Conformation of the Acyl Chains in Diacylphospholipid Gels by IR Spectroscopy

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An IR study of the conformational disorder in the part of the hydrocarbon chain nearest the ester group in gel-phase lipid bilayers is presented. The infrared method for determining the conformation of the CH_2-CH_2 bond attached to the ester group is discussed and applied to a number of representative gel-phase 1,2-diacylphosphatidylcholine fully hydrated dispersions, including symmetric and mixed-chain PCs in their gel and subgel phases. The effect on chain conformation of the headgroups PC, PE, and PA^- was explored. Only two conformations of the alkyl chains were found: a planar (all-trans) chain and a nonplanar chain that is all-trans except for the CH_2-CH_2 bond nearest the ester group. Concentrations of the two conformers were also determined. The overall concentration of the planar form always exceeds 50% but varies significantly depending on the lipid involved. In two cases, the concentration of the planar conformer was estimated for both the SN1 and SN2 chains.

I. Introduction

Studies on the structure of model lipid bilayers, especially the benchmark diacylphosphatidylcholine (PC) bilayer, have provided invaluable insight into the structural elements of natural biomembranes. The fully hydrated PC dispersions exist in a number of phases. The primary phases, in order of increasing disorder, are the subgel, gel, ripple, and liquid-crystal (fluid). Disorder, especially conformational disorder, is ubiquitous among all phases and plays a prominent role in the dynamics of the chains. The characterization of this disorder remains a current challenge. Of the various techniques available for studying conformational disorder in the hydrocarbon chains, vibrational spectroscopy is the most direct.

This paper focuses on conformational disorder in the gel phase, which, along with the subgel phase, is the most highly ordered phase. In this study, we have employed infrared (IR) spectroscopy, because it is especially effective for determining a low concentration of disorder in an otherwise ordered system. We are interested in establishing the conformation of the CH_2-CH_2 bonds in the alkyl portion of the acyl chain in the region nearest the ester group for fully hydrated 1,2-diacylphospholipids in the gel phase. X-ray diffraction studies on single-crystal lipids have shown that the conformations of the CH_2-CH_2 bonds of the alkyl chains are in general either all-trans or else all-trans except for the CH_2-CH_2 bond adjoining the ester group. This bond is gauche. A typical structure, shown in Figure 1, is the B form of crystalline di-C_{14}PC.[1] For this structure, the dihedral angle associated with the first CH_2-CH_2 bond (designed as τ_4) is trans (t) for the SN1 chain and gauche (g) for SN2 chain. The tg combination (that is, $t_{SN1}g_{SN2}$) occurs in many lipid crystals. However, the tt and gt combinations also occur, as may be seen in Table 1, which lists the values of τ_4 for the SN1 and SN2 chains for a number of relevant phospholipids.[2] The dihedral angles τ_2 and τ_3 are also listed. Unlike τ_4, the latter angles, which define the conformation of the ester group, do not significantly affect the frequencies of the bands employed in our analysis.

Earlier studies have shown that nearly all the CH_2-CH_2 bonds in the alkyl chains in the gel phase are trans. The gauche bonds tend to be concentrated near the ends of the alkyl chains. The concentration and distribution of the gauche bonds nearest the methyl group as determined by IR methods[3] are in keeping with recent results obtained from MD modeling.[4] Of the few studies on the conformation at the ester end of the chain, perhaps the most relevant is the neutron diffraction measurements reported by Zaccai et al.[5] on hydrated di-C_{16}PC. Two samples were used in their study: in one, the methylene group nearest the ester group of the SN1 chain was deuterated; in the second, the corresponding methylene in the SN2 chain was deuterated. The

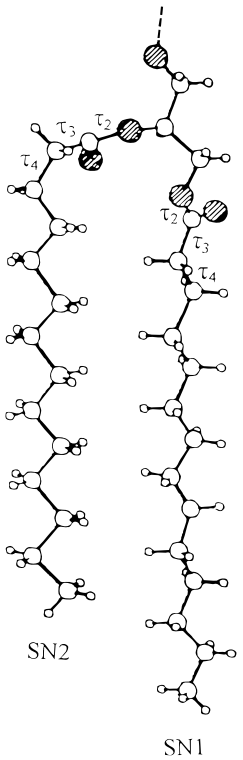

Figure 1. X-ray determined structure of the crystalline phase of di-C_{14}PC dihydrate.[1] The dihedral angles are numbered as indicated. The first CH_2-CH_2 bond of the SN2 chain is gauche.

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TABLE 1: Skeletal Dihedral Angles for the Ester-Group Region of the Acyl Chains in Representative Membrane Lipids As Determined by X-ray Single Crystal Analyses

		dihedral angles[a]					
		SN1			SN2		
acyl chain	headgroup	τ_2	τ_3	τ_4	τ_2	τ_3	τ_4
di-C_{12}	PA^-	178	−77	70 (g)	174	168	173 (t)
	PE	173	179	−171 (t)	179	−179	65 (g)
	PEM_1	−167	−170	−172 (t)	178	−67	180 (t)
	PEM_2	−167	166	175 (t)	173	−57	176 (t)
di-C_{14}	PC (1)[b]	168	−173	178 (t)	172	−81	45 (g)
	PC (2)	176	180	180 (t)	179	−134	67 (g)
	PA	180	−119	73 (g)	172	164	179 (t)

[a] The values in this table are from ref 2. The locations of the dihedral angles are defined in Figure 1. [b] The numbers 1 and 2 refer to the two conformationally unique molecules that make up crystal structure.

authors found the first methylene of the SN2 chain to be about 1.8 Å closer to the bilayer surface than the corresponding methylene in the SN1 chain, which is in keeping with the single-crystal X-ray structure determined for the dihydrate of di-C_{14}-PC.[1] However, the aqueous lipid dispersion they used contained only about 6% water by weight, so that each hydrated lipid molecule is associated with 2−3 water molecules. This water content is much closer to that of the dihydrate di-C_{14}PC crystal than to the fully hydrated gel, in which there are about 10 water molecules per lipid,[6] and may account for the fact that the structure found in the neutron scattering study is similar to that found for the dihydrate di-C_{14}PC by X-ray diffraction.

II. Materials and Procedures

Materials. *Phospholipids*. These were obtained from Avanti Polar Lipids, Inc., and each had a stated purity exceeding 99%. These lipids together with the abbreviated names used for them here follow: The symmetric phosphatidylcholines, 1,2-diacyl-*sn*-glycero-3-phosphocholine (di-C_nPC, for $n_{even} = 12-24$); two mixed-chain phosphatidylcholines, 1-acyl-2-acyl-*sn*-glycero-3-phosphocholines (1,2-C_mC_nPC, for m, $n = 18$, 14 and 14, 18); two perdeuterated dimyristoylphosphatidylcholines, one with a perdeuterated SN1 acyl chain, 1-myristoyl-d_{27}-2-myristoyl-*sn*-glycero-3-phosphocholine (1,2-C_{14}^D C_{14}^HPC), and another with a perdeuterated SN2 chain 1-myristoyl-2-myristoyl-d_{27}-*sn*-glycero-3-phosphocholine (denoted as 1,2-C_{14}^H C_{14}^DPC), 1,2-diacyl-*sn*-glycero-3-phosphatidylethanolamines (di-C_nPE, for $n = 14$, 18, and 20), and monosodium 1,2-diacyl-*sn*-glycero-3-phosphates (di-C_nPA, for $n = 14$, 18, and 20).

Fatty Acid Methyl Esters. $CH_3(CH_2)_{n-2}CO(O)CH_3$ (C_n-ME, for $n_{even} = 12-24$) were purchased from Aldrich Chemical Co. The stated purities were ≥98%.

Triglycerols. Three monoacid 1,2,3-triglycerols, trilaurin (tri-C_{12}G), trimyristin (tri-C_{14}G), and tripalmitin (tri-C_{16}G), were obtained from Sigma Chemical Co. The stated purities were listed as approximately 99%.

Experimental Procedures. The dispersions were prepared by first dissolving the lipid powder in a $CHCl_3$/MeOH solvent, and then drying under a stream of N_2 and then under vacuum to form films. These were hydrated by adding highly deionized H_2O (the H_2O-to-lipid weight ratio is 2:1). Complete dispersion was achieved using a vortex mixer with the sample temperatures above the gel-to-liquid transition temperature (T_m). To attain equilibrium, the dispersion cycle was repeated at least five times.

Samples for IR measurement were prepared by sandwiching the aqueous dispersions between two CaF_2 windows separated by a 6 μm Teflon spacer. The sandwich was held in a brass block sample-holder placed inside a thermostated chamber. The temperature was controlled to within ±0.1 °C with a Neslab refrigerated circulation bath. The lowest temperature attainable with this arrangement was about −20 °C. Temperatures as low as −40 °C were reached using a Thermal-Electric Cooler (TEC) in series with the circulation bath.

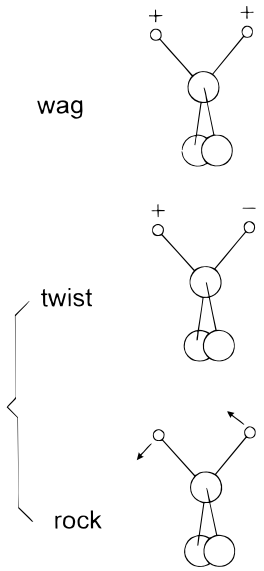

Figure 2. Wagging, twisting, and rocking motions of a methylene group.

Thin films of the fatty-acid methyl esters for IR measurements were prepared by melting the sample between KBr windows and then cooling slowly. The spectra were measured at a resolution of 1 cm^{-1} with a Nicolet Magna 550 FTIR spectrometer equipped with an MCT/B detector.

III. IR Method for Determining Alkyl Chain Conformation

The method used depends on the variation of frequencies of the methylene wagging and twisting/rocking IR bands with chain conformation. This dependence, which we discuss below, makes it possible to distinguish between planar (all-trans) and nonplanar chains. First, we will consider the wagging bands. Such bands appear in the 1370−1170 cm^{-1} region of the IR spectra of systems having all-trans polymethylene chains of a given length. The wagging motion for a single methylene group is depicted in Figure 2. Because the wagging of each methylene is highly coupled with its nearest neighbor methylenes, all the methylenes are equally involved in the wagging modes. The result is a progression of regularly spaced modes, whose number equals the number of methylenes, found between 1370 and 1170 cm^{-1}. Each mode of the progression corresponds to a standing wave that extends over the alkyl part of the chains.[7-9] The wagging progression is clearly evident in the IR spectra of the PC gels.

The intensities of the wagging bands vary greatly between systems. In the Raman spectrum, their intensity is always small. However, in the IR, their intensities can be relatively large depending on the end groups. Although the intensities are not completely understood, in general they increase with the polarity of the end groups: for example, the wagging bands for all-trans *n*-alkanes are much weaker than those for the alkyl methyl ester of the same chain length. Our normal coordinate calculations on the crystalline methyl palmitate ester reveal a strong correlation between the relative intensities of the wagging bands and the relative contribution to their normal coordinates of the

wagging of the methylene nearest the ester group. This indicates that the intensity enhancement originates from this methylene and that the electronic nature of this methylene and thus its dipole derivative depends on the group at the end of the chain.

The one other progression of importance to us is the methylene "twisting" progression. This is made up of bands associated with modes that involve both methylene twisting and rocking in a ratio depending on the particular band.[8] (Methylene twisting and rocking motions are illustrated in Figure 2.) The lowest frequency band in the twisting progression is nearly pure methylene rocking, whereas the highest frequency band is nearly pure methylene twisting. Bands at intermediate frequencies are associated with a mixture of both rocking and twisting motions.[7,8] The bands of the twisting progression appear at frequencies between 1300 and 1160 cm^{-1}, and therefore overlap the wagging bands, which occur in the 1360−1170 cm^{-1} frequency region. The band spacing is about the same for the two progressions, which adds to the difficulty of separating and assigning the bands.

In going from a planar to a nonplanar chain, the frequencies of the wagging and twisting are shifted in a systematic way provided the number of gauche bonds is very small, say 1 or 2, so that the pattern of the wagging and twisting progressions is still recognizable. The frequencies and relative intensities of the bands undergo modest changes. It is important to note that large changes of the dihedral angles τ_2 and τ_3, which are associated with the ester group (Figure 1), do not much affect the twisting and rocking bands. This is observed in the spectra and is supported by our normal coordinate calculations.

To round out this discussion, we note that, if the concentration of gauche bonds is high, such as in a liquid *n*-alkane, the IR spectra show the bands that represent short sequences of C−C bonds associated with specific non-all-trans conformations such a gg bond pair or gtg′ "kink" sequence.[10] These bands, which have characteristic frequencies, can be used to measure the concentration of these sequences.

IV. Band Assignments, Conformation, and Conformer Concentration: The Symmetric PC Gels

A. Assignments and Conformation. Figure 3 shows the IR spectra in the 1400−1150 cm^{-1} region for the fully hydrated gels of a series of diacylphosphatidylcholines di-C_nPC, n_{even} = 12−24. The sample temperatures for n_{even} = 12−16 and n_{even} = 18−24 were −40 and −20 °C, respectively. The subcell packing for the n_{even} = 12−18 gels is that of the usual distorted orthorhombic ($L_{\beta'}$) phase, but for n_{even} = 20−24, the packing is that of the more highly ordered *n*-alkane-like orthorhombic perpendicular phase, described in refs 11 and 12. The measured PC spectra all show an intense band near 1230 cm^{-1}, which is due to the antisymmetric P−O stretching mode of the PO_4^- group. This band has been removed from the spectra shown in Figure 3 by subtracting the fluid phase spectra, which shows essentially only the PO_4^- band, from the gel phase spectra.[13] The shape and frequency of this band are nearly independent of temperature and chain length. Removing the PO_4^- band, however, results in some increased uncertainty as to the location of the spectral baseline.

Most of the bands in Figure 3 represent all-trans alkyl chains. These bands and the type of chains that give rise to them are assigned the letter A. The remaining bands are marked in the figure and represent nonplanar chains. The nonplanar chains and their bands are designated as B. The wagging bands are numbered sequentially (k = 1, 2, 3, ...) beginning with the lowest-frequency band. For the simplest possible chain imaginable, identical harmonic oscillators with nearest-neighbor coupling, the integer k has a well-defined meaning, and it indicates the number of antinodes in the standing wave associated with each vibration. This description is still approximately valid for more complex, ordered chains.[7,8]

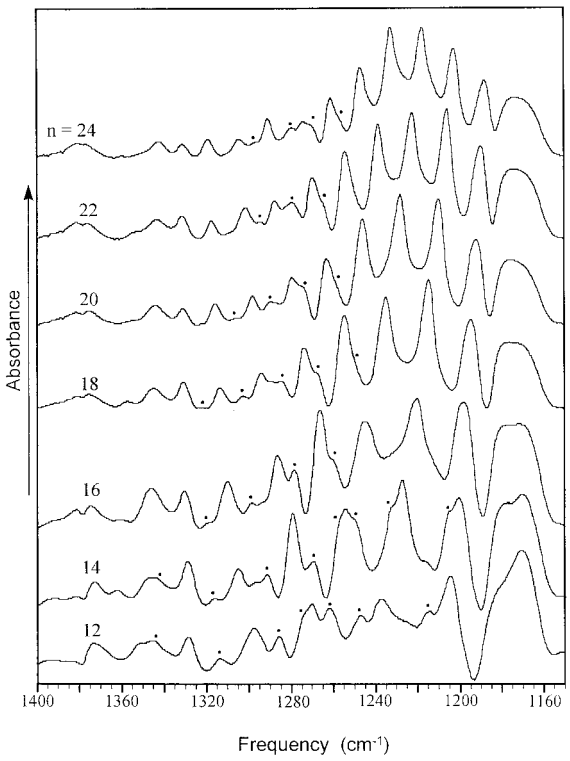

Figure 3. IR spectra of di-C_nPC for n_{even} = 12−24. The sample temperatures for n_{even} = 12−16 are −40 °C and for n_{even} = 18−24 are −20 °C. The intense phosphate band near 1230 cm^{-1} has been removed from the measured spectra (see text). The bands associated with B chains are marked. The detailed assignments are indicated in Figure 5.

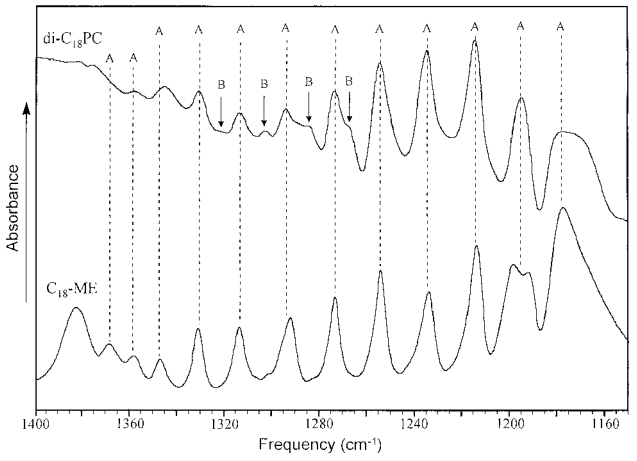

Figure 4. IR spectra of the di-C_{18}PC gel and crystalline methyl stearate (C_{18}-ME) at −20 °C. The bands representing methylene wagging modes of the planar (all-trans) and nonplanar chains are marked A and B, respectively.

For the PC gels, the wagging bands of type A (all-trans, planar chains) are relatively easy to distinguish from B type bands because the intensities of the A bands are generally greater and their frequencies are very near those of the corresponding crystalline fatty-acid methyl esters, whose chains are planar.[14−16] It is apparent in Figure 4 that the bands in the spectrum of di-C_{18}PC, which have been tentatively assigned to A chains, have

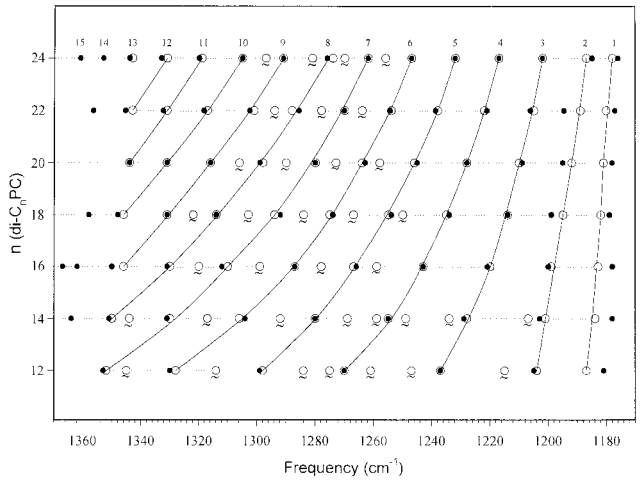

Figure 5. IR band frequencies for the gels of di-C_nPC (○) and for the corresponding crystalline fatty acid methyl esters (●). Bands of the planar (A) chains assigned the same *k* are connected. The underlined bands belong to nonplanar (B) chains.

frequencies that correspond to those of crystalline C_{18}-ME, which are known to be A bands. The assignments of the A bands in the PC spectrum are therefore confirmed. Nearly all the bands in the di-C_{18}PC spectrum not associated with A chains represent B chains.

The frequencies observed for the PC even-numbered gels and the fatty-acid methyl esters are displayed together in Figure 5. The assignments (the values of *k*) of the A bands are shown. The frequency difference between the corresponding bands for the PC gels and the esters are generally less than 3 cm^{-1}, the $k = 1$ and 2 bands excepted. The B bands, which are observed only for the gels, are underlined in Figure 5.

To establish the conformation of the chains that give rise to B bands in the spectra of the PC gels, and, in addition, to assign other IR bands in the 1360−1170 cm^{-1} region, we have relied heavily on the vibrational analysis reported by Yano et al.[17] for the crystalline triglycerols, tri-C_{14}G (trimyristin) and tri-C_{16}G (tripalmitin). From X-ray crystal structures determined from shorter homologues, as will be discussed below, it can be safely assumed that two of the three chains in the triglycerols are all-trans, that is, are A chains. The conformation of the third chain is all-trans except for a gauche CH_2-CH_2 bond nearest the ester group.

Nearly all of the bands in the 1360−1170 cm^{-1} region of the spectra of the di-C_{14}PC and di-C_{16}PC gels coincide with bands in the spectra of the crystalline triglycerols with corresponding chain lengths, and thus the B chains in the PC gels indeed have the conformation of the nonplanar chain in the triglycerols.

The band assignments for both the wagging and twisting bands of the PCs follow those made by Yano et al.[17] on the basis of their extensive IR polarization measurements on single crystals of the triglyerols. These assignments are listed in Table 2 and Table 3. As in the case of the wagging bands, there are two sets of twisting bands, one (designated A′) associated with the planar chain and the other (designated B′) with the nonplanar chain.

The wagging (B) and twisting (B′) bands associated with the nonplanar chains in the gel-phase PCs are regularly spaced and form a pattern similar to that of the wagging and twisting bands of the planar chains. This is seen in Figure 6. We note that there are many more B bands than B′ bands.

The progressions associated with the A, B, A′, and B′ bands are depicted separately in Figure 7. These are for crystalline tri-C_{14}G and tri-C_{16}G using the assignments proposed by Yano et al.[17] Figure 7 suggests that for both the wagging and twisting bands the effect on introducing a gauche bond at the ester end of the all-trans chain is not so much an increase in the spacing of the bands in the progression—as might be expected if the gauche bond in effect reduced the chain length—but rather a shift of the entire progression to higher frequencies. This shift is probably closely related to the "phase shift" that results from changing the end group of the all-trans chain, a topic discussed in refs 18 and 19.

B. Concentrations of the A and B Chains. The molar concentrations of A and B chains for the PC gels can be estimated from the integrated intensity ratios of selected A bands and B bands. To estimate the overall concentration of A type chains for the PC gels, we used the relation:

$$X_A/X_B = \kappa\left[\sum_{i=1}^{m_i} I_{A,i} \Big/ \sum_{j=1}^{m_j} I_{B,j}\right] \qquad (1)$$

The intensity summations involve a set of selected A and B bands. The proportionality constant κ, relating the intensity ratio to molar concentration ratio, is estimated from the spectrum of crystalline tri-C_{14}G whose X_A to X_B ratio is known. Then a set of A and B bands selected from the spectrum of the PC gel is used to evaluate the X_A/X_B ratio for the PC gel.

However, there is a complication stemming from the fact that crystalline tri-C_{14}G has two phases, β and β', that differ in the packing of the alkyl chains.[20−22] A satisfactory X-ray structure determination of the β' phase is not available.[20] However, infrared spectra show that each phase consists of just A and B chains. However, the relative intensities of the B and A bands of each phase are quite different. The intensity ratio B to A is much smaller for the β' phase and is, in fact, very near that observed for the PC gels. This may be seen in Figure 8, which shows the spectra of tri-C_{14}G in the β and β' phase and di-C_{14}-PC in the gel phase.

The remainder of this section concerns choosing a proper intensity standard. Part of the problem is that neither the structure of the β' nor β phase of tri-C_{14}G has been determined by X-ray diffraction. The spectra show, however, that the β phase of tri-C_{14}G has the same structure as that of the β phase of tri-C_{10}G[23] and tri-C_{12}G,[24] which have been determined. After taking into account the chain length difference, it was found that the spectra of the β phases of tri-C_nG with $n = 12$, 14, and 16 are nearly the same (Figure 9), indicating these crystalline triglycerols are isostructural.

Chain packing for the PC gels is much more like that of the β' phase than the β phase of the triglycerols. The acyl chains for both the gels and the β' phase of the triglycerols pack in an orthorhombic perpendicular subcell containing two chains.[20−22] This is evident in the shapes of the methylene scissor bands for the PC gels and β' triglycerols, which show a splitting characteristic of orthorhombic packing. No splitting is observed for the β phase of the triglycerols, which indicates triclinic chain packing.[25]

The differences in the intensity ratios of B to A chains for the β and β' phases are most likely attributable to differences in the dihedral angles τ_2 or τ_3, more likely the latter (Figure 1). Our normal coordinate calculations show that the wagging and twisting band frequencies are nearly independent of τ_2 and τ_3. This predication is consistent with the fact that the A and B band frequencies for the β and β' phases of tri-C_{14}G do not change in going from one phase to the other. On the other hand,

TABLE 2: Observed Frequencies and Assignments of the IR Bands in the 1170−1370 cm^{-1} Region of the Spectra of the Crystalline Fatty-Acid Methyl Esters (C_{14}-ME, and C_{16}-ME), the Crystalline Triglycerols (Tri-C_{14}G and Tri-C_{16}G), and Aqueous Dispersions of Di-C_{14}PC and Di-C_{16}PC in the Gel Phase

	band frequencies (cm^{-1})[a,b]							
k	A_k			B_k		A'_k	B'_k	
	C_{14}-ME	tri-C_{14}G	di-C_{14}PC	tri-C_{14}G	di-C_{14}PC	tri-C_{14}G	tri-C_{14}G	di-C_{14}PC
1	1178	1182	1184	1199		1174	1185	
2	1203	1202	1200	1228			1210	1207
3	1229	1228	1228	1255	1249	1225	1237	1234
4	1255	1257	1255	1276	1269	1246	1260	1259
5	1280	1280	1280	1296	1292	1273	1280	
6	1304	1307	1306	1319	1317		1290	
7	1331	1331	1330	1339	1344			
8	1351	1351	1350					
	C_{16}-ME	tri-C_{16}G	di-C_{16}PC	tri-C_{16}G	di-C_{16}PC	tri-C_{16}G	tri-C_{16}G	
1	1178	1181	1183	1197		1174	1186	
2	1197[c]	1200	1199	1217			1205	
3	1221	1220	1220	1246		1213	1228	
4	1243		1243	1262	1259	1239	1249	
5	1266	1269	1267	1282	1278	1261	1278	
6	1287	1287	1287	1301	1299			
7	1312	1314	1310	1321	1320			
8	1331	1332	1330	1340				
9	1350	1350	1346					

[a] The esters were measured at −40 °C, the triglycerols at 24 °C, and the PCs at −40 °C. [b] The assignments for the β phase of tri-C_{14}G and tri-C_{16}G are those in ref 17. [c] This frequency is the average of the components of the doublet at 1194 and 1200 cm^{-1}.

TABLE 3: Assignments of Methylene Wagging and Twisting Bands for Gel-Phase Diacylphosphatidylcholines

		no. of carbons in the acyl chains[a]						
progression	k	12	14	16	18	20	22	24
A_k	1	1187 (m)[b]	1184 (m)	1183 (m)	1182 (m)	1181 (m)	1180 (m)	1178 (m)
	2	1204 (m)	1200 (m)	1199 (m)	1195 (m)	1192 (m)	1189 (m)	1187 (m)
	3	1237 (m)	1228 (s)	1220 (m)	1214 (m)	1210 (m)	1205 (m)	1202 (m)
	4	1270 (m)	1255 (m)	1243 (m)	1235 (m)	1228 (m)	1222 (m)	1217 (m)
	5	1298 (m)	1280 (m)	1267 (m)	1255 (m)	1246 (m)	1238 (m)	1232 (m)
	6	1328 (m)	1306 (w)	1287 (w)	1274 (w)	1264 (w)	1254 (m)	1247 (m)
	7	1352 (w)	1330 (w)	1310 (w)	1294 (w)	1280 (w)	1270 (w)	1262 (w)
	8		1350 (w)	1330 (w)	1314 (w)	1298 (w)	1288 (w)	1274 (w)
	9			1346 (w)	1331 (w)	1316 (w)	1301 (w)	1291 (w)
	10				1346 (w)	1331 (w)	1317 (w)	1305 (w)
	11					1344 (w)	1331 (w)	1319 (w)
	12						1343 (w)	1331 (w)
	13							1343 (w)
B_k	3	1261 (w)	1249 (m)					
	4	1284 (w)	1269 (w)	1259 (w)	1250 (w)			
	5	1314 (vw)	1292 (w)	1278 (w)	1267 (w)	1258 (w)		
	6	1345 (vw)	1317 (vw)	1299 (vw)	1284 (w)	1273 (w)	1264 (w)	1256 (w)
	7		1344 (vw)	1320 (vw)	1303 (vw)	1290 (vw)	1278 (w)	1270 (w)
	8				1322 (vw)	1306 (vw)	1294 (vw)	1281 (w)
	9							1297 (vw)
B'_k	2	1215 (w)	1207 (m)					
	3	1247 (w)	1234 (s)					
	4	1275 (m)	1259 (m)					

[a] n = 12−16 were measured at −40 °C, and the rest, at −20 °C. [b] Frequencies in cm^{-1}. The letter inside parentheses denotes the relative intensity of the band: s, strong; m, medium; w, weak; vw, very weak.

changes in τ_3 (the dihedral angles associated with the C(O)−CH_2 bond) would change the intensities of the methylene wagging bands, as a result of changing the orientation of the −C(O)− group relative to the first methylene group. We attribute the change in intensity in the β' phase entirely to the change in the angles and make the simplest possible assumption for the ratio of A to B chains, namely, that this ratio is 2:1 as it is in the β phase.

Other explanations for the difference in the intensity ratio were also considered. A simple one is to assume that the observed increase in the A/B intensity ratio in going from β to β' reflects an increase in the A/B concentration. On this assumption, we can estimate the A/B ratio for the β' phase, since we know both the concentration and the intensity ratios in the β phase. This gives the concentration ratio in the β' phase as 4:1, which implies that the β' phase is made up of at least two different conformers of tri-C_{14}G. One possibility is that the A to B ratio is 2:1 for one conformer and 3:0 for the other. To make the observed intensity ratio, this would require concentrations of these conformers to be 0.6 and 0.4, respectively. This seems unlikely. (We note that for the gel phase, there is no corresponding stoichiometric requirement.)

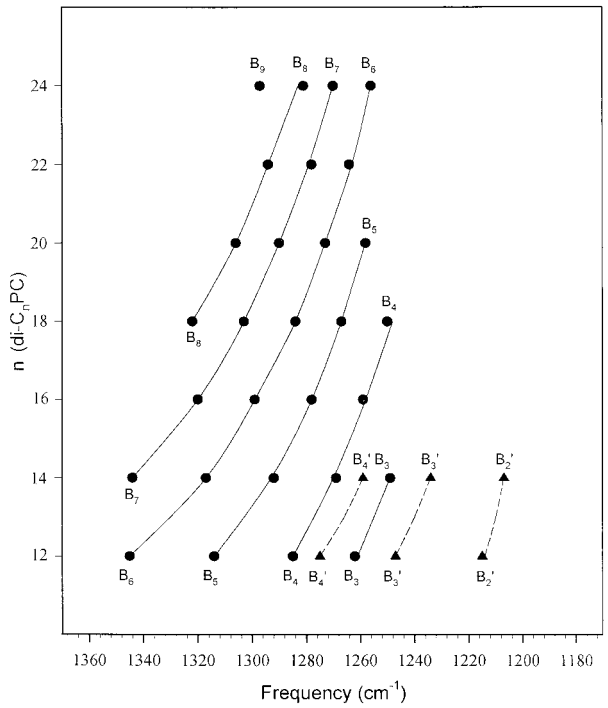

Figure 6. Observed frequencies of bands associated with the nonplanar chains. The bands belonging to wagging and twisting modes are designated B and B′, respectively. The subscripts give the *k* values.

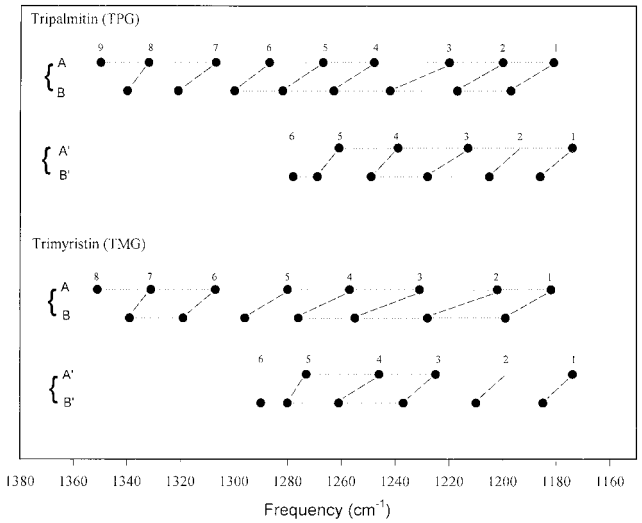

Figure 7. Observed frequencies of the IR bands of the A, A′, B, and B′ progressions for the β phase of tri-C_{14}G and tri-C_{16}G. The assignments (*k*) are from ref 17.

Another explanation might be that the films of tri-C_{14}G are highly oriented, in which case the observed intensity difference might be a polarization effect. To check this, the spectrum of a polycrystalline sample, randomly dispersed in a pressed KBr pellet, was compared to that of a melt-quenched film. The two spectra were found to be nearly identical. We also measured the spectrum of a tri-C_{14}G film whose plane was tilted about 30 degrees away from the usual orientation perpendicular to the IR beam. The resultant change in the B to A intensity ratio was found to be less than 5%. These various alternate explanations are thus rejected.

V. Results

A. Symmetric PC Gels. In the section above, relations between the spectra and chain conformation for the symmetric

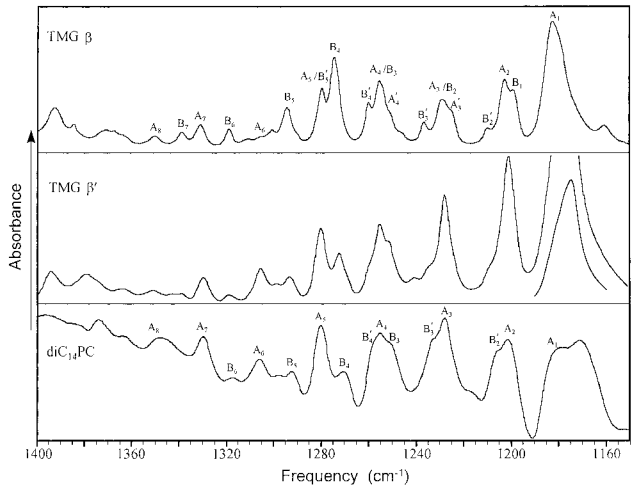

Figure 8. IR spectra of the β and β' phases of tri-C_{14}G (trimyristin) together with that of gel-phase di-C_{14}PC. Note the similarity between the spectrum of the β' form of tri-C_{14}G and that of the gel phase of di-C_{14}PC.

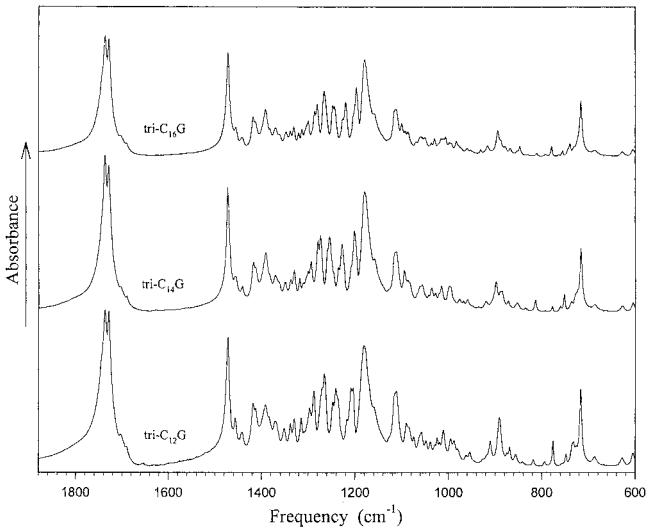

Figure 9. Spectra of polycrystalline tri-C_nG for n = 12, 14, 16 in pressed KBr disks at room temperature. Except for small, systematic band frequency shifts, which are dependent on chain length, the spectra are virtually identical. The crystals of the β phase of these triglycerols can therefore be assumed to be isostructual.

PC gels were established. We found that the alkyl chains exist in one of two conformations: planar (all-trans) chains (A) and nonplanar chains (B) that are all-trans except for the CH_2-CH_2 bond nearest the ester group which is gauche. We identified and assigned the IR bands associated with the A and B type chains.

The concentrations of these conformers are the next topic. Overall molar concentrations of the A chains (X_A) were estimated using eq 1. We used a value for κ that was obtained from the IR spectrum of the triglycerol, tri-C_{14}G in its β' phase, assuming $X_A/X_B = 2$. The bands selected for use in eq 1 were those located in the least crowded spectral region. Even then we could not avoid serious band overlap. When necessary, we separated the bands by fitting the spectral contour with a series of Lorentzian bands. The parameters used and the results obtained are summarized in Table 4.

The overall molar concentration of A chains for the di-C_{14}-PC gel was estimated to be 0.71 ± 0.16. The value of X_A for crystalline di-C_{14}PC is 0.50.[1] The concentration of A chains

TABLE 4: Estimated Molar Concentrations of All-Trans Alkyl Chains (X_A) and Values of the Parameters Used in the Determination

		data used to evaluate κ[a]						
			bands selected[d]				results	
gel	quantity determined	ref	A	B	X_A/X_B	κ	R_{int}^{obs} [b]	X_A^{est} [c]
di-C_{14}PC	$X_A^{overall}$	tri-C_{14}G (β')	1319	1331	2[f]	0.79 ± 0.06	3.03 ± 1.50[e]	0.71 ± 0.16
			1339	1350				
1,2-C_{14}^H C_{14}^DPC	X_A^{SN1}	tri-C_{14}G (β')	1319	1331	2	0.79 ± 0.06	4.87 ± 0.27	0.79 ± 0.02
			1339	1350				
1,2-C_{14}^D C_{14}^HPC	X_A^{SN2}	tri-C_{14}G (β')	1319	1331	2	0.79 ± 0.06	1.47 ± 0.31	0.54 ± 0.05
			1339	1350				
1,2-$C_{14}C_{18}$PC	X_A^{SN1}	di-C_{14}PC	1200	1207	2.39 ± 1.20[g]	1.23 ± 0.20	2.48 ± 0.88	0.75 ± 0.10
			1228	1249				
	X_A^{SN2}	di-C_{18}PC	1215	1268	2.39 ± 1.20[h]	0.61 ± 0.28	6.68 ± 3.20	0.80 ± 0.14
			1274	1284				
1,2-$C_{18}C_{14}$PC	X_A^{SN1}	di-C_{18}PC	1215	1268	2.39 ± 1.20	0.61 ± 0.28	10.28 ± 5.30	0.85 ± 0.08
			1274	1284				
	X_A^{SN2}	di-C_{14}PC	1200	1207	2.39 ± 1.20	1.23 ± 0.20	1.61 ± 0.15	0.66 ± 0.04
			1228	1249				

[a] κ is the proportionality constant in eq 1. [b] $R_{int}^{obs} = \sum I_{A,i}/\sum I_{B,j}$, is the intensity ratio of the A band sum to the B band sum. [c] Estimated molar concentration. [d] For the A and B chains. [e] Error estimated from the uncertainties in the measured values of the intensity ratios. [f] Ratio for tri-C_{14}G. [g] Evaluated molar ratio for di-C_{14}PC. [h] The molar ratio X_A/X_B for di-C_{18}PC is assumed equal to that for di-C_{14}PC.

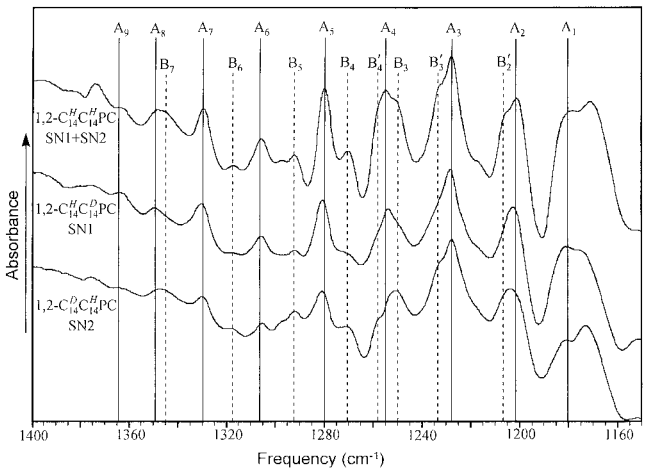

Figure 10. IR wagging mode region spectra of the di-C_{14}PC gel and of gels whose acyl chains are deuterated at the SN2 or else SN1 position at -40 °C. Assignments are indicated.

therefore increases substantially in going from the crystalline phase to the gel phase.

The average concentrations of A chains at the SN1 and SN2 positions were also determined for the di-C_{14}PC gel using a variant of the method described above. Two samples were measured. In one sample, the acyl chain at the SN2 position was replaced by its perdeuterated counterpart. The second sample was the same except the replacement was at the SN1 position. Since the wagging bands of a deuterated polymethylene chain appear at much lower frequencies ($900-850$ cm^{-1}) than those for the hydrogenated chains, the $1370-1170$ cm^{-1} region shows bands representing either the SN1 chain or the SN2 chain, but not both. The spectra of these 1,2-C_{14}^H C_{14}^DPC (HD) and 1,2-C_{14}^D C_{14}^HPC (DH) gels are displayed in Figure 10 along with that of the di-C_{14}PC gel. It can be seen that for the di-C_{14}PC gel the concentration of A chains at the SN1 position is slightly higher than the overall concentration of A chains; at SN2 it is lower. More quantitatively, X_A^{SN1} and X_A^{SN2} are 0.79 ± 0.02 and 0.54 ± 0.05, respectively, whereas the overall concentration is 0.71 ± 0.16. The values of the parameters involved in this estimation are given in Table 4. We note that our results imply the existence of a significant concentration of tt pairs. This is in agreement with a recent MD calculation that will be discussed in section VI.

B. Mixed-Chain PC Gels. In mixed-chain PC gels, the two acyl chains differ in length. We would like to know to what extent this asymmetry affects chain conformation. Of special interest are the mixed-chain gels that exhibit interdigitation. In an interdigitated bilayer the longer chains of opposing monolayers penetrate into the other monolayer, so that the short chains from one side tend to be collinear with the long chains from the other monolayer. This can increase the stability by reducing void volume. Two complementary mixed-chain diacylphosphatidylcholines, 1,2-$C_{14}C_{18}$PC and 1,2-$C_{18}C_{14}$PC, were studied. Mason and Huang report that for 1,2-$C_{14}C_{18}$PC gel the chains pack in the same manner as for the symmetric PC gels, whereas interdigitation occurs for 1,2-$C_{18}C_{14}$PC.[26,27]

The spectra of the mixed chain PCs along with those of di-C_{14}PC and di-C_{18}PC are shown in Figure 11. Essentially all the bands in the spectra of the mixed-chain gels correspond to the A, B, A′, and B′ bands found for the symmetric di-C_{14}PC and di-C_{18}PC gels. The chains in the mixed gels are therefore either A or B types. However, their concentrations differ from those of the symmetric gels. The values of X_A for the SN1 and SN2 chains for the two mixed-chain gels were estimated using eq 1. The di-C_{14}PC and di-C_{18}PC gels served as intensity references. We assumed that X_A is the same for both the di-C_{14}PC and di-C_{18}PC gels, although, as mentioned earlier, it appears that X_A for di-C_{18}PC may be slightly larger than for di-C_{14}PC. The parameters used in this analysis are given in Table 4.

The concentrations of A chains at the SN1 and SN2 positions for the 1,2-$C_{14}C_{18}$PC gel, which, as noted above, is not interdigitated, are 0.75 ± 0.10 and 0.80 ± 0.14, respectively. The SN1 value is nearly equal to and the SN2 value is much larger than that found at the corresponding position in the di-C_{14}PC gel. The concentration of A chains in the 1,2-$C_{18}C_{14}$PC gel, which is interdigitated, is 0.85 ± 0.08 for SN1 and 0.66 ± 0.04 for SN2, respectively. Thus, the value of X_A^{SN2} is significantly smaller for the interdigitated gel 1,2-$C_{18}C_{14}$PC.

C. Subgel Phase. We have studied the low-temperature phases of the di-C_nPC gel for $n = 12$, 14, and 16. Transformations to these phases were induced by incubating the gel phases between -10 and $+1$ °C. The transitions were monitored by

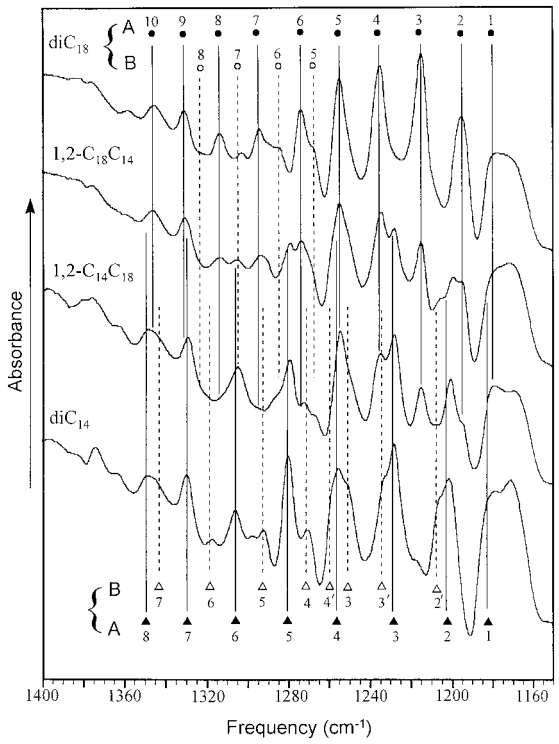

Figure 11. IR spectra and band assignments for the mixed-chain phosphatidylcholine gels, 1,2-$C_{18}C_{14}$PC and 1,2-$C_{14}C_{18}$PC together with those for the di-C_{18}PC and di-C_{14}PC gels.

following changes in the shape of the scissors band.[28] In the gel phase the chains pack in an orthorhombic perpendicular subcell. This leads to a splitting of the scissors band.[25] However, in the subgel phase the chains pack in a triclinic subcell.[28] In this case the scissors band is not split.[25]

The subgels of di-C_{14}PC and di-C_{16}PC were obtained by incubating the gels for 10 h at −5.0 and +0.3 °C, respectively. The scissors bands, which are shown in Figure 12, indicate the transition is essentially complete. The subgel obtained is the same as that previously reported in refs 28 and 29.

The spectrum of this subgel shows the alkyl chains have the A and B conformations as in the gel (Figure 13). However, the overall concentrations of A chains for the subgels are significantly greater than for the gel. The value of X_A for the di-C_{14}PC subgel is estimated to exceed 0.90, whereas for the gel it is about 0.71. This finding and the observation that the IR bands of the subgel are generally narrower than those of the gel support the conclusion from X-ray studies[30,31] that the subgel is significantly more ordered than the gel.

The situation for di-C_{12}PC is quite different. Incubating the gel for 2 h at −10 °C induced a transformation, but the IR spectrum of the resulting solid differs substantially from that of the subgel phase of di-C_{14}PC and di-C_{16}PC. This solid will be referred to as the LT (low-temperature) phase. The scissors band of this phase, shown in Figure 12, peaks at 1472 cm^{-1} rather than at 1474 cm^{-1}, which is observed for the subgels, and is also considerably broader. It appears then that the new phase may have a structure intermediate between that of the gel and the subgel.

The LT phase of di-C_{12}PC consists of planar (A) and nonplanar (B) chains. The conformation of the planar chains are nearly identical to the A chains of the PC gels studied here because the frequencies of these bands for the two systems are within a few wavenumbers and that the relative intensities of these bands are nearly the same (Figure 13).

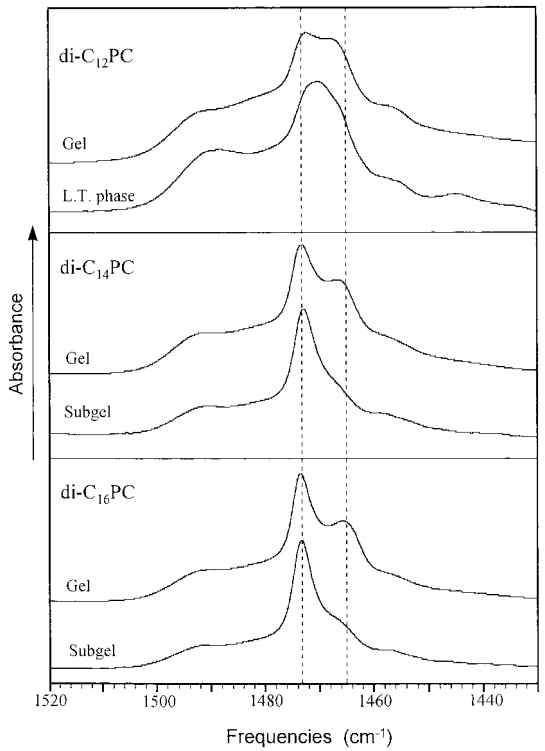

Figure 12. Methylene scissor bands for the di-C_nPC gels, $n = 12$, 14, and 16, the subgels, $n = 14$ and 16, and the solid obtained from incubating di-C_{12}PC. The latter is referred to as the LT (low-temperature) phase.

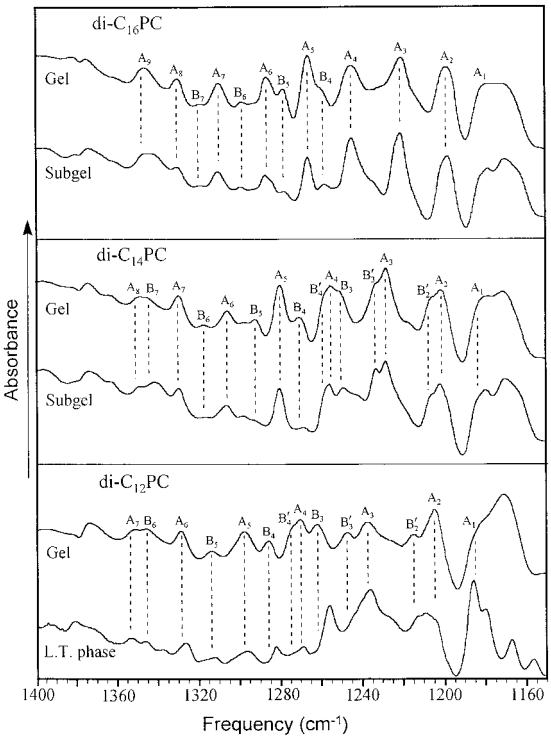

Figure 13. Spectra of the di-C_nPC gels, $n = 12$, 14 and 16, the subgels for $n = 14$ and 16, and the LT (low-temperature) phase of di-C_{12}PC.

The situation for the nonplanar chains is different. In the frequency region 1360−1300 cm^{-1}, where only wagging bands appear, the B bands of the LT phase of di-C_{12}PC occur at nearly the same frequencies as those for the gel phase. However, below 1300 cm^{-1}, where both wagging and twisting are present, the frequencies of the B bands in the LT phase spectrum are often significantly different from the gel phase spectrum. The same

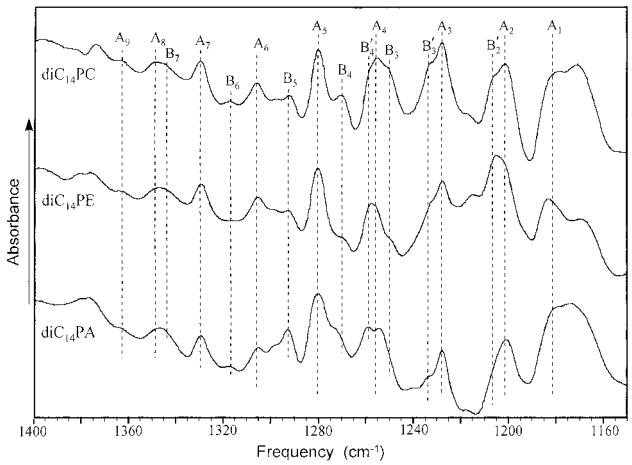

Figure 14. IR spectra of di-C_{14}PC, di-C_{14}PE, and di-C_{14}PC gels, all at −40 °C. The assignments to all-trans (A) and nonplanar (B and B′) acyl chains are indicated.

is true for the B′ bands. In addition, the LT phase bands in the 1180−1150 cm^{-1} region do not match those found for the PC gel or for either the β or β' phases of tri-C_{12}G. Thus, the nonplanar chains in LT-phase di-C_{12}PC differ in some degree from the B chains previously found.

A subgel phase of the mixed-chain bilayer, 1,2-$C_{14}C_{18}$PC was also prepared. Its spectrum is similar to the spectra of the di-C_{14}PC and di-C_{16}PC subgels. However, for 1,2-$C_{18}C_{14}$PC, no subgel formed after annealing the gel for over 20 h at 0 °C.

D. Other Headgroups. We found that the conformation of the alkyl chains is nearly independent of the headgroup among the gels of di-C_{14}PC, di-C_{14}PE, and di-C_{14}PA. Their spectra, which are shown together in Figure 14, are strikingly similar in the wagging region. The chains are therefore nearly the same conformation, and from the shapes of their scissors bands (not shown), the chain packing is identical.

We note that the same series of gels, but with an acyl chain length of 18 and 20 carbons, have the spectra similar to those of the 14 carbon series, after allowing for the difference in chain length; that is, the structures of the gels having a common headgroup appear to be the same for acyl chains of 14, 18, and 20 carbons.

The spectrum of the PE gel differs just slightly from those of PC and PA^-. The intense band of PE near 1170 cm^{-1} has a somewhat lower frequency and is relatively a little less intense than the corresponding band for PC and PA^-. It appears that the PE gel may be marginally more ordered than the others, in that the overall concentration of planar chains is greater.

VI. Summary and Conclusions

We have characterized, by means of IR spectroscopy, the conformation of the alkyl chains nearest their headgroup in fully hydrated phosphatidylcholine bilayers in the gel phase at temperature −20 °C or lower. Little information has been available. It has sometimes been assumed that the chain conformation in the gel is similar to that found for single crystals of the corresponding lipid hydrate, if the crystal structure is known through X-ray diffraction studies.

To identify chain conformation, we have developed an IR technique for distinguishing the chains that are all-trans or nearly all-trans, a method that is especially well suited to the gel phase. It is based on the frequency and intensity sensitivity of IR bands associated with the methylene wagging and twisting vibrations that appear in the mid-IR region between 1360 and 1180 cm^{-1} to small conformational changes in a highly ordered chain assembly. To find relations between the IR bands and specific conformers, we relied heavily on a recently reported vibrational analysis of certain relevant crystalline triglycerols[17] that are structurally related to the PC gels studied here. In addition we carried out a normal coordinate vibrational analysis for methyl palmitate. This calculation suggests an explanation for the frequency shifts observed when the conformation of a highly ordered chain is modified at its ends. In short, it appears that the shifts are a result of changes in the forms of the normal coordinates, which may be described in terms of wavevector or phase shift.[18,19] An alternative explanation that has been sometimes invoked is that the introduction of a gauche bond causes, in effect, a chain length "shortening" that results from a purported chain disconnect that occurs at the site of a gauche bond.

This IR method has been applied to various representative phosphatidylcholines: symmetric and mixed-chain PCs in their gel, and sometimes subgel, phases. For phospholipid gels with different headgroups, the conformations of the alkyl chains have been identified and the conformer concentrations estimated.

Two alkyl chain conformations were found for the gels and subgels: planar (all-trans) and nonplanar. The latter is all-trans except for a gauche CH_2-CH_2 bond next to the ester group. The spectra sometimes indicate small differences between gels due to small variations in the dihedral angle of trans or gauche bonds. Such variations are more pronounced for the nonplanar chains and may in fact reflect conformational changes associated with the neighboring ester group.

For the di-C_{14}PC gel, the overall concentration of planar chains, and the separate concentration at the SN1 and SN2 positions, were measured. The overall planar-chain concentration for the di-C_{14}PC gel was estimated to be around 0.71. The concentrations for the SN1 and SN2 chains are 0.79 and 0.54, respectively. The average of these two values is 0.67, in good agreement with the (independently) determined overall concentration of 0.71.

A complementary pair of mixed-chain gels—1,2-$C_{14}C_{18}$PC (usual PC packing) and 1,2-$C_{18}C_{14}$PC (interdigitated packing)—were measured. These are of interest because their chain packing is different and because of the chain reversal at the SN1 and SN2 positions. Unfortunately, due to the limited data from our measurements, it is difficult to distinguish between the effects of chain packing and chain reversal. For the 1,2-$C_{14}C_{18}$PC gel, the planar-chain concentrations are 0.75 for C_{14} (at SN1) and 0.80 for C_{18} (at SN2). These values are similar to each other, in contrast to dissimilar values found for the symmetric PC, di-C_{14}PC. However, the planar-chain concentrations for 1,2-$C_{18}C_{14}$PC are 0.85 for C_{18} (at SN1) and 0.66 for C_{14} (at SN2), values that are close to the values of 0.79 and 0.54 found at the corresponding positions for symmetric di-C_{14}PC.

The spectra of the subgel phases of di-C_{14}PC and di-C_{16}PC, which were obtained by low-temperature incubation of the gel phase, indicate the subgel is more ordered than the gel, in keeping with earlier studies.[30,31] We can add that the concentration of planar chains in the subgel is significantly larger than that for the corresponding gel. Incubating the di-C_{12}PC gel produced a different phase from the subgel phase derived from the di-C_{14}PC and di-C_{16}PC gels. The spectrum of this low-temperature phase of di-C_{12}PC tells us that the conformation of the nonplanar chains is somewhat different from that found in the other PC gels and subgels we have previously considered.

To explore the effect of headgroups on conformation, we measured the IR spectra for the symmetric phospholipid gels with PC, PA^-, and PE headgroups but with the same chain

TABLE 5: Measured and MD-Calculated Concentrations of All-Trans Chains

chain position	molar fraction of all-trans chains		
	measured (di-C_{14}PC)		MD calcd (di-C_{16}PC)
	gel[a]	crystalline[b]	gel[c]
SN1	0.79	1	1.00
SN2	0.54	0	>0.98

[a] Our results for the sample at −40 °C. [b] Dihydrate di-C_{14}PC crystal at 13 ± 3 °C.[1] [c] Simulation for the bilayer at 19 °C.[32]

lengths, $n = 14$, 18, or 20. The spectra for this series, display only a minor difference, indicating the headgroup has little effect on chain conformation. The spectrum of the PE gel was found to differ marginally from the other two.

Opportunities for comparing our results with those obtained in other ways are quite limited. Table 5 lists the concentration of trans bonds for the first CH_2-CH_2 bond for the SN1 and SN2 chains. Three pairs of values are listed: ours for di-C_{14}-PC; those associated with the crystalline dihydrate of di-C_{14}-PC, whose structure has been determined by X-ray diffraction;[1] and those for the MD-simulated di-C_{16}PC gel.[32]

For the SN1 and SN2 chains of the di-C_{14}PC dihydrate, there are equal numbers of trans and gauche bonds. However, for other related crystalline lipids gauche/trans and trans/trans pairs also occur. Therefore, averaging over all studies the fraction of trans bonds will exceed 0.5. For the gel phase, this is also the case.

The calculated trans bond conformation concentrations listed in Table 5 for di-C_{16}PC at 19 °C are from the gel-phase bilayer MD simulations reported by Tu et al.[36] This model predicts values of τ_4 corresponding to trans bonds for both SN1 and SN2 chains. However a comparison with our results, which indicate a sizable gauche fraction, must take into account the temperature difference between the two gels of about 60 °C: −40 °C for the IR measurements and 19 °C for the MD simulation.

To make the comparison, we have determined, at least roughly, the temperature dependence of the concentration of planar chains for the di-C_{14}PC and di-C_{16}PC gels. Quantitative estimates are difficult because the widths of the IR wagging bands increase with temperature, and their intrinsic intensities, as reported earlier, decrease with temperature and vanish entirely at T_m.[11,13]

For the di-C_{14}PC gel we found that with increasing temperature (from −40 to +17 °C) the fraction of planar chains increases substantially: The ratio of planar to nonplanar chain is increased by a factor of about 5.5 ± 0.1. However, the planar chain concentration for di-C_{16}PC gel over approximately the same temperature range (−40 to ∼+30 °C) is nearly constant. This is most likely due to the higher value of T_m for di-C_{16}PC (23 and 41 °C, respectively, for di-C_{14}PC and di-C_{16}PC). It appears that as the rotational disorder increases with temperature, the planar chains become increasingly more stable than the nonplanar forms. This is consistent with our spectral observation of lipids in their hexagonal phase—the α phase tri-C_{16}G, for example.

In summary, it appears that the predictions (trans bonds for both the SN1 and SN2 chains) agree with experiment for the di-C_{14}PC gel just below T_m, but not for the same gel at −20 °C or for di-C_{16}PC at temperatures below 30 °C.

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