Vibrational Spectra of Liquid Crystals. IV. Infrared and Raman Spectra of Phospholipid–Water Mixtures[1,2]

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Abstract: Infrared (600–4000 cm^{-1}) and Raman (100–4000 cm^{-1}) spectra of four phospholipid–water mixtures (20% water) have been measured as a function of temperature. The infrared spectra are insensitive to phase transitions, except for a methylene deformation vibration of the fatty acid chains, at 1470 cm^{-1}. A plot of its intensity, relative to that of another band in the spectrum, as a function of temperature, provides a sensitive method for detecting the phase transitions. In the Raman spectrum, a change in relative intensity of symmetric and asymmetric C–H stretches is observed. In the C–C stretching region a collapse of the symmetric and asymmetric stretch into a broad, central maximum is observed. The spectral changes are discussed with reference to the polyethylene system.

Many models exist for the arrangement of protein and lipid in biomembranes. An important difference between these models is the degree to which lipid bilayers, with ordered fatty acid chains, play a role in the structure. It has been speculated that increased knowledge about mobility in these fatty acid chains would lead to an understanding of the significance of the lipids in determining membrane properties. To this end, a number of physical techniques, including nuclear magnetic resonance,[3] electron spin resonance,[4] differential thermal analysis,[5] and infrared spectroscopy,[6] have been employed. Nonetheless, only a partial picture has emerged.

In this paper, we present results from infrared and Raman spectroscopy which indicate that molecular vibrations can be a sensitive probe of order in the fatty acid chains of phospholipids. The number of bands observed, the relative intensities of the bands, and the band widths all yield information on this subject. The ability to probe these bilayer systems without introducing any chemical perturbation, using small amounts of sample (*ca.* 8 nl), with simultaneous examination of all parts of the phospholipid, makes infrared and Raman spectra important methods for future studies of biomembranes. Preliminary results for the infrared spectra of phosphatidylethanolamine–water bilayers have already been reported.[6a]

Results

Figure 1 shows the infrared spectrum of lysophosphatidylethanolamine (LPEA) suspended in 20% water. This is not a homogeneous phase, and the spectrum obtained is identical with that seen in a sample of the pure solid in Nujol, with the exception of the strong absorption due to water near 1640 cm^{-1}.

One notes that the spectrum contains a number of sharp, well-defined bands, characteristic of the infrared spectra of pure solids.

When the above sample is sonicated for about 5 sec, a gel forms. The spectrum of this gel is shown in Figure 2. The differences between these two spectra may be summarized as follows: (1) there are fewer distinct bands in the spectrum of the gel than in that of the solid; (2) nearly all bands in the gel appear much broader than those of the solid. An exception to (2) is the band at 1470 cm^{-1}. This methylene deformation vibration is quite as sharp in the gel as it is in the solid.

The spectra in Figures 1 and 2 are characteristic of phospholipid–water systems. Identical changes have been observed with phosphatidylethanolamine (PEA), lecithin (L), and lysolecithin (LL).

When these gels are heated, the spectra remain unchanged, with the exception of the band at 1470 cm^{-1}. Its peak intensity is observed to decrease relative to the other bands in the spectrum. Plots of this relative absorbance *vs.* temperature are shown in Figure 3 for all four systems. These plots reveal at least one phase transition, and, in some cases, several distinct transitions, for each system. All points in these plots represent equilibrium measurements, averaged for several runs.

Infrared spectral measurements conceal certain features which are revealed by Raman spectra. The foremost among these arises from the intense infrared spectrum of water, which obscures the 3000-cm^{-1} region (C–H stretching) as well as the lower frequency region (<700 cm^{-1}). While the Raman spectrum of water is quite weak, carbon–carbon stretching vibrations appear relatively intense. These bands give rise to very weak infrared absorption.

The complete Raman spectra for the phospholipid–water systems have been examined as a function of temperature. Again, as in the infrared spectra, many of the bands are insensitive to temperature variation and hence to the phase transition. For example, Figure 4 shows the phosphate vibration near 700 cm^{-1} in lecithin, which undergoes no significant change as the temperature is raised.

Changes do occur, however, in the spectral regions associated with the fatty acid chains. Figure 5 shows this for lecithin and lysolecithin in the C–H stretching

(1) Paper III of this series: B. J. Bulkin and K. Krishnan, *J. Amer. Chem. Soc.*, **93**, 5998 (1971).
(2) Presented at the 161st National Meeting of the American Chemical Society, Los Angeles, Calif., April 1971.
(3) D. Chapman and D. F. H. Wallach, "Biological Membranes. Physical Fact and Function," D. Chapman, Ed., Academic Press, New York, N. Y., 1968.
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(6) (a) B. J. Bulkin and N. Krishnamachari, *Biochem. Biophys. Acta*, 211, 592 (1970); (b) D. Chapman in "The Structure of Lipids," Methuen, London, 1965.

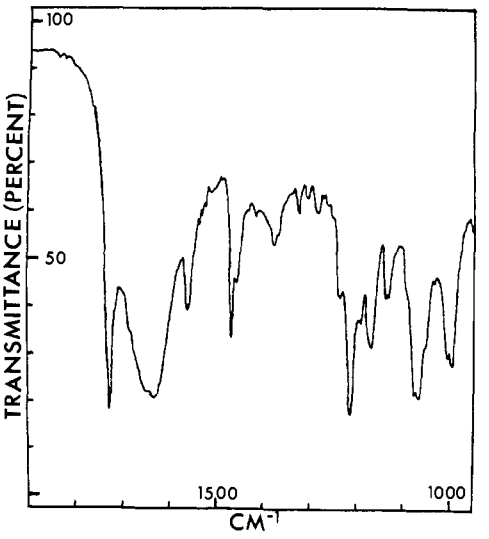

Figure 1. Infrared spectrum of lysophosphatidylethanolamine (solid powder)–water mixture, unheated and unsonicated.

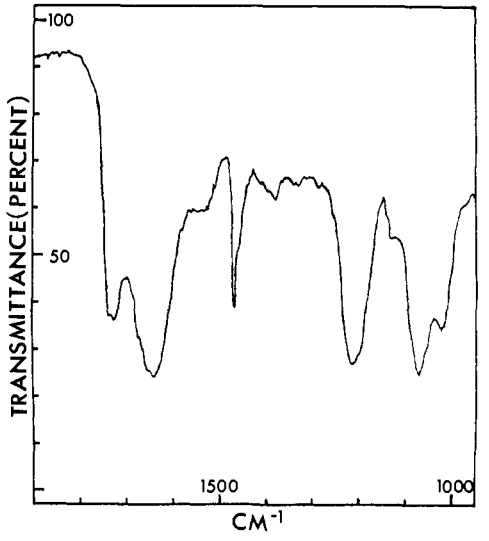

Figure 2. Infrared spectrum of same mixture shown in Figure 1 after brief (*ca.* 5 sec) sonication.

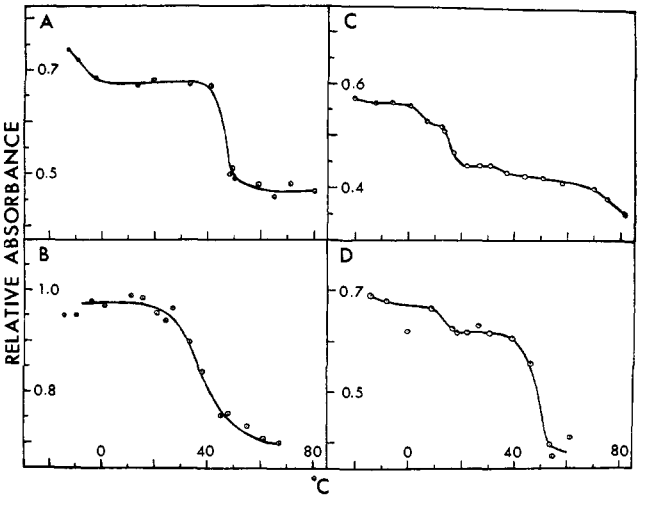

Figure 3. Change in absorbance (relative to another band in the spectrum) of infrared band at 1470 cm^{-1}, as a function of temperature, for (A) lecithin, (B) lysolecithin, (C) phosphatidylethanolamine, and (D) lysophosphatidylethanolamine, gels containing 20% water.

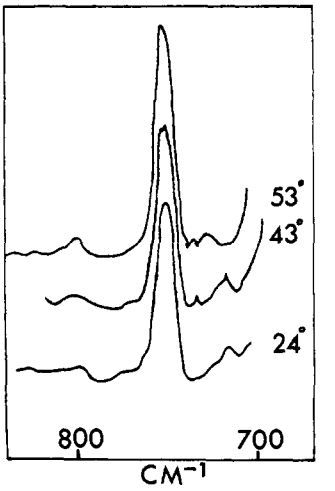

Figure 4. Temperature dependence of Raman spectrum of a lecithin–water gel near 700 cm^{-1}.

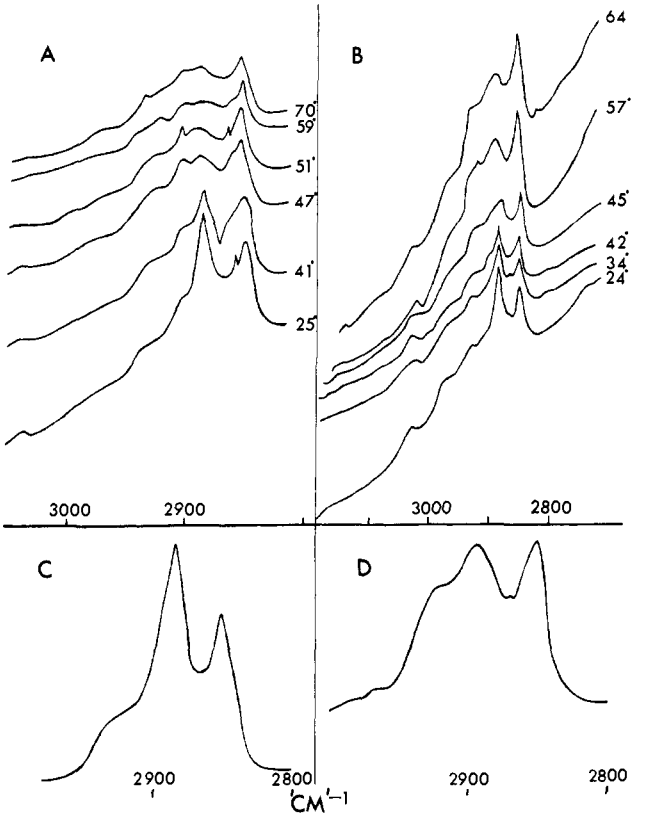

Figure 5. Temperature dependence of Raman spectrum of (A) lecithin–water and (B) lysolecithin–water gels in the C–H stretching region. (C) and (D) are spectra of solid and molten polyethylene, respectively, in the same region (redrawn from ref 7).

region. Identical changes to those shown for L and LL are seen in the spectra of PEA and LPEA. The two intense bands at 2840 and 2875 cm^{-1} are symmetric and asymmetric vibrations of the methylene groups. As the temperature is raised, these bands change in relative intensity. Comparison with the spectrum of acetylcholine indicates that the C–H stretches of the methyl groups attached to nitrogen occur to higher frequency from those of the methylene groups. Figure 5 shows the Raman spectrum of solid and molten polyethylene, redrawn from a paper by Brown.[7]

(7) R. G. Brown, *J. Chem. Phys.*, 38, 221 (1963).

The C–C stretching region also changes with temperature. This is illustrated for L, LL, and PEA in Figure 6. We have not been able to obtain high quality spectra for LPEA in this region because of high background scattering. It appears that three bands collapse to a broad, central maximum as the temperature is raised. The analogous spectra in solid and molten polyethylene, again redrawn from ref 7, have been included for comparison.

Discussion

Chapman[6b] has shown that when a solid phospholipid in a KBr pellet is heated to 140°, the bands broaden and a number of bands disappear. This is similar to the change seen between Figures 1 and 2. Thus at room temperature, in the gel, the phospholipid appears to be in a liquid-like state with respect to the time scale of molecular vibrations. The broad bands seen in Figure 2 are typical of systems in which a number of slightly different environments exist. This is undoubtedly the case for the lipid glycerol backbone and phosphorylcholine end, with its water sheath.

The methylene deformation vibration at 1470 cm^{-1} remains as sharp in the gel as it was in the solid. This can be interpreted as indicative of a crystal-like environment for the methylene groups of the fatty acid chains. As discussed below, the Raman spectra yield more detailed information on this point.

As the temperature is raised, this environment changes, and the changes are reflected in a change in the intensity of the band at 1470 cm^{-1}. The decreasing intensity observed could mean that the overall dipole change for this transition is decreasing. One can speculate that this is due to a more random orientation of the chains. Alternatively, the less ordered chains may be shifting intensity into the wings of the absorption band, away from the center. This would appear as a decrease in the peak intensity at 1470 cm^{-1}. It is this latter quantity which is plotted in Figure 3.

One notes from Figure 3 that the intensity change is a sensitive method for detecting phase transitions in the phospholipid–water systems. It is difficult to make comparisons with transition temperatures from the literature, as these have not always been measured with pure phospholipids, or with the same fatty acid chains present. However, Table I summarizes the results from several key references and compares them with this work. As expected, some differences are found. This is usually the case for measurements made by widely different techniques.

Table I. Transition Temperatures for Phospholipid–Water Systems[a]

	Temp, °C	
	This work	Lit.
Dipalmitoyllecithin	43	41[b]
Lysolecithin	27	35[c]
Phosphatidylethanolamine	12, 33	20,[c] 25–35,[d] 55[c,d]
Lysophosphatidylethanolamine	9, 43	

[a] See Experimental Section for description of the phospholipids used in this study. [b] B. D. Ladbrooke and D. Chapman, *Chem. Phys. Lipids*, **3**, 304, (1969). [c] F. Reiss-Husson, *J. Mol. Biol.*, **25**, 363 (1967). [d] E. Junger and H. Reinauer, *Biochem. Biophys. Acta*, **183**, 304 (1969).

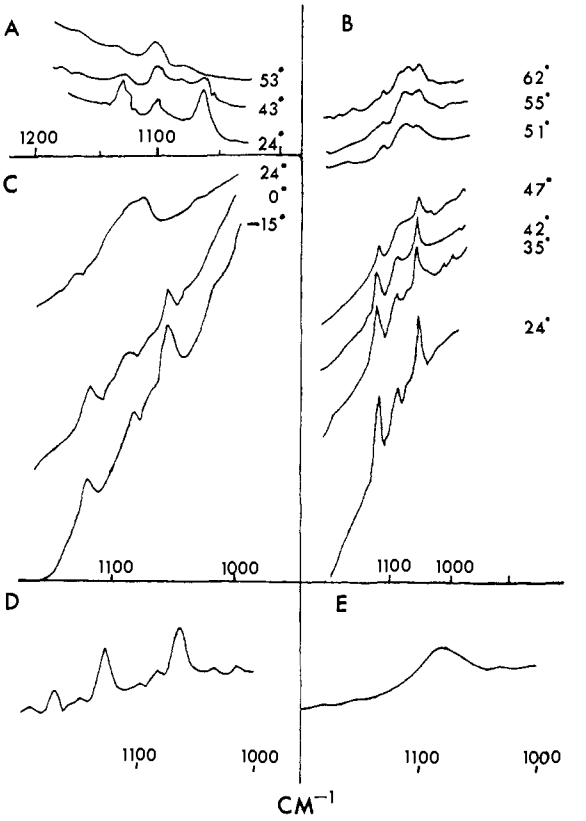

Figure 6. Temperature dependence of Raman spectrum of (A) lecithin–water, (B) lysolecithin–water, and (C) phosphatidylethanolamine–water gel in the C–C stretching region. (D) and (E) are solid and molten polyethylene, respectively (redrawn from ref 7).

It is important to point out that the infrared method shown here is nondestructive to the system, sensitive to small changes (*e.g.*, the several PEA transitions), requires little sample, and involves no chemical perturbation. As with many spectroscopic techniques, it measures the transition as it affects one portion of the system, the CH_2 groups.

The transition temperatures measured here correlate well with the electron spin resonance data of Hubbell and McConnell.[4] This can be seen by comparing the results for lecithin–water from infrared spectra (Figure 3) with those in ref 4 (Figures 7 and 9). Not only is the transition temperature the same, but the shape of the curves is similar as well. It is important to point out this agreement, as questions have been raised concerning the extent to which a spin label perturbs the bilayers. In the case of lecithin we find no significant perturbation.

The Raman spectra, because of poorer signal to noise ratios than infrared spectra, are not useful for measurements of transition temperatures. Nevertheless, a considerable amount of information about the transitions can be gleaned from these spectra. Whereas in the infrared spectra only the CH_2 deformation vibrations appear to be sensitive to the thermal phase transitions, in the Raman spectrum changes appear in the C–H stretching and C–C stretching regions as well.

As in the infrared spectra, one finds that all changes in the Raman spectra observed at thermal phase transitions are in bands associated with the methylene groups of the fatty acid chains. No difference appears in the environment of the phosphate or choline methyl groups.

This general result is consistent with that of other techniques.

The changes which do appear are relatively easy to understand. As seen in Figures 5 and 6, they can all be described as relative intensity changes, some involving complete disappearance of one or more bands.

In the C–H stretching region, the initially different relative intensities of two modes, a symmetric and asymmetric stretch, become almost the same in a higher temperature phase. These bands appear at approximately the same frequency in crystalline polyethylene and, as seen in Figure 5, undergo a similar change in relative intensity in that case. Although this effect has not been previously discussed, it seems clear that it is associated with a change from an extended, crystalline methylene chain to one which is kinked. Further, in molten polyethylene there is considerable motional freedom for the chains. It would not be unexpected for either of these changes to alter the relative intensities of the modes. In both cases the ratio of the polarizability derivatives for different stretches should change. It is not possible to predict, based on our current knowledge of Raman intensities, the direction of this change.

It is interesting that the transition manifests itself here only in intensity and not in frequency shifts. These modes do not involve long-range coupling between adjacent methylene groups. Indeed, symmetric and asymmetric motions could appear even in a single CH_2 moiety. Thus the relative intensities of the two bands, which are an average over the whole chain, are sensitive to chain conformation, while the frequencies, which probably reflect no more than a three carbon coupling, remain constant.

The C–C stretching region complements these observations. Again, analogous changes are observed in the lipids to those in polyethylene. This is clearly seen in Figure 6. Three bands are observed in the lipid spectra, even at the lower temperatures. These have frequencies of 1130, 1090, and 1060 cm^{-1}. In crystalline polyethylene only two of the bands are found, those at 1130 and 1060 cm^{-1}. These have been assigned as the asymmetric and symmetric C–C stretching, respectively. In molten polyethylene, one sees only a broad C–C stretching band centered near 1080 cm^{-1}. The lipids also show an increase in the intensity of this central band relative to the two side bands. However, the center band is initially more intense in the lipids than in polyethylene, and, at least in the LL/water case, there is evidence that the side bands do not completely disappear as they do in polyethylene.

The collapse of symmetric and asymmetric C–C stretches into a central maximum means that an ordered, crystalline chain has become kinked with a broad distribution of different structures. When this happens the distinction between symmetric and asymmetric stretching is lost.

The presence of the band at 1090 cm^{-1} even in the lower temperature phase of the lipids may be evidence for some disorder at these temperatures. However, this band could also arise from a breakdown of translational symmetry selection rules ($k \neq 0$ modes become allowed) which has been previously noted for crystalline alkanes in the literature.[8] It is not easy to distinguish between these possibilities. Further measurements at lower temperatures are in progress in the hope of elucidating this point. Additional work on these and natural membrane systems is in progress.

Experimental Section

Infrared Spectra. Ir spectra were obtained on a Perkin-Elmer Model 521 dual grating spectrometer. The instrument was run at a spectral slit width of *ca.* 1 cm^{-1}. The sample was a film about 10 μ thick, held between Irtran-2 plates.

A Barnes Engineering Model VTC-1 variable temperature chamber was used to obtain spectra as a function of temperature. Spectra were obtained from -20 to 80°, with temperatures recorded using a thermocouple and potentiometer. In the plots shown in Figure 3 the computed uncertainty in a given absorbance ratio is ± 0.005, using propagation of errors theory. The estimated uncertainty in the temperature is ± 1°.

Raman Spectra. The Raman instrument used was a Spex Industries Model 1401 double monochromator, Spectra-Physics helium–neon laser (*ca.* 50 mW power at the sample) and photon counting detection. Spectra were run at *ca.* 5 cm^{-1} spectral slit width. A Corning 2-62 filter was used to eliminate a Lyman ghost at 2902 cm^{-1}.

The unit for obtaining variable temperature spectra on this instrument has been described previously.[9] Samples were held in thin capillaries, with a sample size of about 1 μl assuring even heating. All spectra were scanned several times, and the plotted results are an average of these scans.

Phospholipids. All phospholipids used were chromatographically pure (tlc). The PEA used was described previously.[6a] LPEA was prepared from this sample and has the same fatty acid characteristics. This was also obtained from Pierce Chemical Co. Lecithin (L) was obtained from Mann Research and was tlc pure L-2-dihexadecylglycerine-1-phosphorylcholine. The lysolecithin used was also pure hexadecyl compound.

Acknowledgments. The support of the American Cancer Society, Grant No. P504a, is gratefully acknowledged.

(8) R. G. Snyder and J. H. Schachtschneider, *Spectrochim. Acta*, **19**, 85 (1963); R. G. Snyder, *J. Chem. Phys.*, **47**, 1316 (1967); R. F. Shaufele and T. Shimanouchi, *ibid.*, **47**, 3605 (1967); M. Tasumi and T. Shimanouchi, *J. Mol. Spectrosc.*, **9**, 261 (1962).

(9) B. J. Bulkin and F. T. Prochaska, *J. Chem. Phys.*, **54**, 635 (1971).