Configuration-Dependent Raman Bands of Phospholipid Surfaces. 1. Carbonyl Stretching Modes at the Bilayer Interface

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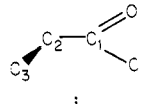
Abstract: Two strong carbonyl stretching modes (~1740 and ~1720 cm^{-1}) are observed in the Raman spectra of anhydrous crystalline dipalmitoylphosphatidylcholine (DPPC), anhydrous dipalmitoylphosphatidylethanolamine (DPPE), and dilaurylphosphatidylethanolamine (DLPE) cocrystallized with glacial acetic acid (HAc). Evidence is presented which convinces us that the two bands are due to conformation differences in the acyl linkages of the two hydrocarbon chains. Evidence is presented which shows that (1) the C=O stretching frequency is sensitive to rotation about the C_2—C_1 carbon–carbon bond, and is not sensitive to rotation about the C_1—O bond; (2) the higher frequency C=O stretching band arises from an approximately gauche conformation (attributed to the β-chain acyl linkage in the crystalline samples); (3) the lower frequency C=O stretching band arises from an approximately trans conformation (attributed to the γ-chain acyl linkage in the crystalline samples). The relative intensities of these two bands are shown to depend upon the state of the phospholipid sample. The Raman spectra of gel-phase aqueous dispersions of phospholipid samples and partially hydrated DPPC have only one strong Raman C=O band, located at the frequency of the gauche conformer band. The use of the relative intensities of these two bands to determine acyl linkage conformation in the β and γ chains and to estimate the packing arrangement of the two chains is discussed. Raman C=O stretch bandwidths were measured for various phospholipid samples and found to vary greatly as a function of the state (hydration and temperature) of the sample. The use of Raman C=O bandwidths to monitor freedom of motion in the acyl region of phospholipids is also discussed.

Introduction

We have observed that the Raman spectra of phospholipids often exhibit two strong bands in the carbonyl region (the C=O stretching region near 1700 cm^{-1}), but that under other experimental conditions one strong band appears in this region. The doublet in the C=O stretch region of anhydrous dipalmitoylphosphatidylcholine (DPPC), of anhydrous dipalmitoylphosphatidylethanolamine (DPPE), and of other phospholipid samples is a very striking feature, which we have investigated in detail. We have found that the observed Raman scattering in this C=O stretch region in phospholipid spectra appears to depend directly on the configuration at the acyl linkage of each of the two fatty acid side chains. The appearance of the two C=O stretching bands apparently signifies that the configurations at the two acyl linkages of the phospholipid molecule are different. Since the nature of the "packing" of hydrocarbon chains in the gel (or nearly all-trans) configuration may depend upon the geometry at the acyl linkage, an ability to readily monitor acyl linkage geometries by measuring relative intensities of these two C=O bands in the Raman spectrum offers an opportunity to obtain important information about hydrocarbon chain "packing" in the gel and in the solid states of phospholipid systems.

The experimental evidence and its interpretation, which we present here, have convinced us that the observed doublet in the C=O stretch region is indeed due to the existence of different conformations in the two acyl linkages of the phospholipid molecule. Our evidence indicates that the C=O stretch frequency *is* sensitive to rotation about the acyl C_2—C_1 bond and that it *is not* sensitive to rotation about the acyl C_1—O bond. We suggest that it is possible to analyze the conformation about the C_2—C_1 bond of each fatty acid acyl linkage (C_2—C_1(=O)O) located at the bilayer interface and to distinguish between gauche acyl linkages (i) which we show are responsible for the higher C=O stretch frequency in the doublet and trans acyl linkages (ii) which



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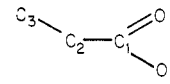

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we show are responsible for the lower C=O stretch frequency. We have used the results from the X-ray diffraction analysis of crystalline DLPE[1] to interpret the Raman spectrum of crystalline DLPE and show that each C=O stretch band in the doublet can in this case be assigned to one of the two fatty acid side chains. We have studied the effect of melting and hydration of the phospholipid on the Raman doublet; these studies show that the total Raman scattering intensity in the doublet is constant but that it redistributes between the two bands according to the experimental conditions. The results of these studies, as we shall show, are consistent with the conclusion that the two bands appear because of the dependence of the C=O stretch frequency on local geometry. We present an interpretation of the intensity redistribution in the two bands in terms of rotation about the acyl C_2–C_1 bond and consider its consequence on lipid hydrocarbon chain packing.

Experimental Section

Synthetic dipalmitoyl-DL-α-phosphatidylcholine (99%, crystalline Sigma grade 1) and synthetic dipalmitoyl-DL-α-phosphatidylethanolamine (98%, crystalline Sigma) were purchased and used without further purification. Synthetic dilauryl-L-α-phosphatidylethanolamine (Sigma) was recystallized from glacial acetic acid to reproduce the crystalline DLPE sample for which the geometry is known from X-ray diffraction studies.[1] Approximately 200-mW power from the 488.0-nm line of a Coherent Radiation CR5 argon ion laser was incident on samples for all spectra. An interference filter for the 488.0-nm laser line was placed before the sample to filter out plasma emission lines. Scattered light was focused with elliptical mirrors (described in ref 2) on the entrance slit of a Spex Ramalog 5 double grating monochromator. A cooled RCA c3104 photomultiplier tube was used to count photons in the Raman scattered light. Anhydrous samples were prepared by heating to ap-

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(1) P. B. Hitchcock, R. Mason, K. M. Thomas, and G. G. Shipley, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3036 (1974).
(2) K. G. Brown, E. B. Brown, and W. B. Person, *J. Am. Chem. Soc.*, **99**, 3128 (1977).

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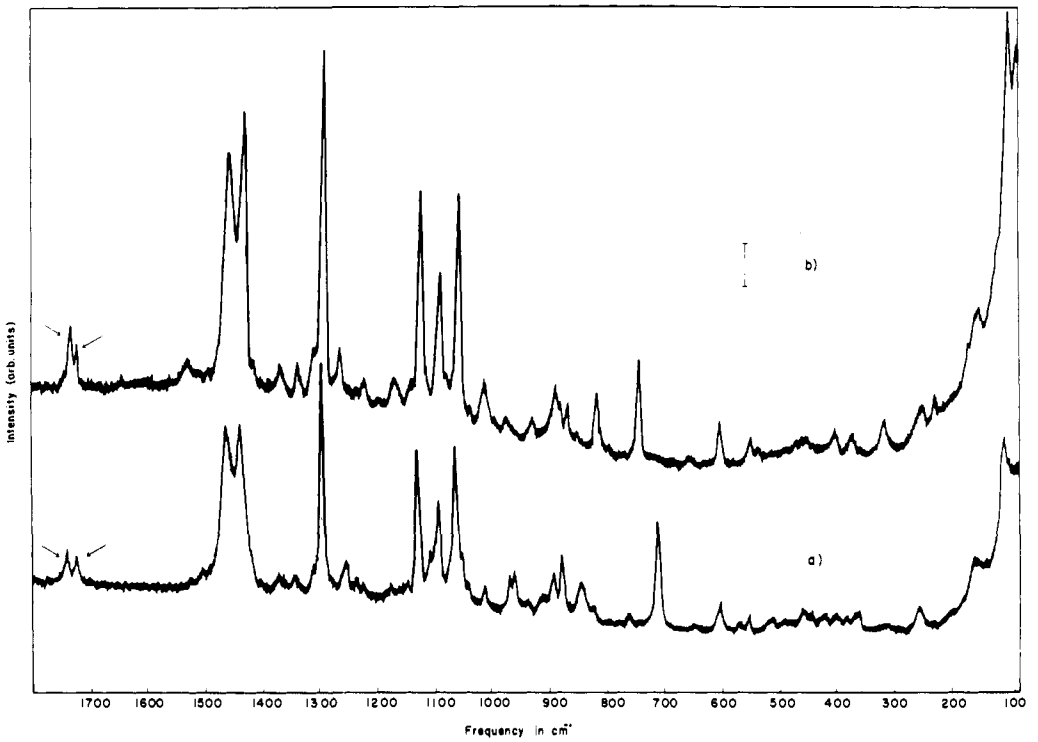

Figure 1. Raman spectra of anhydrous phospholipids. (a) Raman spectrum of anhydrous crystalline dipalmitoylphosphatidylcholine at 22 °C. (b) Raman spectrum of anhydrous crystalline dipalmitoylphosphatidylethanolamine at 22 °C. The carbonyl region for both spectra is marked by arrows. Instrumental parameters are described in the text.

proximately 100 °C under vacuum for 24 h. A sample having the melting behavior of DPPC monohydrate (or α_1 form DPPC) as discussed by Chapman et al.[3] was prepared by exposing a sample of anhydrous DPPC to an atmosphere of approximately 45% relative humidity for 10 h. The transition temperatures of the anhydrous ($T > 95$ °C) and the monohydrate ($T \simeq 65$ °C) DPPC were measured by Raman temperature studies of the side chain C–H stretch and skeletal C–C stretch regions, and the melting temperatures were found to agree with published values for these types of samples.[3] Samples were sealed in glass capillaries to study the Raman spectra. A thermocouple was attached to the capillary at the site of the incident laser beam, and the temperature was measured immediately prior to and after the recording of each scan while the laser beam was blocked to prevent the beam from striking the thermocouple. The temperature was found to remain constant (within 1 °C) at 22 °C during each scan. The spectrum of each sample was measured three times for comparison. Each spectrum was measured at the rate of 0.1 cm^{-1}/s with a pen period of 10 s. The band-pass was 4 cm^{-1}.

Results and Discussion

Carbonyl Stretch Doublet. A characteristic doublet appears in the Raman spectrum in the carbonyl stretching region of solid anhydrous DPPC and of solid anhydrous DPPE, as shown in Figure 1. The carbonyl stretch doublet has been observed previously[4-6] and it has been suggested that the doublet may reflect the different packing arrangements of the two C=O groups.[6] For solid DPPC, the bands are at 1741 and 1723 cm^{-1}, are approximately equal in intensity, and are separated by approximately 18 cm^{-1}. For solid DPPE, the higher wavenumber band appears at 1736 cm^{-1} and is about one and one-half as intense as the lower wavenumber band, at 1726 cm^{-1}, which is separated by approximately 10 cm^{-1}. The Raman scattering in the skeletal stretching region (shown in Figure 1) and also the C–H stretching region indicates that the hydrocarbon side chains themselves are in the rigid, planar backbone configuration.[4,7-12] Other spectral changes from one system to another that can be seen in Figure 1 are discussed elsewhere.[13] The Raman bands in the C=O stretch region were also measured for the cocrystallized DLPE–glacial acetic acid sample. Two bands of approximately equal intensity were observed at 1734 and 1720 cm^{-1}, separated by 14 cm^{-1}.

The most striking feature about the carbonyl stretching region is that there are *two* bands observed in these samples. There are, of course, two different carbonyl groups in the lipid molecule—one in the γ chain and one in the β chain. However, the two C=O bonds are chemically very similar and would not be expected to have frequencies that differ by 10 cm^{-1}.

It is possible that the two C=O bonds are located in the bilayer in different environments (for example, one in a strong and one in a weak hydrogen bonding site). Levin and Spiker have observed a single C=O stretch band in the spectrum of hydrated DPPC which shifts about 20 cm^{-1} to lower frequency when the lipid is prepared in a clay film.[14] They believe that this shift is due to environmental interactions. However, we are studying pure anhydrous lipid samples. We do not believe that the doublet is due to environmental interactions because the carbonyl bonds in crystalline DLPE, for example, do not appear from the X-ray diffraction studies[2] to be located in such different environments that one is more likely to be hydrogen bonded than is the other. In addition, hydration of the phospholipid causes most of the intensity of the lower frequency band at 1720 cm^{-1} to shift to the higher frequency band at 1737 cm^{-1}. The direction of this shift is opposite to that expected if the frequency shift were due to exposure of the carbonyl to a stronger hydrogen-bonding environment.[24]

(3) D. Chapman, R. M. Williams, and B. D. Ludbrooke, *Chem. Phys. Lipids*, **1**, 445 (1967).
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(11) R. C. Spiker, Jr., and I. W. Levin, *Biochim. Biophys. Acta*, **388**, 361 (1975).
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(13) E. B. Brown, K. G. Brown, and W. B. Person, to be submitted to *Biochim. Biophys. Acta*; E. B. Brown, K. G. Brown, and W. B. Person, to be submitted to *Chem. Phys. Lipids*.
(14) R. C. Spiker and I. W. Levin, *Biochim. Biophys. Acta*, **455**, 588 (1976).

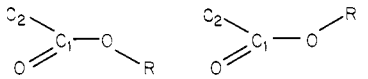
Another possible explanation for the appearance of two C=O bands was proposed by Abe and Krimm[15a] and by Moore and Krimm[15b] to explain the splitting of carbonyl bands in crystalline acid dimers and in polypeptides. They suggested that the C=O stretching mode in these molecules is split because of dipole–dipole interactions between adjacent, oriented, carbonyl bonds. Recently Bosi and Zerbi have demonstrated that this explanation requires postulated magnitudes for the vibrational transition dipole moments for the C=O stretch that are unreasonably large.[16] Use of reasonable values with this model predicts negligibly small splittings. Therefore, we believe that this explanation is unlikely for the doublet observed in the carbonyl region of the Raman spectra of these phospholipids, even if some arrangement of the phospholipid molecule can be found that orients the two C=O bonds so they are adjacent to each other.

The only other explanation we can think of to explain *two* carbonyl bands is the one we believe is correct, namely, that the two bands in the spectrum arise from differences in local geometry about the two phospholipid C=O bonds because each is associated with a chain with a different configuration about the carbonyl ester linkages. We believe that this suggestion is consistent with previous experimental work on similar systems. In particular Hayashi and Umemura have observed two C=O stretching bands due to different conformations in fatty acids.[17] They assigned them to coexisting cis and trans $C_3—C_2—C_1(=O)O(H)$ isomers resulting from migration of the hydroxyl protons between the two acids in the dimer,[17] as described in more detail below.

If the two bands in the carbonyl region are due to C=O stretching vibrations from C=O groups associated with different conformers, then the difference between the intensity ratios found in DPPC and in DPPE can be explained. Namely, the different relative intensities of the two bands for the two different samples (1.1 for DPPC and 1.5 for DPPE) are observed because different relative populations of the two configurations occur in the two samples.

We shall now examine different conformations that might exist around the acyl group to determine whether any of them can be expected to show differences in the C=O stretching frequency. Conformations about two possible bond sites must be considered for the conformation dependence of the C=O stretch frequency ($\nu_{C=O}$): (1) the conformation about the $C_1—O$ carbon–oxygen bond and (2) the conformation about the $C_2—C_1$ carbon–carbon bond. Figure 2 shows examples of different conformers about both bonds.

Insensitivity to Conformations about the C–O Carbon–Oxygen Bond. The $C_2—C_1(=O)OR$ group is believed to be held planar by the partial double bond character of the $C_1—O$ carbon–oxygen bond; therefore, conformations about the $C_1—O$ bond are just cis and trans isomers. Simple esters are found predominantly to exist in the trans (iii) conformation.[18] The cis (iv) isomer may



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sometimes coexist with the trans (iii) isomer in esters with bulkier substituents (e.g., *tert*-butyl formate, 2,2-diethylpropyl formate, and triphenylmethyl formate) which contribute steric interactions in the trans conformer, thereby destabilizing it.[18] However, only *one* C=O stretching mode is observed by Ōki and Nakanishi in the infrared spectra of several esters which are known to exist with both the cis and trans conformations present.[18]

A normal coordinate analysis of methyl propionate conformations[19] supports the experimental IR results of Ōki and Nakanishi,[18] since the calculated C=O frequencies for the cis and trans conformations about the C—O bond are identical. These

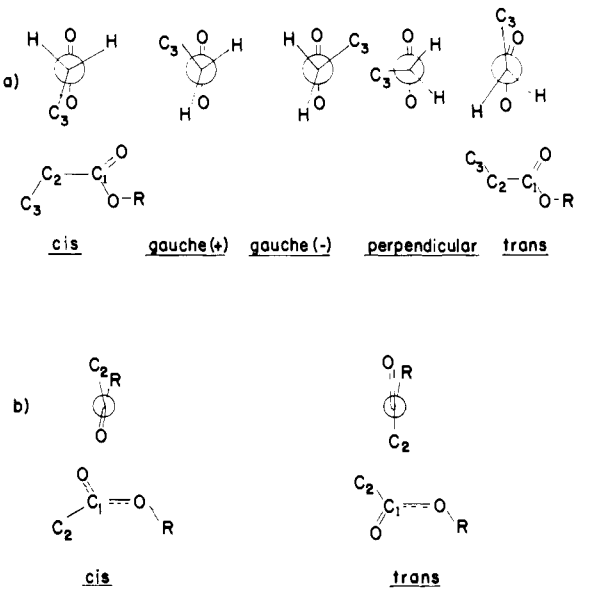
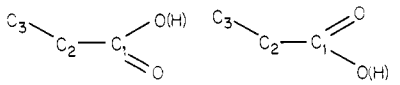


Figure 2. Definition of conformers in the acyl region. (a) Conformers are defined for the C_2–C_1 bond of the acyl linkage. The dihedral angle is defined by the C_2–C_3 bond and the C_1–O bond linked to the glycerol backbone (R). Rotamers are shown looking down the C_2–C_1 bond. Dihedral angles are incremented by clockwise rotations of the C_3–C_2 bond. (b) Cis and trans conformers are defined for the C_1–O bond linked to the glycerol backbone (R). The partial double bond character of the C_1–O bond is indicated by a dashed line.

calculations support the arguments by Ōki and Nakanishi that a single IR C=O peak observed for coexisting cis and trans isomers about the $C_1—O$ bond is not due to resolution difficulties and is consistent with mixed populations of conformers about the $C_1—O$ bond. We believe that the C=O vibrations from the ester linkages in the phospholipids will be similar to those studied by Ōki and Nakanishi,[18] so that the doublet observed in our Raman spectra is not due to coexisting cis and trans conformers about the $C_1—O$ bond, but instead to conformation differences about another bond.

Sensitivity to Conformations about the C_2–C_1 Carbon–Carbon Bond. Having argued that cis–trans isomerism about the $C_1—O$ bond is not expected to produce a shift in the C=O stretching frequency, we shall now consider the possible dependence of $\nu_{C=O}$ on conformation about the $C_2—C_1$ bond. In infrared polarization studies of well-oriented crystalline fatty acid layers, Hayashi and Umemura observed four carbonyl bands which were assigned to factor group splittings of two temperature-dependent bands: a band around 1700 cm^{-1} which was assigned to the cis (v) con-



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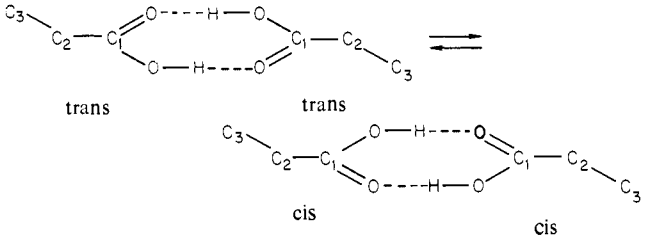
formation about the C_2–C_1 bond and a band around 1710 cm^{-1} which was assigned to trans (vi) conformation about the C_2–C_1 bond.[17] Unlike the phospholipids we are examining, the crystalline fatty acids (which exist there as dimers) could convert from the trans conformer to the cis conformer without rotation about the C_2–C_1 bond simply by exchanging hydroxyl protons:



trans ⇌ trans

cis cis

Thus, the *only* conformation change possible in these fatty acid

(15) (a) Y. Abe and S. Krimm, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2788 (1972); (b) W. H. Moore and S. Krimm, *ibid.*, **72**, 4933 (1975).
(16) P. Bosi and G. Zerbi, *Chem. Phys. Lett.*, **38**, 571 (1976).
(17) S. Hayashi and J. Umemura, *J. Chem. Phys.*, **63**, 1732 (1975).
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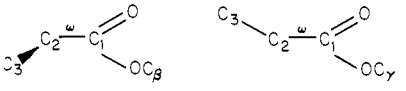
crystals is between trans and cis conformations about the C_2–C_1 bond. The difference in ν_{CO} (10 cm^{-1}) observed by Hayashi and Umemura for these two conformations[17] is comparable to the differences in the ν_{CO} which we observe in the phospholipid spectra presented in Figure 1.

In addition to this experimental evidence for the dependence of ν_{CO} on the conformation about the C_2–C_1 bond, our normal coordinate analysis[19] of the methyl propionate rotamers also shows that ν_{CO} is expected to depend on the rotation about the C_2–C_1 bond. The energy difference for the rotamers calculated in the normal coordinate treatment[19] is in good agreement with the 10–18-cm^{-1} separations observed for the C=O bands in the fatty acids[17] and also in our phospholipid spectra (Figure 1). By analogy with both these calculated results and the experimental results of Hayashi and Umemura, we assign the lower frequency C=O stretch observed in phospholipids to an approximately trans conformer and the higher frequency C=O stretch to an approximately cis or gauche conformer at the C_2—C_1 bond in the β and γ hydrocarbon chain acyl linkages in phospholipids. Since a cis conformer in phospholipids is highly unlikely for steric reasons and since the normal coordinate treatment[19] shows relatively little difference in C=O stretch frequency between a cis conformer and a gauche conformer, we believe that the lower frequency C=O stretch observed in phospholipids is due to a geometry more similar to a gauche conformer than a cis conformer.

Carbonyl Stretch Region and Acyl Geometry in Crystallized DLPE. It is probable that the two conformations about C_2–C_1 generally coexist in each molecule of the solid lipids. An X-ray diffraction study[1] of crystalline dilaurylphosphatidylethanolamine (DLPE) shows that the conformation in the ester group vii bonded



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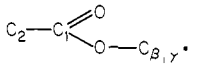
to the glycerol β carbon is nearly gauche (ω_β = 227°) about the C_2–C_1 bond, while the ester group bonded to the glycerol γ carbon is trans (viii) about the C_2–C_1 bond (ω_γ = 176°). This geometry has been called the "parallel chain–planes" arrangement.[1,21] (It should be noted that in both γ and β chains the conformation about the C_1–O bond remains trans (ix).) Thus, according to our



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premise, we expect to observe *two* different C=O stretches of *equal intensity* in the Raman spectrum of crystalline DLPE: a lower frequency band corresponding to the trans (ii) conformation at the acyl linkage of the γ hydrocarbon chain and a higher frequency band corresponding to the gauche (i) conformation at the β-chain acyl linkage.

To investigate this premise, we crystallized DLPE from glacial acetic acid (DLPE·HAc) and measured the Raman spectrum of this sample below its transition temperature. The C=O stretch region, shown in Figure 3a, has two bands at 1734 and 1720 cm^{-1}, which are of approximately equal intensities.

Melting of Crystalline DLPE. Raman spectra of the C=O stretch region for crystalline DLPE·HAc below and above the transition temperature are compared in Figure 3. Below the transition, two C=O stretches of equal intensity are observed in Figure 3a. Above the melting temperature, the higher frequency C=O stretch band is very strong while the lower frequency C=O stretch band is very weak, as seen in Figure 3b. This result indicates that in the liquid-crystalline form both acyl linkages of the DLPE side chains spend most of their time in the gauche (i) conformation. The strong gauche band is greatly broadened above the transition point, suggesting that there may be more rotational freedom about the C_2–C_1 bonds, although the average conformation about the C_2–C_1 bonds is predominantly gauche. Both these results, the increased intensity in the gauche conformer band and the rotational broadening in this band above the transition

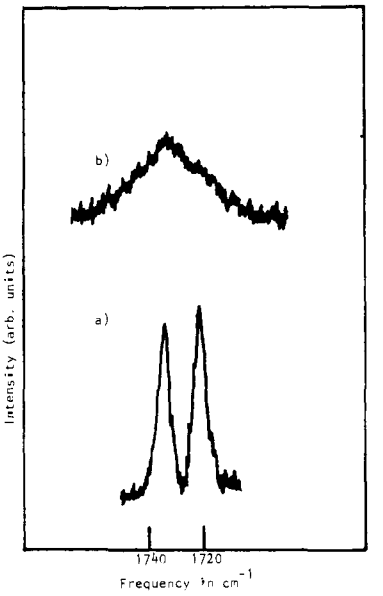


Figure 3. C=O stretch region Raman spectra of cocrystallized DLPE–glacial acetic acid: (a) solid; (b) melted.

temperature, are consistent with the melting behavior of phospholipids and therefore support our interpretation of the C=O doublet in phospholipids. These results show that the Raman spectrum C=O stretch region of phospholipids may be monitored to obtain information about the melting behavior in a specific region of the phospholipid molecule—namely, changes occurring about the C_2–C_1 bonds.

Relative Intensities of the C=O Stretches and Populations of Acyl Rotamers. We have observed that the relative intensities of the two carbonyl stretch bands can vary from sample to sample. The Raman spectrum in the C=O region has been used as an analytical tool in studies of polymers,[20] because the C=O stretch is relatively pure and its intensity seems to be a fairly accurate measure of the relative concentration of a carbonyl-containing moiety in a mixture. Hence, we expect that the observed variation in the relative intensity in the C=O region is an accurate measure of the changes in relative populations of the two conformers responsible for the two different bands. Since we observe, for example, that the two C=O stretches are of equal intensity in anhydrous DPPC (as they were also in crystalline DLPE), we propose that the two acyl linkages in the anhydrous molecule DPPC are like those of crystalline DLPE·HAc: that the β chain is approximately a gauche rotamer about the C_2–C_1 bond and that the γ chain is approximately a trans rotamer.

Table I summarizes some measurements of relative intensities of the two C=O stretches in phospholipid samples (DLPE, DPPC, and DPPE) prepared under various conditions. All Raman spectra were measured at temperatures *below* the melting transition of the samples, and all spectra show Raman C–H and C–C stretch regions typical for phospholipids with rigid, ordered side chains. In crystalline DLPE·HAc and anhydrous DPPC two C=O bands of equal intensity appear. Hence, we believe that in each of these samples the acyl linkages are found with the β chain in the gauche acyl rotamer and the γ chain in the trans acyl rotamer. In other samples, such as the anhydrous DPPE sample, the total intensity of the two C=O stretch bands measured relative to the 1300-cm^{-1} methylene bending mode is the same as for crystalline DLPE, but the relative intensities have changed. In the anhydrous DPPE sample, the higher frequency (gauche) band with relative intensity 1.2 is more intense than the lower frequency (trans) band with relative intensity 0.8.

This result suggests that 60% of the acyl linkages are gauche about the C_2–C_1 bond and 40% of the acyl linkages are trans about the C_2–C_1 bond. We believe that most of the DPPE molecules

(20) J. Štokr and B. Schneider, *J. Appl. Polym. Sci.*, **23**, 3553 (1979).

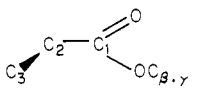
Table I. Relative Intensities in the Carbonyl Doublet Observed in the Raman Spectra of Phospholipids

sample	C=O stretch intensities[a] high frequency	C=O stretch intensities[a] low frequency	acyl linkage C_2–C_1 conformer % gauche	acyl linkage C_2–C_1 conformer % trans	% of the lipid molecules with these C_2–C_1 conformers (β chain acyl gauche) γ chain acyl trans	% of the lipid molecules with these C_2–C_1 conformers (β chain acyl gauche) γ chain acyl gauche
cryst DLPE[b]	1.0	1.0	50	50	100	0
anhydrous DPPC	1.0	1.0	50	50	100	0
DPPC monohydrate	~2	<0.2	>90	<10	<20	>80
anhydrous DPPE	1.2	0.8	60	40	80	20
DPPC in aqueous dispersion at room temp, gel form	~2	<0.2	>90	<10	<20	>80
DPPE in aqueous dispersion at room temp, gel form	~2	<0.2	>90	<10	<20	>80

[a] Each intensity was measured relative to the intensity of the 1300-cm^{-1} methylene bending mode in the same spectrum and normalized to the relative intensity of the anydrous DPPC higher frequency C=O stretch band. [b] Prepared as described in the text. DLPE = dilaurylphosphatidylethanolamine; DPPC = dipalmitoylphosphatidylcholine; DPPE = dipalmitoylphosphatidylethanolamine; HAc = glacial acetic acid.

are present with the β chain in the gauche acyl conformation and with the γ chain in the trans acyl conformation. However, in some DPPE molecules in this sample a further rotation about the γ chain C_2–C_1 bond has occurred so that for these molecules β chain and γ chain linkages are gauche about the C_2–C_1 bond. If 80% of the DPPE molecules have the β chain in the gauche acyl conformation and the γ chain in the trans acyl conformation while 20% of the DPPE molecules have both chains in the gauche acyl conformation, then we would expect to see the relative C=O stretch intensities occur in the relative intensity ratio observed in the Raman spectrum of anhydrous DPPE. We have noted that other anhydrous samples of DPPE have nearly equal C=O intensities, which suggests that the populations of the two geometries may depend upon the method of preparation of the sample.

The Raman C=O stretch region of the gel form aqueous dispersions (80% w/w lipid–water) of DPPC and DPPE show that nearly all of the C=O intensity lies in the higher frequency band (see Table I). If the doublet in anhydrous DPPC were indeed due to differences in the environment, the addition of water to the interface would be expected to increase the cross section of the molecule and consequently increase the exposure of the oxygens of the β chain to the polar region[22] and would therefore cause the C=O stretch frequency of one band to decrease. However, the opposite result is observed. Most of the intensity of the lower frequency band is shifted to the higher frequency band. This result is consistent with and supports our interpretation of the frequency dependence of the C=O region. This result indicates that the complete hydration of the lipid causes the γ chain to rotate about the C_2–C_1 bond so that both linkages are in the gauche (x)



x

conformation. Presumably the remainder of the C–C bonds in the side chains are still in the planar all-trans C–C backbone configuration.

C=O Stretch Intensities and Lipid Chain Packing. The X-ray diffraction study of crystalline DLPE[1] shows that after the bend in the β chain the plane of the β chain carbon–carbon backbone is almost parallel to the plane of the γ chain carbon–carbon backbone. In this configuration, the β chain acyl linkage is a gauche (vii) conformer about C_2–C_1 and the γ chain acyl linkage is a trans (viii) conformer; the Raman spectrum shows a C=O stretch doublet with equal intensities. Rotation about the γ chain C_2–C_1 bond from a trans to a gauche conformer, which is our interpretation of the change in the Raman spectrum in the C=O region from a two-band to a one-band spectrum, would be expected also to rotate the plane of the γ chain carbon–carbon backbone about its longitudinal axis, leading to a tilted arrangement of the two chain planes with respect to each other (see Figure 4). This "tilted chain-planes" packing is thought to be energetically favored over the parallel chain-planes packing observed in crystalline DLPE. McAlister, Yathindra, and Sundaralingam[21] calculated

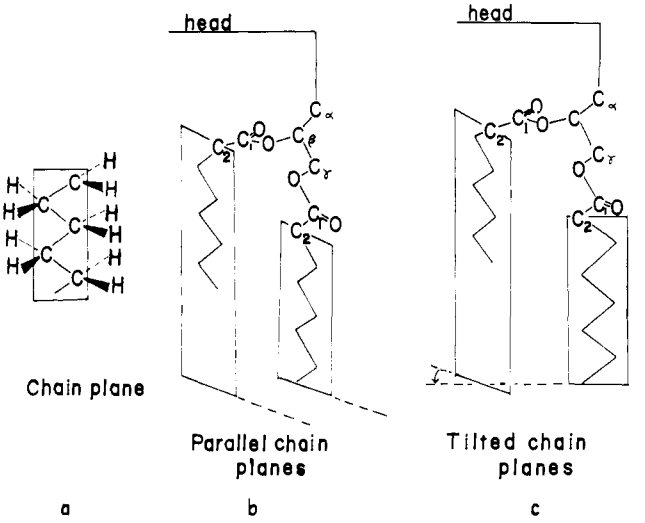


Figure 4. Packing arrangements of hydrocarbon side chains. (a) The chain-plane is defined as the backbone of the carbon–carbon bonds in the all-trans configuration. (b) The parallel chain-planes arrangement observed in cocrystallized DLPE·HAc (ref 1). In this arrangement, the conformation about the γ chain C_2–C_1 bond is approximately trans as defined in Figure 2a and the conformation about the β chain C_2–C_1 bond is approximately gauche. (c) The tilted chain-planes arrangement which we believe is assumed when the γ chain rotates about the C_2–C_1 bond from a trans to an approximately gauche conformer.

two minimum-energy conformations for rigid phospholipids, determined mainly by the hydrophobic interactions of the hydrocarbon chain methylene groups. In both conformations the hydrocarbon chain-planes are "tilted", in order to optimize interchain interactions. The dihedral angle between the planes is 72° in the lower energy conformation. Thus, the change from the parallel chain-planes configuration, with two carbonyl stretching bands (trans and gauche), to a tilted chain-planes configuration, with only one strong carbonyl stretching band (gauche), would optimize interchain interactions according to Sundaralingam's[21] calculations.

Thus, the change in the Raman spectrum from two C=O stretch bands to one doubly intense C=O band on changing from anhydrous DPPC or DPPE to the aqueous dispersion below the transition temperature suggests the possibility that the hydrocarbon chains have changed relative orientation from the "parallel chain-planes" to the energetically favored "tilted chain-planes arrangement". This change might be expected to occur since hydration of head groups may reduce the favorable energetic interactions in the head group, allowing (or requiring) the more favorable interactions of the "tilted chain-planes" packing to control the phospholipid conformation.

(21) J. McAlister, N. Yathindra, and M. Sundaralingam, *Biochemistry*, **12**, 1189 (1973).
(22) R. H. Pearson and I. Pascher, *Nature (London)*, **281**, 499 (1979).

Table II. Bandwidths of the Carbonyl Doublet Observed in the Raman Spectra of Phospholipids

sample	frequency, cm^{-1}	bandwidth, cm^{-1}
anhydrous DPPC	1741[a]	12
	1723[b]	12
DPPC, aqueous dispersion[c]	1737[a]	30
anhydrous DPPE	1736[a]	11
	1726[b]	12
DPPE, aqueous dispersion[c]	1737[a]	30
cocrystallized DLPE-HAc solid	1734[a]	11
	1720[b]	13
melted[c]	1734[a]	28

[a] Gauche conformer i Raman band. [b] Trans conformer ii Raman band. [c] Weak intensity in the lower frequency band precluded measurement of the bandwidth. Abbreviations are defined in Table I.

Raman C=O Bandwidths and Motional Freedom. The Raman C=O bandwidths (full width at half peak height) vary considerable for different phospholipid states, as shown in Table II. Sensitivity of the C=O stretch frequency to conformation in the acyl region would be expected to lead to broadening in the Raman C=O stretch band belonging to a particular conformer as the motional freedom in that conformer increases. Because we believe that we have shown that the C=O stretch frequency is sensitive to the C_2—C_1 bond dihedral angle, we expect that, as freedom of motion about the C_2—C_1 bond increases for a particular acyl conformer, the Raman C=O band for that conformer will broaden.

The anhydrous crystalline samples (DPPC, DPPE, and DLPE·HAc) show relatively narrow Raman carbonyl bandwidths ($\sim$12 cm^{-1}) for each band in the carbonyl doublet. We believe that the bandwidths are narrow because the C_2–C_1 bond dihedral angles are distributed within a narrow range of values for each of the two acyl linkage conformers present.

In the Raman spectra of aqueous dispersions of DPPC and DPPE below the transition temperature, the one strong C=O band (the higher frequency gauche conformer C=O stretch) is considerably broader (30 cm^{-1}) than the C=O bands in the anhydrous phospholipid spectra. We believe that this result is due to the greater freedom of motion about the C_2–C_1 bonds in the hydrated phospholipid, leading to a greater distribution of rotamers about the most probable conformer.

The bandwidth of the strong gauche conformer C=O stretch of a melted DLPE·HAc sample is considerably broader than that of the crystalline sample. Again, we believe that this band broadening occurs because the distribution of conformers around an approximately gauche conformer broadens.

The apparent sensitivity of the Raman C=O stretch bands to motional freedom or population distribution around a particular acyl conformer suggests that Raman measurements of C=O stretch bandwidths may provide information about freedom of motion in the phospholipid acyl region.

Summary

We have related evidence which leads us to believe that the phospholipid acyl C=O stretch frequency depends on acyl geometry (specifically the rotation about the C_2—C_1 bond) and that the relative intensities of the two Raman C=O stretch bands observed in the Raman spectra of various phospholipid samples may be used to estimate the relative populations of two types of acyl linkage conformers: (1) a conformer which is approximately trans about the C_2—C_1 acyl bond for which the Raman C=O stretch appears at about 1720 cm^{-1} and (2) a conformer which is approximately gauche about the C_2—C_1 bond, for which the Raman C=O stretch appears at about 1740 cm^{-1}. We conclude that the presence of a doublet in the Raman C=O region indicates that the β-chain acyl linkage is in the approximately gauche conformation vii and produces the higher frequency Raman C=O band, while the γ-chain acyl linkage is in the approximately trans conformation viii and produces the lower frequency C=O band.

An increase in intensity in the higher frequency C=O stretch band accompanied by a decrease in intensity in the lower frequency C=O stretch band suggests that rotation about the C_2—C_1 band in the γ chain has occurred in a fraction of the lipid molecules. We observe this intensity redistribution occurring (1) upon melting of anhydrous lipids, (2) upon dispersion of lipids in aqueous media below the transition temperature, and (3) upon partial hydration of crystalline DPPC. The direction of this intensity shift (1720 $\rightarrow$ 1737 cm^{-1}) is opposite that expected for exposure of C=O groups to water if the doublet were due to differences in hydrogen bonding[24] or polarity of the environment, and suggests that indeed a conformation change causes the C=O stretch shift. For samples in which the side chains are in the rigid gel form, we propose that rotation about the γ chain C_2—C_1 bond is probably a mechanism by which rotation of the γ chain backbone plane about its longitudinal axis can occur. This rotation would bring the two chain planes of a lipid molecule from a packing array in which the two chain planes are parallel with respect to each other to an array in which the two chain planes are tilted with respect to each other while remaining perpendicular to the plane of the bilayer.

The Raman C=O stretch bandwidths are sensitive to the state of the phospholipid sample. It appears that freedom of motion in the acyl region causes considerable broadening in the Raman C=O stretch bands. Broadening in the Raman C=O stretch band of phospholipids was observed upon melting of crystalline DLPE·HAc and upon hydration of DPPC and DPPE.

Thus, the C=O stretch affords a valuable means of monitoring (1) conformation about the acyl linkage C_2—C_1 bond of each phospholipid side chain, (2) hydrocarbon chain packing arrangements in the gel lipid state, and (3) freedom of motion about the C_2—C_1 bond from band broadening. (We have learned that Levin[23] has a different interpretation of the C=O stretch doublet.)

Acknowledgments. We are grateful for support from General Research Support Grant RR-07051, for partial support from National Science Foundation Grant CHE74-21471, and for National Science Foundation Grant NSF GP-42692 for support in purchasing the laser Raman spectrometer at the University of Florida.

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