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THE GEL PHASE OF DIPALMITOYL PHOSPHATIDYLCHOLINE

AN INFRARED CHARACTERIZATION OF THE ACYL CHAIN PACKING

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Summary

It is shown, by infrared spectroscopy, that the packing in the gel phase of fully-hydrated dipalmitoyl phosphatidylcholine is not uniform over a large temperature range. With decreasing temperature, starting at that of the pre-transition, there is a gradual change in the molecular packing of the acyl chains, from near hexagonal to orthorhombic or monoclinic.

The structure and dynamics of the gel and liquid-crystalline phases of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) multibilayers have been the subject of extensive spectroscopic studies. While the highly-disordered nature of the liquid-crystalline phase is well-documented [1,2], the gel phase is less well-characterized. Although the latter is often referred to as a solid-like phase, recent ^{2}H-NMR studies have demonstrated the presence of considerable motional disorder [2]. The motion responsible for this disorder is thought to be a reorientation about the long axes of the acyl chains, the rate of which decreases as the temperature is lowered [2]. Similar conclusions with regard to motional disorder were also reached from X-ray diffraction studies [3—5] which indicate that at temperatures close to that of the pre-transition, the acyl chains are packed in a slightly distorted hexagonal form and tilted with respect to the plane of the bilayer. With decreasing temperature, the angle of tilt increases and the hexagonal packing becomes progressively more distorted [4,5]. The above studies generally conclude that throughout the gel phase, the acyl chains are in an all-*trans* conformation.

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Abbreviations used: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, 1,2-diperdeuteropalmitoyl-*sn*-glycero-3-phosphocholine.

Temperature-dependent changes in the gel phase of DPPC were also observed by Raman spectroscopy [6,7]. A different interpretation was placed on these results in that most of the temperature-dependent changes were attributed to variations in the number of *gauche* conformers of the acyl chains. However, Yellin and Levin [6] found evidence for crystal-packing (inter-chain) effects at low temperatures, but were unable to separate these from intra-chain effects. More recently, Gaber et al. [7] concluded from Raman difference spectroscopy that the increasing hexagonal distortion results from the introduction of triclinic packing into the acyl chain region of the bilayer.

In this report, we present the results of an infrared study which clarifies the temperature-dependent modification of the crystal packing of hydrated DPPC in the gel phase.

A fully hydrated 6 μm thick multibilayer dispersion of DPPC (Sigma, St. Louis), was prepared in a CaF_2 cell as described in detail elsewhere [8]. The cell was placed in an evacuable low-temperature chamber located in the sample compartment of a Perkin-Elmer 180 infrared spectrophotometer. Spectra were recorded at a resolution of 1.5 cm^{-1} at temperatures ranging from –100 to 30°C; the temperature was monitored by a copper-constantan thermocouple located against the edges of the cell windows.

As shown in Fig. 1, the infrared spectrum of the gel phase of DPPC changes drastically in the CH_2 scissoring region as the temperature is varied. At 30°C, just below the pre-transition temperature, the spectrum consists of a strong narrow band at ~1468 cm^{-1}, superimposed on several weak bands. The 1468 cm^{-1} band results from the scissoring mode of the methylene groups in all-*trans* acyl chains in which the phase difference between adjacent groups is 180°, while the underlying bands arise from scissoring modes of glycerol and choline methylenes and methyl bending modes.

As the temperature is progressively lowered, the 1468 cm^{-1} band splits into two clearly-resolved components while the underlying bands remain con-

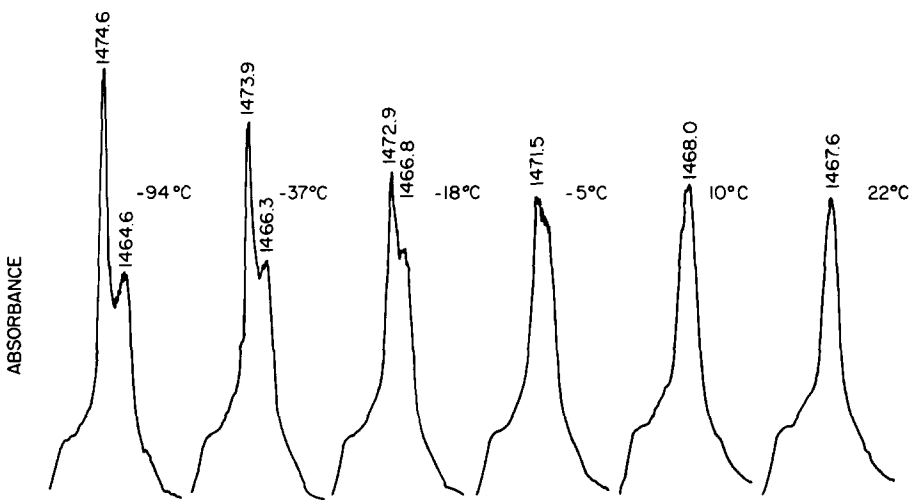

Fig. 1. Infrared spectra in the CH_2 scissoring region of a fully-hydrated dispersion of dipalmitoyl phosphatidylcholine in the gel phase at –94, –37, –18, –5, 10 and 22°C.

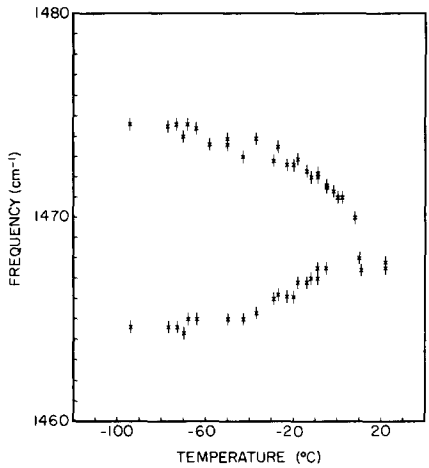

Fig. 2. Temperature-dependent splitting of the acyl chain CH_2 scissoring mode in dipalmitoyl phosphatidylcholine in the gel phase. The lack of lower-frequency points between 0 and 10°C results from the fact that in this range the lower-frequency band is only evident as an unresolved shoulder.

stant in frequency and intensity. This splitting is readily monitored by plotting the frequencies of the two components as a function of temperature (Fig. 2). The onset of the splitting is evident at 10°C; it increases in magnitude rapidly between 10 and -20°C, becoming constant at temperatures below -60°C. However, at lower temperatures there are still considerable reductions in the width of the two components, as evidenced by the increasing depth of the valley between the bands.

The observation of this splitting provides the key to the understanding of the gel phase changes observed by the various other spectroscopic techniques. The splitting is due to a crystal field effect, resulting from inter-chain interactions, and has been previously observed in the spectra of *n*-alkanes [9], fatty acids [10] and an anhydrous film of DPPC [11]. It only occurs when the acyl chains are packed in an orthorhombic or monoclinic crystal lattice (Fig. 3A), which have similar subcells in the *ab* plane [9]. On

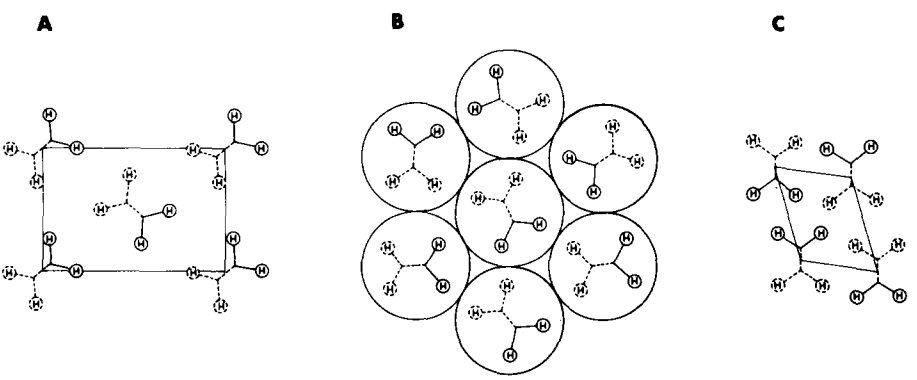

Fig. 3. Acyl chain crystal-packing patterns: A, orthorhombic or monoclinic; B, hexagonal and C, triclinic. In all cases, the long axes of the chains are projecting from the page. In the case of hexagonal packing, the torsion about the long axes is such that the orientations of chains relative to each other at a given moment are random.

the other hand, a single CH_2 scissoring mode indicates hexagonal (Fig. 3B) or triclinic (Fig. 3C) packing. On the basis of the X-ray and Raman studies, it is clear that the packing is hexagonal at 30°C, albeit slightly distorted [4], and not triclinic.

Consequently, the infrared data demonstrate that in the gel phase of fully hydrated DPPC, the packing of the acyl chains changes from a hexagonal or near hexagonal lattice at temperatures near the pre-transition, to an orthorhombic or monoclinic lattice at lower temperatures. The frequency plot in Fig. 2 demonstrates that this is a continuous process, extending over a wide temperature range. This is in marked contrast with the behaviour shown by odd-numbered *n*-alkanes, where the same transition occurs within a temperature interval of less than 1°C [9].

We may now consider these data in relation to the X-ray diffraction, Raman and ^{2}H-NMR studies mentioned in the introduction. Our infrared data demonstrate that the crystal lattice of DPPC undergoes a continuous change from near-hexagonal at 30°C to a grossly-distorted hexagonal form, i.e., orthorhombic or monoclinic, at low temperatures. In this system, such packing also requires an increase in the angle of tilt of the chains with respect to the bilayer surface and thus brings the distorted hexagonal model, proposed from X-ray diffraction studies [4,5], in complete accord with our data.

The determination of the nature of the crystal packing in the gel phase of DPPC also facilitates the understanding of the temperature-dependent effects observed in the ^{2}H-NMR spectra of DPPC-d_{62} [2], i.e., DPPC with fully-deuterated acyl chains. In the hexagonal lattice, the acyl chains undergo rapid motions about their long axis, the motions being torsional rather than rotational due to the restrictions imposed by the head group. The introduction of orthorhombic or monoclinic packing, sufficient to cause crystal field splitting of the CH_2 scissoring mode, restricts the angular amplitudes of such torsional motions and should lead to an increase in intensity at ±63 kHz in the ^{2}H-NMR spectrum. This value of the quadrupolar splitting for C^2H_2-groups is characteristic of the absence of motion on the ^{2}H-NMR time scale. Indeed, the large increase in spectral width observed by Davis [2] on lowering the temperature from 20 to −7°C reflects the restriction of motion imposed by the large change in the packing of DPPC, as detected by the infrared spectra over this temperature range.

Finally, the temperature-dependent changes in the Raman spectra, which have been previously attributed to increases in the *gauche* conformer population, are also observed in the orthorhombic to hexagonal phase transition in *n*-$C_{17}H_{36}$ [7]. As we have now established that such a transition occurs in DPPC, we believe that the Raman data must be critically re-evaluated with this in mind.

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References

1 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839—4845
2 Davis, J.H. (1979) Biophys. J. 27, 339—358
3 Tardieu, A., Luzzati, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711—733
4 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Biol. Chem. 254, 6068—6078
5 Harlos, K. (1978) Biochim. Biophys. Acta 511, 348—355
6 Yellin, N. and Levin, I.W. (1977) Biochim. Biophys. Acta 489, 177—190
7 Gaber, B.P., Yager, P. and Peticolas, W.L. (1978) Biophys. J. 21, 161—176
8 Cameron, D.G., Casal, H.L. and Mantsch, H.H. (1979) J. Biochem. Biophys. Methods 1, 21—36
9 Snyder, R.G. (1961) J. Mol. Spectrosc. 7, 116—144
10 Koyama, Y., Yanagishita, M., Toda, S. and Matsuo, T. (1977) J. Colloid. Interface Sci. 61, 438—445
11 Fookson, J.E. and Wallach, D.F.H. (1978) Arch. Biochem. Biophys. 189, 195—204