Phospholipids Chiral at Phosphorus: Fourier-Transform Infrared Study on the Gel–Liquid Crystalline Transition of Chiral Thiophosphatidylcholine†

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ABSTRACT: Fourier-transform infrared spectroscopy (FT-IR) was used to study the structural properties of R_P, S_P, and $R_P + S_P$ isomers of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine (DPPsC), in comparison with those of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). For the vibrational modes of acyl chains, isomers of DPPsC show similar temperature and phase dependence to DPPC. However, the R_P isomer of DPPsC exhibits several unique properties: the CH_2 symmetric stretching band is unusually weak, the CH_2 asymmetric stretching band is unusually narrow, and the CH_2 wagging bands do not disappear completely at temperatures above the main transition. These differences could imply a tighter packing and be responsible for the unique phase-transition property of (R_P)-DPPsC. For the vibrational modes of the thiophosphodiester group, the frequency of the P–O stretching mode of DPPsC suggests that the POS^- triad exists predominantly in the mesomeric form. This is in contrast to the structure of nucleoside phosphorothioates where charge localization at sulfur has been demonstrated [Iyengar, R., Eckstein, F., & Frey, P. A. (1984) *J. Am. Chem. Soc. 106*, 8309–8310]. This suggests that the different biophysical properties between isomers of DPPsC are not due to different charge distribution in the POS^- triad or different geometry of charge distribution on the membrane surface. Instead, factors such as size or hydration property of oxygen and sulfur, as well as the different configuration at phosphorus, could be responsible for the differences in the conformation and packing of acyl chains, as revealed by the different properties in the CH_2 stretching and wagging modes of DPPsC.

The R_P and S_P isomers of DPPsC[1] (Figure 1), in which a chiral phosphorus center is created by substituting a nonbridging oxygen with a sulfur atom (Bruzik et al., 1983; Jiang et al., 1984), are useful models in studying the roles of the phosphate group of phospholipids in the structure and function of membranes. In summary, DPPsC isomers show large differences in the hydrolysis catalyzed by phospholipase A_2 (Tsai, T.-C., et al., 1985). They also form small unilamellar vesicles of different sizes (Tsai et al., 1984). In the liquid crystalline phase, differences in the chemical shift anisotropy in ^{31}P NMR and in the quadrupolar splitting in ^{14}N NMR have been observed (Tsai et al., 1983). The most dramatic of these is the recent finding that while (S_P)-DPPsC in the multilamellar phase shows a pretransition (T_{pt}) at 43.7 °C (ΔH = 1.6 kcal/mol) and a main transition (T_m) at 45.0 °C (ΔH = 7.1 kcal/mol), the R_P isomer shows a broad transition at 45.6 °C with a large ΔH (14.7 kcal/mol). Addition of the S_P isomer to the R_P isomer converted the thermotropic property of the latter to normal behavior. For example, ($R_P + S_P$)-DPPsC shows T_{pt} at 43.8 °C (ΔH = 1.7 kcal/mol) and T_m at 44.8 °C (ΔH = 6.8 kcal/mol) (Tsai, M.-D., et al., 1985).

Infrared and Fourier-transform infrared spectroscopy (FT-IR) have become increasingly useful tools in studying the detailed conformational and structural properties of synthetic and biological membranes [for recent reviews, see Cameron et al. (1979), Wallach et al. (1979), Fringeli & Günthard (1981), and Cameron & Dluhy (1985)]. In an effort to understand the thermotropic properties of DPPC and DPPsC isomers at the structural level, we have performed an FT-IR study of these compounds at temperature ranges from gel to liquid crystalline phases.[2] The results reveal differences and similarities between DPPC and DPPsC and between different isomers of DPPsC, in the gel phase and the liquid crystalline phase. Further, the data on the P–O stretching frequency suggest that the negative charge at the POS triad of DPPsC is fully delocalized, in contrast to that of nucleoside phosphorothioates where charge localization at sulfur has been demonstrated (Frey & Sammons, 1985; Iyengar et al., 1984).

Materials and Methods

Materials. Isomers of DPPsC were prepared as described previously (Bruzik et al., 1983). The diastereomeric purity of (R_P)- and (S_P)-DPPsC was 97% and >98%, respectively, as determined by ^{31}P NMR in CH_3OD at 121.5 MHz, with high resolution and high signal/noise ratios. DPPC was

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[1] Abbreviations: ADPαS, adenosine 5′-(1-thiodiphosphate); AMPS, adenosine 5′-monothiophosphate; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPsC, 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine; DSC, differential scanning calorimetry; FT-IR, Fourier-transform infrared spectroscopy; UMPS, uridine monothiophosphate; T_m, main transition temperature; T_{pt}, pretransition temperature.

[2] It is known that DPPC exhibits a third transition termed "subtransition" at 18 °C after the sample has been incubated at 0 °C for several days (Chen et al., 1980). Cameron and Mantsch (1982) demonstrated that the thermal history (length of incubation at 2 °C) affects the spectral behavior of DPPC, particularly in the C=O stretching band. The subtransition property of DPPsC is still under investigation in our laboratories, and this paper deals mainly with the main transition. All samples were preheated at 50–60 °C immediately before use to ensure the same thermal history. No attempt was made to resolve the pretransition since it is close to the main transition within 1 °C in DPPsC, and the temperature control in the sample cell is accurate only within ±0.5 °C.

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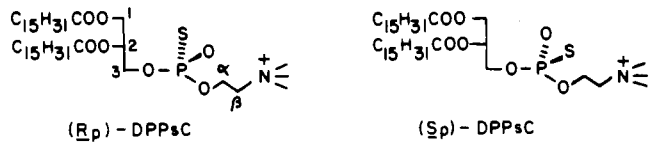


FIGURE 1: Structure and configuration of chiral thiophosphatidylcholine.

Table I: Methylene C–H Stretching Frequencies and Intensity Ratios of Acyl Chains

	temp (°C)	DPPC	DPPsC R_P	DPPsC S_P	DPPsC $R_P + S_P$
		Frequency (cm^{-1})			
asym stretch	24	2916	2917.5	2917.5	2917
	57	2922	2922	2922	2922
sym stretch	24	2848	2849	2849	2849
	57	2852	2852	2852	2952
		Intensity Ratios			
I_{asym}/I_{sym}[a]	24	1.3	2.6	1.4	1.4
	57	1.6	2.6	1.6	1.5
R_{asym}/R_{sym}[b]	24	2.3	3.6	2.3	2.2
	57	2.5	3.6	2.6	2.5

[a] I represents peak absorbance intensity measured from peak height. [b] R represents integrated absorbance intensity measured from band areas.

purchased from Avanti. The synthetic compounds were purified by repetitive precipitation from acetone/ethanol. All compounds were pure on the basis of ^1H NMR (200 MHz), ^{31}P NMR (121.5 MHz), and thin-layer chromatography on silica gel (Bruzik et al., 1983), as well as differential scanning calorimetry (Tsai, M.-D., et al., 1985).

Sample Preparation. Phospholipid samples were prepared by mixing 4 mg of dry lipid and 36 μL of doubly distilled water through a small aperture at 50–60 °C, by repetitive centrifugation. If not used immediately, the sample was stored in a freezer but was heated at 50–60 °C for 5 min immediately before use. Thus, all samples should have the same thermal history. The sample was then suspended between two pieces of KRS-5 windows (TlBr, TlI) spaced with a 35-μm poly(vinyl chloride) spacer. The entire cell assembly was described in detail in Chang (1985) and Alben and Fiamingo (1984). A thin thermocouple constructed from copper and constantan wires was placed between the windows, in direct contact with the sample. The cell path length was measured as described in Alben and Fiamingo (1984).

FT-IR Spectroscopy. Spectra were recorded on a Digiliab FTS-14D Fourier-transform infrared spectrometer (Alben & Fiamingo, 1984), equipped with a HgCdTe detector. The resolution was kept at 1 cm^{-1} and 512 interferograms were signal-averaged for each spectrum. The base line of the spectrum was obtained by subtracting the water absorbance spectrum from the sample spectrum at the same temperature. Frequencies and bandwidths were measured directly from the absorption spectra. The integrated absorption intensity of a given absorption band was obtained by cutting and weighing the expanded spectra. The possible error in the temperature control of the sample cell was ±0.5 °C.

RESULTS

Methylene C–H Stretching Modes. FT-IR spectra were obtained at 10–14 different temperatures from 24 to 57 °C for DPPC and (R_P)-, (S_P)-, and (R_P + S_P)-DPPsC. The methylene C–H stretching frequencies of DPPC and isomers of DPPsC in the gel phase (24 °C) and the liquid crystalline phase (57 °C) are listed in Table I, and the corresponding spectra are shown in Figure 2. It has been well established

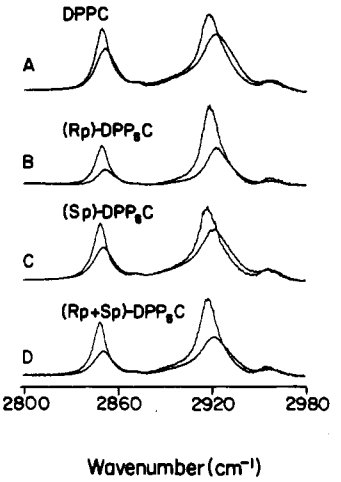


FIGURE 2: FT-IR absorbance spectra of the C–H stretching region of DPPC (A), (R_P)-DPPsC (B), (S_P)-DPPsC (C), and (R_P + S_P)-DPPsC (D) in the multilamellar phase, at 24 (gel) and 57 °C (liquid crystal).

that, as a result of increasing proportions of gauche conformation, a temperature increase results in a reduction of peak height and intensity, an increase in half-width, and a shift in frequency to higher wavenumbers, with all the parameters showing maximal discontinuities at the main transition temperature (Cameron et al., 1979, 1980; Cameron & Dluhy, 1985). These properties have also been observed for isomers of DPPsC, except that the pretransition was not always detectable due to its closeness to the main transition and that the T_m was ca. 45 °C and was always broader for (R_P)-DPPsC, in consistence with the recent DSC results (Tsai, M.-D., et al., 1985). The detailed plots of intensity, bandwidth, and frequency vs. temperature are presented in Chang (1985). The results suggest that the behavior of acyl chains of DPPsC in the phase transition is similar to that of DPPC.

There are, however, notable differences between isomers of DPPsC. As shown in Figure 2 and the data summarized in Table I, the ratio of peak height between the two CH_2 stretching bands, I_{asym}/I_{sym}, lies between 1.3 and 1.6 throughout the temperature range studied, for DPPC and (S_P)- and (R_P + S_P)-DPPsC. The ratio of the R_P isomer, however, is 2.6 at all temperatures.

Another difference lies in the bandwidth. While DPPC and isomers of DPPsC have approximately the same bandwidth in the CH_2 symmetric stretching band, the R_P isomer of DPPsC shows significantly smaller bandwidth in the CH_2 asymmetric stretching band. The temperature dependence of the bandwidth of this mode is also different among the compounds studied, as shown in Figure 3. On the other hand, isomers of DPPsC show little difference in the temperature dependence of the frequencies of both CH_2 stretching bands. Despite the narrower bandwidth in the CH_2 asymmetric stretching mode of (R_P)-DPPsC, the ratio of the integrals, R_{asym}/R_{sym}, is still considerably larger in (R_P)-DPPsC than in others (Table I, last row), although the difference between (R_P)-DPPsC and others is somewhat smaller in R_{asym}/R_{sym} than in I_{asym}/I_{sym}.

Methylene Scissoring Modes and Wagging Band Progression. Figures 4–7 show the FT-IR spectra from 900 to 1500 cm^{-1} for DPPC, (R_P)-DPPsC, (S_P)-DPPsC, and (R_P + S_P)-DPPsC, respectively, at 24 (A) and 57 °C (B) and the difference spectrum (C). Although the spectral patterns in the phosphate region are quite different between DPPC and DPPsC, the difference spectra are very similar, which suggests similar conformational changes upon phase transition. The

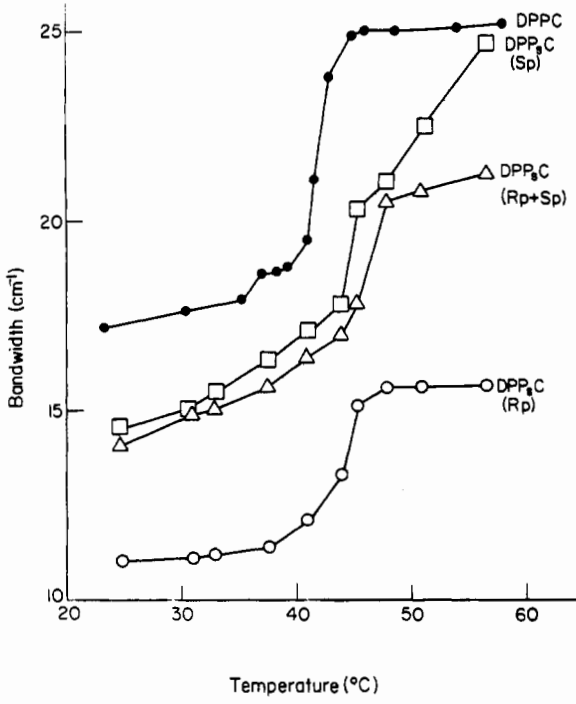


FIGURE 3: Temperature dependence of the bandwidth (at half-height) of the CH_2 asymmetric stretching mode of DPPC (●), (R_P)-DPPsC (○), (S_P)-DPPsC (□), and (R_P + S_P)-DPPsC (△).

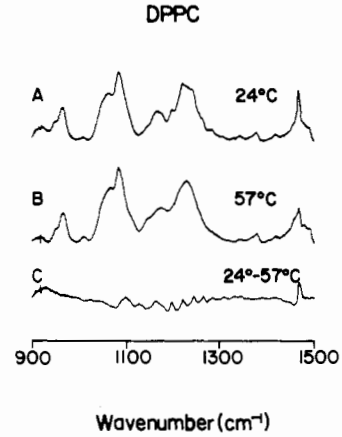


FIGURE 4: FT-IR absorbance spectra of the fingerprint region of multilamellar DPPC: (A) gel phase, 24 °C; (B) liquid crystalline phase, 57 °C; (C) 24 °C – 57 °C.

peak assignments are summarized in Table II. The three weak bands at 1418, 1378, and 1341 cm^{-1}, the weak band at 1013 cm^{-1}, and the strong band at 968 cm^{-1} are assigned on the basis of Fringeli and Günthard (1981). These bands remain almost unchanged throughout the whole temperature range, except a shift of 2 cm^{-1} for the C–N asymmetric stretching mode of (R_P)-DPPsC, which is accompanied by a decrease in intensity (Figure 5C). However, detailed analysis in Figure 8 indicated that the change occurs between 37.7 and 41.0 °C, before the main transition. Whether this represents an additional transition involving changes in the environment of the $(CH_3)_3N^+$ group requires further investigation.

The CH_2 scissoring mode at 1468 cm^{-1} loses intensity upon increasing temperature, which is accompanied by an increase in the intensity of shoulder peaks in the region 1440–1480 cm^{-1}, in consistence with the report of Cameron et al. (1980). This is typical of the behavior of these modes on the melting of the acyl chains (Nielsen & Hathaway, 1963; Synder, 1967), and the same behavior was observed for R_P, S_P, and R_P + S_P isomers of DPPsC, as shown in Figures 5–7.

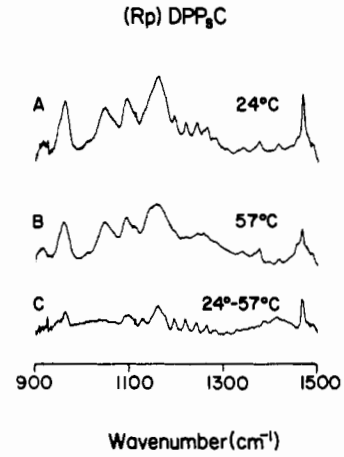


FIGURE 5: FT-IR absorbance spectra of the fingerprint region of multilamellar (R_P)-DPPsC: (A) gel phase, 24 °C; (B) liquid crystalline phase, 57 °C; (C) 24 °C – 57 °C.

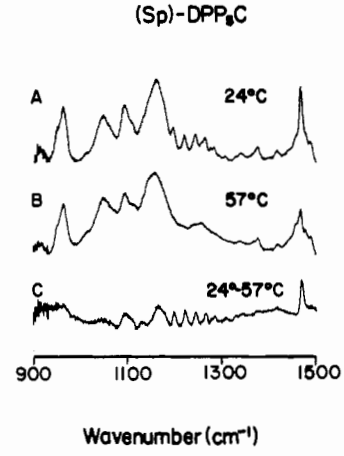


FIGURE 6: FT-IR absorbance spectra of the fingerprint region of multilamellar (S_P)-DPPsC: (A) gel phase, 24 °C; (B) liquid crystalline phase, 57 °C; (C) 24 °C – 57 °C.

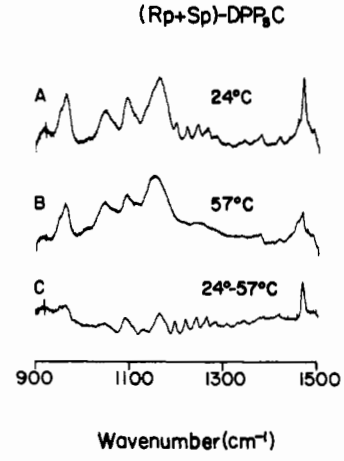


FIGURE 7: FT-IR absorbance spectra of the fingerprint region of multilamellar (R_P + S_P)-DPPsC: (A) gel phase, 24 °C; (B) liquid crystalline phase, 57 °C; (C) 24 °C – 57 °C.

The eight CH_2 wagging bands were identified from the difference spectra in Figures 4–7. The frequencies are almost identical for DPPC and isomers of DPPsC. Disappearance of these CH_2 wagging bands at temperatures above the main transition has been well documented by Cameron et al. (1980). However, in Figure 5B, residuals of some wagging bands can be identified, which were reproducible at two other temperatures (48 and 51 °C) above T_m. The successive difference spectra, as shown in Figure 8, clearly show the existence and

Table II: Summary of Assignments on the Fingerprint Region[a]

vibrational modes	frequency (cm^{-1})			
	DPPC	R_P	S_P	$R_P + S_P$
$N-CH_3$ asym C–N stretching	968	965 (963)	965	964
C–C stretching	1013 (1012)	1012 (1013)	1013	1012
C–OP stretching[b,c]		1049	1049	1049
		1095 (1096)	1095	1094 (1095)
C–O–CO sym stretching[b,c]	1066 (1068)			
C–O–CO asym stretching	1168 (1172)	[f]	[f]	[f]
P–O stretching[b]	1088 (1086.5) (sym)	1164 (1160)	1163 (1158)	1161 (1154)
	1230 (asym)			
CH_2 wagging[d]	1199	1198	1198	1198
	1222	1221 (1221)	1222	1221
	1244	1244 (1244)	1245	1244
	1265	1265	1266	1265
	1284	1284	1284	1285
	1309	1309	1308	1308
	1330	1331	1330	1330
	1343	1342	1343	1342
CH_2 twisting[d,e]	(1341)	(1341)	(1340)	(1340)
terminal CH_3 sym scissoring	1378	1378	1377	1377
CH_2 scissoring (adj to CO)	1418	1418	1418 (1419)	1418
CH_2 scissoring	1468	1468	1468	1468

[a] The reported frequencies were measured from the spectra obtained at 24 °C, whereas those in parentheses were measured at 57 °C. The difference in frequencies between 24 and 57 °C may not be significant, unless further characterized by different spectra and their dependence on temperature, as described in the text. [b] These bands are broad and possibly contain more than one component. The frequencies were measured at the apparent peak maxima. [c] The assignments of these bands are based on literature as described in the text. However, they should be considered tentative, since there are significant differences between DPPC and DPPsC. [d] The frequencies of the CH_2 wagging bands in the gel phase are obtained from the difference spectra in Figures 4C to 7C. Those in the liquid crystalline phase of (R_P)-DPPsC were obtained from the successive difference spectra in Figure 8. [e] At 24 °C, this band overlaps with one of the CH_2 wagging bands. [f] This band of DPPsC is buried under the strong P–O stretching band.

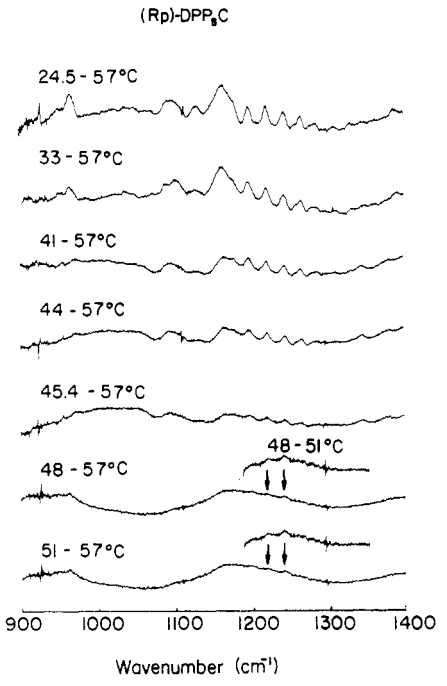


FIGURE 8: Successive difference spectra of (R_P)-DPPsC. The y scales of the insets are 2 times expanded.

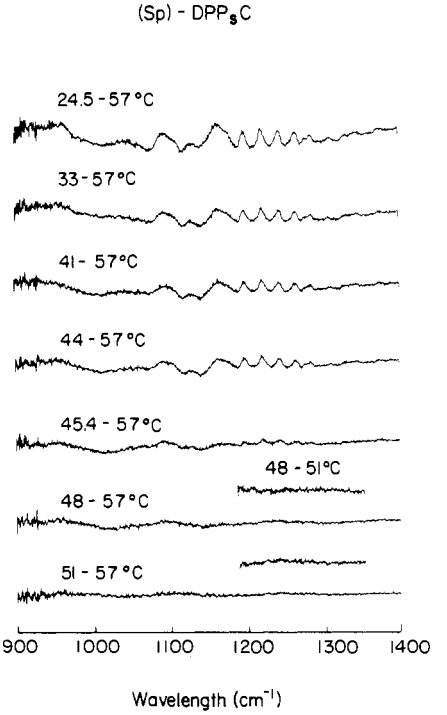


FIGURE 9: Successive difference spectra of (S_P)-DPPsC. The y scales of the insets are 2 times expanded.

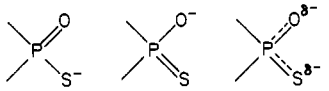
continual decrease of at least two wagging bands (1221 and 1244 cm^{-1}) above T_m for (R_P)-DPPsC. The corresponding plots for the S_P isomer (Figure 9), $R_P + S_P$ isomer, and DPPC show no detectable wagging bands above T_m. This suggests that a small percentage of trans conformation still exists in the acyl chains of (R_P)-DPPsC in the liquid crystalline phase.

P–O and C–O Stretching Modes. The P–O and C–O stretching modes for DPPC are assigned according to Fringeli and Günthard (1981) and Mendelsohn and Mantsch (1985). The spectra of isomers of DPPsC consist of three major bands in the 1050–1200-cm^{-1} region. For the R_P isomer (Figure 5A), the band at 1049 cm^{-1} could be assigned to the C–OP stretching (Mendelsohn & Mantsch, 1985). The band at 1095 cm^{-1} also belongs to the vibrations of the P–O–C group adjacent to the POS^- triad, as suggested by the study of various salts of diethyl thiophosphate (Kabachnik et al., 1965). The strong absorption at 1164 cm^{-1} can be assigned to the P–O stretching mode of the POS^- triad (Kabachnik et al., 1965; Thomas & Chittenden, 1970; Corbridge, 1969). The weaker C–O stretching band of esters is probably buried under the strong P–O stretching band in the spectra of DPPsC. As shown in Table II, there are observable shifts in the apparent frequencies of these modes. Temperature-dependent plots of

these bands suggest that the shifts correspond to the main transition temperatures (Chang, 1985). However, most of these bands are broad and may contain more than one component. It is difficult to distinguish band-frequency shifts from a change in absorptivity of an underlying component in the band envelope. In any case, the positions of the P–O stretching mode suggest that the negative charge is almost fully delocalized in the POS^- triad, as discussed in the next section.

Discussion

Charge Distribution in the POS Triad. The distribution of electron density in the thiophosphate group of DPPsC can approximate one of the following three limiting structures, the thiol form (I), the thione form (II), and the mesomeric form (III):



I II III

After reviewing the literature data on vibrational spectroscopy, ^{18}O isotope effect in ^{31}P NMR, and others, Frey and Sammons (1985) and Iyengar et al. (1984) suggested that the thiol form I would be the preferred structure of nucleoside phosphorothioates in general (e.g., AMPS, ADPαS, etc.), except the crystal structure of endo-2′,3′-cyclic UMPS (Saenger & Eckstein, 1970) which prefers the mesomeric form (Frey & Sammons, 1985). It has also been established that coordination by a soft metal (e.g., Zn^{2+}, Cd^{2+}) will favor the thiol form, whereas the thione form may be favored in the complexes of hard metal ions such as Mg^{2+} or Ca^{2+} (Kabachnik et al., 1965; Jaffe & Cohn, 1979; Pecoraro et al., 1984).

The most sensitive parameter to this problem seems to be the P–O stretching frequency. According to the calculation by Matrosov (1967), the expected P–O stretching frequencies of I–III are 1376, 952, and 1141 cm^{-1}, respectively. The P–S stretching frequency also varies, but to a much smaller extent and nonlinearly (549, 613, and 601 cm^{-1} for I–III, respectively). In the KBr pellet of solid powders of DPPsC, the P–O stretching band is located at 1178 and 1183 cm^{-1} for the R_P and the S_P isomers, respectively (Bruzik et al., 1983). This suggests that in the solid state the charge is slightly polarized to the sulfur. In the gel and the liquid crystalline phases, the P–O stretching mode is shifted to ca. 1160 cm^{-1}. Thus, in contrast to nucleoside phosphorothioates, the mesomeric form III seems to be the preferred structure of chiral thiophospholipids. Such a structure could be a result of ionic interactions between the quarternary ammonium group and the thiophosphate of neighboring molecules, since the mesomeric form is also the favored structure in the tetramethylammonium salt of $(C_2H_5O)_2POS^-$ (Kabachnik et al., 1965) and in the triethylammonium salt of endo-2′,3′-cyclic UMPS mentioned above. Such an ionic interaction has also been demonstrated by ^{31}P NMR for small unilamellar vesicles and micelles, on the basis of the nuclear Overhauser effect between the *N*-methyl protons and the ^{31}P nucleus (Yeagle et al., 1975, 1976; Burns et al., 1983).

Comparison between DPPC and Isomers of DPPsC. Compared to other analogues of phospholipids that are used as structural probes (e.g., spin-labels, fluorescent labels, modified acyl chains or head groups), thiophospholipids seem to meet the criteria of a good probe: minimal structural change and maximal perturbation. Previously, we have demonstrated the similarity between DPPC and DPPsC in terms of the formation and function of unilamellar and multilamellar vesicles (Tsai et al., 1984, 1983). On the other hand, striking differences have been observed between isomers of DPPsC in the thermotropic property and in the reaction catalyzed by phospholipases A_2, C, and D (Tsai, M.-D., et al., 1985; Tsai, T.-C., et al., 1985; Jiang et al., 1984; Bruzik et al., 1983).

The results of FT-IR again establish, on the structural basis, the similarity between DPPC and DPPsC in the macroscopic properties of membranes, as well as some subtle differences between isomers of DPPsC that could be responsible for large differences in certain functions. The temperature dependences of vibrational properties reported previously by Cameron et al. (1979, 1980) have all been observed for isomers of DPPsC. The differences in the vibrational frequencies between DPPC and DPPsC are minimal, except in the bands related to the thiophosphate group and its close proximity. On the other hand, the R_P isomer of DPPsC exhibits several unique properties: the I_{asym}/I_{sym} ratio and R_{asym}/R_{sym} ratio (peak height and integral, respectively) are unusually large, the bandwidth of the CH_2 asymmetric stretching band is unusually small, and the CH_2 wagging bands do not disappear completely at temperatures above T_m. While the difference in intensity ratio cannot be readily explained, the narrower CH_2 asymmetric band may suggest a higher degree of structural uniformity and imply more "trans" conformation in the R_P isomer throughout the whole temperature range. This is well supported by the existence of CH_2 wagging bands above T_m. These differences could be responsible for the unique phase transition property of (R_P)-DPPsC. The S_P and $R_P + S_P$ isomers of DPPsC behave very similarly both in the phase transition and in the vibrational spectroscopic properties.

It should be noted that this study does not involve the effect of low-temperature incubation on the spectral properties of phosphatidylcholine (Cameron & Mantsch, 1982). Future investigation on the subtransition properties (Chen et al., 1980) of DPPsC by DSC and FT-IR may further enhance our understanding in the structural properties of DPPsC.

Thiophospholipids as Models of Phospholipids. We have previously raised a question of possible "stereospecific interaction" between the phosphate group of phospholipids and other chiral molecules of membranes. In other words, the charge of the phosphate group of phospholipids could be localized as O=P—O^- or $^-$O—P=O under certain conditions. The isomers of DPPsC were suggested as good models for these forms (Tsai et al., 1983, 1984). An excellent example is the interaction of DPPC with phospholipase A_2, which involves a stereospecific binding of Ca^{2+} to the *pro-S* oxygen of DPPC. Due to the preference of Ca^{2+} for an oxygen ligand over a sulfur ligand, the enzyme shows a high degree of stereospecificity toward the R_P isomer of DPPsC (Tsai, T.-C., et al., 1985).

The results of this work suggest that the situation is different in the biophysical properties of bilayer membranes. Since the P–O stretching frequency is nearly the same for R_P, S_P, and $R_P + S_P$ isomers of DPPsC, there should be little difference in the charge distribution among isomers of DPPsC. Further, since the predominant structure of DPPsC is the mesomeric form, there is no geometrical difference in the charge distribution in the bilayers of different isomers of DPPsC. Thus, in the absence of proteins, DPPsC isomers are *not* good models for charge-localized DPPC (O=P—O^-, $^-$O—P=O, or racemic mixture). In terms of charge distribution, DPPsC should be very similar to the bilayers of DPPC, which is more likely to be in the delocalized form, $^{\delta-}$O—P—$O^{\delta-}$. Thus, the differences in the biophysical properties between isomers of DPPsC should *not* be due to different geometry in charge distribution. Other factors, such as the sizes of oxygen and sulfur atoms and the

different hydration and hydrogen-bonding properties between O and S, should be responsible for the differences between DPPC and DPPsC. The different geometry of these factors should be responsible for the differences between isomers of DPPsC. Such a "geometric difference" at the phosphate group apparently could induce differences in the conformation and packing of acyl chains, as revealed by the results of this paper.

Conclusions. The structural properties of R_P, S_P, and $R_P + S_P$ isomers of DPPsC, at temperatures above and below the main transition temperature, were investigated by FT-IR and compared with those of DPPC. The temperature dependence of the vibrational modes of the acyl chains of DPPsC parallels that of DPPC. However, (R_P)-DPPsC exhibits several unique properties, which imply tighter packing in the acyl chains of this isomer, consistent with its distinct phase transition behavior. All isomers of DPPsC exist predominantly as the mesomeric form, which suggests that factors other than charge distribution are responsible for the different biophysical properties between isomers of DPPsC.

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