glucocerebroside samples suggest that this exothermic transformation does not involve a major rearrangement of the hydrocarbon chains.

The existence of metastable phases and irreversible processes in association with the phase behavior of supramolecular lipid structures has received little attention, and their biological implications are still unknown. Recently, however, Estep et al. (1980) have presented evidence of a metastable gel phase in *N*-stearoylsphingomyelin and studied the thermodynamics and structural characteristics of these conformations. These authors concluded that the gel phase of these lipids is more highly ordered than that of other phospholipids and undergoes a gel–liquid-crystalline transition that is not rapidly reversible. The resulting liquid-crystalline phase supercools below the phase-transition temperature, giving rise to a metastable gel phase that slowly converts to a highly ordered gel phase. It must be noted, however, that the pattern of transitions obtained for *N*-stearoylsphingomyelin differs from that of glucocerebroside. In the case of *N*-stearoylsphingomyelin, the metastable phase is also capable of undergoing the gel–liquid-crystalline transition as demonstrated by the appearance of a second endothermic peak associated with the melting of the metastable phase (Estep et al., 1980). In the case of glucocerebroside the metastable phase transforms into the stable gel phase during the exothermic transition. These differences in behavior suggest that the sources of metastability for these two systems are different. In the case of glucocerebroside, the metastable behavior is most likely associated with the head groups, as deduced from the calorimetric data and by the fact that both natural glucocerebroside and synthetic *N*-palmitoylglucocerebroside show this behavior. On the other hand, it is quite conceivable that the origin of the metastable behavior in *N*-stearoylsphingomyelin is associated with the packing of the hydrocarbon chains, since *N*-palmitoylsphingomyelin and *N*-lignocerylsphingomyelin do not show a metastable behavior (Estep et al., 1980).

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# Characterization of the Pretransition in 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine by Fourier Transform Infrared Spectroscopy†

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ABSTRACT: Fourier transform infrared spectroscopy has been used to study the infrared-active acyl chain vibrational modes of fully hydrated multibilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (L-DPPC) over the temperature range 0–55 °C. Frequencies, bandwidths, and other spectral parameters were measured as a function of temperature for the methylene scissoring, rocking, and wagging modes, as well as for the C–H stretching modes, and they were used to monitor the packing of the acyl chains. Particular emphasis was placed on determining the nature of the pretransition event. It is shown that between 36 and 38 °C the spectral changes are indicative of a phase change in the acyl chain packing from an orthorhombic to a hexagonal subcell. It is also concluded that in the gel phase, at all temperatures below the main transition, the acyl chains are predominantly in all-trans conformations and that the temperature-dependent variations of spectral parameters result from changes in interchain interactions.

The phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (L-DPPC) forms bilayer structures when hydrated, and has been used extensively as a model for more complex biomembranes. The bilayer exists in two distinctly different phases. The nature of the liquid-crystalline phase, particularly the state of the acyl chains at temperatures above 42 °C, has been well characterized by vibrational spectroscopy (Wallach et al., 1979, and references therein) and by $^2$H NMR (Seelig & Seelig, 1974; Davis, 1979). In this phase, the acyl chains contain large numbers of gauche conformers, which vary rapidly in number and location, leading to the description of it as a fluid state.

The detailed organization of the bilayer in the gel phase is less clearly understood. Extensive studies on multibilayers by techniques such as X-ray diffraction (Tardieu et al., 1973;

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Rand et al., 1975; Janiak et al., 1976, 1979; Stamatoff et al., 1979; Pearson & Pascher, 1979), calorimetry (Hinz & Sturtevant, 1972; Peterson et al., 1975; Silvius et al., 1979), $^2H$ NMR (Seelig & Seelig, 1974; Davis, 1979), and vibrational spectroscopy (Bulkin & Krishnamachari, 1972; Asher & Levin, 1977; Gaber & Peticolas, 1977; Mendelsohn & Taraschi, 1978; Sunder et al., 1978) have determined that the acyl chains are extended and packed in a regular lattice. However, the exact structure of the lattice in the gel phase at various temperatures, particularly the nature of the endothermic event between 32 and 38 °C, generally referred to as the pretransition, is still the subject of discussion.

In an earlier publication (Cameron & Mantsch, 1978), we demonstrated that the problems encountered when working with aqueous samples in the infrared could be largely surmounted by means of Fourier transform spectral techniques, and we presented preliminary data on the gel to liquid-crystal phase transition. We have also recently characterized the packing of the acyl chains in L-DPPC at temperatures below 10 °C (Cameron et al., 1980). We describe here a study of the infrared active acyl chain vibrations of L-DPPC over the temperature range 0 to 55 °C which covers both transitions. The nature of the gel-phase lattice packing, and of the changes in the lattice at the pretransition temperature, is characterized.

## Materials and Methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine was purchased from Sigma Chemical Co. Its purity was checked by thin-layer chromatography. Thin films and gels were prepared for infrared spectroscopy according to the detailed methods reported earlier (Cameron et al., 1979). The following samples of L-DPPC were employed: 6-$\mu$m thick samples hydrated with double-distilled $H_2O$ in $BaF_2$ cells; a 6-$\mu$m sample hydrated with double-distilled $H_2O$ in a ZnSe cell; 25-$\mu$m samples hydrated with double-distilled $H_2O$ in $BaF_2$ cells.

Spectra were recorded on two different Nicolet 7199 Fourier transform infrared spectrometers and on a Digilab FTS-11 Fourier transform infrared spectrometer. Mercury cadmium telluride (Infrared Associates, New Brunswick, NJ) and deuterated triglycine sulfate detectors were used. In the case of the sample in the ZnSe cell, a low-frequency band-pass filter (10.0–20.0 $\mu$m) was used in order to study the $CH_2$ rocking band at 720 $cm^{-1}$, superimposed on the strong $H_2O$ rotational band in this region. With this cell, despite wedging of the windows, high-frequency interference fringes were observed. The effects of these fringes were minimized by smoothing with an 11-point quadratic function, which reduced the resolution of this band to $\sim$3 $cm^{-1}$ (Savitzky & Golay, 1964). Interferograms were collected with an optical retardation of 1 cm, apodized with a Happ-Genzel function (Nicolet) or triangular function (Digilab), and Fourier transformed to yield a resolution of 0.9 $cm^{-1}$. The collection time, selected according to the desired signal-to-noise ratio, varied from 5 to 30 min. The absolute frequency accuracy and reproducibility of the digitized spectra are each 0.01 $cm^{-1}$.

Temperature control was achieved by flowing a thermostated methanol/water mixture through a hollow cell mount. Temperature was monitored by a copper-constantan thermocouple located against the edge of the cell windows, a continuous record being obtained on a printer via a Newport digital pyrometer. The spectrometer controlled the complete operation of recording a spectrum, printing and incrementing the temperature, waiting for temperature equilibration, and then repeating the sequence.

Spectra were processed according to the techniques described previously (Cameron et al., 1979). Frequencies and bandwidths were measured directly from absorbance spectra. Difference spectra were generated by taking the absorbance of the ratio of a higher temperature single beam spectrum to that at a lower temperature. Provided the concentration, cell path length, and all instrumental parameters are kept constant, this method directly yields a spectrum comprising all changes in absorbance resulting from the temperature variation. This procedure leads to positive deviations from the base line where the absorbance has increased with increasing temperature and negative deviations where it has decreased. Two parameters, $\Delta B$ and $\Delta A$, were determined from the difference spectra. Changes in integrated intensity ($\Delta B$) were measured by subtracting the negative difference band from a given peak from the positive difference band and were divided by the temperature increment to yield $\Delta B/°C$. $\Delta A$ is a parameter measuring the maximum change in absorbance in the region of a given band (see Figure 1); when normalized with respect to the corresponding temperature increment, it yields $\Delta A/°C$, useful in monitoring phase changes in lipids (Mantsch et al., 1980).

As the application of Fourier transform infrared difference spectroscopy to the study of biomembranes is a relatively recent innovation, an added measure of confidence in the technique is available from the fact that identical results were obtained from three different instruments. For a more detailed discussion of the precision and use of Fourier transform infrared spectroscopy and infrared difference spectroscopy, see Cameron et al. (1979) and references cited therein.

## Results

*Acyl Chain Scissoring and Rocking Modes.* The infrared spectra of L-DPPC in the $CH_2$ scissoring and rocking regions are shown in Figure 1, parts A and B, respectively. Each part shows in the bottom section a set of five absorbance spectra taken at different temperatures and in the upper section the four difference spectra generated from these absorbance spectra. The temperatures are selected so as to bracket the various stages of the temperature-dependent behavior of L-DPPC: the liquid-crystalline phase, the gel to liquid-crystal phase transition, the pretransition, and the gel phase below the pretransition.

The dominant features in Figure 1 are strong fundamentals at 1468 and 721 $cm^{-1}$ resulting from the out-of-phase scissoring and rocking of the methylene groups in all-trans segments of the acyl chains (Snyder, 1961). Other weak bands in the $CH_2$ scissoring region result from head group and terminal methyl absorptions, but we shall restrict the present discussion to the acyl methylene bands.

In the liquid-crystalline phase, these bands are relatively insensitive to temperature variations; however, they undergo abrupt changes in intensity during both transitions. Below the temperature of the pretransition, the principal changes in the absorbance spectra are broadenings of the band contours as the temperature is decreased. In the case of the $CH_2$ scissoring mode, this occurs primarily on the high-frequency side, and at 0 °C (Figure 1A, broken line) the band contour is comprised of two barely resolved components, at about 1472 and 1467 $cm^{-1}$. In the case of the $CH_2$ rocking mode, the band maximum has shifted to lower frequency, but the band contour is now asymmetric as a result of the broadening occurring solely on the high-frequency side of the peak maximum (Figure 1B, broken line).

In the difference spectra of Figure 1, more subtle effects are evident. Throughout the liquid-crystalline phase (55–43 °C), only minor reductions in intensity occur as the temperature is raised, while the main transition (43–38 °C) produces

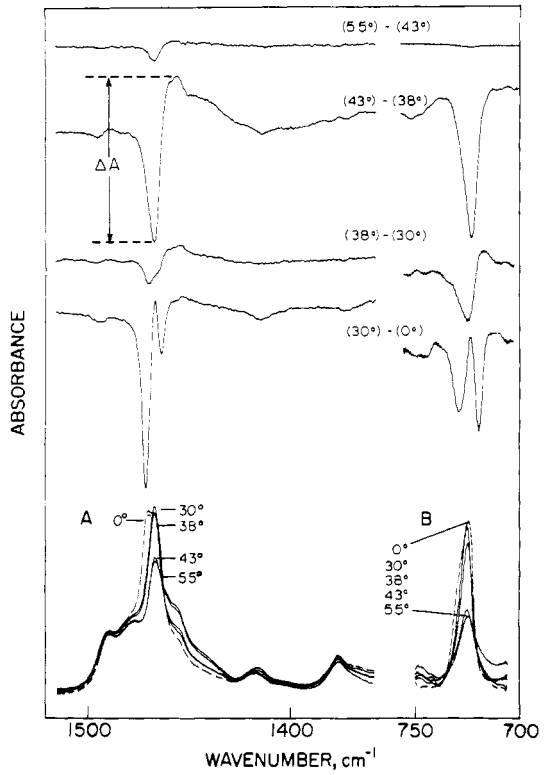


FIGURE 1: Fourier transform infrared absorbance (bottom) and corresponding difference spectra (top, ×2.5) of the $CH_2$ scissoring (A) and $CH_2$ rocking (B) regions of DPPC. The spectra in A were recorded in a 6-μm $BaF_2$ cell and were not smoothed. The spectra in B were obtained in a 6-μm ZnSe cell and were smoothed with an 11-point function. In addition, base lines in B were corrected by removing the slope from the underlying broad water fundamental.

large intensity decreases. In the case of the $CH_2$ scissoring band, the intensity loss is accompanied by an increase in the intensity in the region 1440–1450 $cm^{-1}$. This is typical of the behavior of these modes on melting of the acyl chains (Nielsen & Hathaway, 1963; Snyder, 1967).

The changes observed in the temperature range covering the pretransition (38–30 °C) are smaller than those in the main transition and also different in nature. Although there are similar reductions in the intensities of the fundamentals, and an intensity increase in the region 1440–1450 $cm^{-1}$, both difference spectra minima are shifted relative to those of the main transition. Furthermore, in the case of the $CH_2$ scissoring mode, a low-frequency shoulder is evident in the negative peak.

The gel-phase difference spectrum (30–0 °C) is completely different. In both the $CH_2$ scissoring and the $CH_2$ rocking regions there are two minima. As the difference spectra are an absolute measure of the absorbance differences resulting solely from the variation in temperature, the observation of the two minima confirms the detection in the absorbance spectra of splitting of the $CH_2$ scissoring mode at 0 °C. They also indicate that the changes in the $CH_2$ rocking mode result from splitting of the band, one component shifting to lower frequency and the second, weaker component appearing at higher frequency. As we have shown earlier (Cameron et al., 1980; Mantsch et al., 1980), this splitting continues progressively with decreasing temperature, and at about −60 °C both the $CH_2$ scissoring and the $CH_2$ rocking mode of L-DPPC are split by about 10 $cm^{-1}$. The simultaneous observation of two bands in each of these regions is assigned to factor group (or crystal field) splitting observed only when the acyl chains are packed in an orthorhombic or monoclinic unit cell, both of which have an orthorhombic subcell the same as the unit cell of orthorhombic polyethylene (Snyder, 1979).

We have characterized the nature of the changes in the methylene scissoring and rocking modes by quantitatively evaluating several spectral parameters. The absorbance and $\Delta A/°C$ (rate of change of absorbance) plots of the $CH_2$ scissoring mode (Figure 2A) and of the $CH_2$ rocking mode (Figure 2B) show similar behavior. Large changes are observed in the main transition at 41.5 °C, while at a slightly lower temperature, 37 ± 1 °C, smaller changes associated with the pretransition are found. Both in the gel phase and in the liquid-crystalline phase, the rates of change are much smaller and nearly constant.

The behavior of the bandwidth of the $CH_2$ rocking mode (Figure 2C) is in contrast to that shown by the above-mentioned absorbance plots. The bandwidth undergoes a large change at the main transition and is constant over the range 40 to 20 °C. It then increases again when the temperature is further reduced, as a result of the factor group splitting. The same trend was observed for the $CH_2$ scissoring band in the gel phase. In addition, no evidence of a pretransition could be obtained from the width of either band.

In an earlier study of L-DPPC multibilayers (Cameron et al., 1980), using a grating instrument, we reported the onset of factor group splitting at temperatures below 10 °C. However, the half-widths of the $CH_2$ rocking and scissoring bands indicate that splitting is present in the range 10 to 20 °C, while the shoulder in the methylene scissoring band in the difference spectrum covering the pretransition range 38–30 °C suggests that splitting occurs at even higher temperatures. In order to observe the onset of the splitting phenomenon, we monitored the positions of the minima in the difference spectra of the $CH_2$ scissoring mode. These frequencies are plotted as a function of temperature in part D of Figure 2. At all temperatures above 37 °C, a single minimum is observed at the same frequency as that of the peak in the absorbance spectrum, demonstrating that in this temperature range there is only one band, at constant frequency, the peak height of which decreases with increasing temperature. Below this temperature, splitting of the $CH_2$ scissoring mode commences and increases in magnitude with decreasing temperature. This is evident from the appearance of a low-frequency shoulder in the difference spectrum, which is resolved into a second minimum below 30 °C, and the simultaneous shift of the stronger minimum to higher frequency. The separation of the two components increases steadily with decreasing temperature until, as discussed above, the splitting in the difference spectra can be clearly related to the splitting in the absorbance band contours.

Thus, these data demonstrate that as the temperature is reduced below 36 °C there is a progressive factor group splitting of the $CH_2$ rocking and scissoring modes of L-DPPC. Of the three possible forms of acyl chain packing, i.e., hexagonal, orthorhombic, or triclinic, such splitting is in agreement only with the assumption of packing in an orthorhombic subcell.

*Methylene Wagging Band Progression.* In the region 1360–1190 $cm^{-1}$ (Figure 3) the major spectral feature is the antisymmetric phosphate stretching band at 1232 $cm^{-1}$. In the gel-phase absorbance spectra there is also evident a progression of eight weak, regularly spaced bands at about 1200, 1220, 1245, 1265, 1290, 1310, 1330, and 1345 $cm^{-1}$. These bands are absent in the liquid-crystalline phase. They are present at all temperatures below the main transition and increase considerably in intensity as the temperature is further reduced. In fact, as is evident from the spectra in Figure 3, the phosphate band is almost invariant with temperature, and

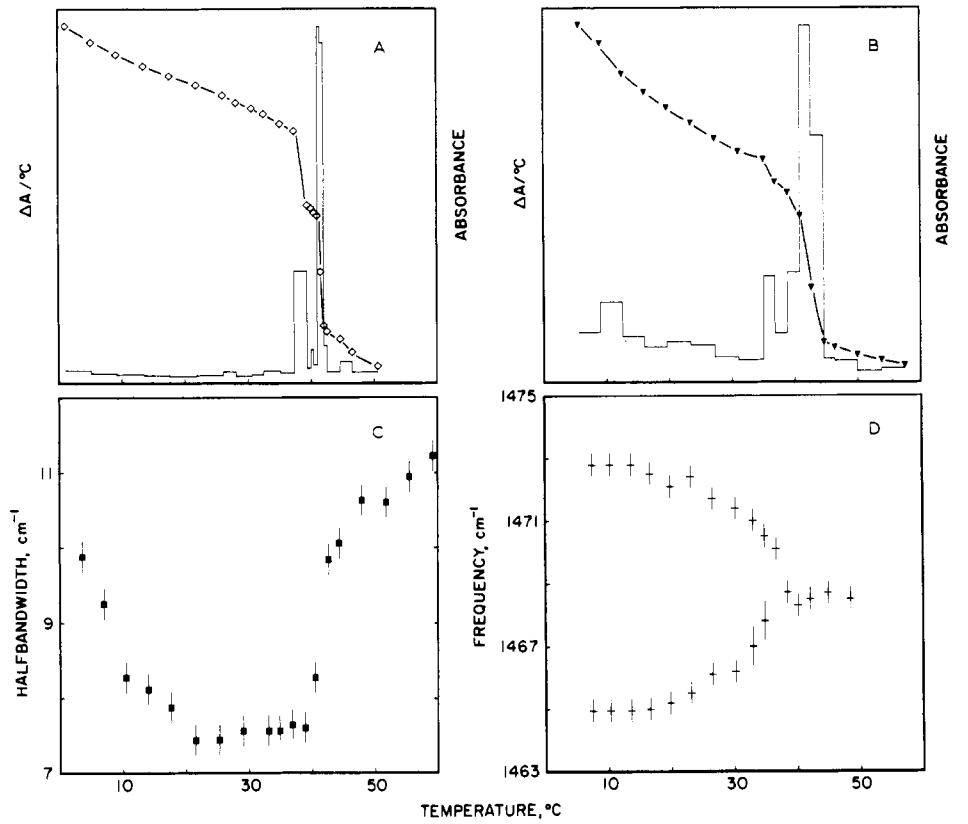


FIGURE 2: Temperature dependence of the $CH_2$ scissoring and rocking modes in the spectra of DPPC: (A) $CH_2$ scissoring mode, as $\Delta A/°C$ (solid line) and A (◇) vs. temperature; (B) $CH_2$ rocking mode, as $\Delta A/°C$ (solid line) and $A$ (▼) vs. temperature; (C) half-bandwidth of $CH_2$ rocking mode vs. temperature; (D) frequency positions of the minima in the difference spectra of the $CH_2$ scissoring mode vs. temperature.

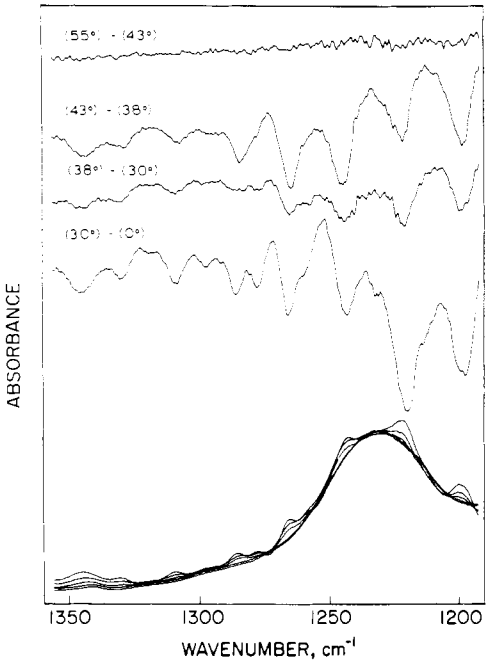


FIGURE 3: Fourier transform infrared absorbance (bottom) and corresponding difference spectra (top, ×7) of the $CH_2$ wagging band progression and of the antisymmetric phosphate stretching mode of DPPC. Spectra are from a 6-μm gel and are not smoothed.

the difference spectra result entirely from changes in the band progression.

The band progression results from the wagging of $n$ coupled $CH_2$ oscillators in an all-trans conformation. The phase differences, $\psi$, allowed between adjacent groups are given by

$$\psi = k\pi/(n + 1)$$

where $k$ is an integer from 1 to $n$ that represents the number of loops in the stationary wave representing the normal mode (Synder & Schachtschneider, 1963). The frequency pattern is specific to the length of the all-trans segment of the chain, and in this case, the frequencies are characteristic of the all-trans palmitoyl chain (Susi & Pazner, 1962; Hayashi & Umemura, 1975).

The difference spectrum for the liquid-crystalline phase is featureless, confirming the absence of the band progression in this phase, as a result of the high proportion of gauche conformers present in the acyl chains. When the temperature is reduced from 43 to 38 °C, large changes are evident in the band progression due to the transition to a predominantly all-trans conformation. Smaller intensity changes are observed in the pretransition range 38–30 °C, and, as the temperature is further reduced, the intensities of the band progression components continue to increase. Also evident in the 30–0 °C difference spectrum is a set of weak bands at about 1265, 1278, and 1300 $cm^{-1}$. We believe this to be the $CH_2$ twisting–rocking band progression, which is considerably weaker than the wagging progression when the chain is terminated by a carbonyl group (Susi & Pazner, 1962).

The way in which the bands change was determined by monitoring $\Delta A/°C$ and the frequencies of the band progression. The $\Delta A/°C$ plots (not shown here) were almost identical with those of the $CH_2$ rocking and scissoring modes, except that above 42 °C, $\Delta A/°C$ is zero, as these bands are absent in the liquid-crystalline phase. The frequencies of the band progression were derived from the positions of the minima in the difference spectra and are listed in Table I. As the individual bands of this band progression show no splitting, the values in Table I correspond to their actual peak positions, except when a shift in frequency is occurring. It is evident that in the range 37 ± 1 °C there is an abrupt shift to higher

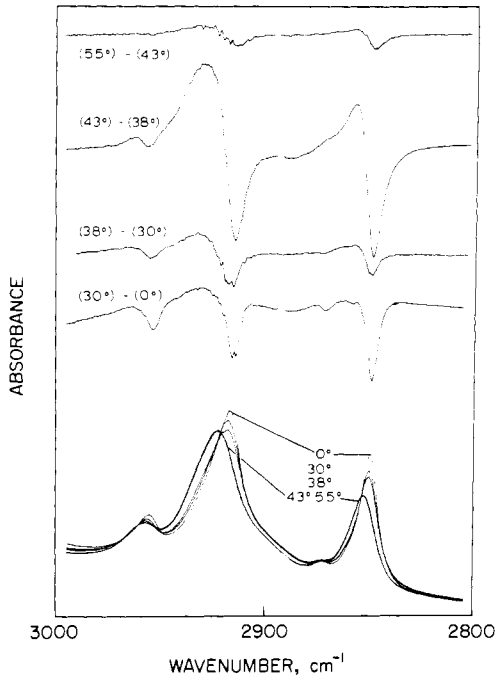


FIGURE 4: Fourier transform infrared absorbance spectra (bottom) and corresponding difference spectra (top, ×4) of the C–H stretching region of a 2.5-$\mu$m fully hydrated film of DPPC at 0, 30, 38, 43, and 55 °C. The spectra at 43 and 55 °C overlap to the extent that they are indistinguishable. The spectra are not smoothed.

frequency of the $k$ = 1, 2, and 3 components as the temperature is raised. Apart from this change, the frequencies are constant.

*C–H Stretching Modes.* The absorption spectra of the CH stretching modes of L-DPPC and the corresponding difference spectra are shown in Figure 4. Four bands are resolved: the methylene antisymmetric and symmetric CH stretching bands at about 2920 and 2850 cm$^{-1}$, respectively, and the asymmetric and symmetric terminal methyl CH stretching bands at about 2956 and 2872 cm$^{-1}$, respectively. In addition, there is a broad band at about 2900 cm$^{-1}$ resulting from a weak Fermi resonance interaction between the symmetric methylene mode and the first overtone of the methylene scissoring mode (Synder et al., 1978).

As is evident from Figure 4, these bands change substantially in the pre- and main transitions, the frequencies of the methylene bands having previously been used to monitor the main transition in DL-DPPC (Asher & Levin, 1977). The antisymmetric and symmetric methylene bands show identical behavior. They are almost invariant in frequency throughout the gel phase, with an abrupt peak height change at the temperature of the pretransition. The main transition results in changes in all band parameters, while in the liquid-crystalline phase changes are minor and restricted to the peak height. In contrast, the methyl bands at 2956 and 2872 cm$^{-1}$ undergo large changes in frequency, bandwidth, and peak height in the gel phase and in the pretransition. This is particularly evident in the corresponding difference spectra.

Detailed data regarding the temperature dependence of these bands are given in Figure 5. The plots of $\Delta A/°C$ (Figure 5A) and $\Delta B/°C$ (Figure 5B) of the methylene symmetric CH stretching mode closely resemble the $\Delta A/°C$ plots of the $CH_2$ scissoring and $CH_2$ rocking modes in Figure 2.

The temperature dependence of the frequencies and bandwidths of the CH stretching modes are shown in Figure 5C and 5D, respectively. In neither plot is there evidence of a pretransition. The frequencies of the methylene modes are invariant (Figure 5C), except at the main transition where the

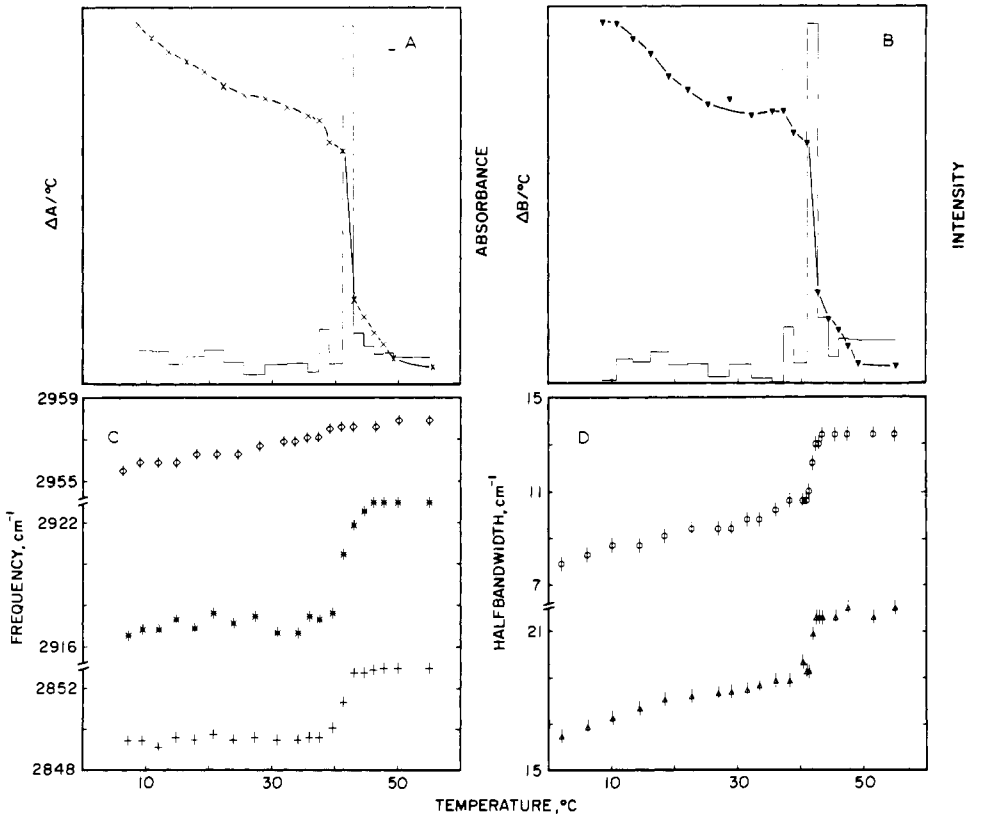


FIGURE 5: Temperature dependence of spectral parameters derived from the C–H stretching modes of DPPC: (A) symmetric methylene stretching vibration as $\Delta A/°C$ (solid line) and as $A$ (×–×) vs. $T$; (B) integrated intensity of the symmetric methylene stretching mode ($B$, ▼) and its rate of change ($\Delta B/°C$, solid line); (C) frequencies—asymmetric methyl CH stretching (♦), antisymmetric methylene stretching (*), and symmetric methylene stretching (+); (D) half-bandwidths—antisymmetric methylene stretching ($\phi$), symmetric methylene stretching (⏃).

Table I: Peak Positions of the First Three Components of the $CH_2$ Wagging Band Progression, Determined from the Indicated Difference Spectra

| | $k$ = | | |
|---|---|---|---|
| temp range (°C) | 1 | 2 | 3 |
| 42–40 | 1200 | 1222.5 | 1246 |
| 40–38 | 1200 | 1222.5 | 1246 |
| 38–36 | 1199 | 1221.5 | 1244.5 |
| 36–33 | 1198 | 1221 | 1244 |
| 33–30 | 1198 | 1221 | 1244 |
| 30–0 | 1198 | 1221 | 1244 |

shifts reflect the acyl chain melting. This also produces the large bandwidth changes at 41 °C (Figure 5D). Below 41 °C there are steady decreases in the methylene bandwidths with inflections at 20 °C, which can be correlated with the factor group splitting effect observed in the $CH_2$ rocking mode. Bandwidths were not monitored for the methyl mode due to overlap with the methylene band, but the corresponding frequency plot shows large changes throughout the gel phase and a relatively minor change at the main transition.

## Discussion

As mentioned in the introductory statement, the main transition at 41.5 °C marks a change from a solidlike to a fluidlike phase. At this temperature, the infrared spectrum of the L-DPPC acyl chain modes exhibits large concomitant variations in peak height, frequency, and bandwidth, similar to the changes observed in the melting of *n*-alkanes (Sheppard, 1959). In the liquid-crystalline phase much smaller changes of the same type are observed as the temperature is raised. This correlates with results of $^2H$ NMR studies (Seelig & Seelig, 1974), which indicate an increase in the average number of gauche conformers per acyl chain as the temperature is raised in the liquid-crystalline phase.

The most specific data from the infrared spectra relate to the gel phase and are obtained from the absorptions due to the methylene groups. Below the pretransition temperature, the dominant factor determining the band shapes and positions is the crystal packing. The resultant effects are first evident at 37 °C in the difference spectra of the $CH_2$ scissoring band (Figures 1A and 2D). At about 20 °C the effects are also evident in the bandwidths of the $CH_2$ stretching, rocking, and scissoring modes. Below 10 °C there is clear evidence for two bands in the absorbance contour of the $CH_2$ scissoring mode, and at lower temperatures both the scissoring and rocking modes are split (Cameron et al., 1980; Mantsch et al., 1980).

The pretransition event at 37 ± 1 °C is characterized by abrupt decreases in peak height of all methylene bands. The only other band parameters observed to change in the temperature interval 36–38 °C are the frequencies of the wagging band progression (Table I). The key to the interpretation of the above results lies in the crystal packing of the L-DPPC acyl chains. The observation of factor group splitting in the infrared spectrum at all temperatures below 36 °C is only compatible with the assumption that the acyl chains are packed in an orthorhombic subcell in an orthorhombic or monoclinic crystal lattice, such splittings being absent in the spectra of compounds packed in triclinic or hexagonal lattices.

Factor group splitting results from interchain coupling of vibrational modes. The temperature dependence of splitting demonstrated here can be rationalized in terms of variations in the interchain coupling resulting from distortions of the orthorhombic subcell probably due to torsional motions about the long axes of the acyl chains. The relatively large changes observed in the methyl asymmetric stretching band support the argument of continuously increasing rates of motion and therefore an increasingly mobile central area of the bilayer in the gel phase as the temperature is raised.

This description of the bilayer structure below the pretransition is in accord with the observation of distorted hexagonal packing as reported by Janiak et al. (1979). These authors also reported an increase in the distortion as the temperature is reduced, accompanied by a decrease in the rate of motion about the long axes of the acyl chains. A similar conclusion with regard to rates of motion about the long axes was reached by Davis (1979), on the basis of $^2H$ NMR results.

We also note that a recent X-ray determination of the structure of the 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (L-DMPC) dihydrate at about 15–20 °C demonstrates a monoclinic unit cell (Pearson & Pascher, 1979) and that, toward the methyl end of the chains, the planes are twisted to a mutually parallel orientation, resembling a triclinic parallel chain packing mode. This illustrates the potential for torsional motions about the long axes, particularly in the fully hydrated system where the packing is less rigid than in the dihydrate (Levine & Wilkins, 1971).

On the basis of X-ray diffraction (Janiak et al., 1976, 1979) and Raman spectroscopic studies (Gaber & Peticolas, 1977; Gaber et al., 1978), the general nature of the pretransition has been reported as a change from a regular close hexagonal lattice above the pretransition to one involving a greater degree of interchain interactions below the pretransition. Our infrared data at temperatures in the range 41.5–38 °C are compatible with close hexagonal packing and below 36 °C with orthorhombic packing; hence we conclude that the pretransition is a crystal-lattice phase change from a hexagonal subcell (above 38 °C) to an orthorhombic subcell (below 36 °C).

Confirmation of this characterization of the pretransition comes from a vibrational study of the odd-numbered alkanes, *n*-$C_{19}H_{40}$ and *n*-$C_{21}H_{44}$ (D. G. Cameron, H. L. Casal, H. H. Mantsch, and R. G. Snyder, unpublished results). These hydrocarbons undergo an abrupt hexagonal to orthorhombic crystal-lattice phase change about 10 °C below their melting points as the temperature is reduced (Schaerer et al., 1955; Nielsen & Hathaway, 1963). We find that the major changes observed in their infrared spectra during this phase change are: the appearance of factor group splitting, slight decreases in frequency and bandwidth of the $CH_2$ stretching modes, relatively large increases in the peak heights of the $CH_2$ stretching modes and of the wagging band progression, a decrease in intensity in the 1450–1440-$cm^{-1}$ region, and shifts to *lower* frequencies of the wagging band progression as the temperature is *decreased* through the phase change. The frequency shifts of the $CH_2$ wagging band progression and the abrupt increases in peak height of all $CH_2$ modes are exactly the changes observed at the pretransition in L-DPPC. The other effects observed in the spectra of the *n*-alkanes, i.e., slight frequency shifts and narrowing of bands, are observed in the L-DPPC spectra as the temperature is reduced below 36 °C, and can be correlated with increased splitting of the $CH_2$ scissoring and rocking bands. Further evidence for an orthorhombic subcell is found in the low-temperature Raman spectrum of DL-DPPC (Yellin & Levin, 1977), where a band is evident at 1420 $cm^{-1}$. This band is only observed when acyl chains are packed in orthorhombic or monoclinic lattices (Boerio & Koenig, 1970).

The exact nature of the crystal lattice, orthorhombic or monoclinic, cannot be determined directly from the infrared spectra. The two unit cells are nearly identical, the difference being in the angle of tilt of the chains with respect to the *ab*

plane, the plane in which the terminal methyl groups lie. In the orthorhombic unit cell the chains are normal to this plane, while in the monoclinic unit cell the chains are tilted with respect to the plane (Snyder, 1979). Generally, the packing of similar compounds such as fatty acids (von Sydow, 1956), fatty acid esters (Aleby, 1962), and dipalmitoylphosphatidylethanolamine (Hitchcock et al., 1974) is monoclinic with orthorhombic packing restricted to *n*-alkanes and polyethylene. X-ray diffraction studies (Janiak et al., 1976, 1979; Stamatoff et al., 1979) of DPPC and DMPC demonstrate substantial tilting of the acyl chains with respect to the plane of the bilayer in completely hydrated samples (water content greater than 40 wt %), while the X-ray determination of the molecular structure of L-DMPC dihydrate shows that the unit cell is monoclinic (Pearson & Pascher, 1979). These data thus favor monoclinic over orthorhombic packing. However, we note that in a partially hydrated sample of DMPC (10 wt %) an orthorhombic unit cell has been reported (Stamatoff et al., 1979).

From this description of the pretransition it is also evident why the addition of perturbants such as anesthetics and cholesterol leads to its disappearance (Asher & Levin, 1977; Mendelsohn & Taraschi, 1978; Pringle & Miller, 1979). The orthorhombic subcell requires a highly specific packing of the acyl chains, especially when compared with the hexagonal phase where the interchain interactions are minimal. The introduction of molecules into the bilayer that cannot pack into the orthorhombic lattice will readily minimize or destroy the potential for such packing and hence alter the pretransition. Similar effects have previously been reported in the case of *n*-alkanes (Schaerer et al., 1955), where the presence of small amounts of impurity change the crystal lattice considerably at a given temperature.

As mentioned above, the Raman spectroscopic studies of Gaber et al. (Gaber & Peticolas, 1977; Gaber et al., 1978) lead to the conclusion that the pretransition is marked by large increases in lateral chain interactions as the temperature is decreased. However, it was concluded that the interactions were typical of a triclinic phase and that as the temperature is decreased to −14 °C there is a gradual increase in the amount of triclinic phase.. Our infrared data point to an increase in the orthorhombic nature of the crystal lattice, which is in apparent conflict with the above conclusion. However, if on reduction of motional freedom about the long axes, as a result of lowering the temperature, the chains pack as they do in the L-DMPC dihydrate, evidence for both orthorhombic and triclinic packings may be accommodated. The "triclinic" phase, if present, is relatively minor as the low-temperature infrared (Cameron et al., 1980) and Raman spectra (Yellin & Levin, 1977) are both characteristic of orthorhombic packing, the main evidence for triclinic packing being a weak unresolved Raman band at 2860 $cm^{-1}$ (Gaber et al., 1978).

A further conclusion reached from studies of Raman spectra is that the presence of gauche conformers in the acyl chains can be monitored by following changes in the peak height of the skeletal optical mode at 1130 $cm^{-1}$. Gaber & Peticolas (1977) derived a parameter, $S_{trans}$, which relates the peak height of the band to the number of trans conformers in the chain, by assuming that the intensity is the sum of intensities from all-trans segments. From the changes in height of this band, the $S_{trans}$ parameter predicts that as many gauche conformers are introduced when the temperature is raised from 15 to 37 °C as are introduced in the main transition, which is estimated to result in the introduction of three-four gauche conformers per chain (Gaber & Peticolas, 1977; Gaber et al., 1978; Mendelsohn & Taraschi, 1978). The introduction of such a large number of gauche conformers should result in several types of change in the infrared spectrum. In particular, we should expect shifts to higher frequency and broadenings of the $CH_2$ stretching bands as observed in the main transition. In fact, both infrared spectral parameters are invariant throughout the pretransition temperature range. As a result of an increased gauche conformer population, one should also observe a shift to lower frequency of the $k = 1$ component of the $CH_2$ wagging band progression (Snyder, 1967). On the contrary, shifts in the opposite direction are observed (Table I). This, however, correlates with shifts observed in the orthorhombic to hexagonal phase change in *n*-alkanes. Below the pretransition small changes in the bandwidths of the methylene stretching modes are observed, which correlate with changes in the acyl chain packing.

The lack of confirmation by infrared spectroscopy of the conclusions reached from Raman data may indicate that the assumptions used in the derivation of the $S_{trans}$ parameter are only partly correct. In this respect, Loshchilova & Karvaly (1978) have shown that the $S_{trans}$ order parameter is quite sensitive to ion/polar head interactions and cannot give unequivocal information on the trans/gauche population of hydrocarbon chains of phospholipids. Recently, Pink et al. (1980) suggested that the integrated intensity rather than the peak height of the 1130-$cm^{-1}$ Raman band more correctly reflects the average number of trans conformers per chain, and that the $S_{trans}$ parameter only provides a general measure of the membrane "fluidity". We are currently investigating the 1130-$cm^{-1}$ skeletal optical mode in the Raman spectra of *n*-alkanes (R. G. Snyder, D. G. Cameron, H. L. Casal, and H. H. Mantsch, unpublished results) in order to determine to what degree the peak height and integrated intensity are sensitive to interchain interactions.

## Conclusions

In accord with previous reports, we find that bilayers of L-DPPC exist in distinctly different phases above and below the main transition. The main phase change was confirmed as a melting of the acyl chains, and evidence was obtained for further disruption of the ordering of the chains as the temperature is raised in the liquid-crystalline phase.

Changes in the gel-phase infrared spectrum of the acyl chain modes of L-DPPC, and primarily the pretransition around 37 °C, mainly reflect changes associated with interchain interactions and with the packing of fully extended all-trans acyl chains. Above 38 °C the infrared spectra are in accord with the chains being packed in a hexagonal subcell with a high degree of motion about their long axes. Reduction of the temperature below 36 °C leads to a progressive reduction in the rate of axial motions and a consequent increase in factor group splitting due to the gradual introduction of orthorhombic packing.

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