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RAMAN ACTIVE VIBRATIONS IN LONG-CHAIN FATTY ACIDS AND PHOSPHOLIPID SONICATES

J. L. LIPPERT AND W. L. PETICOLAS

Department of Chemistry, University of Oregon, Eugene, Oreg. 97403 (U.S.A.)

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SUMMARY

The Raman active vibrations of saturated and unsaturated fatty acids are examined. The all-*trans* chain lengths of the saturated carbon chains in both types of homogeneous fatty acid samples can be determined in this way. The side-chain melting transition of L-α-dioleoyl lecithin suspension is studied and it is found that although the interior of the multilayer is more mobile than the region closer to the polar surface, both regions are in a random configuration at room temperature.

INTRODUCTION

A recent study from this laboratory has illustrated the utility of laser Raman spectroscopy in obtaining the conformation of the hydrocarbon chains in dipalmitoyl lecithin multilayers[1]. From measurements of the Raman frequencies, it is a simple matter to distinguish between the all-*trans* configuration and the random configuration of the saturated hydrocarbon chains. However, since naturally occurring membranes contain derivatives of unsaturated as well as saturated fatty acids, the question arises as to whether it is possible by means of Raman spectroscopy to examine the configuration of unsaturated fatty acids in a similar manner. In particular, it is of interest to examine the saturated hydrocarbon chains on each side of the double bond in an unsaturated fatty acid to see if it is possible to differentiate between that portion of the side chain close to the polar surface of a membrane and that portion in the hydrophobic interior.

This paper outlines Raman spectral assignments of long-chain fatty acids which can be used to determine all-*trans* chain length as well as the position and configuration of double bonds in homogeneous fatty acid samples. In addition, the carbon–carbon stretching region of phospholipids and fatty acids containing double bonds shows two intense bands which are assigned, respectively, to the all-*trans*-methylene chain between the acid moiety and the double bond (bound chain) and the methylene chain extending from the double bond to the terminal methyl group (free chain). These bands are used to study the side chain melting transition of L-α-dioleoyl lecithin suspensions. Both free and bound chains transform from the all-*trans* to a random configuration at the same temperature, but the Raman intensities appear to indicate that the interior of the multilayer is more mobile than the region closer to the polar surface.

EXPERIMENTAL

High-purity long-chain saturated and unsaturated fatty acids were purchased from Applied Science Laboratories. The samples were sealed in melting point capillary tubes and Raman spectra were obtained in a special variable temperature Raman cell controlled by flowing nitrogen gas. L-α-Dioleoyl lecithin was obtained from P-L Biochemicals and used as obtained. Our Raman spectrometer consists of a Spectra Physics 165 Ar^+ ion laser, specially built optics and a Spex 1401 double monochromator. A cooled ITT FW 4013 phototube detects the analyzed light and is amplified by a combination of RIDL and Ortec counting equipment. A mixture of lecithin and water (1:4, w/w) was allowed to swell for 24 h and then sonicated in a Heat Systems ultrasonic cleaner (40 W) for 20 min. The sonicate was sealed in a melting point capillary tube and Raman spectra were run in the flowing nitrogen Raman cell.

THEORETICAL

The vibrational spectra of long, straight-chain hydrocarbons has been analyzed in detail by several authors[2-6] in terms of the frequency–phase angle relationship of a polymethylene zig-zag chain. In general[5], for a free end polymer with n atoms per translational repeat unit and M repeat units, there are $3Mn$ vibrations (including 6 zero-frequency vibrations). These vibrations are distributed in $3n$ frequency branches at M phase angles:

$$\phi_k = k\pi/M, \qquad k = 0, 1, 2, \ldots M - 1 \tag{1}$$

The 18 frequency branches of all-*trans*-polyethylene in the Brillouin zone corresponding to the translational unit cell (C_2H_4) are shown in Fig. 1. The two methylene groups of the translational unit cell are related to each other by inversion *plus* half unit translation. This means that the 18 branches are grouped in pairs which connect at phase angle π. More commonly the frequency–phase angle relationships are represented with the coupled branches unfolded so that there are only 9 branches in the enlarged Brillouin zone corresponding to the chemical repeat unit CH_2.

For an infinite polymer, it can be shown that only those vibrations which remain invariant under any primitive translation along the chain axis will be active in either the infrared or Raman. These vibrations correspond to those with $k = 0$, *i.e.* those with identical nuclear displacements in each unit cell.

The allowed vibrations of polyethylene and their symmetries are shown at the $k = 0$ edge of Fig. 1. In oligomers, this selection rule is no longer exactly true and hence all vibrational frequencies are in principal allowed either in the infrared or Raman. However, those frequencies closest to $\phi = 0$ are most intense. (Typically $k = 0, 1, 2$ are observed.) Because of the appearance of $k = 1$ and 2 vibrations, vibrational spectra and dispersion curves may be analyzed to obtain the all-*trans* chain length of polymethylene chains.

The $\phi_k = 0$, $k = 0$ vibrational frequency of all modes is independent of chain length. For any all-*trans* chain these frequencies lie on the ordinate of Fig. 1. However, in view of Eqn 1, the frequency of the $k = 1$ mode (or any $k \neq 0$ mode) will be a function of the chain length, M and can be obtained from a dispersion curve (Fig. 1)

at the phase angle, $\phi = k\pi/M$, $k = 1$. If the frequency branch is sufficiently disperse, *i.e.* if there is considerable variation of frequency with phase angle, then the frequencies of varying lengths of all-*trans* chain can be distinguished. Conversely, from a knowledge of the dispersion curve, measured frequencies, and Eqn 1, all-*trans* chain lengths can be studied in physically interesting situations.

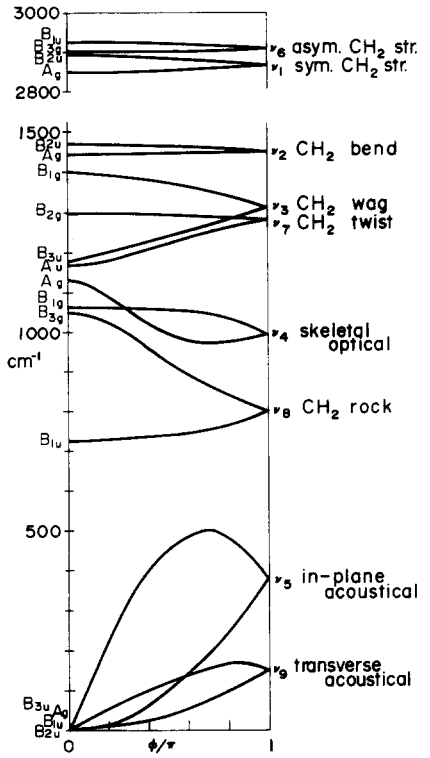

Fig. 1. Phonon dispersion curve of a polyethylene zig-zag chain. Based on the calculation of ref. 3. The Brillouin zone is that of the translational group C_2H_4.

The branches which are expected to be the most valuable in this regard for carbon chains (Fig. 1) are ν_5, the C–C chain longitudinal acoustical mode; ν_4, the C–C chain optical mode; and ν_8, the CH_2 rocking mode. The latter is important in the infrared spectroscopy of hydrocarbons but is very weak in the Raman effect.

The two carbon chain modes (ν_4 and ν_5) are the most important in the study of structure in polymethylene chains by Raman spectroscopy. Since the backbone is directly involved in these vibrations, substantial spectral changes are expected whenever the conformation of the backbone changes. Schaufele has studied the low frequency, $k = 1$, ν_5 vibration in long-chain crystalline and melted paraffins[5-6] as well as[8] in $C_{34}H_{68}$, a cyclic hydrocarbon which crystallizes with two C_{15} chains. Peticolas and coworkers[9-10] have found very low frequency, Raman active vibrations in polyethylene single crystals corresponding to the longitudinal mode (ν_5) to decrease in frequency when the crystals are annealed at higher temperatures. We have used the optical mode ν_4 to study the effect of cholesterol on dipalmitoyl lecithin model membranes[1].

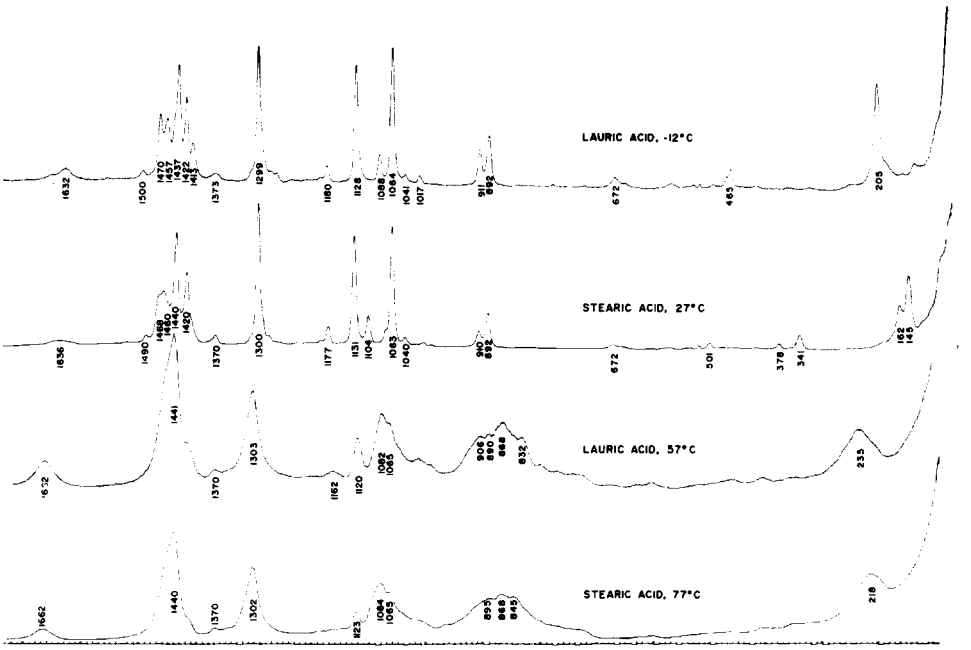

Fig. 2. Photographed Raman spectra of the powder (low temperature) and melted forms of the typical saturated fatty acids, lauric and stearic acid (C_{12} and C_{18} straight chain). The acoustical modes at 145 cm^{-1} and 162 cm^{-1} in the spectrum of polycrystalline stearic acid correspond to the vibrations of all-*trans* C_{18} and C_{16} chains, respectively. This may be due to incomplete separation of stearic and palmitic acid in our commercial samples. Spectra were obtained with 800 mW laser power and 250-μm slits.

The present work deals with the question: What effect do end groups (in particular the acid group) have on the carbon chain skeletal vibrations? Can individual C_n lengths be determined in saturated and unsaturated fatty acids? Can changes in conformation of fatty acids and phospholipids be readily observed by Raman spectrocopy? These questions are easily answered for the saturated fatty acids but are more complicated for the unsaturated acids.

Long-chain fatty acids

Fig. 2 shows the Raman spectra in the crystalline powder and liquid states of two typical long-chain saturated fatty acids. Fig. 3 shows the Raman spectra in the crystalline powder and liquid states of the *cis* and *trans* isomers of the unsaturated fatty acid 9-octadecenoic acid. Evident in Figs 2 and 3 are the similarities between the high temperature spectra, which are typical of all the liquid fatty acids. In particular, notice the broad bands around 230 and 1100 cm^{-1}. Recent studies[2,3,7] have assigned these vibrations to the acoustical and optical skeletal modes of polymethylene chains containing random *gauche* rotations. These liquid bands serve as the fingerprints of randomly oriented fatty acid side chains.

Evident in Fig. 3 (and also in Fig. 5, below) are certain bands which are exclusive to the *cis* or the *trans* form of the polycrystalline solid unsaturated fatty acids and can be used to distinguish between those isomers. These bands are listed in Table I.

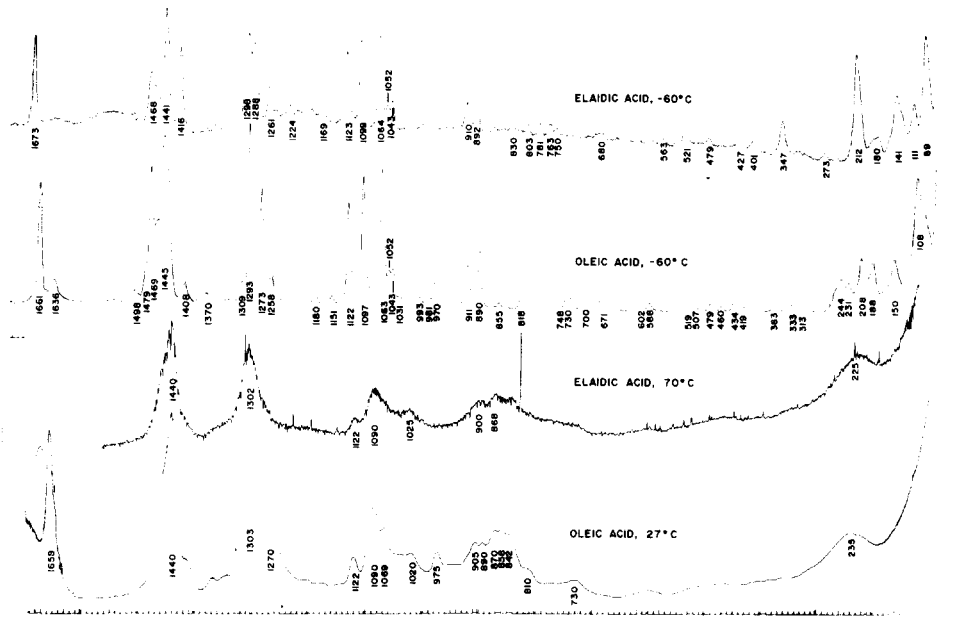

Fig. 3. Photographed Raman spectra of the powder (low temperature) and melted forms of the typical unsaturated fatty acids, elaidic acid (*trans*-9-octadecenoic acid) and oleic acid (*cis*-9-octadecenoic acid). Spectra were obtained with 800 mW laser power and 250-μm slits. The spectrum of liquid elaidic acid shows an instrumental noise problem which is occasionally experienced.

TABLE I

RAMAN SPECTRAL FEATURES OF *cis* AND *trans* DOUBLE BONDS IN UNSATURATED FATTY ACIDS

Frequency (cm^{-1})		*Assignment**
Cis	*Trans*	
1650–1665 s	1670–1680 s	C = C stretch
Very weak	1410 m	CH in-plane bend
970 w	810 w	CH out-of-plane bend

* By comparison with olefinic assignments in ref. 16, *Laser Raman Spectroscopy*, Wiley–Interscience, 1970.

In Fig. 4 the Raman spectra of the even-number straight-chain fatty acids between C_8 and C_{22} are plotted. Regular changes in vibrational frequency with chain length are readily observed, particularly below 500 cm^{-1} and in the 1030–1130-cm^{-1} region. The frequencies of these spectra correspond very closely to those predicted for polymethylene chains with the same number of carbons except that now the terminal acid and methyl group vibration are also observed. In the longer-chain fatty acid spectra bands corresponding to fatty acid contaminants, two carbons longer or shorter, are also resolved in the acoustical region.

Fig. 5 is a plot of the Raman spectra of a number of unsaturated fatty acids. These spectra are much more complex than those of the saturated fatty acids. This is no doubt because of the strong perturbations to an all-*trans*-polymethylene chain and increased interactions caused by the double bond. Nevertheless, the principle peaks

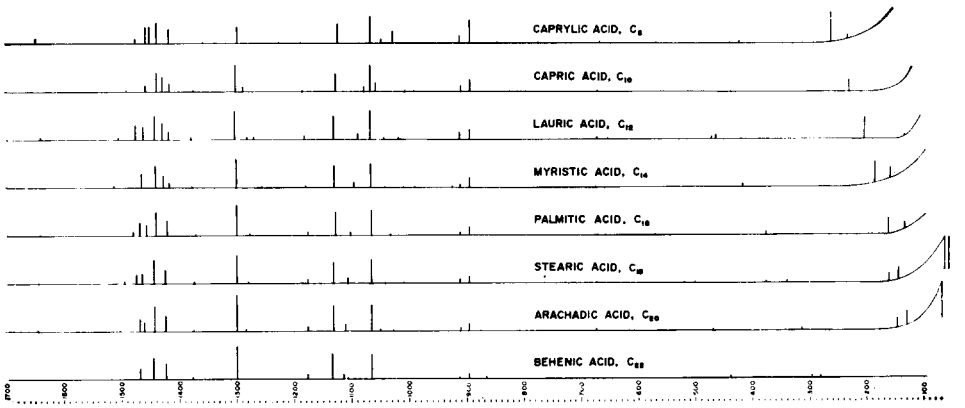

Fig. 4. Summary of the Raman spectra of even number, saturated fatty acids.

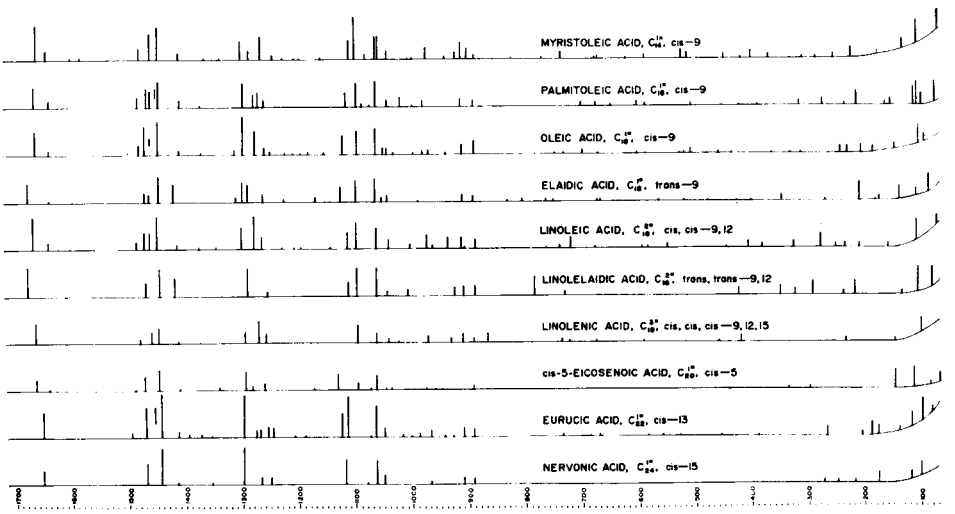

Fig. 5. Summary of the Raman spectra of various unsaturated fatty acids, $C_n^{a=}$, $i - b$: where n is the number of carbons, a is the number of double bonds, i is *cis* and *trans* isomer, and b is the position of the double bond.

($k = 0$) due to polymethylene chain vibrations remain the strongest in the spectrum. And, particularly in the 1000–1130-cm^{-1} region, relations between vibrational frequency and the length of the separate bound and free hydrocarbon chains (acid terminal and methyl terminal respectively) are also observed. For example, peaks occur at 1044 and 1099 cm^{-1} whenever a C_9 bound chain is present, and at 1112 and 1005 whenever C_6 free chain is present.

In Figs 6 and 7 the frequencies of vibrations in the skeletal acoustical and optical branches are plotted against the inverse of chain length and compared with the dispersion curves obtained from linear hydrocarbons. While the points in the optical region are unambiguous for both saturated and unsaturated acids, only those of the saturated chains are clearly defined in the low-frequency region (Fig. 5). Several Raman active vibrations of the unsaturated fatty acids appear in the region 250–400

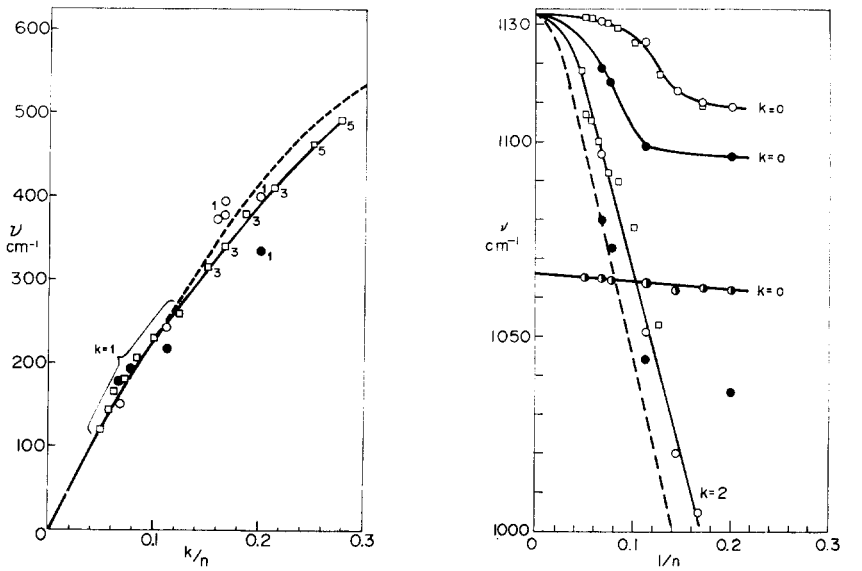

Fig. 6. Vibrational frequencies of all-*trans*, *n*-carbon chain in polycrystalline saturated and unsaturated fatty acids in the longitudinal acoustical region plotted against k/n where $k = 0, 1, 2, \ldots$ as in Eqn 1. □, vibrations in saturated fatty acids; ○, those of the methyl-terminal carbon chain of unsaturated fatty acids; ●, those of the carboxyl-terminal carbon chain of unsaturated fatty acids. The dashed line is the acoustical branch of *n*-hydrocarbons measured in ref. 4.

Fig. 7. Vibrational frequencies of all-*trans*, *n*-carbon chains in the optical skeletal region of polycrystalline saturated and unsaturated fatty acids plotted against the reciprocal of the number of carbons. *k* values after Eqn 1 are listed. Labels as in Fig. 6 except that ◑ correspond to points obtained for the $k = 0$, 1163–1165-cm^{-1} skeletal optical mode in all saturated and unsaturated fatty acids. The dashed line shows the $k = 2$ frequencies of the optical skeletal mode of *n*-hydrocarbons taken from ref. 2.

cm^{-1} where the acoustical vibrations of short chains ($n < 10$) are expected to occur. The frequencies plotted in Fig. 6 generally belong to the most intense band in that to region of Fig. 5.

It is clear that the skeletal chain vibrational frequencies can provide a useful probe to the *trans* chain length in long-chain fatty acids. The vibrational frequencies of carbon methylene chains in unsaturated fatty acids are very close to the separate vibrational frequencies of equally long, straight-chain segments in all-*trans* hydrocarbons. This supports the concept of decoupled all-*trans* chain segments suggested by Peticolas and coworkers for polyethylene single crystals[9].

The way in which the all-*trans* chain is terminated does, however play a role in variations of the chain mode frequencies. It is easy to show that while the selection rules for free polyethylene chains are $\phi_k = k\,\pi/M$, $k = 0, 1, 2, \ldots, M-1$; a polyethylene chain with both ends fixed has allowed frequencies $\phi_j = j\pi/M+1$, $j = 1, 2, 3, \ldots, M$ on the same dispension curve. If a particular *k* mode is allowed in the Raman for a free polyethylene chain, then the $j = k + 1$ vibration is allowed for the fixed end chain.

We had originally felt that substitution of an acid moiety for a methylene would tend toward a fixed end in polyethylene chains. As shown in Fig. 6, the fre-

quencies of the n-carbon saturated fatty acids in the acoustical region are slightly lower than those for the same n-carbon hydrocarbon. The frequencies of the fatty acids in the optical region are higher than the equivalent hydrocarbons. This represents a decrease in phase angle in both regions from a lighter end to a heavier end polyethylene chain. The theoretical derivation, however, predicts a change from $\phi = k\pi/m$ to $(k + 1)\pi/(m + 1)$ from the free to fixed end model, or an increase in phase angle. The explanation of this discrepancy is unclear.

The Raman spectra of unsaturated fatty acids in Figs 3 and 5 show two intense bands in the optical region between 1100 and 1130 cm^{-1}. These vibrations are assigned to the $k = 0$, ν_4 modes of the bound and free chains, respectively. The frequencies of these vibrations are plotted against chain length in Fig. 7. The differences between the various frequencies of the $k = 0$ mode must be an indication of partial breakdown of the concept of applying dispersion relations to short carbon chains not terminated by methyl groups. The changes are really quite small, however, and do not represent severe perturbations on the concept. These vibrations provide a means of distinguishing changes in chain orientation in which the hydrophobic end of the carbon chain differs from the polar acid end of the chain because the $k = 0$ mode of of the carboxyl-terminal chain is slightly lower in frequency and can be distinguished from the methyl-terminal end in unsaturated fatty acids. We have applied this probe to the suggestion that in biological membranes, the interior carbons are more free to move than those nearer the polar phosphate surface.

L-α-Dioleoyl lecithin sonicate

The Raman spectrum of L-α- dioleoyl lecithin sonicate, 20 % by weight in water shown in Fig. 8 at —62 °C and 27 °C. The spectrum at —62 °C can be compared directly with low-temperature spectrum of oleic acid (Fig. 3) except for new bands at 720 cm^{-1} and 1089 cm^{-1}. In the low-frequency region at low temperature, the 218-cm^{-1} and perhaps the 248-cm^{-1} acoustical modes of the extended all-*trans* chains are observed. At room temperature the low-frequency region is obscured by water.

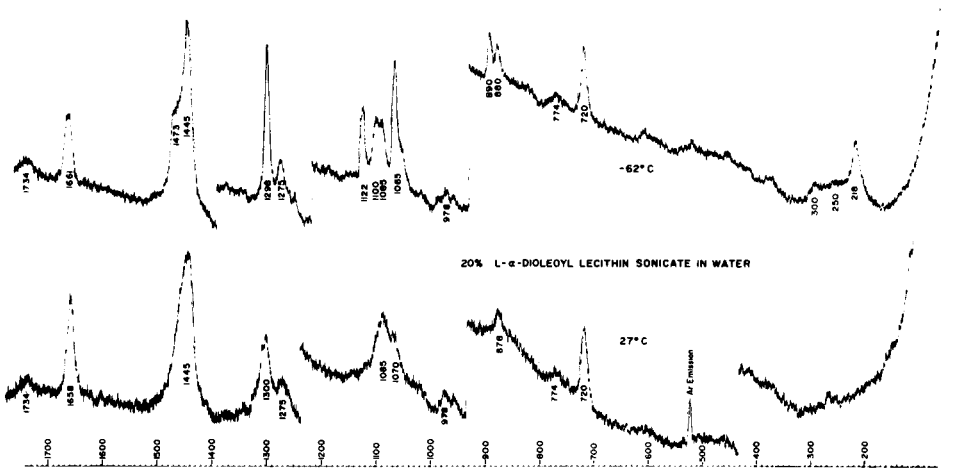

Fig. 8. Photographed Raman spectra of 20% dioleoyl-L-α-lecithin sonicated in water, below and above the chain melting temperature. Spectra were obtained with 1 W laser power and 300 μm slits.

The 720-cm^{-1} band is assigned to the phosphate diester symmetric stretch. We had earlier tentatively assigned the 1089-cm^{1} band in dipalmitoyl lecithin to the 16-carbon, all-*trans* vibration in the optical chain stretching mode with $k = 2$ (see Fig. 7). It now appears more likely to be the phosphate O–P–O symmetric stretch[11]. The conclusions reached in the earlier paper are not changed by this change in assignment.

The room-temperature spectrum of dioleoyl lecithin is reminiscent of that of the liquid phase of oleic acid. In particular, notice the disappearance of the all-*trans* carbon chain peaks at 1064 cm^{-1} ($k = 0$, free *plus* bound chains), 1101 cm^{-1} ($k = 0$, bound), and 1124 cm^{-1} ($k = 0$, free). A broad band at 1088 cm^{-1}, presumably a combination of the O–P–O symmetric stretch and the randomly oriented polymethylene chain vibration seen in melted fatty acids, is now the most intense band in the region.

The disappearance of the all-*trans* peaks, relative to the 1089-cm^{-1} peak height, is plotted in Fig. 9 as a function of temperature. The chain vibrations decrease in

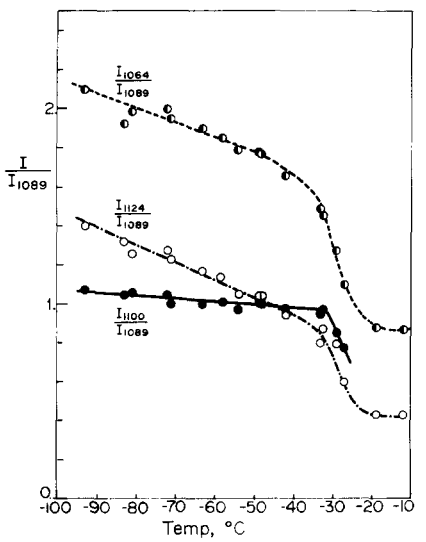

Fig. 9. Changes with temperature of peak heights relative to the peak height of the 1089-cm^{-1} phosphate band, of the bands in the skeletal optical region of 20% L-α-dioleoyl lecithin sonicated in water. ○, the 1124-cm^{-1}, $k = 0$ band of the methyl-terminal carbon chain; ●, the 1100-cm^{-1}, $k = 0$ band of the carboxyl-terminal carbon chain; ◐, the 1064-cm^{-1}, $k = 0$ band which contains both methyl-terminal and carboxyl-terminal carbon chains.

intensity quite sharply in the range —33 to —25 °C. Such a change has previously been shown[1] to manifest the gel–liquid crystal phase transition in dipalmitoyl lecithin sonicates. The phase transition is a change from the all-*trans* configuration of the fatty acid side chains to a random configuration[12]. The —33 to —25 °C transition in dioleoyl lecithin is presumably the equivalent change.

From the changes in the Raman spectra around —33° to —25 °C, it is clear that in dioleoyl lecithin multilayers at room temperature both the internal carbon chain (free chain between the double bond and terminal methyl) and the carbon chain closer to the polar phosphate surface (bound chain) are in an essentially liquid-like random structure. A carbon backbone structure in which all fatty acids contain

a single *gauche* rotation at the same carbon should still give one or more sharp peaks above 1089 cm^{-1}, *i.e.* $k = 0$ for a shorter all-*trans* chain.

The changes in height of the all-*trans* vibrational peaks below the transition temperature (Fig. 9) may, however, be indicative of greater freedom of motion in the free chain. Below the transition temperature, the decrease in height of the 1064-cm^{-1} and 1124-cm^{-1} bands with increasing temperature is accompanied by a roughly proportional broadening of those bands and is probably due to increasing molecular motion.

In contrast, the height of the 1101-cm^{-1} band changes very little with temperature below −33 °C. One possible explanation is that the 1088-cm^{-1} phosphate-*plus*-random chain band may extend to 1101 cm^{-1} and change in shape to compensate for an actual decrease in the all-*trans* peak. This seems unlikely since at the lower temperatures both bands are fairly well resolved but maintain the constant height ratio. The overlapping nature of the 1101-cm^{-1} and 1088-cm^{-1} bands precludes measurement of their band widths.

We favor the explanation that the motions of the chain closer to the polar phosphate group are more restricted and thus do not decrease in height as the temperature is raised. This explanation is supported by the ESR experiments of Hubbell and McConnell[13] and of Jost *et al.*[14], which show that nitroxide spin labels at various positions along the hydrocarbon chain have more restricted motion closer to the polar end than near the hydrophobic center of egg-lecithin model membranes. Recent ^{13}C NMR experiments[15] show a much longer relaxation time T_1 for the interior carbons of lecithin vesicles than those near the polar surface.

With these results we see that laser Raman spectroscopy can provide information concerning both the structure and dynamics of model membrane systems.

ACKNOWLEDGEMENTS

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REFERENCES

1 J. L. Lippert and W. L. Peticolas, *Proc. Natl. Acad. Sci. U.S.*, 68 (1971) 1752.
2 R. G. Snyder and J. J. Schachtschneider, *Spectrochim. Acta*, 19 (1963) 85.
3 R. G. Snyder, *J. Chem. Phys.*, 47 (1967) 1316.
4 M. Tasumi, T. Shimanouchi and T. Miyazawa, *J. Mol. Spectrosc.*, 9 (1962) 261.
5 R. F. Schaufele and T. Shimanouchi, *J. Chem. Phys.*, 47 (1967) 3605.
6 R. F. Schaufele, *J. Chem. Phys.*, 49 (1968) 4168.
7 R. Zbinden, *Infrared Spectroscopy of High Polymers*, Academic Press, New York, 1964.
8 R. F. Schaufele and M. Tasumi, *Polymer J.*, in the press.
9 W. L. Peticolas, G. W. Hibler, J. L. Lippert, A. Peterlin and H. Olf, *Appl. Phys. Lit.*, 18 (1971) 87.
10 A. Peterlin, H. Olf, W. L. Peticolas, G. W. Hibler and J. L. Lippert, *J. Polymer Sci. B*, 9 (1971) 583.
11 T. Shimanouchi, M. Tsuboi and Y. Kyogoku, *Adv. Chem. Phys.*, 7 (1964) 435.
12 D. Chapman, R. M. Williams and B. D. Ladbrooke, *Chem. Phys. Lipids*, 1 (1967) 445.
13 W. L. Hubbell and H. M. McConnell, *J. Am. Chem. Soc.*, 93 (1971) 314.
14 P. Jost, L. J. Libertini, V. C. Herbert and O. H. Griffith, *J. Mol. Biol.*, 59 (1971) 77.
15 J. C. Metcalf, N. J. M. Birdsall, J. Feeney, A. G. Lee, Y. K. Levine and P. Partington, *Nature*, 233 (1971) 199.
16 M. C. Tobin, *Laser Raman Spectroscopy*, Wiley-Interscience, New York, 1970.