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Infrared spectroscopic measurements of phosphatidyl ethanolamine–water liquid crystals

It is well known that phospholipids can exist in liquid crystalline form. CHAPMAN[1] examined the infrared spectrum of DL-α-dipalmitoyl ethanolamine in a KBr disc as a function of temperature from —186 to +140°. The spectrum at low temperature resembled that of a crystalline solid, showing splitting of bands in various spectral regions. This splitting disappeared as the temperature was raised to 140°. At 140° the spectrum resembled that of a fluid rather than a solid phase.

Recent studies[2–4] on many phospholipid–water systems by X-ray diffraction and electron microscopy indicate that these systems exist in a number of well-defined mesomorphic phases (lamellar, hexagonal, cubic). In particular, studies on the phase transitions observed for the phosphatidyl ethanolamine–water system show the existence of the following mesomorphic phases: (1) a lamellar phase at 20°; (2) an inverted hexagonal phase at 55°; (3) at 25–35° simultaneous coexistence of the lamellar and hexagonal phases over a wide concentration range.

In this paper, we report the first infrared spectra of phosphatidyl ethanolamine–water phases (20 % water). The phosphatidyl ethanolamine was supplied by Pierce Chemical Co. (bacterial, chromatographically pure), a white powder which darkens at 170°. It was primarily a mixture of palmitic and oleic acid esters, with small amounts of stearic and linoleic acid chains also present. The average molecular weight was 725, slightly lower than that reported by REISS-HUSSON[3], who did not specify hydrocarbon chain composition but only reported a mean molecular weight of 748. Results from electron microscopy[2] have not indicated which phosphatidyl ethanolamine was used in that study, making comparison of results somewhat uncertain. The spectral results obtained lend further strong support to the existence of liquid crystalline phases in these systems, and give some specific evidence regarding the nature of these phases.

Infrared spectra were obtained using a Perkin–Elmer Model 521 double beam grating spectrophotometer. The sample, sandwiched in a thin film (about 10 μ) between Irtran plates, was examined as a function of temperature using a Barnes

Engineering VTC-1 variable temperature chamber. Spectra were obtained from —20 to 80°, with temperatures recorded using a thermocouple and potentiometer.

Fig. 1 shows the spectrum of the phosphatidyl ethanolamine–water mixture at room temperature. When this spectrum is compared with those of anhydrous phosphatidyl ethanolamine[1], it closely resembles the spectrum recorded at high temperatures (140°) for the dry solid. The splittings characteristic of crystalline solids are not present, indicating that the sample is in a liquid or liquid-like state. When the sample is cooled to —20°, there is no substantial increase in the crystalline character, as judged by the appearance of the infrared spectrum. It appears that no separation into ice and lipid phases occurs at low temperature. The infrared spectra thus confirm other techniques in their indication of liquid–crystalline behavior of phosphatidyl ethanolamine–water mixtures at room temperature.

When the temperature of a sample is raised, and the infrared spectrum is carefully examined as a function of temperature, almost all bands remain unchanged. However, in the 1450–1500-cm^{-1} region considerable changes in the intensity of one band, relative to others in the spectrum, are observed. This band is a -CH_2 scissoring motion of the hydrocarbon chains in the phospholipid.

When a plot of the peak absorbance of this band (relative to another band in the spectrum) *vs.* temperature is made, the curve shown in Fig. 2 is obtained. In this plot the computed uncertainty in a given absorbance ratio is ± 0.005, using propagation of errors theory, and in a given temperature, ± 1°.

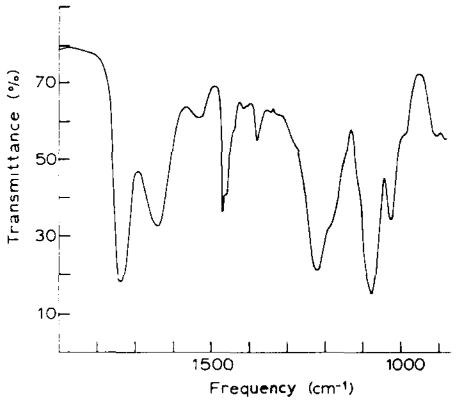

Fig. 1. Infrared spectrum of the phosphatidyl ethanolamine–water mixture at 22°.

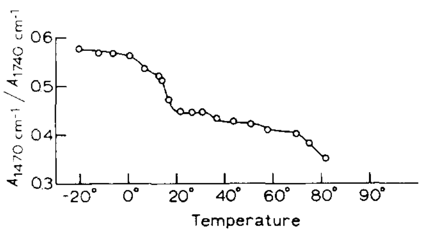

Fig. 2. A plot of $A_{1470\ \text{cm}^{-1}}/A_{1740\ \text{cm}^{-1}}$ (ratio of peak absorbances) *vs.* temperature for a phosphatidyl ethanolamine–water mixture.

As the temperature is raised from —20°, several intensity changes are observed. The most dramatic one occurs between 0 and 10°, followed by two less prominent transitions (which are, however, quite reproducible with different samples) centered about 35 and 55°. We believe that these intensity changes in the infrared spectrum are closely associated with the crystal and liquid crystal phase transitions for phosphatidyl ethanolamine–water mixtures. Above 70° the sample appears to break down and many spectral changes, probably including loss of water, are observed.

The variations in the infrared spectrum of phosphatidyl ethanolamine–water mixtures as a function of temperature offer important new evidence regarding the nature of the phases. They indicate that a change in the environment of the hydrocarbon chains is probably the most important effect which the phospholipid experiences during the phase transitions.

A further result of this study is the indication that the infrared spectroscopic technique may be of considerable utility in observing phase transitions involving mesomorphic phases. This has already been shown for simple organic liquid crystals[5]. The development of new techniques for this purpose is important, as existing techniques either are not sufficiently sensitive with standard sample sizes (*e.g.* differential thermal analysis), require pretreatment of the sample (electron microscopy) or lengthy analysis of the data before results can be obtained (X-ray diffraction). The infrared spectroscopic method not only minimizes all of these factors, it also provides a specific probe of the changes taking place in the system. Further work on this and other systems is now in progress.

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