METASTABILITY AND POLYMORPHISM IN THE GEL PHASE OF 1,2-DIPALMITOYL-3-*SN*-PHOSPHATIDYLCHOLINE

A Fourier Transform Infrared Study of the Subtransition

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ABSTRACT Fourier transform infrared spectroscopy was used to study the metastability of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) at temperatures near 0°C. It was found that when DPPC is incubated at 2°C for three days the two-dimensional acyl chain packing changes from one resulting in spectra typical of an orthorhombic subcell to one resembling that found in triclinically packed acyl systems. This transition proceeds in two stages. The first step, requiring less than one day, approximates first-order kinetics; the second stage proceeds with second- or higher-order kinetics. Comparison of spectra recorded at −36°C with and without prior incubation at 2°C shows that there are two stable low temperature forms of DPPC; that is, DPPC is metastable only within a narrow temperature range. A study of the thermotropic behavior in the range 0–45°C shows that the subtransition near 15°C is a transition from the alternate form to one with orthorhombic characteristics. Spectral changes at the pretransition and the main phase transition demonstrate that there are differences in behavior that are related to the thermal history of the sample.

INTRODUCTION

Fully hydrated multibilayer assemblies of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) exhibit a gel-to-liquid-crystalline phase transition at 41.5°C (T_m), and a change in the acyl chain packing at 34–36°C, commonly referred to as the pretransition (T_p). Both these transitions are now well understood, at least in terms of the hydrophobic region of the bilayer (1–11). However, Chen et al. (12) have recently reported a third phase transition in phosphatidycholines. This "subtransition" (T_s) is observed only after extensive incubation (>8 h) at low temperatures (~0°C) and, in the case of DPPC, is observed at 14.9°C with a width of 1.8°C. As the calorimetric behavior of the incubated sample above T_s was the same as that of a sample exhibiting only two transitions (T_p and T_m), it was concluded that the gel phase was metastable below 6°C, converting slowly to an alternate stable low temperature form, and that the transition could be completely reversed by raising the temperature of the sample to just above T_s.

In this paper we report on the results of an infrared study of the transition to the alternate form at 2°C, and on the nature of the subtransition. We conclude that the subtransition represents a change in the acyl chain packing, and that there are two stable low temperature forms of DPPC. We also demonstrate that the spectral behavior in the range T_s to T_m is dependent on the thermal history of the sample, there being substantial differences at T_p depending on whether or not the system exhibits a subtransition.

EXPERIMENTAL

Materials

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, stated purity 99%, was purchased from Sigma Chemical Company, St. Louis, MO, and exhibited a single spot when analysed by thin-layer chromatography. An aqueous bilayer dispersion was prepared by adding 80 mg of heavy water to 12 mg of DPPC. The sample was agitated at 50°C for 2 min, then cooled to 20°C. This cycle was repeated three times. The gel was then assembled into a 25-μm thick cell equipped with BaF_2 windows.

Spectroscopy

Spectra were recorded on a nitrogen-purged Digilab FTS-15 Fourier transform infrared spectrometer equipped with a wide range (400-cm^{-1} low-frequency cutoff) mercury cadmium telluride detector (Digilab, Cambridge, MA). 1,000 scans were averaged, with a maximum optical retardation of 0.5 cm, triangularly apodized, zero-filled once, and Fourier transformed to yield a resolution of 2 cm^{-1} and an encoding interval of 1 cm^{-1}.

Temperature control was achieved by locating the cell in a thermostated cell mount (13). Temperatures were stable to within 0.05°C.

Frequencies were determined by measuring the center of gravity of the topmost 2-cm^{-1}-wide segment of the CH_2 scissoring band, and the

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topmost 6-cm^{-1}-wide segment of the C=O stretching band. Bandwidths were determined relative to linearly interpolated base lines extending from 1,530 to 1,400 cm^{-1} (CH_2 scissoring band) and 1,795 to 1,650 cm^{-1} (C=O stretching band) (14). Difference spectra were generated by subtracting low temperature spectra from high temperature spectra, or spectra collected after short periods of time from those collected after longer periods, and normalized with respect to the temperature or time interval. As the path length and, to a high approximation, the concentration are fixed, a scaling factor of 1 was used in all subtractions.

Thermal History

Following the preparation outlined above, the sample was placed in the cell mount, and was not touched or moved throughout the course of the experiment.

In the first stage, the sample was cooled to 2°C, and maintained at 2 ± 0.05°C for a period of 88 h. During this period, spectra were recorded every 2 h. The sample was then, within 30 min, cooled to −36°C, at which temperature a spectrum was recorded every 2 h for a period of 48 h. The sample temperature was then raised to 0°C, and an automated study (15) was carried out, with small increments (1°C) being used in the vicinty of T_s, T_p, and T_m.

The sample was then, within 1 h, cooled to −36°C, at which temperature a spectrum was recorded every 2 h during a 48-h period. The sample temperature was then raised to 0°C, and a study carried out in the range 0–45°C. The sample was then again cooled to 2°C and maintained at 2°C for 3 d. It was then warmed to 20°C, maintained at 20°C for 1 d, after which a study of T_p and T_m was carried out.

In separate experiments the sample was incubated at 2°C with H_2O as the hydrating agent, and was not cooled to −36°C. No differences from the changes in the above studies were observed. We also note no significant changes in the spectrum on freezing the sample excepting the water bands, in accord with previous vibrational spectroscopic studies of this system (4, 5, 8–11).

RESULTS

Incubation at 2°C

Structural Changes. Fig. 1 shows the fingerprint region of the infrared spectrum of DPPC at 2°C, 2 h after cooling from 45°C, and after being maintained at 2°C for 88 h. Superimposed is the difference spectrum, the features resulting from bands associated with the head group being indicated with an asterisk while those resulting from acyl chain bands are unmarked.

In general, incubation at 2°C results in a reduction in bandwidth and an increase in peak height, which indicates a large reduction in the mobility of the various functional groups. Major changes are evident in the bands resulting from the ester linkage (1,740; 1,150; and 1,060 cm^{-1}), the methylene wagging band progression (1,360–1,190 cm^{-1}), the acyl chain C—C stretching bands (1,140–1,100 cm^{-1}), and the methylene scissoring and rocking bands at 1,470 and 720 cm^{-1}, respectively (see references 16 and 17 for detailed assignments). Relative to the changes in the CH_2 bands, the choline bands at 1,490; 1,020; and 970 cm^{-1} are almost invariant, as are the O=P=O stretching bands near 1,230 and 1,090 cm^{-1}. There are, however, large increases in the intensities of the O—P—O stretching bands near 830 and 770 cm^{-1}.

Fig. 2 *A* and *B*, show, respectively, the C=O stretching and CH_2 scissoring bands of DPPC after periods of 2, 24, 48, and 88 h at 2°C. 2 h after cooling to 2°C, the CH_2 scissoring band (Fig. 2*B*) exhibits a broad maximum, reflecting the summation of two bands. These bands result from temperature-dependent factor group splitting of the scissoring mode (18, 19), analogous to the splitting observed in the spectra of simpler acyl systems, when the acyl chains are packed in an orthorhombic subcell.

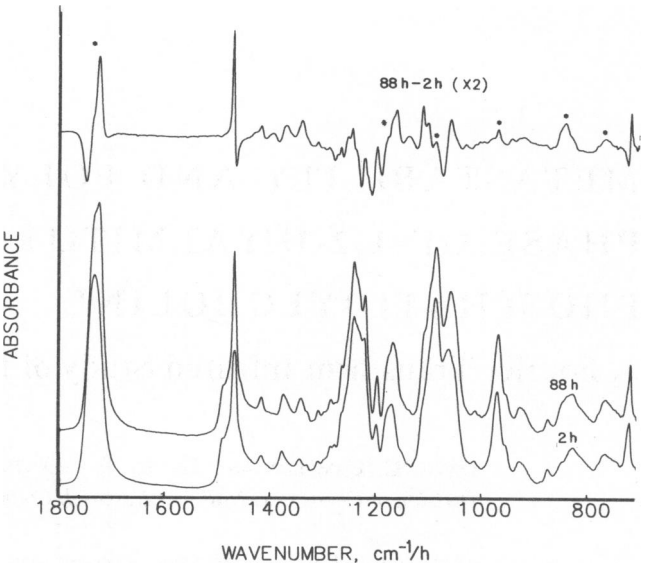

FIGURE 1 Fingerprint region of the infared spectrum of a 25 μm thick sample of DPPC hydrated with D_2O, in a BaF_2 cell at 2°C. *Bottom*, spectrum after periods of 2 and 88 h. The spectrum of D_2O in a BaF_2 cell has been removed by subtraction. *Top*, difference spectrum resulting from subtraction of the spectrum recorded after 2 h from that recorded after 88 h. Features marked with an asterisk result from bands associated with head group vibrational modes.

As the sample is maintained at 2°C, the higher frequency component, near 1,473 cm^{-1}, progressively increases in intensity, whereas that near 1,462 cm^{-1} decreases. This results in a narrowing of the band contours and a shift of the maximum to higher frequency. After 88 h the 1,473-cm^{-1} band dominates the spectrum, and only a weak shoulder is evident near 1,462 cm^{-1}.

After 2 h the C=O stretching band is slightly asymmetric, the contour being typical of the gel phase of phosphatidylcholines (20, 21). After 24 h, there is evidence of two component bands, with the higher frequency component being the stronger. During the second and third days of incubation, however, the lower frequency component steadily increases in intensity, and the width of the band contour decreases.

The nature of the changes in the component bands are more evident in Fig. 3, which shows a series of spectra of the carbonyl band contour following Fourier self-deconvolution with a 20-cm^{-1}-wide Lorentzian line (22, 23). At all times the band is composed of two principal components, at 1,741 and 1,727 cm^{-1}, which have been assigned to the C=O stretching modes of the *sn*-1 and *sn*-2 carbonyl groups, respectively (21). Examination of the spectra

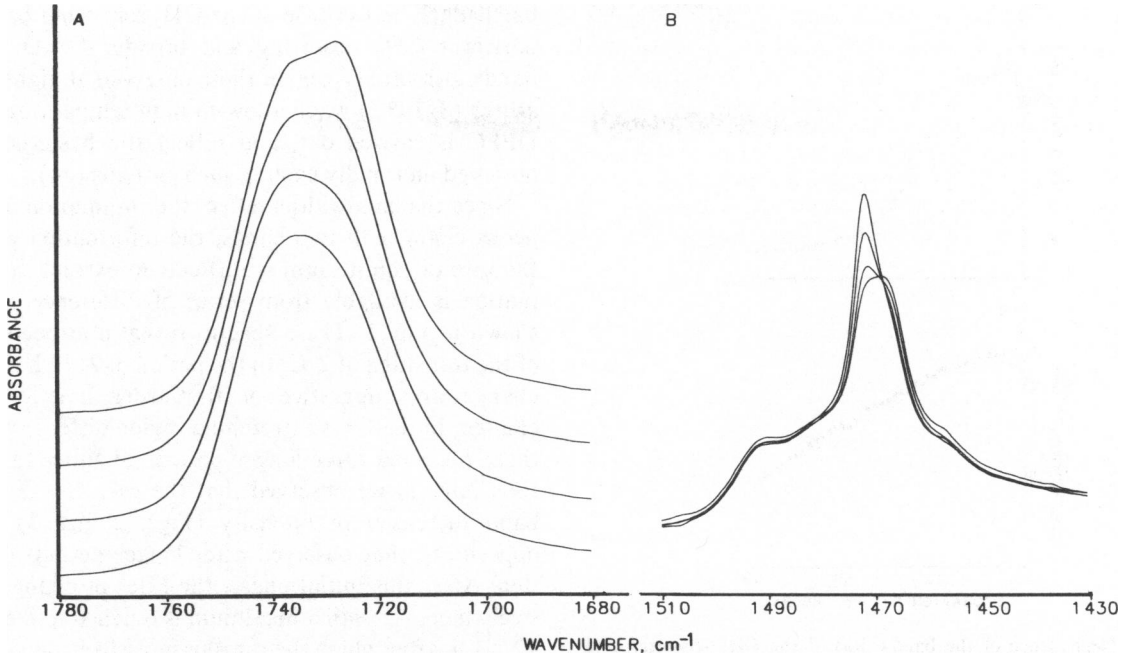

FIGURE 2 Infrared spectra of DPPC after periods of 2, 24, 48, and 88 h at 2°C. In the region of the maxima, spectra are shown from bottom to top in order of increasing incubation period. *A*, C═O stretching band. Spectra have been displaced relative to each other to permit a better comparison. *B*, CH_2 scissoring band superimposed on weak bands resulting from choline and acyl chain methyl groups. Spectra are plotted exactly as recorded, i.e., there is no displacement.

reveals an increase in the intensity of the band at 1,741 cm^{-1} in the period 2–9 h, which results in the shift to higher frequency of the band contour maximum after a period of 1 d (Fig. 2*A*). After this initial effect, the 1,727-cm^{-1} band increases in intensity relative to the 1,741-cm^{-1} band, the peak heights being equal after 42 h and the 1,727-cm^{-1} band being ~25% more intense than the 1,741-cm^{-1} band after 88 h (Fig. 3). Also evident in the deconvoluted spectra with increasing incubation period is an increase in the depth of the high-frequency negative lobe, and the appearance of a negative lobe on the low-frequency side. These artifacts result from the use of too wide a band in the deconvolution procedure (22). The initial overestimation is probably related to the fact that the 1,741-cm^{-1} band is actually composed of several bands lying very close together in frequency (21). The changes indicate that the widths of both the *sn*-1 and *sn*-2 bands decrease with increasing incubation period.

We also monitored the frequencies of the component bands. No changes larger than those expected to result from variations in the degree of overlap were observed.

Kinetics. In Fig. 4 *A* and *B*, we show, respectively, the width at nine-tenths peak height of the CH_2 scissoring band and the width at half-height of the C═O stretching band as a function of the period of incubation at

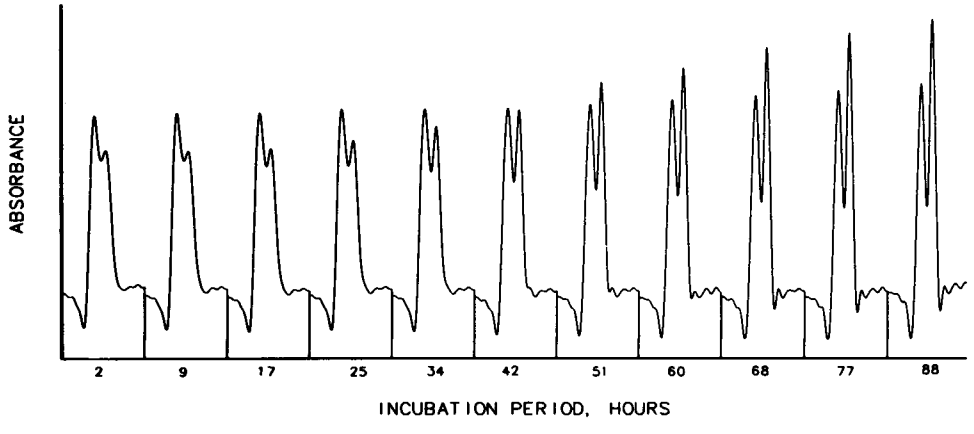

FIGURE 3 Fourier self-deconvoluted spectra of the C═O stretching band of DPPC at 20°C after various incubation periods. Each spectrum covers the region 1,770–1,690 cm^{-1}. Spectra have been deconvoluted with a 20-cm^{-1} full-width at half-height Lorentzian line, and smoothed so that precise matching of bandwidth will result in a line width of 8 cm^{-1} (see references 14 and 15).

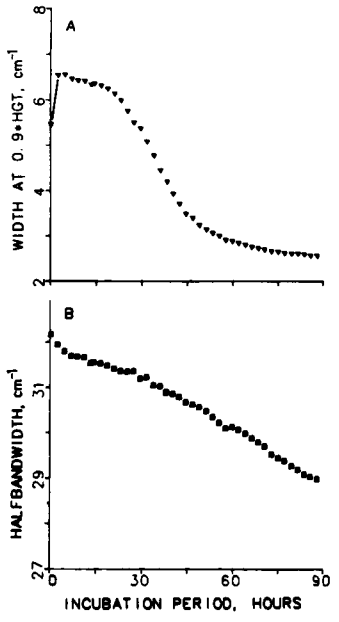

FIGURE 4 Dependence of the bandwidths of the CH_2 scissoring and C═O stretching bands of DPPC on the period of incubation at 2°C. *A*, full-width at nine-tenths peak height (0.9*HGT) of the CH_2 scissoring band. *B*, full-width at half-height of the C═O stretching band.

2°C. The continuous nature of the changes during the incubation is clearly evident. Also demonstrated is the fact that even after 88 h complete equilibrium has not been achieved (12). We note that the first spectrum, recorded within the first hour at 2°C, exhibits quite different bandwidths from those in the spectrum recorded after 2 h, particularly in the case of the CH_2 scissoring band. These narrower CH_2 scissoring and broader C═O stretching bandwidths are typical of those observed at higher temperatures (8–14°C) when a low-to-high temperature study of DPPC is carried out, and reflect the hysterisis in time observed on rapidly cooling such systems (24).

Since the bandwidths reflect the summation of simultaneous changes in two bands, the information concerning the rate of equilibrium is difficult to extract. Such information is available from series of difference spectra, as shown in Fig. 5. These spectra reveal unexpected aspects of the transition at 2°C. In the period of 2–17 h the rates of change are suggestive of first-order kinetics and the changes in the C═O stretching region differ in form from those observed after longer periods of time. In the same time interval we observed that the *sn*-1 C═O stretching band increased in intensity (Figs. 2 and 3), behavior opposite to that observed after longer periods of incubation. After this initial phase, the rates of change progressively increase until a maximum is reached in the period of 42–51 h, after which the rates progressively decrease. That is, the system exhibits second- or higher-order kinetics during the incubation at 2°C.

Incubation at −36°C

After being maintained at 2°C for 88 h, the DPPC sample was rapidly cooled to −36°C to compare the spectrum with that obtained by cooling the DPPC rapidly from 45°C ($T>T_s$, T_p, T_m) to −36°C. The sample cooled from 45°C equilibrated immediately, whereas that cooled from 2°C required 4 h to reach equilibrium. In both cases no

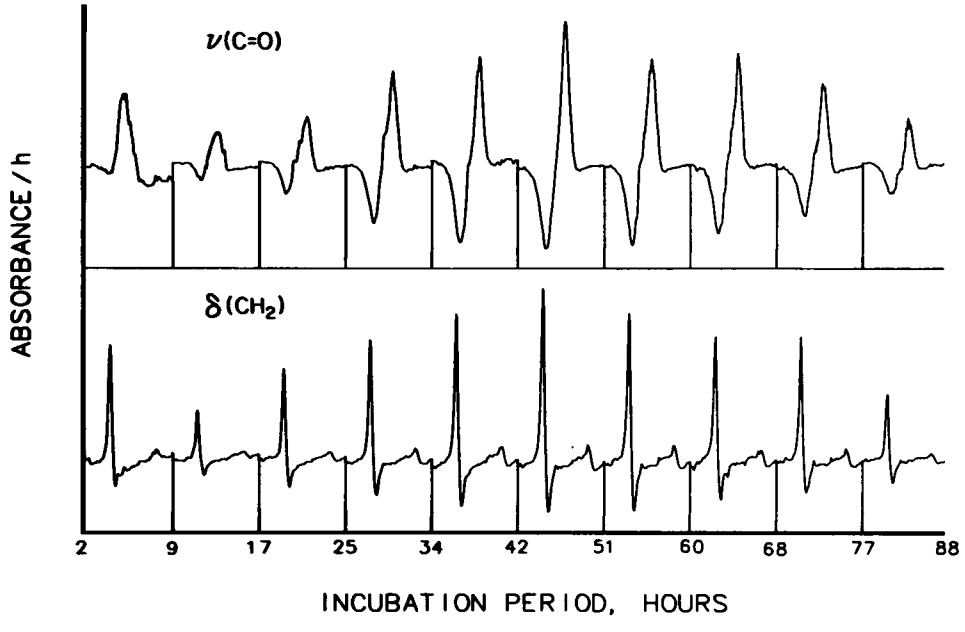

FIGURE 5 Infrared difference spectra in the regions of the C═O stretching (*top*) and CH_2 scissoring (*bottom*) bands during the incubation at 2°C. Spectra have been normalized with respect to the time interval used to generate the spectra. The times used in the subtraction are indicated on the bottom axis; e.g., the left-most difference spectra were generated by subtracting the spectrum recorded after 2 h from that recorded after 9 h, and dividing the result by 7.

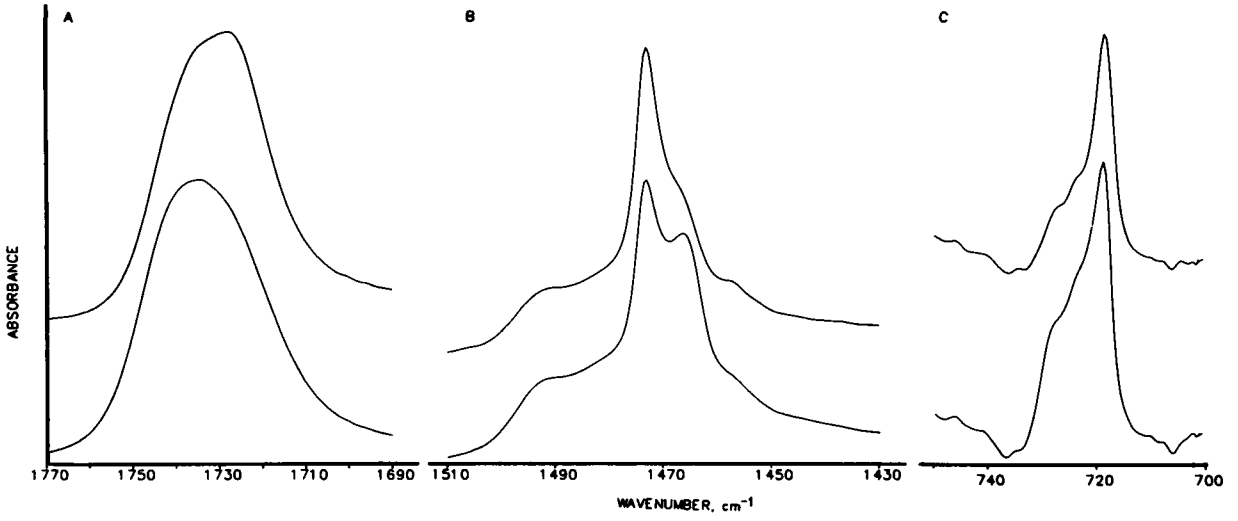

FIGURE 6 Spectra of DPPC at −36°C resulting from 88 h incubation at 2°C followed by 48 h incubation at −36°C (*top*), compared with spectra resulting from 48 h incubation at −36°C after rapid quenching of the temperature from 45°C (*bottom*). *A*, C═O stretching region; *B*, CH_2 scissoring region; *C*, CH_2 rocking region.

further changes in the spectra were observed in the period of 4–48 h.

The C═O stretching, CH_2 scissoring, and CH_2 rocking bands resulting from the two experiments are shown in Figs. 6 *A*, *B*, and *C*, respectively. Differences in other regions of the infrared spectrum merely represent an extension of those shown in Fig. 1, and hence are not shown.

The C═O stretching band contours at −36°C (Fig. 6*A*) are similar to those observed after periods of 2 and 88 h at 2°C (Fig. 2*A*). This suggests major differences in the glycero region of the head group. The correspondence of the C═O stretching band in the spectrum of the sample cooled to −36°C from 45°C with that recorded after 2 h at 2°C also confirms that this slightly asymmetric band is the standard C═O stretching contour for the orthorhombic form of DPPC. Consequently, this demonstrates that the increase in intensity of the *sn*-1 C═O stretching band in the first 24 h of incubation at 2°C (Figs. 2*A* and 3) is part of the process of the transformation to the alternate form, and not a residual hysterisis effect.

The differences in the CH_2 scissoring (Fig. 6*B*) and CH_2 rocking (Fig. 6*C*) bands reflect differences in the acyl chain packing. The quenching from 45°C results in factor group splitting of both the rocking and scissoring modes. At lower temperatures the splitting is even greater and the CH_2 rocking band is also resolved into two distinct bands (8, 9, 11).

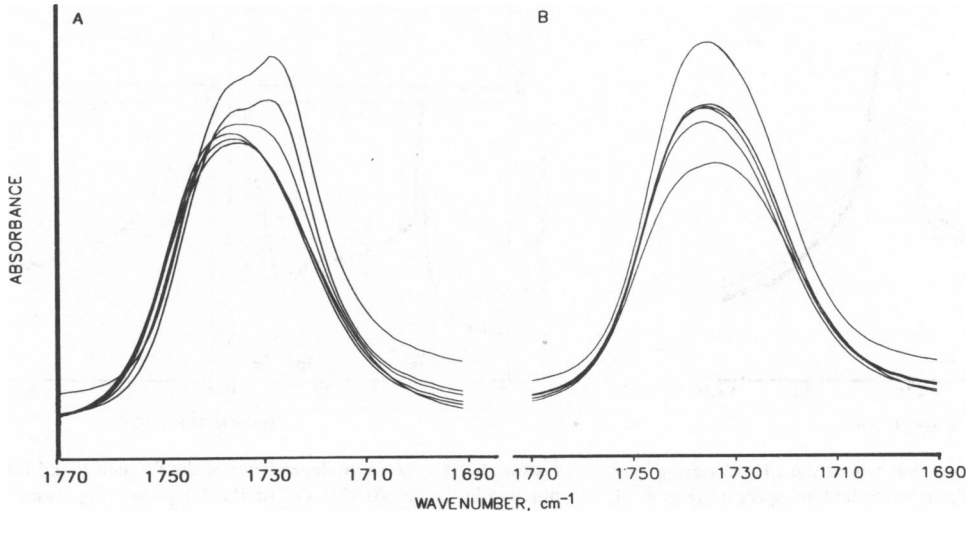

FIGURE 7 Temperature-dependent behavior of the C═O stretching band of DPPC in the range 0–45°C. From top to bottom, spectra are at 4, 11, 15.5, 24, 37, and 43°C. *A*, spectra after 88 h incubation at 2°C followed by 48 h incubation at −36°C. *B*, spectra after rapid quenching from 45 to −36°C, and 48 h incubation at −36°C.

In contrast, the spectra obtained when the sample is quenched from 2°C (top spectra, Fig. 6*B* and *C*) have strong fundamentals near 1,473 and 719 cm^{-1} but the components near 1,462 and 730 cm^{-1} are greatly reduced in intensity.

We note two additional points. First, it is not necessary to quench from the liquid-crystalline phase, since the same result is obtained from any temperature, except when the sample has been incubated for a long period at a temperature near 0°C. Second, the weak bands near 746, 740, 735, and 725 cm^{-1} (Fig. 6C) are the P_7 and P_5 components of the CH_2 rocking progression (25) and hence expected in the spectra.

Thermotropic Behavior

The temperature-dependent behavior of the C=O stretching bands of the DPPC following incubation at 2 and −36°C and incubation only at −36°C is shown in Fig. 7 *A* and *B*, respectively, while the corresponding plots for the CH_2 scissoring bands are shown in Fig. 8 *A* and *B*.

In the range 0–14°C the spectrum of the sample previously incubated at 2°C exhibits progressive decreases in the peak heights of the *sn*-2 C=O stretching band at 1,727 cm^{-1} and of the 1,473 cm^{-1} CH_2 scissoring band, a reversal of the behavior evident in the second and third days of incubation (Fig. 2). At 14–16°C the subtransition results in abrupt shifts in the positions of the maxima of both bands, whereas at higher temperatures the pretransition and main transition result in further changes. In contrast, the spectrum of the sample cooled rapidly from 45 to −36°C exhibits no abrupt changes at 14–16°C.

The changes in the range 0–45°C were quantitatively monitored via the frequencies and bandwidths of the CH_2 scissoring and the C=O stretching bands, the data being shown in Figs. 9 and 10, respectively. Below 16°C the data from the two series of spectra are completely different. These differences correlate with the differences observed at −36°C (Fig. 6), with a reduction in the magnitude of the differences resulting from the increased mobility at temperatures > −36°C. In particular, at higher temperatures the mobility in the subcell giving orthorhombic-like spectra results in a reduction in the factor group splitting and the consequent observation of a maximum near 1,468 cm^{-1} (Fig. 8*B*).

In the temperature range encompassing the subtransition abrupt changes are observed in all parameters derived from the spectrum of DPPC after incubation at 2°C. Particularly interesting is the change in the width at nine-tenths peak height of the CH_2 scissoring band (Fig. 9*A*). At T_s the band becomes extremely broad and, as

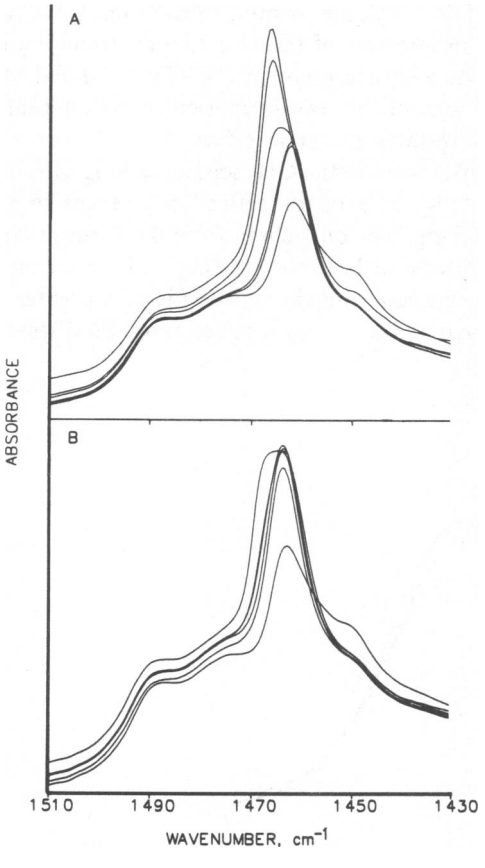

FIGURE 8 Temperature-dependent behavior of the CH_2 scissoring band of DPPC in the range 0–45°C. From top to bottom, spectra are at 4, 11, 15.5, 24, 37, and 43°C. *A*, spectra after 88 h incubation at 2°C followed by 48 h incubation at −36°C. *B*, spectra after rapid cooling from 45 to −36°C, and 48 h incubation at −36°C. The broad peak is that obtained at 4°C.

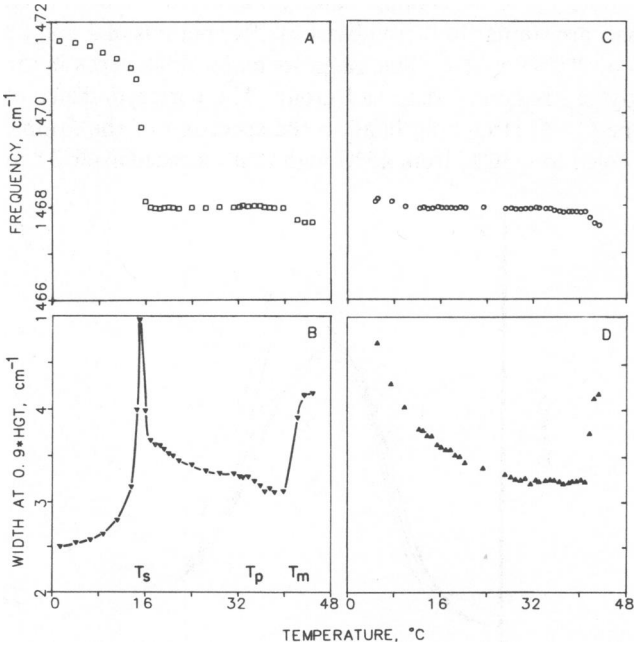

FIGURE 9 Temperature dependence of the frequency and full-width at nine-tenths height (0.9*HGT) of the CH_2 scissoring band of DPPC. Frequency (*A*) and bandwidth (*B*) determined after 88 h incubation at 2°C, and 48 h incubation at −36°C. Frequency (*C*) and bandwidth (*D*) determined after quenching from 45 to −36°C, and 48 h incubation at −36°C.

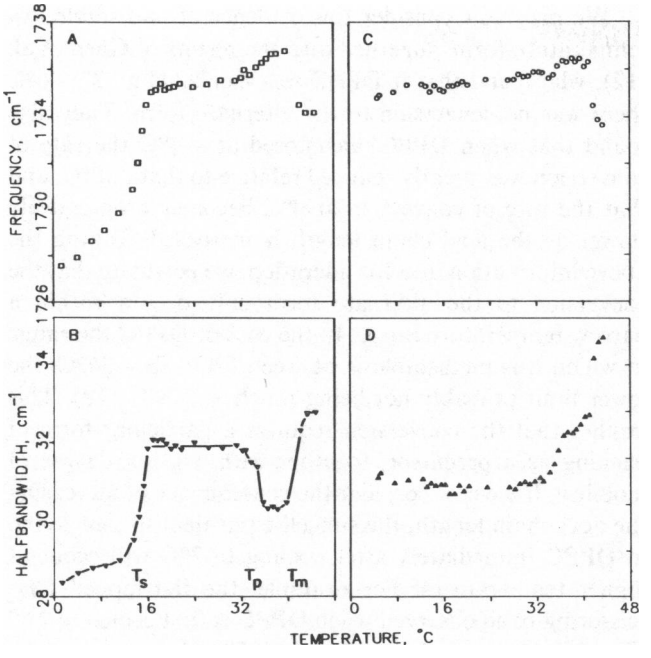

FIGURE 10 Temperature dependence of the band maximum and full-width at half-height of the C=O stretching band of DPPC. Frequency (*A*) and bandwidth (*B*) determined after 88 h incubation at 2°C, and 48 h incubation at −36°C. Frequency (*C*) and bandwidth (*D*) determined after rapid cooling from 45 to −36°C, and 48 h incubation at −36°C.

shown in Fig. 8*A*, flat-topped. This indicates that there are two components of approximately equal intensity present at 15°C. As T_s is somewhat noncooperative (12), this results from the simultaneous presence of two species of acyl chain packing within this temperature range.

Above T_s the frequency and bandwidth plots obtained from the CH_2 scissoring band in the two studies are almost identical. There are, however, small differences at T_p. In the frequency plots we observe a slight decrease at T_p after incubation only at −36°C (Fig. 9*C*), but not in the plot derived when the DPPC was incubated at 2°C (Fig. 9*A*). In the case of the bandwidths, an inflection is also evident in only one plot, that obtained when the sample was incubated at 2°C (Fig. 9*B*).

Rather more dramatic differences at T_p and T_m are evident in the plots obtained from the C=O stretching band. In the frequency plots (Figs. 10*A* and *C*) there are differences in the magnitudes of the changes at the two transitions. In the bandwidth plot derived when the DPPC was first incubated at 2°C, there is a large increase at T_s, indicating a considerable increase in the mobility of the ester linkages during this transition. Above T_p, a comparison of the two bandwidth plots (Figs. 10*B* and *D*) demonstrates absolute differences at all temperatures, and a dependency of the change at the pretransition on the thermal history of the DPPC sample. Although a decrease in bandwidth at T_p is observed when DPPC is first incubated at 2°C (Fig. 10*B*), an increase is observed when the sample is only incubated at −36°C.

Fig. 11 shows a series of difference spectra in the region of the CH_2 scissoring band. In the bottom series, derived from spectra of DPPC that was only incubated at −36°C, two minima arising from the simultaneous variation in the two bands resulting from the factor group splitting are evident at all temperatures below T_p. In contrast, the spectra in the series obtained when the sample was first

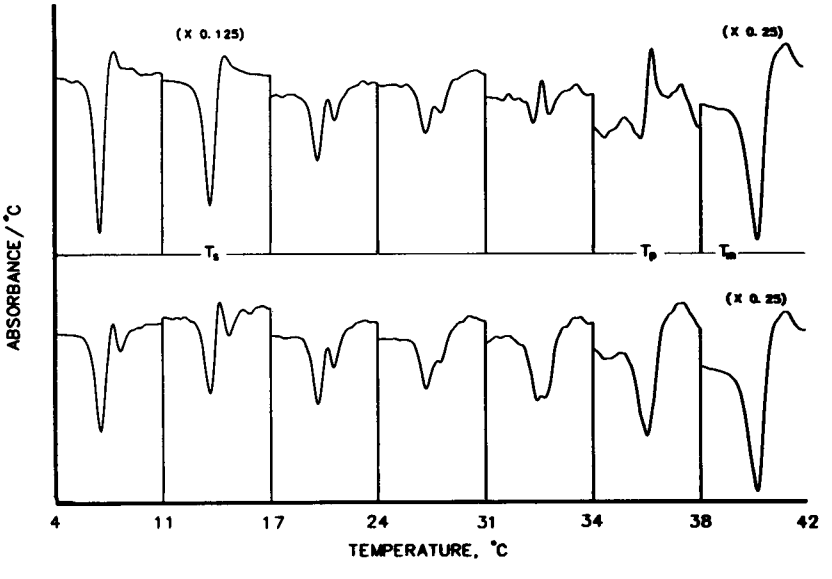

FIGURE 11 Infrared difference spectra in the region of the CH_2 scissoring band as a function of temperature in the range 0–42°C. Spectra have been normalized with respect to the temperature used to generate the spectra. Temperatures used in the subtraction are indicated on the bottom axis; e.g., the left-most difference spectra were generated by subtracting the spectra recorded at 4°C from those recorded at 11°C. Note that at T_s and T_m the difference spectra have been, respectively, reduced by factors of 8 and 4. *Top*, series obtained after 88 h incubation at 2°C followed by 48 h incubation at −36°C. *Bottom*, series obtained after quenching from 45 to −36°C, and 48 h incubation at −36°C.

incubated at 2°C (Fig. 11, *top*), exhibit a single minimum below T_s, indicating that in the range 0–15°C only a single band is changing. Between T_s and T_p the difference spectra show two minima, similar to those spectra obtained when the sample is not incubated at 2°C (see also Mantsch et al. [11], Fig. 1). This confirms that T_s is a solid-solid transition to a subcell in which the acyl chains have high mobility about the long axis, and which results in factor group splitting in the infrared spectrum.

At T_p the spectra in the two series (Fig. 11) are quite different. The spectra obtained when the sample was not incubated at 2°C exhibit a large change, as expected from the changes in Fig. 9. In contrast, only a weak derivative-shaped feature is obtained when the sample is incubated at 2°C. The data thus demonstrate that in the temperature range $T_s<T<T_p$, the two-dimensional form of the acyl chain packing is the same, regardless of the thermal history. Nonetheless, there are distinct spectral differences that indicate differences in the three-dimensional packing in this temperature range.

In a further experiment, DPPC was incubated at 2°C for 2 h, then at 20°C for 24 h. After this, the temperature dependency in the range 20–45°C was studied. The results were essentially identical to those shown in Figs. 9 and 10. In particular, the decrease in width of the C=O stretching band at T_p (Fig. 10*B*) was reproduced.

DISCUSSION

This study demonstrates that the subtransition is a transition between two alternate forms of DPPC. As shown by the experiments at −36°C, both forms are within a reasonable time frame, stable at low temperatures.

Immediately after a reduction of the temperature to any value below T_p, the acyl chain bands show factor group splitting and the carbonyl band is typical of a fully hydrated sample. On incubation at 2°C there is a reduction of the mobility of all functional groups, and a transformation to an alternate form, which transformation follows second- or higher-order kinetics. In the spectrum of this form the C=O stretching band resembles that of DPPC monohydrate (26) and anhydrous DPPC (21). Therefore, the data suggest a partial dehydration of the carbonyl and, by inference, the head group, during the incubation period. The loss of intensity of the CH_2 scissoring and rocking bands at 1,462 and 728 cm^{-1} results in a final spectrum composed of two major bands at 1,473 and 720 cm^{-1}. This spectrum is close to that observed in the spectra of simple acyl compounds packed in the triclinic subcell, in which factor group splitting is absent (18, 19). However, there is residual intensity at 1,462 and 728 cm^{-1}. If we consider the simplest forms of packing, orthorhombic and triclinic, the data could be explained in terms of a substantial reduction in the angle between the carbon skeletal planes of adjacent chains, from a value of 80–90° in the orthorhombic subcell, towards a value of 0° in the triclinic subcell (see Cameron et al. [8], Fig. 3).

We may now consider this evidence of two stable low temperature forms together with the results of Chen et al. (12), who found that if DPPC was incubated at $T > 6$°C there was no conversion to the alternate form. They also found that when DPPC was stored at −8°C the rate of conversion was greatly reduced relative to that at 0°C, and that the rate of conversion at 0°C becomes progressively slower as the acyl chain length is increased. Taking the above information into consideration, we postulate that the conversion to the alternate form only occurs within a narrow temperature range. In the case of DPPC the range in which it is metastable is between $6°C>T>-36°C$, the lower limit probably not being much < -8°C (12). This implies that the conversion requires a particular form of packing as a precursor, together with a given degree of mobility. If we now consider the consequence of increasing the acyl chain length, the subcell equivalent to that found in DPPC immediately after cooling to 2°C will occur at higher temperatures. For example, the flat-topped CH_2 scissoring band observed when DPPC is first cooled to 2°C (Fig. 2*B*) is observed at ~10 and 30°C in the spectra of the distearoyl and dibehenoyl phosphocholines, respectively (this laboratory, unpublished results), differences in temperature roughly corresponding to the differences in the temperatures of their main transitions. Consequently, we predict optimum rates of conversion will occur at ~40°C below the main transition temperature; that is, a reduced temperature rather than an absolute temperature. The most likely explanation for the transformation is that, on incubation, gel phase DPPC forms a coagel. Relative to the gel, semicrystalline coagels are poorly hydrated, the mobility is greatly decreased, and their infrared spectra resemble those of anhydrous solids (27–29). Metastable gels are well known in surfactant systems (27, 28) and the observation of a coagel in a lipid system would not be unexpected.

One can also consider the postulate that there is a change in the head group conformation. Seelig and Seelig (30) pointed out that there are two possible conformations of the head group of DPPC, of near equal energy but with a considerable steric barrier between them. Although they have now demonstrated that the simultaneous occurrence of the two conformations is not the cause of the two deuterium NMR signals they observed (personal communication cited by Büldt and Wohlgemuth [31]), the possibility that T_s involves conversion from one conformation to another should be given consideration because of the thermotropic behavior of DPPC at $T>T_s$. Chen et al. (12) had concluded that at $T>T_s$ the thermal behavior was independent of the thermal history of the sample. However, our data indicate that the three-dimensional packing is dependent on the thermal history. Although the subcell always results in factor group splitting in the range 16–34°C, the CH_2 scissoring bands show substantially different behavior at T_p (Figs. 8, 9, and 11), while the carbonyl band parameters show a high dependence on

whether or not the sample has been incubated near 0°C (Fig. 10).

These observations suggest that some of the changes in the region of the glycero moiety induced by the incubation at 2°C are retained throughout the gel phase, the principal change at T_s being a large increase in the mobility. The fact that the conversion to the configuration which gives rise to "normal" spectral changes in the infrared spectrum is extremely slow at 20°C supports a high energy barrier, in keeping with the suggestions of Seelig and Seelig (30).

Polymorphism is well known in related compounds such as *n*-alkanes, fatty acids, esters, and alcohols (32, 33). Frequently, several stable forms, mixed crystals, and, indeed, metastable forms can be obtained, depending on factors such as the thermal history and the solvent used in crystallization. We also note that two gel phases have been reported for an aqueous stearoyl sphingomyelin dispersion (34), although in that case one form is only metastable, as distinct from DPPC where polymorphism is exhibited at low temperatures.

Finally, we would reiterate a point made previously (9). In simpler systems, such as *n*-alkanes and fatty acids, the preferred form of packing of the acyl chains is in rigid orthorhombic or triclinic lattices. The high degree of mobility encountered in the gel phase of phospholipids and the temperature dependence of the factor group splitting indicate that the energetically most favored conformations of the two lipid components, the head group and the acyl chains, are incompatible. Consequently, the bilayer structure at any given temperature and chain length represents a compromise between the two, and will be different from that obtained with the same chain length at different temperatures, or at the same temperature with different chain lengths. Within such a framework, it is not surprising that the transition to the alternate form may only be induced within a certain temperature range, a range within which the overall conformation is that required to effect the transformation.

APPENDIX

After submission of this article for publication, two x-ray studies of the subtransition have been reported (35, 36), the results of which agree with our conclusions. However, these and other x-ray structural studies of lipids (37, 38) demonstrate that the acyl chains probably pack in complex hybrid subcells. Hence, while providing a basis for comparison and discussion of the characteristics of spectra, the triclinic and orthorhombic subcells may not be encountered in lipids, and in any case can only be determined from x-ray studies.

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