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Infrared characterization of conformational differences in the lamellar phases of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine

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Fourier transform infrared spectroscopy was used to characterize the lamellar phases of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (1,3-DPPC), a positional isomer of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (1,2-DPPC). The molecule exists in three distinct phases over the temperature interval 0–70°C In the low-temperature (L_C) phase, the spectra are indicative of acyl chains packed in an orthorhombic subcell, while the carbonyl groups and phosphate ester at the head group show evidence of only partial hydration. The transition from the low-temperature (L_C) phase to the intermediate-temperature (L_β) phase at 25°C corresponds to a temperature-induced head-group hydration in which the hydration of the phosphate and carbonyl ester groups results in the reorganization of the hydrocarbon chain-packing subcell from orthorhombic to hexagonal The transition from the intermediate (L_β) to the high-temperature (L_α) phase at 37°C is a gel-to-liquid-crystalline phase transition analogous to the 41.5°C transition of 1,2-DPPC The spectra of the acyl-chain carbonyl groups show evidence of significant differences in molecular conformation at the carbonyl esters in the L_C phase. In the L_β and L_α phases, the carbonyl band contour becomes much more symmetric. However, two components are clearly present in the spectra indicating that the *sn*-1 and *sn*-3 carbonyls experience slightly different environments. The observed differences are likely due to a preferred conformation of the phosphocholine group relative to the glycerol backbone. Indications from the infrared spectra of differences in the structure of the C=O groups provide a possible explanation for the selection of the *sn*-1 chain of 1,3-DPPC by phospholipase A_2 on the basis of a preferred head group conformation.

Introduction

Vibrational spectroscopic techniques have been used with considerable success in recent years in elucidating the molecular and conformational properties of membrane phospholipids In particular, due to its enhanced sensitivity and precision over dispersive infrared spectroscopy, Fourier transform infrared spectroscopy (FT-IR) has allowed the precise study of pure lipids and the interaction of these molecules with ions, cholesterol, membrane proteins, and other lipids [1–4]

In the present study we have investigated the thermotropic behavior of the positional isomer 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (1,3-DPPC) using FT-IR While not naturally occurring, these 1,3-phospholipids have been shown to

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Abbreviations DPPC, dipalmitoylglycerophosphocholine, FT-IR, Fourier transform infrared spectroscopy

act as substrates for phospholipase A_2 [5,6] and the question arises as to what particular aspects of molecular conformation common to 1,3- and 1,2-DPPC correlate with substrate binding [7–9] Differential scanning calorimetric studies reveal the occurrence of two reversible phase transitions, at 25 and 37°C, with the higher-temperature transition having an enthalpy characteristic of a gel-to-liquid-crystalline transition (Chowdhry et al., unpublished data) X-ray diffraction studies confirm this description, and demonstrate that the low-temperature phase is semicrystalline in nature [9]

Materials and Methods

Materials 1,3-Dipalmitoyl-*sn*-glycero-2-phosphocholine was purchased from Fluka (Hauppage, NY) Purification and calorimetry of the purified material were as described [10] ^2H_2O was purchased from Merck, Sharp & Dohme (Montreal)

Sample preparation Multilamellar lipid dispersions were prepared by the addition of the appropriate amount of doubly deionized H_2O or ^2H_2O to the dry lipid The samples were then heated to 50°C and vortexed for 10 min The heating cycle was repeated several times in order to ensure complete dispersion Typically, the final concentration of lipid was 50 mM

Fourier transform infrared spectroscopy Samples were prepared for infrared spectroscopic analysis in 50-μm-thick demountable cells (Harrick Scientific, Ossining, New York) with Mylar spacers and CaF_2 windows Spectra were recorded on a Digilab FTS-15 Fourier transform infrared spectrometer equipped with an HgCdTe detector 250 interferograms, collected with an optical velocity of 1 26 cm s^{-1} and a maximum optical retardation of 0 25 cm, were coadded, apodized with a triangular function, and Fourier transformed with one level of zero filling to yield a resolution of 4 cm^{-1} and data were encoded every 2 cm^{-1}

Temperatures were controlled by a thermostatted EtOH-H_2O mixture flowing through a hollow cell mount and monitored by a copper-constantan thermocouple placed against the edge of the cell window [11]

Frequencies and bandwidths were determined with an uncertainty of less than $\pm 0\,01$ cm^{-1} by using a center-of-gravity alogorithm [12]

Results

Acyl chain absorptions

Figs 1 and 2 show, respectively, the 3000–2800 cm^{-1} and the 1500–1150 cm^{-1} regions of the infrared spectrum of hydrated 1,3-DPPC at 20, 33 and 43°C These regions encompass the principal absorption bands associated with the hydrophobic acyl chains, and at these temperatures the spectra are those of the L_c (20°C) L_β (33°C) and L_α (43°C) phases

The low-temperature L_c phase spectrum is typical of highly ordered, long acyl chains The most striking feature is the two sharp CH_2 scissoring bands at 1472 and 1462 cm^{-1} The splitting of the scissoring band results from interchain interactions when the acyl chains are rigidly packed in a three-dimensional lattice [13,14] In the simpler *n*-alkanes and fatty acids the subcell is orthorhombic In lipids it is more likely to be a complex hybrid [15,16]

Additional evidence for rigid packing comes from the strong series of CH_2 wagging bands stretching from 1180 to 1350 cm^{-1} (superimposed on the broad asymmetric PO_2^- stretching band) The band progression results from the wagging of *n*-coupled CH_2 oscillators in an all-*trans* conformation The phase differences, ψ, allowed between the *n* different groups are given by

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

where *k* is an integer from 1 to *n* and corresponds to the number of loops in the stationary wave that represents the normal mode [17] The frequencies of the components of the progression seen in the L_c phase spectrum of 1,3-DPPC confirm the presence of all-*trans* chains, while the intensities indicate rigid packing when compared with those in the L_β phase spectrum (see below)

Finally, the antisymmetric and symmetric CH_2 stretching bands are observed at 2916 2 and 2848 0 cm^{-1}, respectively, typical of all-*trans* hydrocarbon chains [18] In addition the asymmetric CH_3 stretching band contour near 2954 cm^{-1} is partially split, as is clearly shown in the deconvolved spectrum (Fig 1, inset) Such splitting occurs when the rotation of the methyl group relative to the adjacent CH_2 is severely restricted, and is typically observed in the spectra of orthorhombic

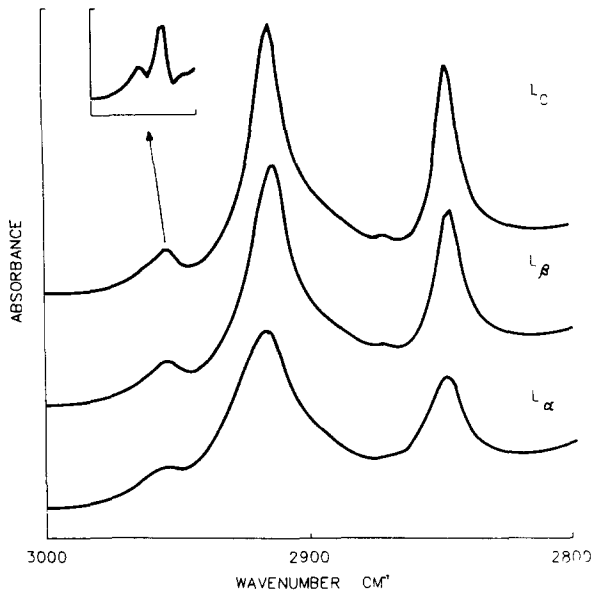

Fig 1 Infrared spectra of a 50-μm-thick sample of 1,3-DPPC in ^2H_2O in the L_C (20°C), L_β (33°C) and L_α (45°C) phases in the region 3000–2800 cm^{-1} that encompasses the C–H stretching bands Inset Fourier self-deconvolution spectrum of the asymmetric CH_3 stretching band at 2954 cm^{-1} in the L_C phase (20°C) The spectrum was deconvoled with a 4 cm^{-1} halfwidth Lorentzian line and smoothed to $K = 2\,0$ with a Bessel function [31 32]

and triclinic crystals of alkanes and fatty acids [19]

The transition of the L_β phase at 25°C results in marked changes in the acyl-chain spectrum The principal change is the collapse of the CH_2 scissoring doublet to a single band at 1468 cm^{-1} This frequency is characteristic of long acyl chains in an hexagonal subcell, with high mobility about the long axis of the acyl chain [13]

The CH_2 wagging progression is still present, but greatly reduced in intensity The CH_2 stretching bands are broader, less intense, and shifted slightly in frequency (see below), while the asymmetric CH_3 stretching band is now a singlet All of these changes are similar in magnitude and direction to those observed in the spectra of *n*-alkanes on transition from the orthorhombic to the "hexagonal" or "rotator" phase [20]

In the L_α phase spectrum above 37°C the intensity of the 1468 cm^{-1} bands is decreased, the wagging progression is absent, and the C-H stretching bands have broadened and shifted to higher frequency These changes indicate the introduction of a high degree of conformational disorder, and are similar to those observed in the spectrum of 1,2-DPPC on transition from the L_β to the L_α phase [4]

Head-group absorptions

Fig 3 shows the 1150–1000 cm^{-1} region of the infrared spectrum of 1,3-DPPC in the L_C, L_β and L_α phases The strong band near 1090 cm^{-1} is the symmetric PO_2^- stretching band, overlapped with a C–O–C stretching band near 1070 cm^{-1} and a weak C–C stretching band near 1080 cm^{-1} In the L_C phase spectrum lines are narrow and reasonably well resolved Transition to the L_β phase results in broadening, a shift to the PO_2^- stretching band from 1090 2 (L_C) to 1086 4 cm^{-1} (L_β) Little change is observed on transition to the L_α phase

The L_C phase spectrum resembles that ob-

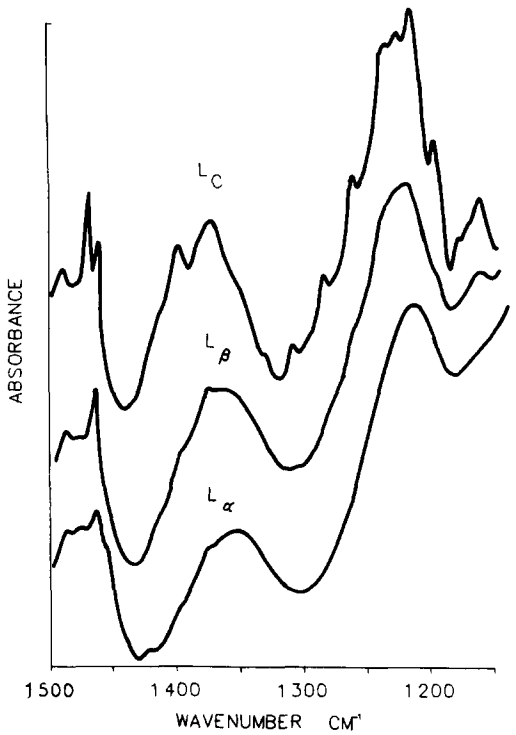

Fig 2 Infrared spectra of a 50-μm-thick sample of 1 3-DPPC in H_2O in the L_C (20°C), L_β (33°C) and L_α (45°C) phases in the region 1500–1150 cm^{-1} which encompass the CH_2 scissoring band ($\approx$1468 cm^{-1}) as well as the CH_2 wagging band progression superimposed on the broad asymmetric $[O-P-O]^-$ stretching band near 1230 cm^{-1}

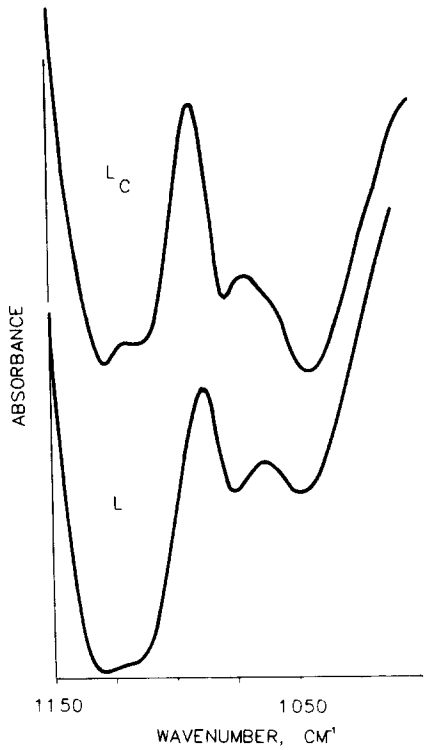

Fig 3 Infrared spectra of a 50-μm-thick sample of 1 3-DPPC in ^2H_2O in the L_C (20°C) and L_β (33°C) phases in the region 1150–1000 cm^{-1} The major feature is the symmetric [O–P–O]$^-$ stretching band (see text)

served in studies of poorly hydrated 1,2-DPPC, while the L_β and L_α phase spectra are typical of the spectra of a fully hydrated lipid [21] Further a shift to lower frequency of the symmetric PO_2^- stretching band on hydration has been demonstrated [22,23], and we conclude that the spectral changes indicate that in the L_C phase the phosphate group is poorly hydrated, and the transition to the L_β phase involves hydration of this group A similar shift would be expected for the asymmetric PO_2^- stretching band but any changes are obscured by the wagging band progression

The C=O stretching band was also monitored as a function of temperature Fig 4A shows the band at several temperatures encompassing the two transitions, while Fig 4B shows the band following Fourier self-deconvolution In the low-temperature phase the band is virtually invariant from 5–20°C The deconvolved spectra demonstrate that it is comprised of two principle bands at about 1742 and 1725 cm^{-1} We note a close agreement of the frequencies of the two components of the 1,3-DPPC carbonyl band contour with those of 1,2-DPPC (1741 and 1727 cm^{-1} in the spectrum of 1,2-DPPC)

The observed frequency and intensity differences in the two components of the carbonyl

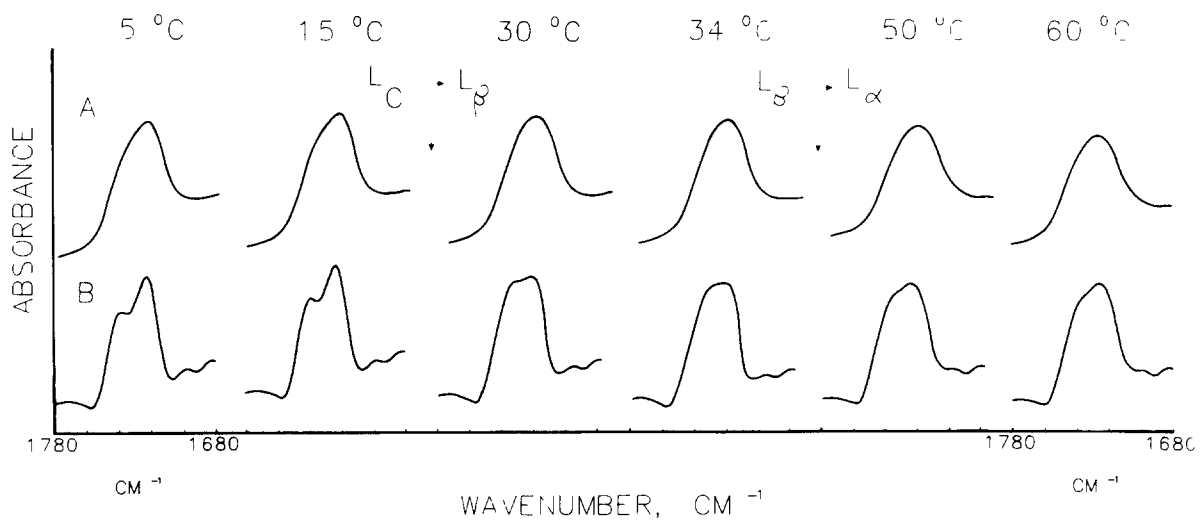

Fig 4 Temperature dependence of the infrared spectra of the C=O stretching region for a 50-μm-thick sample of 1 3-DPPC in ^2H_2O The spectra in (B) have been Fourier self-deconvolved with a 16 cm^{-1} halfwidth Lorentzian line smoothed to $K = 1\,8$ with a Bessel function [31 32]

spectrum in the L_c phase can be correlated with an increase in the dielectric constant of the medium [24] The dehydration of the hydrophilic region in the L_C phase leads to a situation in which the carbonyl groups are deshielded from the electronic charge of the negatively charged phosphate group [25,26], as a consequence, the preceived dielectric constant at the carbonyl increases resulting in an increase in band intensity

The splitting of the carbonyl band suggests that both carbonyls do not experience identical environments in the L_C phase In this case, the 1742 cm^{-1} carbonyl reflects a more hydrophobic environment, as indicated by the higher vibrational frequency

During the transition to the L_β phase, the 1725 cm^{-1} band loses intensity and shifts to higher frequency, suggesting decreased intramolecular interactions resulting from rehydration of the head group The transition to the L_β phase results in a single asymmetric band contour This asymmetry indicates that there are at least two discrete bands present The deconvolved spectra also demonstrate that the principal changes resulting from the transition are band broadening and a shift to higher frequency of the 1725 cm^{-1} band Little change results from transition to the L_α phase at 37°C, the presence of two components in the carbonyl band contour again suggests that the *sn*-1 and *sn*-3 C=O adopt slightly different conformations in the L_α phase

Cooperativity

In order to obtain an indication of the cooperativity of the transitions the frequency and full-width at three-quarters peak height of the asymmetric CH_2 stretching band at 2920 cm^{-1} were monitored as a function of temperature and are shown in Figs 5 and 6 Both transitions are clearly evident The $L_\beta \rightarrow L_\alpha$ transition is highly co-operative, occurring within a 1 Cdeg temperature interval The large increases in bandwidth and frequency are typical of those resulting from the introduction of a large population of *gauche* rotamers

The $L_C \rightarrow L_\beta$ transition occurs in the range 20–30°C The overall changes in frequency (0 3 cm^{-1}) and bandwidth (2 cm^{-1}) are similar to those observed in studies of the orthorhombic → hexagonal transition of alkanes [20] However, during the transition the bandwidth reaches a

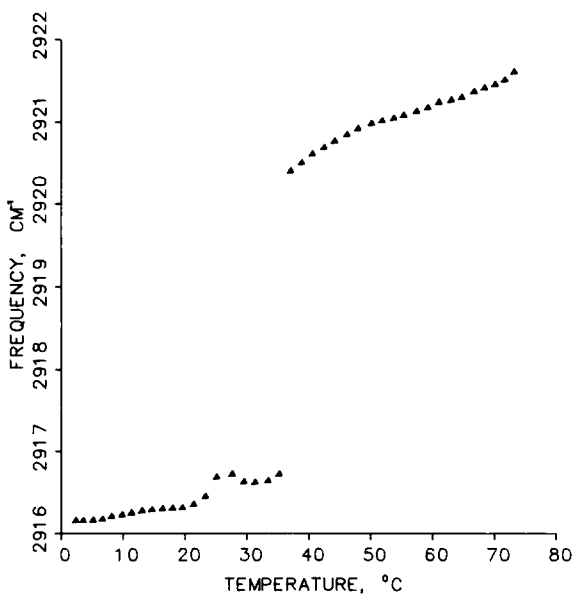

Fig 5 Plot of the frequency vs temperature for the asymmetric CH_2 stretching band at 2920 cm^{-1} in 1,3-DPPC multilayers in ^2H_2O Frequencies were determined by using a 3-point center-of-gravity algorithm [12]

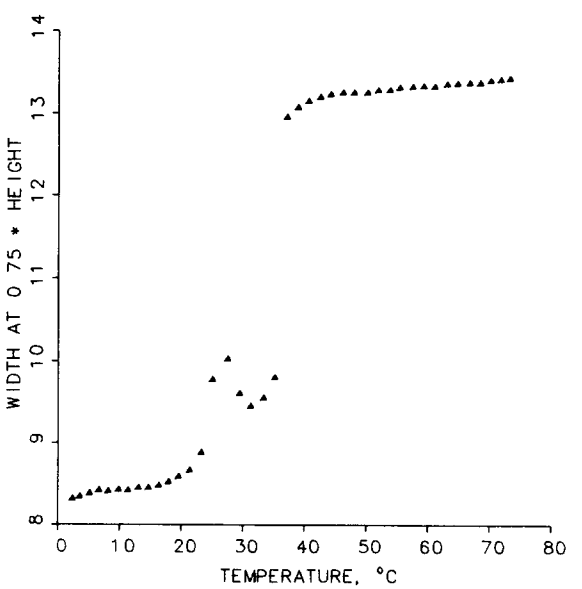

Fig 6 Plot of the full width at three-quarters maximum peak height for the asymmetric CH_2 stretching band at 2920 cm^{-1} in 1 3-DPPC multilayers in ^2H_2O

maximum value and then decreases, the maximum occurring at a temperature lower than that of the maximum rate of change of frequency This behavior has been observed in studies of surfactants, lipids and natural biomembranes [1,27,28] It results from the overlap of two simultaneously varying absorption bands, and hence is indicative of the co-existence of two phases during the transition Similar confirmation of a low-cooperativity transition was obtained from plots of the width of the C=O stretching band (not shown)

Discussion

The data presented here confirm that the $L_\beta \rightarrow L_\alpha$ transition is highly co-operative, while the $L_c \rightarrow L_\beta$ transition has a low degree of co-operativity, occurring over a 5–10 Cdeg range In this regard, our data are in agreement with the results of X-ray studies, which showed that during the $L_c \rightarrow L_\beta$ transition two phases are present in temperature-dependent proportions [9]

Considerable insight can also be gained into the nature of the acyl-chain packing and head-group conformation in the various phases In the L_c phase the acyl chains are highly ordered with severely restricted mobility about the long axis in all positions The packing is such that interchain interactions giving rise to factor group splitting of the CH_2 scissoring mode take place This indicates that the packing is highly crystalline, in agreement with X-ray data [9], with the subcell being orthorhombic or one of the complex hybrid subcells

In the L_β phase the acyl chains are still conformationally highly ordered However, the spectra indicate that the packing is hexagonal, with high mobility about the acyl-chain long axis X-ray studies are in agreement, and further indicate that in this phase the chain layers may be interdigitated [9] No information on this aspect can be gained from the infrared spectra

Finally, the L_α phase spectrum indicates high conformational disorder, and is typical of the liquid-crystal spectra of other phospholipids [4]

Turning to the head-group modes, the L_c phase spectra are characteristic of a poorly hydrated system, while the L_β and L_α phase spectra closely resemble those of fully hydrated 1,2-DPPC Particularly interesting in the various phases is the shape of the C=O stretching band contour In the L_c phase spectrum there are clearly two major components present In terms of the relative intensities, frequencies and band shapes, this spectrum closely resembles that of partially dehydrated 1,2-DPPC [21] The general trends are characteristic of a C=O group experiencing an increase in the effective dielectric constant of the medium as H_2O is removed from the glycerophosphocholine region and the polar groups are brought into closer proximity [25,26] Although the structure of 1,3-DPPC suggests a priori that both carbonyl groups have an equal probability of interacting with the phosphate group, the infrared spectra (Fig 4) reveal that one chain experiences a higher dielectric constant, giving rise to the lower-frequency band We also note that due to the crystal lattice the hydrocarbon chains are tilted in the L_c phase [15], since the crystal lattice is rigid and hence regular, it is not unexpected that the head groups also adopt a regular form of packing

Unlike the interfacial region in 1,2-DPPC, in which the *sn*-1 and *sn*-2 carbonyls are in different environments (and hence have different vibrational frequencies) due to the structural inequivalence of the two acyl chains [25,26], the *sn*-1 and *sn*-3 acyl chains in 1,3-DPPC have been shown to be structurally equivalent [7,8] Therefore, a different mechanism is necessary to interpret the observed carbonyl band contour in 1,3-DPPC

An assignment of the carbonyl bands of 1,3-DPPC to a particular acyl chain is possible in view of the enzymatic activity of phospholipase A_2 This enzyme catalyzes the stereospecific hydrolysis of the carbonyl ester at the *sn*-1 chain of 1,3-DPPC [29] The stereospecificity of phospholipase A_2 activity arises from the specific nature of the active site [34], in particular the ternary enzyme-Ca^{2+}-phospholipid complex The role of Ca^{2+} appears to be that of an intermediary between the negative charge of the phosphate and the ester carbonyl oxygen in the group undergoing clevage [34] The implication in the case 1,3-DPPC is that the stereospecific hydrolysis of the *sn*-1 ester would require the preferred orientation of the phosphocholine group relative to that of the *sn*-1 carbonyl thus giving rise to inequivalent environments for the two carbonyls and, therefore, different vibrational frequencies The relative orientation of the phos-

phocholine head group towards the *sn* − 1 carbonyl would also increase the effective dielectric constant experienced by that C=O, thereby giving rise to a lower-frequency infrared band On this basis, we assign the 1725 cm^{-1} band to the *sn*-1 C=O and the 1742 cm^{-1} band to the *sn*-3 C=O

In the L_β and L_α phase spectra the C=O contour is much more symmetric than in the L_c phase spectrum However, in the spectra of the lipid in both phases, there are still clearly two components present (Fig 4) This indicates that the C=O groups are in slightly different environments This could be attributed to a slight tilt of the glycero group relative to the bilayer surface, and/or a preferred conformation of the phosphocholine group relative to the glycero group A recent ^{2}H-NMR study concluded that there was no major difference between the mobility of the 2-CH_2 groups of the *sn*-1 and *sn*-3 chains of 1,3-DPPC [7] This indicates that there is no substantial intrachain difference in conformation about the C_2–C_1 bond of the C_3–C_2–C_1–O structural unit These results are consistent with the interpretation stated above of the infrared data as a preferred tilt of the phosphocholine group relative to the glycerol backbone that results in different environments for the *sn*-1 and *sn*-3 carbonyls The indications from the infrared spectra of differences in the structure of the C=O groups provide a possible explanation for the selection of the *sn*-1 chain of 1,3-DPPC by phospholipase A_2 on the basis of a preferred head group conformation

Transition to the L_β phase of 1,3-DPPC at 25°C produces changes in the spectra of the phosphate group The frequency of the symmetric PO_2^- stretching band shifts from 1090 2 cm^{-1} to 1086 4 cm^{-1}, indicating a rehydration of the phosphate ester The effect of the binding of H_2O to the phosphate group is to separate and shield the polar carbonyl groups from the charged phosphate As a consequence of the perceived decrease in the dielectric constant, the C=O stretching band of the *sn*-1 acyl chain looses intensity and shifts to higher frequency The result is a single asymmetric contour for the C=O stretching band

The binding of additional H_2O to the phosphate group has implications for the acyl chain packing characteristics in the L_β phase As has been shown for the cholesterol-DMPC interaction, a decrease in carbonyl intensity suggests a loosening of the lipid crystal lattice which leads to a less densely packed bilayer [25] As seen in Fig 2 and discussed above, the transition to the L_β phase results in a collapse of the factor group splitting of the CH_2 methylene scissoring band at 1472 and 1464 cm^{-1} and the appearance of a single band at 1468 cm^{-1} This frequency is characteristic of hydrocarbon chains packed in a hexagonal or near-hexagonal lattice in which the acyl chains have increased freedom to undergo rapid torsional motions about the long axis [3]

In view of the above data, the $L_c \rightarrow L_\beta$ phase transition of 1,3-DPPC at 25°C corresponds to a temperature-induced hydration of the head-group phosphate and carbonyl esters which results in the reorganization of the hydrocarbon chain lattice from orthorhombic (L_c phase) to hexagonal (L_β phase) It is also clear that the transition of of 1,3-DPPC at 25°C corresponds, in general terms, to the sub-transition of 1,2-DPPC [11,30] That is, there is hydration, low cooperativity, and transition to hexagonal packing The principal difference lies in the specific form of the packing in the L_c phase

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