Vol. 83, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1978 Pages 886-892

THE PHASE TRANSITION OF 1,2-DIPALMITOYL-sn-GLYCERO-3-PHOSPHOCHOLINE AS SEEN BY FOURIER TRANSFORM INFRARED DIFFERENCE SPECTROSCOPY [1]

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Received June 19,1978

SUMMARY: The potential of Fourier transform infrared difference spectroscopy for biochemical applications is demonstrated by the gel to liquid crystal phase transition of the title compound. While the changes occurring in the vibrational pattern of the hydrophobic palmitoyl chains are easily monitored, this technique also discriminates between no change in the choline moiety and a small yet significant change in the carbonyl moiety, both located in the hydrophylic head group.

INTRODUCTION: The structures of biological membranes are currently under investigation by a variety of spectroscopic techniques. Unlike Raman spectroscopy, conventional IR has had limited success, because of the intense water absorptions. However, the instrumental limitations, largely a consequence of the limited dynamic range of grating spectrometers and the difficulty of carrying out computational manipulations on the data, have been greatly reduced by the recent advent of FT-IR spectrometers. Their effect on IR spectroscopy is analogous to that on the NMR technique in the last decade; FT-NMR has since undergone an explosive growth, while FT-IR is still in an early stage, with novel chemical and biochemical applications in the process of development (1,2,3).

For the biochemist, FT-IR opens up the possibility of infrared studies of complex biomolecules in the aqueous phase. In particular, FT-IR difference spectroscopy permits the simultaneous monitoring

[1] N.R.C.C. No. 16713
Abbreviations: DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; FT-IR: Fourier transform infrared.

0006-291X/78/0833-0886$01.00/0

of subtle spectral changes in individual group vibrations as a result of small changes in pH, temperature, concentration or other parameters.We describe here a temperature study of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine multibilayers, to demonstrate the potential of FT-IR difference spectroscopy for such applications.

EXPERIMENTAL: Multibilayer dispersions of DPPC (Sigma) in the presence of excess water were obtained by a modification of the technique employed in ESR spectroscopic studies (4). 500 µg of solid DPPC was placed in the central area of a 25 mm diameter zinc selenide window and sufficient chloroform added to dissolve the DPPC. The chloroform evaporated rapidly, leaving a glassy deposit. Residual chloroform was removed under vacuum, a few drops of doubly distilled water added and after 15 minutes the plate was assembled into a thermostated Harrick cell, using 6 µ mylar spacers. Prior to the measurements, the multibilayer dispersions were twice taken through the phase transition. The temperature was monitored with a copper-constantan thermocouple in close contact with the edges of the two cell windows.

The FT-IR spectrometer used was a Nicolet 7199 system equipped with a DTGS detector, operating under a NIC-1180 data system. Typically, 100 scans were accumulated and averaged. The 16 K interferograms were zero filled once, apodized with a Happ-Genzel apodization function and Fourier transformed to yield a resolution of 1.5 cm^{-1}. The FT-IR difference spectrum was obtained from the ratio of the higher temperature absorption spectrum to the lower temperature spectrum; this procedure leads to positive peaks if the absorption increases with temperature.

RESULTS AND DISCUSSION: The FT-IR spectrum of DPPC multibilayers below the phase transition is shown in Fig. 1. The upper trace shows the FT-IR difference spectrum between 42 and 39°C. Since the main gel to liquid crystal phase transition of this glycerophospholipid occurs at 41.5°C, this difference spectrum reflects all the changes in the vibrational pattern of DPPC multibilayers between the two phases. The extremely strong absorptions around 3370 and 1640 cm^{-1} are due to bulk water, well in excess of the 40% needed for fully hydrated multibilayers. However, as the latter cancels in the difference spectrum, quantitative monitoring of minor absorption changes can be carried out in the mid infrared.

The largest changes are indicated by the strong features at

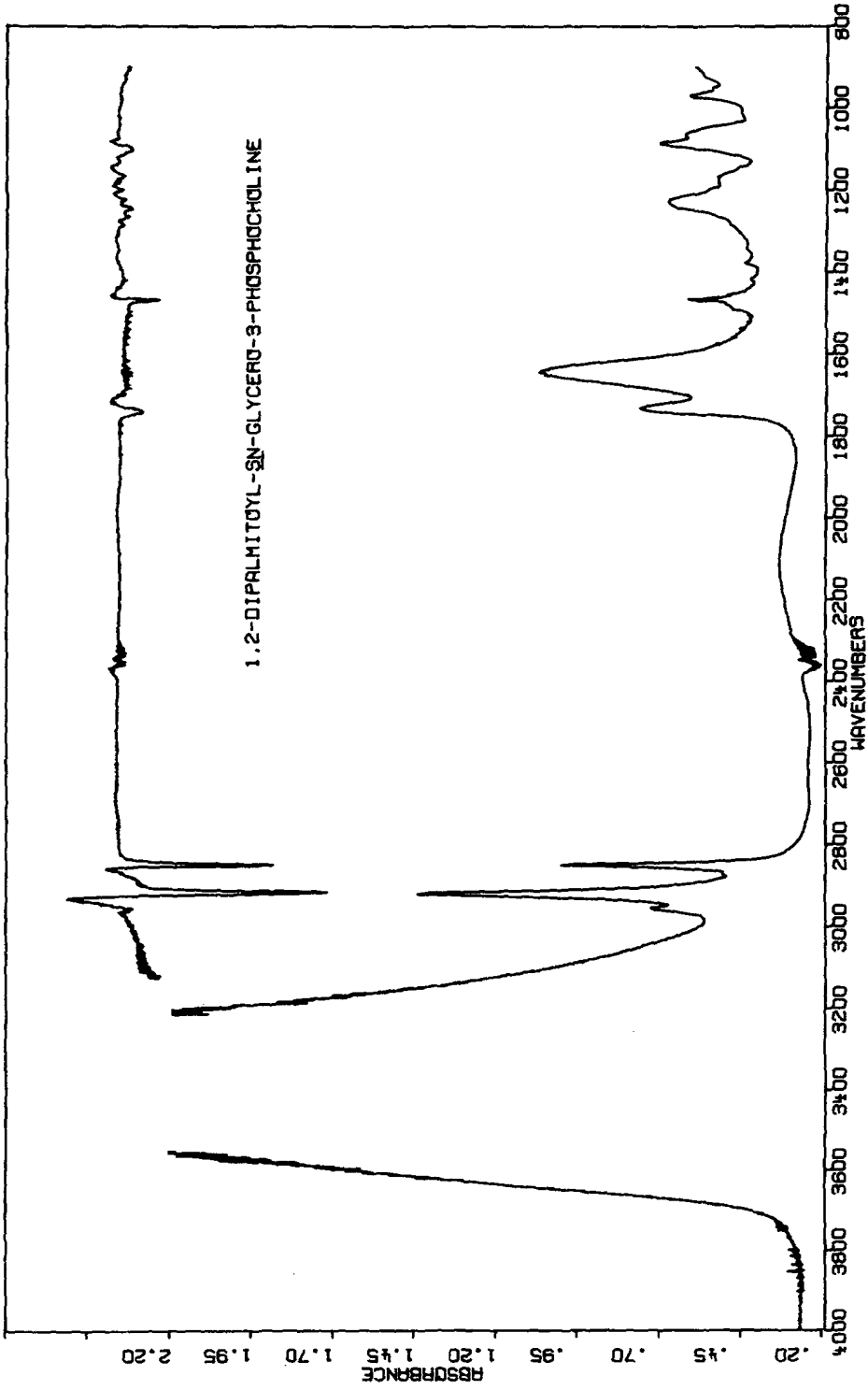

Fig. 1. Bottom: FT-IR absorbance spectrum of a 6 μ DPPC gel at 39°C at a resolution of 1.5 cm^{-1}. Top: FT-IR difference spectrum obtained by subtracting the 39°C spectrum from that obtained under identical conditions at 42°C.

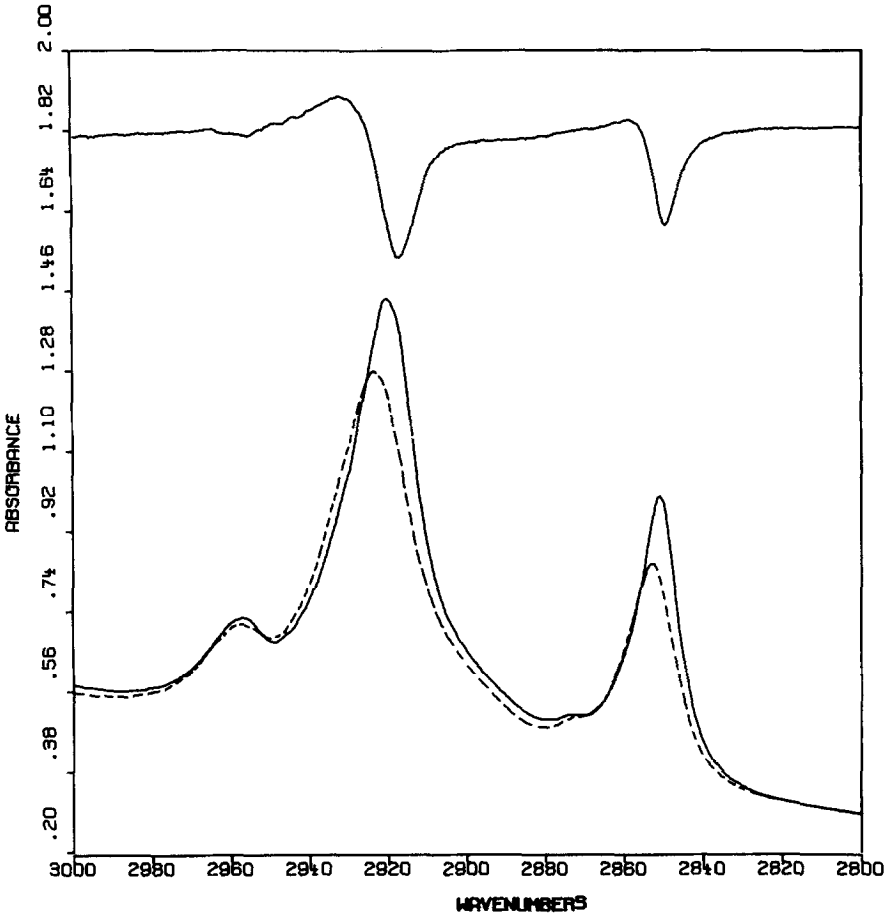

Fig. 2. FT-IR spectra of DPPC multibilayers in the C-H stretching region at 39°C (solid line), at 42°C (broken line) and FT-IR difference spectrum (top).

2920 and 2851 cm^{-1}, corresponding respectively to the antisymmetric and symmetric CH_2 stretching modes of the palmitoyl chains. These have been used in Raman and conventional IR spectroscopy to monitor phase changes (5-10). An expansion of the 3000-2800 cm^{-1} region is shown in Fig.2. The difference spectrum reveals a considerable reduction in the integrated peak intensities, particularly in the symmetric CH_2 stretching mode.Upon phase transition the antisymmetric stretching mode of the terminal palmitoyl CH_3 group at 2956 cm^{-1} also undergoes a reduction in peak height and

a small wavenumber shift.In the gel phase the narrow band at 1468 cm^{-1}, due to CH_2 deformation modes, is similar in band shape and identical in frequency to that observed in solids and reflects a highly ordered state. It undergoes a considerable reduction in peak height in the liquid crystal state. However, the broad band centered at 1480 cm^{-1}, due to the antisymmetric CH_3 deformation mode of the choline headgroup (11), undergoes no change in any of the band parameters.

Despite the considerable overlap of the C=O stretching mode of the palmitoyl carbonyls at 1736 cm^{-1} with the strong water absorption, an increase in the integrated intensity of the C=O fundamental is clearly discernable in the FT-IR difference spectrum. The derivative shape indicates a shift to lower frequency, confirmed by comparison of the two absorbance spectra. These changes indicate that the palmitoyl carbonyls are involved in stronger hydrogen bonding to water molecules in the liquid crystalline phase of DPPC multibilayers than in the less mobil gel phase, suggesting higher water penetration in the former.

The expanded fingerprint region is displayed in Fig.3. It is most interesting that the prominent features in this region, associated with the antisymmetric and symmetric PO_2 stretching modes at 1232 and 1089 cm^{-1}, and with the complex C-O and C-N stretching modes at 1069 and 972 cm^{-1}, show no significant changes in peak heights, frequencies or integrated intensities. However, a distinct pattern of intensity changes is observed with minima about 22±3 cm^{-1} apart at 1285, 1266, 1222, 1199 and 1178 cm^{-1}, which can be correlated with a number of weak shoulders in the gel phase spectrum. This series of weak bands is absent in the liquid crystal phase of DPPC multibilayers and is assigned to a $(CH_2)_n$ wagging progression due to the palmitoyl chains, a pattern observed

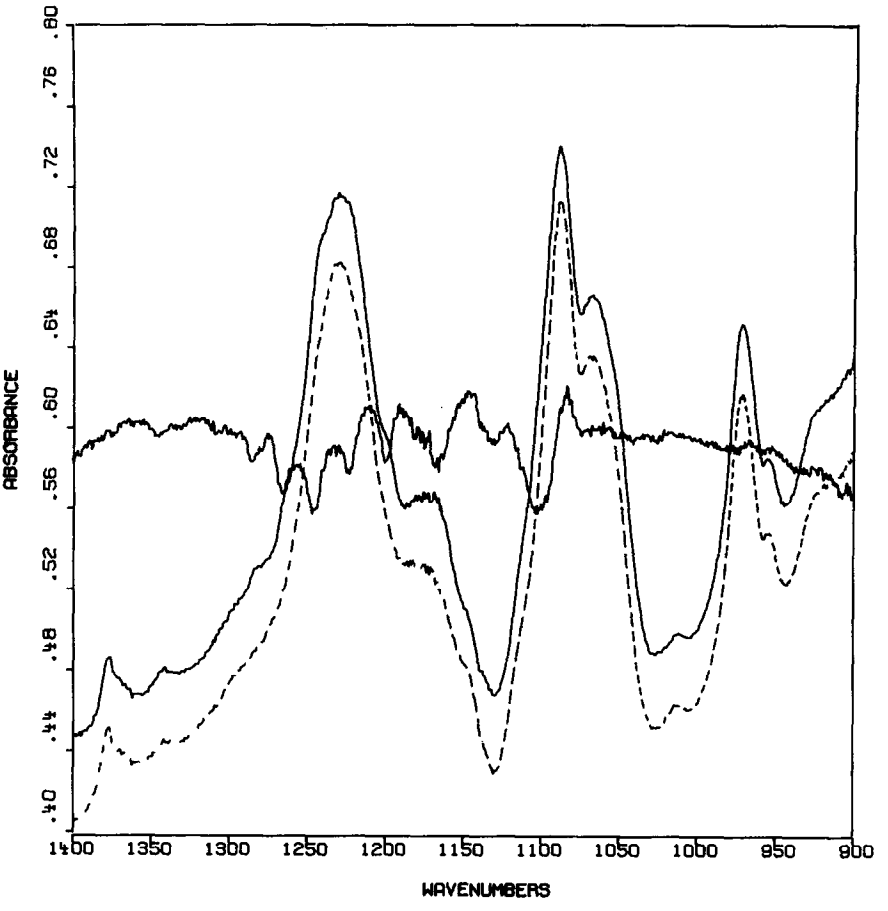

Fig. 3. FT-IR spectra of DPPC multibilayers in the fingerprint region at 39°C (solid line), at 42°C (broken line) and superimposed on both, the FT-IR difference spectrum.

in the IR spectra of highly ordered aliphatic hydrocarbon chains (12).

CONCLUSIONS: FT-IR difference spectroscopy is shown to provide a powerful tool for the simultaneous monitoring of small changes in the vibrational pattern of various functional groups, occuring during the phase transition of glycerophospholipid multilamellar dispersions. Most severely affected by the phase transition are the absorption bands related to the hydrophobic palmitoyl chains

as indicated by changes in the CH stretching and the CH_2 deformation region.This technique also reveals small changes in the vibrational pattern of certain functional groups located in the hydrophylic head group such as the C=O group, while others, particularly the choline group, are not affected at all. Although thermodynamic evidence of the phase transition in multibilayer dispersions comes from calorimetric measurements, only molecular spectroscopic studies can show which parts of the phospholipid are involved in this change. Further specific data can be obtained from ^2H labeled molecules as indicated by recent studies performed in our laboratory using selectively deuterated DPPC and Acholeplasma laidlawii membranes.

ACKNOWLEDGEMENT: We are grateful to the Standards Branch of Consumer and Corporate Affairs Canada for providing access to their Nicolet 7199 FT-IR system, with particular thanks to F.R.S. Clark and D.J. Moffat for their assistance.

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