kcal/mol of DLPC. A linear least-squares fit of the total enthalpy values of 35 different scans taken on the 10 different sample concentrations, as a function of W/L, gives the value for the intercept (at $W/L = 0$) of 6.59 (±0.6) kcal/mol and a positive slope of 1.54 (±0.5) · 10^{-3} (kcal/mol)/(mol water/mol DLPC). Thus, there may be a slight increase in total enthalpy change as

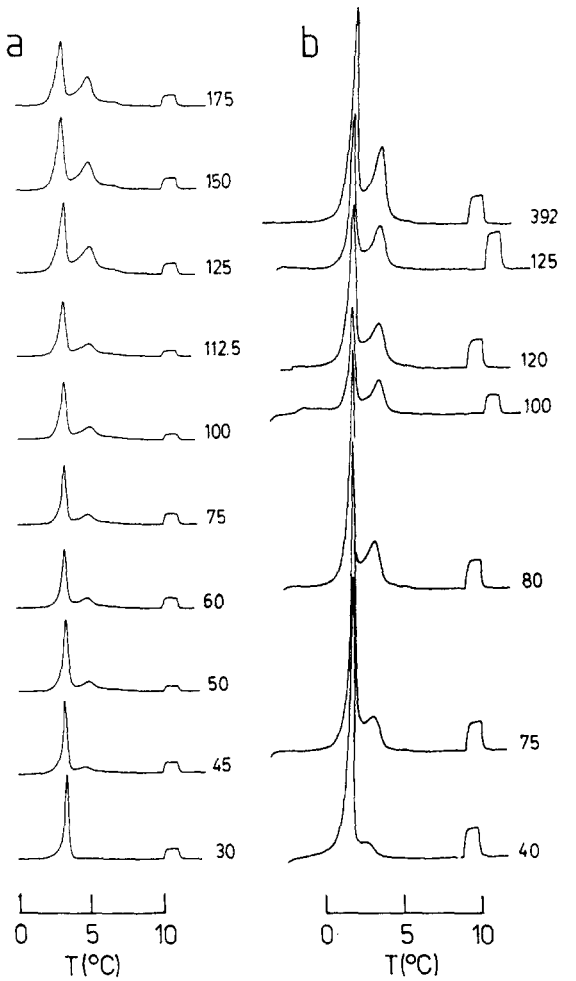

Fig. 2. High sensitivity DSC scans of (a) proteated DLPC at the water to lipid ratios shown, and (b) chain perdeuterated DLPC at the indicated water to lipid ratios. All traces shown were the third or fourth heating scan on a particular sample, and were taken after at least 6 h incubation at temperatures below −6 °C. The scan rate was approx. 7 K/h in all cases. Note the slight difference in the temperature scales for parts (a) and (b).

water content is increased, but the change has the same magnitude as the scatter in the enthalpy values observed at any one concentration, approx. ±3%.

DSC samples made from the deuterated lipids synthesized in our own laboratory display similar behaviour. The B and C features show the same variation with water concentration, as illustrated in Fig. 2b. The only fundamental difference between the DSC scans of the chain perdeuterated DLPC, Fig. 2b, and the commercial proteated DLPC, Fig. 2a, is the downward shift, by about 1 K, in the temperatures of the observed peaks. This shift is an isotope effect and is similar in magnitude, though smaller, to those seen in chain perdeuterated DMPC and DPPC. DSC scans of α-deuterated DLPC do not exhibit this isotope effect since there are only two deuterium atoms per chain. They do exhibit all of the interesting properties, however.

In order to check the lamellar nature of the sample through the transition, X-ray diffraction experiments were performed on DLPC multilamellar vesicles suspended in 50 mM phosphate buffer at $W/L \approx 100$. There was no evidence for non-bilayer phases. At +8 °C, small-angle reflections associated with a regular bilayer spacing were seen. The wide-angle reflections associated with chain ordering were diffuse, suggesting a normal fluid lamellar phase. At −8 °C, the wide-angle reflections sharpened, corresponding to ordered chains, but the sharp small-angle reflections were broadened into a diffuse band, suggesting disorder in the bilayer spacing. It was possible to cycle between these states reversibly.

Calorimetry alone cannot determine the origin of the three prominent features, A, B and C. Surely the melting of the hydrocarbon chains occurs within the temperature range spanned by these three events, but the total enthalpy is much larger than that expected by extrapolation of the enthalpies of the chain melting transitions of longer chain diacylPC [8]. ^{2}H nuclear magnetic resonance spectra are exquisitely sensitive to the melting of the hydrocarbon chains, so, by comparison of NMR with DSC, we would at least be able to associate the chain melting with one or more of the thermal events observed by calorimetry.

Selected ^{2}H-NMR spectra of the α-deuterated DLPC at $W/L = 84$ are shown in Fig. 3. At −10 °C, the spectrum has a shape and width which is characteristic of the nearly rigid lattice, i.e., it exhibits the maximum quadrupolar splitting. This behaviour is typical of disaturated PC bilayers at temperatures below 0 °C. As the temperature is slowly increased (at an effective scan rate of about 0.25 K/h, but in a stepwise fashion),

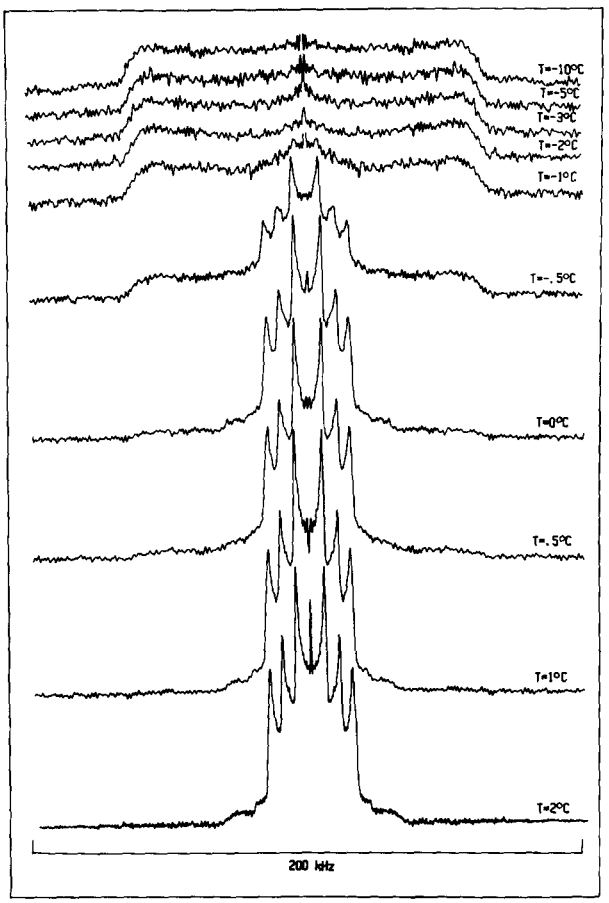

Fig. 3. The temperature dependence of the ^{2}H-NMR spectrum of 1,2-di[2,2-$^2H_2$]lauroyl-*sn*-glycero-3-phosphocholine (α-deuterated DLPC) at $W/L = 84$. These spectra are representative of a series of spectra obtained while increasing the temperature in a step-wise fashion at an effective scan rate of 0.25 K/h.

no changes are observed until above $-1°C$. Specifically, there is no evidence of chain melting or reorientation at $-2°C$. However, between -1 and $-0.5°C$, we observe a partial melting of the system. The melting occurs over a rather wide temperature range, not reaching completion until somewhere between $+1$ and $+2°C$. At $2°C$ and above, the spectra are characteristic of the liquid-crystalline bilayer phase. Normally, for diacylPC, we observe a very sharp chain melting transition, as expected for a pure phospholipid in a fully hydrated state.

Further insight into the nature of the B and C transitions can be obtained by considering the temperature dependence of perdeuterated DLPC spectra and spectral moments. These display the same type of behaviour in the transition region as the spectra of specifically deuterated DLPC. On warming through the temperature range of the B transition, between about $-0.3°C$ and $-0.25°C$, much of the ordered phase is converted into a liquid-crystalline phase. The C transition, extending from $-0.25°C$ to $1°C$, appears to convert the remaining gel-like phase to liquid crystal. Throughout the region of the B and C transition, the spectra are a superposition of a gel-like component and a liquid-crystalline component, implying that at least two phases coexist through this region. This argues against either peak being due to a continuous phase change.

The relationship between the DSC and NMR views of the B and C transitions is illustrated in Fig. 4. The DSC trace for multilamellar vesicles of DLPC at $W/L = 125$, Fig. 4a, displays characteristic B and C transitions. The negative of the integrated DSC scan, Fig. 4b, shows the two step changes in the enthalpy corresponding to the B and C transitions. Fig. 4c shows M_1 for perdeuterated DLPC at $W/L = 40$, 60 and 100. M_1 is proportional to the average quadrupolar splitting for axially symmetric spectra and, more generally, gives the average spectral width. The two-step behaviour of M_1 indicates that both transitions decrease average chain order. Because the shape of the M_1 vs. T plot is similar to an integrated DSC scan, variation of the C transition with water content does not stand out as clearly. Still, the height of the second step, corresponding to the C transition, does increase observably with water content.

Spectra were taken at a very slow effective scan rate (0.25 K/h) leading to an apparent discrepancy between the temperatures of chain melting observed by NMR and the three thermal events seen by DSC. (Note: portions of all NMR samples were run on the DSC and all calorimetry results were consistent with those reported in Fig. 2.) While we were unable to run the calorimeter at rates slower than about 7 K/h, we were able to perform the NMR experiments at different effective scan rates, though always in a step-wise fashion. Using the perdeuterated DLPC, we were able to increase the temperature at effective rates of 8

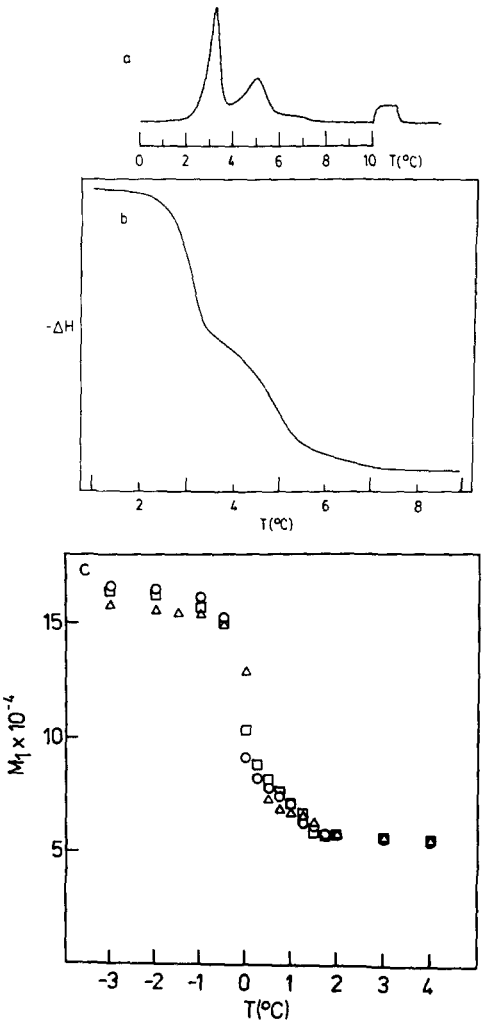

Fig. 4. Comparison of NMR and DSC indicators of the phase changes in DLPC. (a) DSC trace for multilamellar vesicles of DLPC, at $W/L = 125$, showing both B and C transitions. (b) The negative integral of (a) showing two step changes corresponding to B and C. (c) The first moment (M_1) of the ^{2}H-NMR spectrum of chain perdeuterated DLPC at (△) $W/L = 60$, (○) $W/L = 80$, and (□) $W/L = 100$.

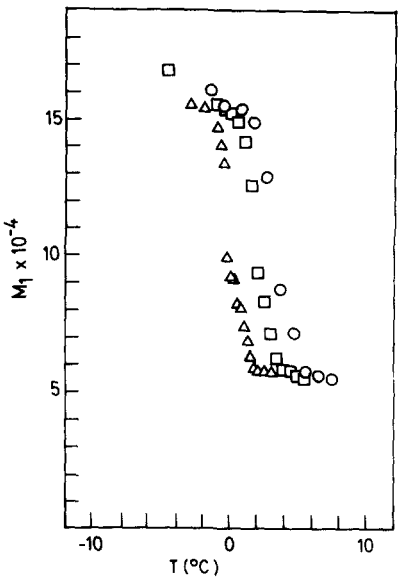

Fig. 5. The temperature dependence of the first moment of the ^{2}H-NMR spectrum of chain perdeuterated DLPC (at 125 waters/lipid). The triangles are for spectra obtained at an effective heating rate of 0.25 K/h. The squares are for an effective heating rate of 8 K/h, and the circles for an effective heating rate of 15 K/h.

and 15 K/h, in addition to the initial slow rate of approx. 0.25 K/h. Fig. 5 shows the plots of the first moments of the spectra taken at these three scan rates. At 8 K/h, the transition region begins at about +1°C and ends at about 4°C. At 15 K/h, the transition region begins at just above +2°C and ends just above 5°C. At the slowest scan rate, 0.25 K/h, the transition for the perdeuterated DLPC begins just below −0.5°C, and ends at about +1.5°C. Thus, the same range of temperatures is spanned by the B and C peaks in calorimetry and by the chain melting transition as observed by NMR when similar scan rates are used (compare Figs. 2B and 5). Finally, it should be mentioned that, even though we never observed any changes in the NMR spectra near −2°C, it is quite possible that the conditions necessary for the occurrence of the A peak were never satisfied in the NMR experiments. Thus, we cannot conclude whether or not the A peak, when it occurs, is associated with chain melting.

While the B and C features in the DSC traces are associated with hydrocarbon chain melting, the large total enthalpy (6.75 kcal/mol) suggests that something more must be happening. The first moment of the ^{2}H-NMR spectrum is a quantitative measure of the degree of order of the chains [5]. At −1°C, which is just below the onset of chain melting for the specifically labelled DLPC, $M_1 = 2.105 \cdot 10^5$ s^{-1} whereas specifically labelled DPPC at 0°C has $M_1 = 1.873 \cdot 10^5$ s^{-1} (for [8,8-

$^2H_2$]DPPC and $M_1 = 1.857 \cdot 10^5$ s^{-1}, (for [4,4-$^2H_2$]DPPC). The perdeuterated DLPC has $M_1 = 1.65 \cdot 10^5 s^{-1}$ at $-2°C$, while perdeuterated DPPC has $M_1 = 1.51 \cdot 10^5$ s^{-1} and perdeuterated DMPC has $M_1 = 1.27 \cdot 10^5$ s^{-1} [5,16] at that same temperature. By contrast, the values of M_1 for DLPC, DMPC, and DPPC, at temperatures just above the chain melting transition, are all of comparable magnitude, even though DLPC is melted by 2°C and DPPC at 38°C. Thus, the chains of DLPC are more highly ordered than either DMPC or DPPC, at -1 or $-2°C$, even though this is just below the chain melting region. The large changes in lipid mobility, which include reorientations about the long axis as well as the *gauche-trans* isomerizations, all occur over this small temperature range. The large total enthalpy observed in features B and C contain contributions from all the molecular degrees of freedom, and not simply the chain melting. The enthalpy of the subtransition of DPPC has been reported to be 3.70 kcal/mol, while that of the pretransition is 1.35 kcal/mol [17], and similar values have been reported for DMPC (1.2 kcal/mol for the pretransition in the absence of a subtransition, and 5.4 kcal/mol for a scan where the subtransition occurs, presumably overlapping the pretransition since no separate pre-transition is visible) [18]. If corresponding processes occur in DLPC, then the total enthalpy observed, 6.75 kcal/mol, less the 5.05 kcal/mol expected for the sub- and pretransitions (on the basis of the DPPC data), leaves 1.7 kcal/mol. This is the value of the enthalpy of the A peak, reported by Mabrey and Sturtevant [7], and is consistent with the expected enthalpy of chain melting for a 12-carbon disaturated phospholipid [8].

While the calorimeter is restricted to scans of increasing temperature, our NMR experiments were not. On cooling the sample slowly (at an effective rate of 1 K/h), the sample remained in a liquid-crystalline state even at $-3°C$. Incubation at this temperature for 3 h resulted in the conversion of the sample from the liquid-crystalline to the 'solid-like' phase. Further cooling to $-10°C$ had no effect on the ^{2}H-NMR spectrum. Subsequent warming finds the onset of chain melting to occur, as reported above, at about $-0.5°C$.

The A feature was observed in the specifically deuterated samples, but was not clearly seen in either the DSC or NMR experiments performed on perdeuterated samples. This negative result is not surprising. The A peak was only seen with a minority of normal DLPC samples and never following prolonged incubation at low temperature. When it appeared, the A peak was close to the lower limit of temperature for our calorimeter. If the A transition were depressed slightly by perdeuteration, as are the B and C peaks, it might not have been observable by DSC in this series of experiments. On a few DSC runs there were indications that an A peak occurred, but the calorimeter had not yet stabilized completely in these cases.

Attempts were also made to observe the A transition in perdeuterated samples by NMR. Using M_1 and inspection of the spectra of perdeuterated DLPC, it was possible to monitor the conversion of liquid-crystal to gel phase on cooling and thus study whether the A transition might result from incomplete conversion of liquid crystal to gel prior to the start of a warming run. Fig. 6

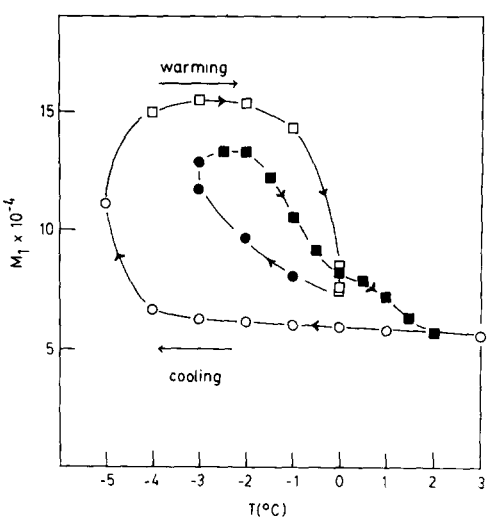

Fig. 6. The temperature dependence of the first moment, M_1, of chain perdeuterated DLPC for runs with temperature cycled in the region of the phase transition. Circles were obtained while cooling and squares while warming. Open symbols represent the first cycle in which warming began before complete conversion to gel. The second cycle began following a waiting period at 0°C and is represented by solid symbols. No evidence for the A transition is observed. Arrows indicate the direction of temperature change.

shows cooling and warming runs in which warming was begun before attainment of the full low temperature M_1. Open circles are for a run with cooling at about 1 K/h. Warming began as soon as significant gel phase component was observed. No evidence was seen for a transition corresponding to A and the B transition occurred near 0 °C as usual. To test whether the broad range of temperatures over which chain melting occurs is due to a kinetic effect, the sample was allowed to incubate for several hours at 0 °C where both liquid-crystalline and solid-like domains are observed. Not only did the spectra appear to be unchanged during this period, but the moments of the spectra also remained constant. Thus, over the time scale of hours, the melting behaviour of DLPC does not appear to be a finite rate effect. The cycle was repeated (solid symbols) with warming again beginning before complete liquid crystal to gel conversion. B and C transitions, although broadened, were evident.

Conclusion

Three peaks, which we have labelled A, B and C, are observed in high sensitivity DSC studies of DLPC, whether in buffer alone or in buffer containing 15% (by volume) ethylene glycol. Sufficient incubation at about −6 °C and/or cycling the temperature from about −6 to +10 °C eliminates the A peak, even when ethylene glycol is present. On continued cycling, the B and C peaks occur reproducibly.

It thus appears that the stable low temperature state of DLPC is the one which melts in the region of the B and C transitions. This is the state obtained with the longest and coldest incubation. The A transition seems to be the outcome of a particular condition which is not obtained predictably or reversibly.

^{2}H-NMR studies show that the chain melting occurs throughout the temperature range spanned by the B and C peaks, when the effective scan rate of the NMR experiments is the same as that of the calorimetry (between 7 and 10 K/h). The slowest scan rate for NMR, 0.25 K/h, shows the same two-step melting, but with everything shifted to lower temperatures by approx. 2 °C. Incubation in the middle of the transition region (at 0 °C) for three hours showed that the two phase coexistence remained stable. Within these experimental limitations, there seems to be two phase coexistence over a roughly 5 K temperature range. This would appear to violate Gibbs phase rule which states that, for a homogeneous equilibrium system in excess water, three phases (water, 'solid-like' and liquid-crystalline lipid bilayer) can coexist at only a single temperature. Varying the water content shows that the relative areas of the B and C peaks change, C increasing with increased water, but that the total enthalpy remains nearly constant. Above 125 waters/lipid, the ratio of B to C remains constant, and the range of temperatures over which two phase coexistence is observed does not change. Only at low water concentration, 30 waters/lipid or below, can we observe a single DSC peak (the B peak). While it seems there is no question that we have excess water, it is still possible that the system is either not in equilibrium or that the system is inhomogeneous.

The first possibility would attribute behaviour in the B/C transition region to co-existence of a metastable low temperature phase and a stable low temperature phase. However, it is difficult to understand why, if a metastable phase is involved, the chain melting should proceed rapidly until midway between the B and C peaks and then remain static and incomplete for as long as three hours at that point, proceeding only when the temperature is raised further. A slightly more promising explanation is the possibility that multilamellar vesicles of DLPC might display an intrinsic inhomogeneity. A possible 'microscopic' inhomogeneity is always present in multilamellar lipid/water dispersions; bilayers near the surface of multilamellar particles may differ significantly from those deeply within. The distinction between the B and C transitions might then be due to the same phase melting in slightly different environments. If the transition in DLPC is more sensitive to interbilayer coupling effects than is normally observed for longer chain phosphocholines, then any difference in interbilayer coupling experienced between the interior and surface of the vesicle might result in a range of thermodynamic behaviour. If this conjecture is correct, then it is possible that the effect of water on the B and C transitions might occur indirectly through its ef-

fect on vesicle size or packing.

It is more difficult to draw any conclusions about the A transition. We have been unable to produce it under the conditions of an NMR experiment and its sporadic occurrence has limited our knowledge of it from DSC. Suppression of B and C in the presence of A argues strongly that the phase immediately above A is fluid. The nature of the low temperature phase giving rise to A is not known. The irreversible loss of A following low temperature incubation, and our inability to recover it following vortexing and short incubation, seems to rule out the involvement of metastable phases. If, however, the thermodynamics of DLPC are sensitive to interbilayer coupling, as suggested above, then the occasional observation of the A transition might be due to non-equilibrium hydration of the bilayers immediately following mixing. The effect of the freeze/thaw cycle might then be to irreversibly influence bilayer coupling by rearranging bilayer packing in the vesicles. Two observations provide indirect support for the role of interbilayer coupling. Our experiments and the work of Mabrey and Sturtevant both suggest that the A transition is more likely to be seen in the presence of ethylene glycol which might be expected to influence interbilayer coupling. The observation that addition of buffer may result in the reappearance of the A peak also supports the possible role of non-equilibrium hydration.

The first moments of the ^{2}H-NMR spectra of DLPC indicate that this lipid is significantly more ordered at $-2°$C, which is just below the chain melting region, than either DMPC or DPPC at that temperature. The total enthalpy of the B and C feature, 6.75 kcal/mol DLPC, is much larger than 1.7 kcal/mol expected from chain melting. However, the combined enthalpy of the sub- and pretransitions of DPPC is 5.05 kcal/mol, while it is 5.4 kcal/mol for DMPC. A similar contribution for DLPC when subtracted from the total of 6.75 kcal/mol leaves just the amount expected for chain melting. Thus, it appears that all of the processes responsible for the reduction in phospholipid mobility observed in the other disaturated PC occur over this narrow temperature range in DLPC. These processes, involving chain melting, molecular reorientation, and changes in lattice packing and symmetry, are somehow coupled.

Clearly, such more needs to be done before we really understand the phase behaviour of DLPC in water. Further studies by NMR and X-ray diffraction will help to determine the nature of the changes occurring over the temperature range of the B and C peaks. The curious behaviour of DLPC should be a challenging test of theories of lipid phase transitions.

Note added in proof: (Received 18 September 1987)

Microdensitometer tracings of the wide-angle region of the X-ray diffraction patterns reveals two clear maxima at temperatures below 0°C. The corresponding spacings are at 4.06 and 4.28 Å, in agreement with spacings observed in the L_c phase of other diacyl PC.

Acknowledgements

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