RAMAN STUDIES OF CONFORMATIONAL CHANGES IN MODEL MEMBRANE SYSTEMS

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SUMMARY: Laser Raman spectra of concentrated samples of phosphatidyl choline and phosphatidyl ethanolamine were taken at approximately 10° intervals over a temperature range of 90°-19°C. The spectral region from 30 to 3300 cm^{-1} was investigated. Several new spectral features were discovered which are correlated to phospholipid liquid crystalline structure. It is shown that 1) frequency shifts occur in the PO_2^- symmetric stretch band which suggest a change in exposure of the PO_2 group to the solvent upon melting, 2) the frequency of the translational hydrocarbon mode around 150 cm^{-1} appears to indicate the degree to which the hydrocarbon chain is extended, 3) the methyl and methylene stretch bands at 2890 and 2850 cm^{-1} very clearly demonstrate hydrocarbon chain melting, and 4) the 720 cm^{-1} band, previously assigned to the symmetric O-P-O diester stretch, appears to be due instead to the symmetric C-N stretch of choline.

Introduction: Lippert and Peticolas (1,2) have utilized Raman spectroscopy to study the melting of the paraffin-like side chains of lipids in the spectral region below 2000 cm^{-1}. In this paper, melting studies of phosphatidyl choline are extended to include the C-H stretching region between 2800 and 3300 cm^{-1}, as well as the CH_2 vibrations in the 1400 cm^{-1} region. The assignments in each of these regions are given in figure 1 of reference 2. In addition, two phospholipids are compared which differ only in substituents on the nitrogen group and in the structure of the phase existing below the melting temperature, T_M (3-6). Comparisons of phosphatidyl ethanolamine and phosphatidyl choline over the spectral region 30 to 3300 cm^{-1} were made to clarify the relation of the spectra to the structure of the lipids.

Materials and Methods: The spectra were taken with the 5145 Å line of a spectra physics 165 Argon laser. The sample holder and

light collection system have been described previously (1). The laser power for all spectra was 120 mW and the slit widths were 350μ.

Dipalmitoyl L-phosphatidyl ethanolamine (PE) and dipalmitoyl L-phosphatidyl choline (PC) were obtained from Calbiochem and used without further purification. Samples were placed in capillary tubes and water was added. The tubes were then sealed and allowed to equilibrate in a water bath at 80°C for two days. Concentrations by weight of the samples were 69% for PC and 79% for PE. To parallel procedures used by X-ray crystallographers, spectra were obtained by first taking high temperature spectra and slowly reducing the temperature, stopping at approximately 10° intervals to take spectra. Samples were reheated and the spectra were run again over the same temperature range. It was observed that all changes are reversible.

Results and Discussion: Spectra of PC and PE taken over a wide temperature range are displayed in figures 1 and 2. As the temperature is lowered through the melting temperature T_M, very pronounced changes in the spectra take place. Most of these changes occur similarly in PE and PC. We will first discuss these similarities. There are also regions which differ in the two phospholipids. These regions indicate structural and conformational differences between the two molecules and will be discussed last.

The bands observed in the 2800 cm^{-1} to 3300 cm^{-1} region are due to C-H stretching modes. Two of these bands are of particular interest since they are quite strong and change dramatically with temperature. We can conclude from studies made of n-paraffins by Snyder (7), that in PC and PE the weak bands at 2960 cm^{-1} and 2929 cm^{-1} are due to the asymmetric CH_3 and antisymmetric

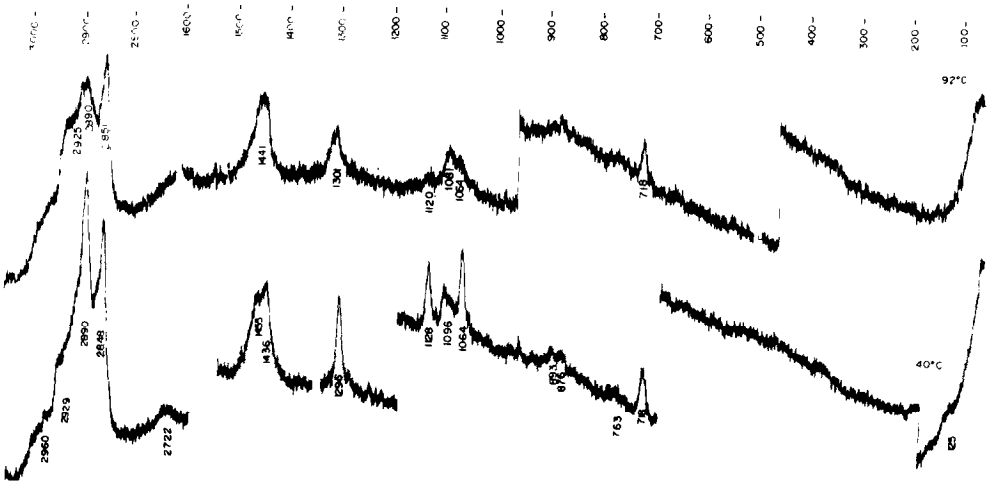

Fig. 1 Raman spectra of dipalmitoyl phosphatidyl choline before and after melting.

CH_2 stretches respectively. The very large band at 2890 cm^{-1} is probably due to overlapping CH_3 and CH_2 symmetric stretching modes. The strong 2850 cm^{-1} band is assigned to symmetric CH_2 stretching. Both the 2890 and 2850 cm^{-1} bands vary in band height inversely with the temperature. However, it is apparent that melting brings about a greater decrease in band height and greater broadening in the 2890 cm^{-1} band relative to the 2850 cm^{-1} band. Broadening of a band shape occurs when a molecule or a molecular group moves more freely. As the hydrocarbon chains are melted, the methyl end groups acquire more freedom of mobility than the average methylene group. Thus, the ratio of the peak height of the 2890 cm^{-1} band to the 2850 cm^{-1} band decreases with hydrocarbon chain melting and can be used to follow changes in the mobility of the paraffin-like side chains. This ratio is plotted in figure 3 and describes a characteristic melting curve with broad pre-melting and post-melting regions.

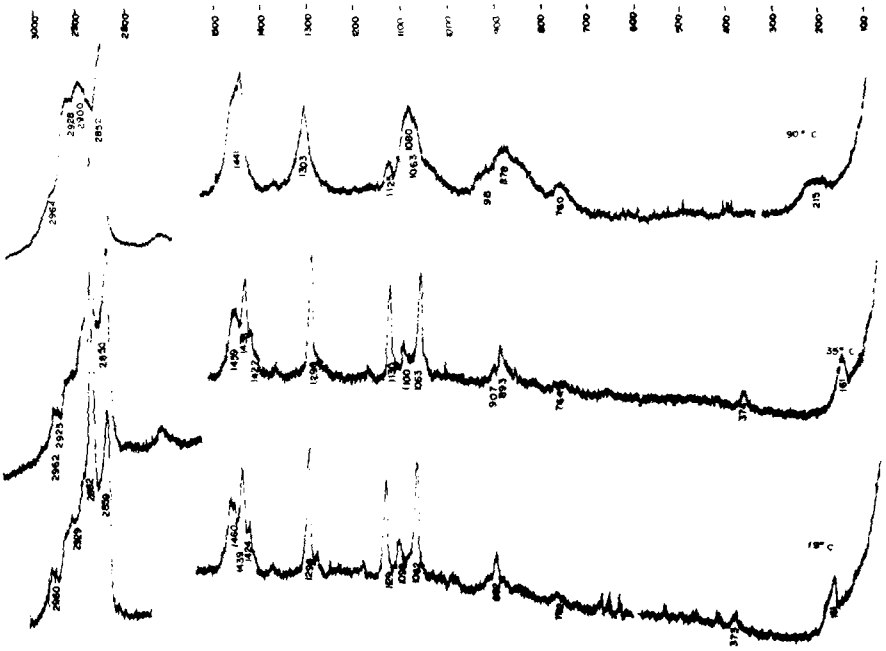

Fig. 2 Raman spectra of dipalmitoyl phosphatidyl ethanolamine.

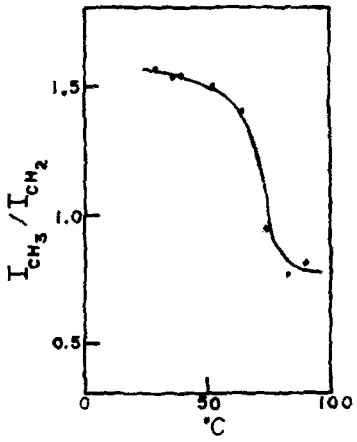

Fig. 3 Ratio of 2890 cm^{-1} band to 2850 cm^{-1} band in PE as a function of temperature.

The observed T_M compares favorably with the literature (5).

Three prominent bands appear in the 1200 to 1500 cm^{-1} region. These bands have all been assigned to modes involving bending and rocking of the methylene groups in the hydrocarbon chain (2). All undergo sudden alterations in shape as the temperature of the sample is lowered past the melting point. The 1299 cm^{-1} band sharpens and increases in band height. Below the T_C two bands exist in the region of 1450 cm^{-1}. One of these bands is a strong sharp band at 1440 cm^{-1}. The other appears as a shoulder at 1460 cm^{-1}. The strong 1440 cm^{-1} band belongs to the A_g CH_2 bending mode and the 1460 cm^{-1} shoulder to the B_{3u} CH_2 bending mode (7). The single peak at 1450 cm^{-1} above the T_M corresponds to the $\phi/\pi = 1$ end of the phonon dispersion curve (2) of a polyethylene zig-zag chain for the CH_2 bend. This seems to imply that the hydrocarbon chains are now totally disordered with no chain symmetry, permitting this point on the Brillouin zone to be observed.

Three bands appear around 1100 cm^{-1}. In spectra taken at temperatures below T_M, bands at 1128 and 1064 cm^{-1} are observed which have previously been assigned to the skeletal vibrations of the hydrocarbon backbone (2). The 1100 cm^{-1} band is thought to be a PO_2^- symmetric stretch. At temperatures above T_M, the intensity of the 1128 cm^{-1} band is markedly decreased and the 1064 cm^{-1} band appears to shifted upward and merges at 1088 cm^{-1} with the PO_2^- band which has shifted downward. This behaviour of the 1128 cm^{-1} and 1064 cm^{-1} bands has been observed previously and is thought to be due to the disruption of the order in the backbone of the hydrocarbon chain (2). The frequency shift in the symmetric PO_2^- band is similar to one observed by Brown and Peticolas in diethyl phosphate and in DNA (8). Diethyl phosphate

exhibits the symmetric PO_2^- vibration at 1097 cm^{-1} in the solid state and at 1077 cm^{-1} in solution. In DNA, this vibration is observed at 1110 cm^{-1} in the dry fiber and at 1094 cm^{-1} in solution. These frequency shifts in diethyl phosphate and in DNA are interpreted as being due to an exposure of the PO_2^- group to the aqueous solvent. Since the symmetric PO_2^- stretch shifts downward with melting, we suggest that as the hydrocarbon chain melts, there is evidently a conformational change which results in increased exposure of the PO_2^- group to the solvent.

A striking difference between the spectra of PC and PE is the presence of a 720 cm^{-1} band in PC which is totally absent in PE. This band has been assigned to the O-P-O diester stretch (2), but this assignment appears to be in error. It is more likely that the 720 cm^{-1} band is due to the $RN^+(CH_3)_3$ group which is present in PC and not in PE. In molecules such as $(CH_3)_4N^+$, a strong Raman band at 750 cm^{-1} is observed which has been assigned to the C-N symmetric stretch (9). The weak band at 763 cm^{-1} is probably due to the O-P-O diester stretch.

The region below 200 cm^{-1} contains the accoustical accordian-like motions of the all-trans hydrocarbon fatty acid chains (2). In a previous search (1) no evidence for such motions could be found in dipalmatoyl phosphatidyl choline (lecithin). In figure 1, a very weak band at 125 cm^{-1} may be due to such motions. However, in figure 2 the sharp band at 161 cm^{-1} must arise from the accordian motion since it falls at exactly the same frequency as that of pure palmitic acid (2). Since the hydrocarbon chains of both lipids studied have the same number of carbon atoms, then the chains are perhaps folded in such a way that the apparent length in PC is greater than that for PE. Reiss-Husson (3) has

demonstrated that for egg PC the pre-melting phase is lamellar while that for egg PE is hexagonal II. In these structures the hydrocarbon chin has a range of freedom of approximately 31 Å for PE and 40 Å for PC. As a result, the hydrocarbon chains of PC could be more extended than the hydrocarbon chains of PE, as our low frequency measurements would seem to indicate.

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