

# Phospholipids Chiral at Phosphorus. Characterization of the Subgel Phase of Thiophosphatidylcholines by Use of X-ray Diffraction, Phosphorus-31 Nuclear Magnetic Resonance, and Fourier Transform Infrared Spectroscopy<sup>†</sup>

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**ABSTRACT:** A recent study using differential scanning calorimetry (DSC) showed that the thermotropic phase behavior of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine (DPPsC) is sensitive to the configuration at phosphorus and that the  $R_P$  isomer displayed only a broad transition at 45.6 °C [Wisner, D. A., Rosario-Jansen, T., & Tsai, M.-D. (1986) *J. Am. Chem. Soc.* 108, 8064–8068]. We have employed X-ray diffraction, <sup>31</sup>P NMR, and Fourier transform infrared (FT-IR) spectroscopy to characterize various phases of the isomers of DPPsC, to compare the structural differences between 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and isomers of DPPsC, and to identify structural factors responsible for the unique behavior of the  $R_P$  isomer. The results from all three techniques support the previous proposal based on DSC studies that ( $S_P$ )- and ( $R_P + S_P$ )-DPPsC undergo a subtransition, a pretransition, and a main transition analogous to those of DPPC, while ( $R_P$ )-DPPsC is quite stable at the subgel phase and undergoes a direct subgel → liquid-crystalline transition at 46 °C. Quantitative differences between DPPC and DPPsC (i.e., the effect of sulfur substitution rather than the configurational effect) in the subgel phase have also been observed in the chain spacing, the motional averaging, and the factor group splitting (revealed by X-ray diffraction, <sup>31</sup>P NMR, and FT-IR, respectively). In particular, DPPsC isomers are motionally rigid and show enhanced factor group splitting in the subgel phase. These results suggest that DPPsC is packed in different subcells relative to DPPC in the subgel phase. Two unique structural features of ( $R_P$ )-DPPsC (relative to other isomers and DPPC) have also been observed in the subgel phase: The bilayer spacing is small, and the C=O stretching band is asymmetric. These features could be responsible for the unusual stability of the subgel phase of ( $R_P$ )-DPPsC.

The  $R_P$  and  $S_P$  isomers of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine (DPPsC)<sup>1</sup> (Figure 1), where a chiral phosphorus center has been created by the substitution of an oxygen with a sulfur atom (Bruzik et al., 1983; Jiang et al., 1984) have been used as models in studying the role of the phosphate group in the structure and function of membranes (Tsai, M.-D., et al., 1983; Tsai, T.-C., et al., 1984, 1985). A recent study using differential scanning calorimetry (Wisner et al., 1986) has shown that ( $S_P$ )-DPPsC shows a pretransition temperature ( $T_{pt}$ ) at 43.7 °C and a main transition temperature ( $T_m$ ) at 45 °C, as well as a subtransition temperature ( $T_s$ ) at 22 °C when the sample is annealed at 4 °C for several days. Such a thermotropic property is similar to that of DPPC (Chen et al., 1980), except that  $T_{pt}$  and  $T_m$  are shifted higher by a few degrees. The mixture of isomers, ( $R_P + S_P$ )-DPPsC,<sup>2</sup> behaves similarly to the  $S_P$  isomer. The  $R_P$  isomer, however, shows only a broad transition at 45.9 °C, even after being annealed at 4 °C for a period of 2 weeks. In separate experiments, Wisner et al. (1986) showed that when ( $R_P$ )-DPPsC was heated at 50–70 °C, cooled to 25 °C quickly, and scanned immediately, a pretransition and a main transition similar to

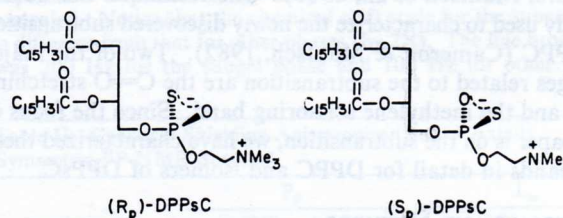


FIGURE 1: Structures of ( $R_P$ )-DPPsC and ( $S_P$ )-DPPsC.

those of the  $S_P$  isomer were observed. These results were interpreted to indicate that ( $R_P$ )-DPPsC is metastable in the gel phase, that it relaxes to the subgel phase rapidly at 25 °C ( $t_{1/2}$  of hours, depending on the isomeric purity), and that it remains in the subgel phase in the heating scan until  $T_m$  is reached. Such results suggest that the configuration at phosphorus is important to the structure of phospholipid bilayers.

Understanding the above configurational effect at the molecular level should enhance our understanding of the structure and function of membranes. The most reasonable steps toward such a goal would be first to confirm the phase assignments

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<sup>1</sup> Abbreviations: 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;  $T_m$ , main transition temperature;  $T_{pt}$ , pretransition temperature;  $T_s$ , subtransition temperature.

<sup>2</sup> For convenience, the mixture of isomers, ( $R_P + S_P$ )-DPPsC, is sometimes referred to as a third isomer.



for all isomers of DPPsC (Wisner et al., 1986) by use of other physical techniques, to compare structural properties of DPPC and DPPsC isomers at subgel and other phases, and to identify unique structural features of ( $R_p$ )-DPPsC that could be responsible for its unique thermotropic behavior. This paper reports the use of X-ray diffraction,  $^{31}\text{P}$  NMR, and FT-IR to study these problems. All three techniques have previously been used to characterize the subtransition of DPPC. A brief background for each technique is described below.

Füldner (1981) and Ruocco and Shipley (1982a) have independently shown by X-ray powder diffraction that the subgel phase of DPPC is characterized by, among other effects, sharp reflections at  $1/4.4$  and  $1/3.86 \text{ \AA}^{-1}$  due to more ordered chain packing. On the basis of more detailed studies, Ruocco and Shipley (1982b) have also proposed a unified structural interpretation of the changes occurring in the two-dimensional acyl chain packing modes during the transitions of the bilayers of DPPC between the  $L_s$  (subgel) phase, the  $L_\beta$  (gel) phase, the  $P_\beta$  (rippled gel) phase, and the  $L_\alpha$  (liquid crystalline) phase.<sup>3</sup>

$^{31}\text{P}$  NMR has been used to study the motion and average orientation of the phosphate group in lipid systems (Seelig, 1978; Griffin, 1981). The "powder pattern" spectra reveal the chemical shielding anisotropy factor, which predominates in the absence of dipolar interactions. Recently, Füldner (1981) and Lewis et al. (1984) showed that the  $^{31}\text{P}$  NMR spectrum of DPPC broadened in the subgel phase, which suggests that the motions are slow and nonaxially symmetric. Thus,  $^{31}\text{P}$  NMR is a good tool to compare the phase behavior between ( $R_p$ )-DPPsC and other isomers.

FT-IR is a useful tool in studying the conformational and structural properties of membranes (Casal & Mantsch, 1984; Cameron & Dluhy, 1985). Before the subtransition was discovered, the pretransition and main transition of DPPC had been characterized by detailed FT-IR studies (Cameron et al., 1980a,b; Mantsch et al., 1980). The technique was subsequently used to characterize the newly discovered subtransition of DPPC (Cameron & Mantsch, 1982). Two of the major changes related to the subtransition are the C=O stretching band and the methylene scissoring band. Since the focus of this paper is on the subtransition, we have characterized these two bands in detail for DPPC and isomers of DPPsC.

## MATERIALS AND METHODS

**Materials.** DPPC was purchased from Avanti and was used without further purification. The isomers of DPPsC were prepared as previously described (Bruzik et al., 1983). The diastereomeric purity was determined by  $^{31}\text{P}$  NMR in  $\text{CD}_3\text{OD}$  on a Bruker WM-300 NMR spectrometer. Both isomers were considered to be >99% pure by the above method. The isomers were chemically purified by repetitive precipitation from acetone/ethanol. The chemical purity of the lipid samples was determined by TLC on silica gel (Bruzik et al., 1983) and differential scanning calorimetry (Wisner et al., 1986).

**X-ray Diffraction Studies.** The sample (3 mg) was hydrated in 0.5 mL of  $\text{H}_2\text{O}$  at 50 °C for 45 min, cooled to 25 °C, and centrifuged at 2000 rpm for 20 min. The excess water was removed by syringe, and the hydrated lipid was transferred to a sample cassette in the space between two pieces of mica window.

The camera used was constructed as described in Hernqvist

(1984); the X-ray source used was  $\text{Cu K}\alpha$  ( $\lambda = 1.5418 \text{ \AA}$ ). The temperature of the sample was controlled by a water bath that circulated water through the sample cassette, as described in Hernqvist (1984). The sample-to-film distance ( $l$ ) was 41 mm. The exposure time was typically 90 min. The film was scanned on a densitometer with a scanning rate of 10 cm/min and a chart speed of 60 cm/min. The distance  $l$  was calibrated by standard crystalline tristearin.

**$^{31}\text{P}$  NMR Spectroscopy.** The lipid samples were prepared by mixing 150–200 mg of dry lipid with 175–225  $\mu\text{L}$  of  $\text{D}_2\text{O}$  in a 5-mm NMR tube. The samples were repetitively heated to 60 °C, vortexed, and cooled until a homogeneous mixture was obtained. The samples were then annealed at 0–4 °C for more than 1 week.

$^{31}\text{P}$  NMR spectra were recorded on a Bruker MSL-300 spectrometer at a frequency of 121.497 MHz. Spectra were obtained by using a Hahn echo pulse sequence (Rance & Byrd, 1983) with a spectral width of 125 kHz, a 90° pulse of 9  $\mu\text{s}$ , a recovery time of 2 s, and broad-band  $^1\text{H}$  decoupling.

**FT-IR Spectroscopy.** The lipid samples were prepared by mixing 6 mg of lipid with 50  $\mu\text{L}$  of  $\text{D}_2\text{O}$ . The samples were then vortexed at 60 °C for 3 min and cooled at 20 °C for 2 min. This was repeated 3 times to assure that the lipid dispersion was fully hydrated. The lipid sample was then placed into a 6- $\mu\text{m}$ -thick transmission cell equipped with  $\text{CaF}_2$  windows. The samples for subgel-phase studies were refrigerated at 4 °C for 10 days and then placed into the spectrometer's sample chamber, which was precooled to  $\sim 0$  °C.

FT-IR spectra were recorded on a dry-air-purged Digilab FTS-15 Fourier transform infrared spectrometer equipped with a narrow-range (800- $\text{cm}^{-1}$  low-frequency cutoff) mercury cadmium telluride detector. The resolution was kept at 2  $\text{cm}^{-1}$ , and 1028 interferograms were signal-averaged for each spectrum. The transmission cell was placed in a thermostated cell mount (Cameron & Jones, 1981) that allowed the temperatures to be stable within 0.1 °C. Frequencies were determined by measuring the center of gravity of the C=O stretching band and the  $\text{CH}_2$  scissoring band. Bandwidths were determined relative to base lines for each band (1800–1650  $\text{cm}^{-1}$  for the C=O stretching band and 1530–1400  $\text{cm}^{-1}$  for the  $\text{CH}_2$  scissoring band).

## RESULTS

**X-ray Diffraction Data of Annealed ( $S_p$ )- and ( $R_p + S_p$ )-DPPsC.** Figure 2 shows the large-angle region of the X-ray diffraction patterns of fully annealed samples at selected temperatures. This region reflects the short spacings of the acyl chains of the phospholipid samples. Comparison of the patterns in Figure 2, parts A–C, reveals that the behavior of the ( $R_p + S_p$ )- and ( $S_p$ )-DPPsC samples closely resembles that of DPPC, in support of the DSC results. Although some differences in the spacings in the  $L_s$  phase are indicated, there are, nonetheless, two reflections: a sharp one at 4.2–4.4- $\text{\AA}$  spacing and a broader one at 3.9–4.0  $\text{\AA}$ . At temperatures above  $T_m$ , the samples gave broad and diffuse reflections. This is indicative of the disorder accompanying chain melting and conversion to the liquid-crystalline phase and is consistent with the pattern observed with DPPC (Luzzati, 1968).

Table I lists the chain and bilayer spacings observed for the samples in each phase. The bilayer spacings are reflected in the small-angle region of the diffraction patterns. On the basis of the very close similarities in the thermotropic properties and the chain and bilayer spacings between DPPC and ( $S_p$ )- and ( $R_p + S_p$ )-DPPsC, these isomers of DPPsC can be placed under the same phase classification as DPPC:  $L_s$ ,  $L_\beta$ ,  $P_\beta$ , and  $L_\alpha$ .

<sup>3</sup> Throughout this paper the primed and unprimed phases are not distinguished.

Table I: Summary of Chain Spacings and Bilayer Spacings<sup>a</sup>

	$L_c$			$L_\beta$			$P_\beta$			$L_\alpha$		
	chain spacing (Å)	bilayer spacing (Å)	temp (°C)	chain spacing (Å)	bilayer spacing (Å)	temp (°C)	chain spacing (Å)	bilayer spacing (Å)	temp (°C)	chain spacing (Å)	bilayer spacing (Å)	temp (°C)
DPPC <sup>b</sup>	4.4, 3.9	60	5	4.2, 4.1 <sup>c</sup>	65	25	4.2		38	4.5		50
	(4.4, 3.9)	(59.1)	5	(4.17, 4.08)	(63.6)	20	(4.19)	(67)	38	(4.5)	(60)	60
$(R_p + S_p)$ -DPPsC	4.2, 4.0	61	5	4.2, 4.1 <sup>c</sup>	65	25	4.2	69	43.5	4.4	66	44
$(S_p)$ -DPPsC	4.2, 4.0	62	5	4.2, 4.1 <sup>c</sup>	66	25	4.2	69	43.5	4.4	67	44.5
$(R_p)$ -DPPsC (heating)	4.4, 4.1	57	5-45							4.5	64	47
$(R_p)$ -DPPsC (relaxation)	4.4, 4.0	56	25	4.3	66	25	4.4	69	44	4.7	63	50

<sup>a</sup>The chain spacings measured for all DPPsC samples have a measurement error of  $\pm 0.05$  Å; the bilayer spacings are accurate to  $\pm 1$  Å. <sup>b</sup>Values for DPPC parentheses were taken from Ruocco and Shipley (1982a). <sup>c</sup>The reflection at ca.  $1/4.1$  Å<sup>-1</sup> is not clearly resolved but is a shoulder on the reflection. This is evident from the asymmetry in that reflection.

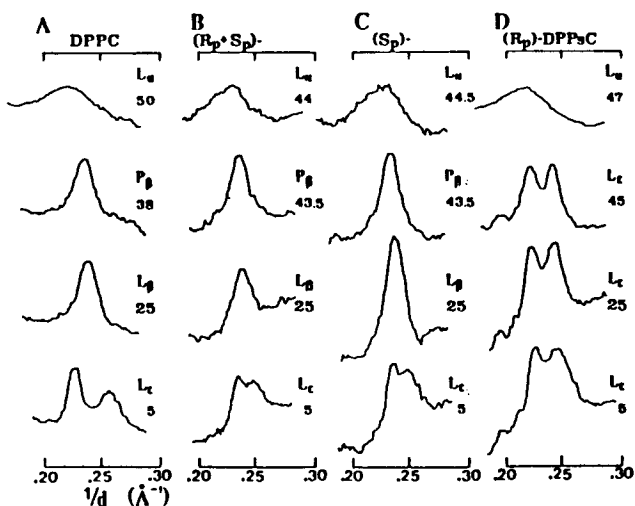


FIGURE 2: Densitometer tracings of the wide-angle X-ray diffraction patterns of DPPC (A),  $(R_p + S_p)$ -DPPsC (B),  $(S_p)$ -DPPsC (C), and  $(R_p)$ -DPPsC (D). The temperature of acquisition and the thermotropic phase are indicated to the right of each trace. The experiment started at the low temperature after the sample had been annealed.

**X-ray Diffraction Results of  $(R_p)$ -DPPsC.** Figure 2D shows that  $(R_p)$ -DPPsC also possesses a low-temperature phase with a pattern similar to the other isomers, but it maintains this phase up to 45 °C, in agreement with the DSC results. The chain and bilayer spacings of  $(R_p)$ -DPPsC from this set of experiments are listed in the fourth row of Table I (with "heating" in parentheses). On the basis of Figure 2D and the data in Table I, it is quite clear that at 47 °C the  $R_p$  isomer exists in the lamellar liquid-crystalline phase,  $L_\alpha$ , whereas at 45 °C or below it exists in the subgel phase. It is also noted that the bilayer spacing of  $(R_p)$ -DPPsC is smaller than that of other isomers and DPPC in the subgel phase.

The  $L_\beta$  and  $P_\beta$  phases of  $(R_p)$ -DPPsC could be observed at 25 and 44 °C, respectively, by collecting the data immediately after the sample was heated to 50 °C for 5 min. This is also consistent with previous DSC results that these two phases of the  $R_p$  isomer are metastable but can be detected by rapid cooling from above  $T_m$  (Wisner et al., 1986). The data of these two phases are listed in the last row of Table I (with "relaxation" in parentheses). The data are fully consistent with those of the  $L_\beta$  and  $P_\beta$  phases of  $(S_p)$ - and  $(R_p + S_p)$ -DPPsC.

**$^{31}\text{P}$  NMR Results.** Figure 3 shows the  $^{31}\text{P}$  NMR spectra of multilamellar DPPC (A),  $(R_p + S_p)$ -DPPsC (B),  $(S_p)$ -DPPsC (C), and  $(R_p)$ -DPPsC (D) at temperatures corresponding to the X-ray diffraction patterns in Figure 2. Note that the chemical shift scale of the subgel phase is different from that of other phases (by 2×). The line shapes of DPPC

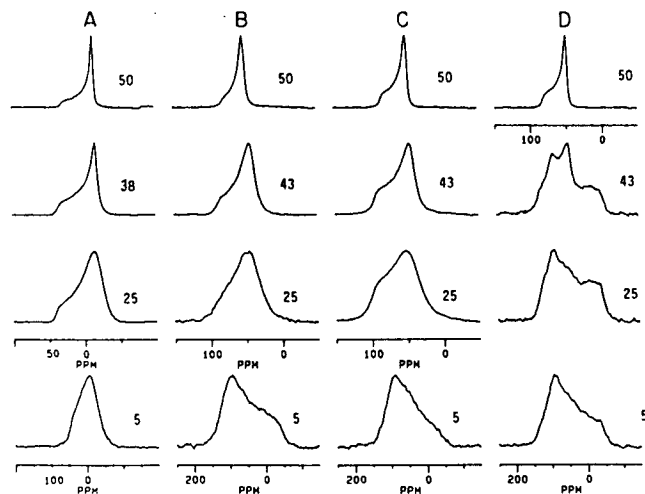


FIGURE 3:  $^{31}\text{P}$  NMR spectra (121.5 MHz,  $^1\text{H}$  decoupled) of DPPC (A),  $(R_p + S_p)$ -DPPsC (B),  $(S_p)$ -DPPsC (C), and  $(R_p)$ -DPPsC (D). The temperature is indicated to the right of each spectrum. The experiment started at the low temperature after the sample had been annealed. Notice that the chemical shift scale for the subgel phase is different from that for other phases (by 2×). The line broadening was 300 Hz for the subgel phase and 100 Hz for other phases.

Table II: Chemical Shielding Anisotropies ( $\Delta\sigma$ ) of Axially Symmetric  $^{31}\text{P}$  NMR Spectra<sup>a</sup>

compound	$P_\beta$		$L_\alpha$	
	temp (°C)	$\Delta\sigma$ (ppm)	temp (°C)	$\Delta\sigma$ (ppm)
DPPC	38	56	50	46
$(R_p + S_p)$ -DPPsC	43	53	50	31
$(S_p)$ -DPPsC	43	56	50	39
$(R_p)$ -DPPsC			50	36

<sup>a</sup>The data are obtained from Figure 3 and have not been corrected for the 100-Hz line broadening. The  $\Delta\sigma$  are measured as the separation between the half-height of the upfield shoulder and the downfield shoulder of the spectrum.

are consistent with previous reports for the subgel phase as well as other phases (Fuldner, 1981; Ruocco et al., 1985). The line shapes of  $(R_p + S_p)$ -DPPsC and  $(S_p)$ -DPPsC also show distinct differences between 5 °C (nonaxially symmetric), 25 °C (slightly axially symmetric), 43 °C (axially symmetric), and 50 °C (axially symmetric, more rapid averaging).  $(R_p)$ -DPPsC, on the other hand, remains nonaxially symmetric at 25 °C. At 43 °C the  $R_p$  isomer shows more than one component, which could be a mixture of the subgel phase and the  $L_\alpha$  phase. At 50 °C the  $^{31}\text{P}$  NMR pattern of the  $R_p$  isomer becomes axially symmetric. Thus, the line shapes in  $^{31}\text{P}$  NMR fully support the previous phase assignments and the unusual

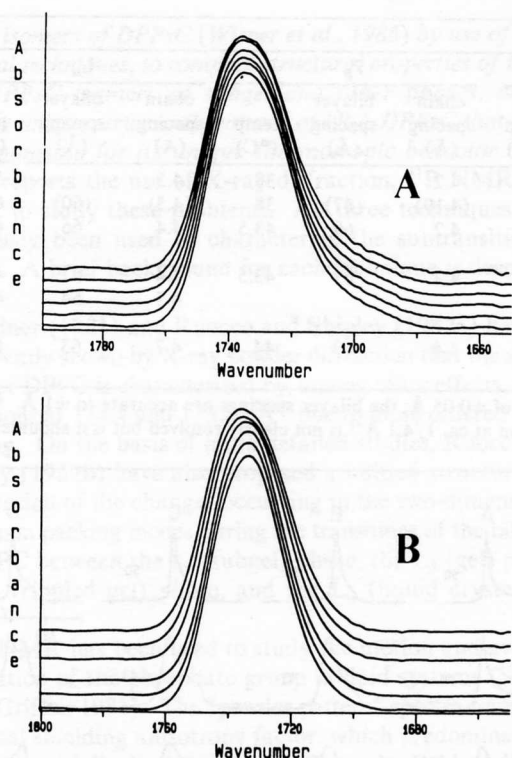


FIGURE 4: Temperature-dependent behavior of the C=O stretching band of ( $R_P + S_P$ )-DPPsC: (A) non-annealed with temperatures, from bottom to top, of 2.0, 6.6, 11.8, 16.7, 18.1, and 28.2 °C; (B) annealed at 4 °C for 10 days with temperatures, from bottom to top, of 1.8, 7.4, 13.4, 15.5, 17.0, and 28.2 °C.

stability of the subgel phase of ( $R_P$ )-DPPsC (Wisner et al., 1986).

The chemical shift anisotropies ( $\Delta\sigma$ ) for the axially symmetric spectra in Figure 3 are listed in Table II. Examination of the spectra in Figure 3 and the data in Table II revealed several interesting points: (a) In the  $L_\alpha$  phase the  $\Delta\sigma$  of DPPsC isomers are significantly smaller than that of DPPC, and there are detectable differences between isomers of DPPsC [ $S_P > R_P > (R_P + S_P)$ ]. These are consistent with the previous paper by Tsai et al. (1983). (b) In the  $P_\beta$  and the  $L_\beta$  gel phases, however, the  $\Delta\sigma$  values are very similar for DPPC, ( $R_P + S_P$ )-DPPsC, and ( $S_P$ )-DPPsC. (c) In the subgel phase, DPPC shows an "amorphous" lineshape at 5 °C due to incomplete motional averaging. Ruocco et al. (1985) showed that upon further cooling DPPC undergoes a reversal in the sign of the chemical shielding anisotropy at -20 °C and approaches the axially asymmetric rigid lattice at -39 °C. The isomers of DPPsC, however, have already approached the axially asymmetric rigid lattice at 5 °C, since the line shapes and the chemical shielding tensors are similar to those of the solid powder of ( $R_P + S_P$ )-DPPsC reported previously (Vasilenko et al., 1982). These results suggest that in comparison with DPPC the phosphate groups of DPPsC isomers are motionally more rigid in the subgel phase and have similar motional properties in the gel phases but undergo more rapid motional averaging in the liquid-crystalline phase.

**C=O Stretching Band of ( $R_P + S_P$ )- and ( $S_P$ )-DPPsC.** FT-IR spectra were taken at 20–23 different temperatures from 0 to 60 °C. The temperature-dependent behavior of the C=O stretching band of ( $R_P + S_P$ )-DPPsC without incubation and with annealing at 4 °C is shown in parts A and B of Figure 4, respectively. Plots of the band frequency and bandwidth (parts A and B of Figure 5, respectively) show a clear shift of the band to a lower frequency and a narrower bandwidth in the subgel phase.<sup>4</sup> These results are consistent with the

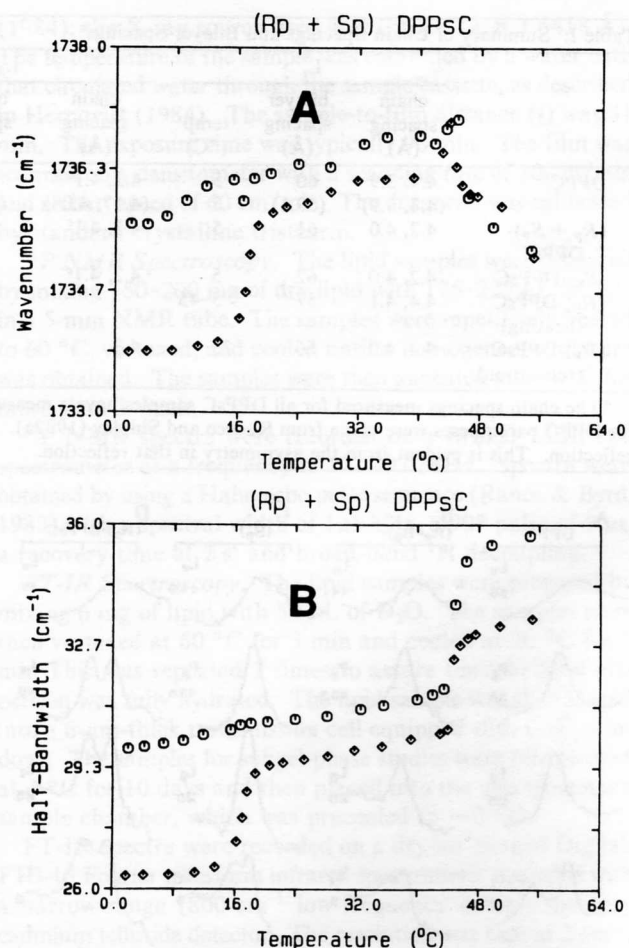


FIGURE 5: Plots of the frequency (A) and bandwidth (B) of the C=O stretching band of ( $R_P + S_P$ )-DPPsC as a function of temperature. The diamond represents the annealed lipid, and the circle represents the nonannealed sample. Note the subtransition of the annealed sample takes place at 16 °C.

previous observation for the gel  $\leftrightarrow$  subgel transition of DPPC (Cameron & Mantsch, 1982). Since this has also been observed for monohydrate (Fringeli, 1981) and anhydrous DPPC (Bush et al., 1980), the results suggest a partial dehydration of the carbonyl region in the subgel phase. On the basis of deconvoluted spectra, Bush et al. (1980) have shown that the C=O stretching band of DPPC consists of two main components at 1741 and 1727  $\text{cm}^{-1}$ , which have been assigned to the *sn*-1 and *sn*-2 carbonyl groups, respectively. When the lipid is in the gel phase, the band at 1741  $\text{cm}^{-1}$  has a greater intensity than the 1727- $\text{cm}^{-1}$  component. When the lipid enters the subgel phase, the band at 1727  $\text{cm}^{-1}$  increases in intensity relative to the 1741- $\text{cm}^{-1}$  band. This change in the relative intensity of the two components causes the decrease in the apparent frequency and the apparent bandwidth of the C=O stretching band. The subtransition ( $T_s$ ) occurs at 16 °C, which differs from the temperature (22 °C) determined by DSC (Wisner et al., 1986). This difference can be explained by the fact that the subtransition is dependent on the scan rate (Chen et al., 1980). The scan rate for the DSC study was 27–29 °C/h, whereas for FT-IR study the acquisition time at each temperature was ca. 1 h.

We have also collected a complete set of data for ( $S_P$ )-DPPsC. The results are essentially the same as shown in

<sup>4</sup> The nonannealed ( $R_P + S_P$ )-DPPsC in Figure 5A shows an additional small frequency shift at ca. 10 °C. The same phenomenon has also been observed for ( $S_P$ )-DPPsC but not for DPPC. The nature of this shift is unclear and has not been pursued.

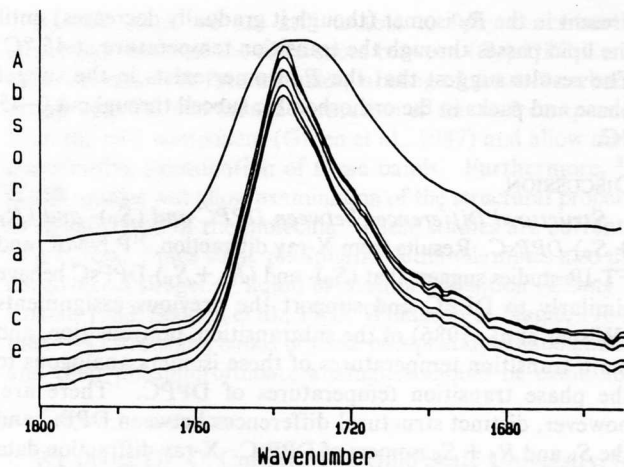


FIGURE 6: Temperature-dependent behavior of the  $\text{C}=\text{O}$  stretching band of  $(R_p)$ -DPPsC without annealing with temperatures, from bottom to top, of 3.4, 13.2, 16.0, 26.8, 42.4, and 46.3 °C.

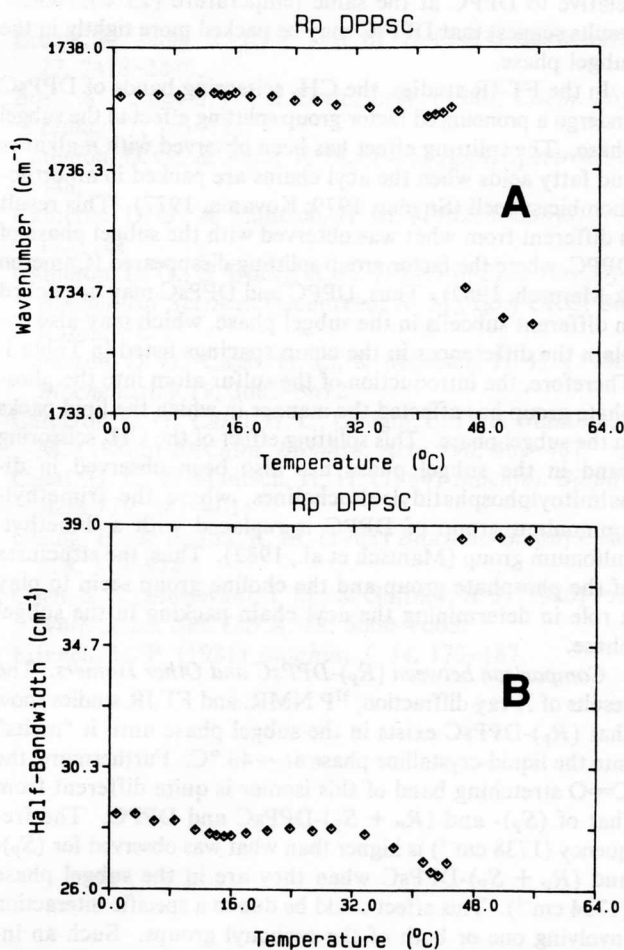


FIGURE 7: Plots of the frequency (A) and bandwidth (B) of the  $\text{C}=\text{O}$  stretching band of  $(R_p)$ -DPPsC as a function of temperature.

Figures 4 and 5; thus, the data are not shown separately.

**$\text{C}=\text{O}$  Stretching Band of  $(R_p)$ -DPPsC.** The temperature-dependent behavior of the  $\text{C}=\text{O}$  stretching band of  $(R_p)$ -DPPsC differs greatly from that of the  $S_p$  isomer. As shown in Figure 6, the peak is very asymmetric until it passes through the main transition temperature at 46 °C, where it broadens out and becomes more symmetrical. As shown in Figure 7, the transition at  $\sim 46.0$  °C causes dramatic changes in the frequency and bandwidth of the  $\text{C}=\text{O}$  stretching band, which is mainly because the *sn*-1 and *sn*-2 bands return to "normal" after the transition. Thus, the results have not only

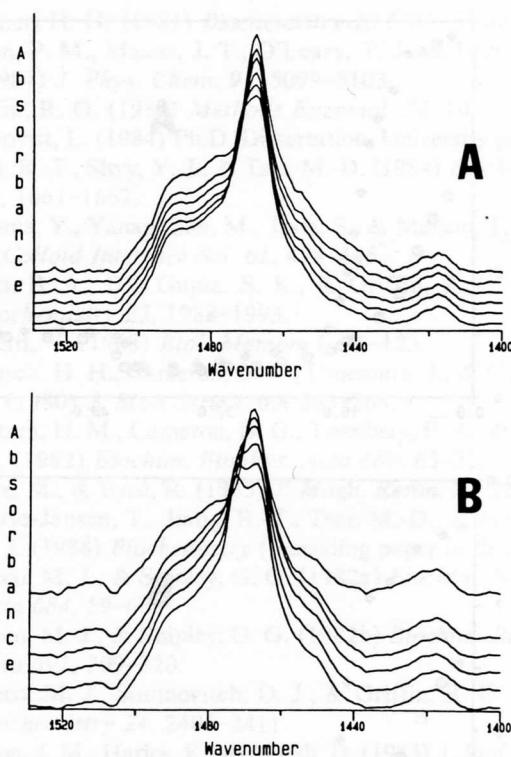


FIGURE 8: Temperature-dependent behavior of the  $\text{CH}_2$  scissoring band of  $(R_p + S_p)$ -DPPsC: (A) nonannealed with temperatures, from bottom to top, of 2.0, 6.6, 11.8, 16.7, 18.1, and 28.2 °C; (B) annealed at 4 °C for 10 days with temperatures, from bottom to top, of 1.8, 7.4, 13.4, 17.0, 18.7, and 28.2 °C.

reconfirmed the phase assignments for  $(R_p)$ -DPPsC but also identified the  $\text{C}=\text{O}$  region as a site of structural difference between this and the other isomers in the subgel phase. The unusual behavior of the  $\text{C}=\text{O}$  region could be at least partially responsible for the unusual stability of  $(R_p)$ -DPPsC.

**$\text{CH}_2$  Scissoring Band of  $(R_p + S_p)$ - and  $(S_p)$ -DPPsC.** Figure 8 shows the  $\text{CH}_2$  scissoring band of  $(R_p + S_p)$ -DPPsC under nonannealed and annealed conditions (parts A and B, respectively). It is evident that when the lipid is in the subgel phase, a pronounced splitting effect occurs, leading to bands at 1472 and 1465  $\text{cm}^{-1}$ , with the band at 1472  $\text{cm}^{-1}$  the more intense. As the lipid passes through the subtransition, the 1472- $\text{cm}^{-1}$  band decreases in intensity and shifts to a lower frequency while the 1465- $\text{cm}^{-1}$  band increases in intensity and shifts to a higher frequency, until there is only one band present at 1468  $\text{cm}^{-1}$ . This splitting effect can also be observed by measuring the apparent bandwidth at  $9/_{10}$  band height (Figure 9A). When the  $R_p + S_p$  mixture is in the subgel phase, the bandwidth becomes broader and a transition to a narrower bandwidth occurs at 16.0 °C. The presence of the two bands in the  $\text{CH}_2$  scissoring mode is known as the factor group splitting effect (Snyder, 1961) and is similar to the splitting effect seen in *n*-alkanes (Snyder, 1979) and fatty acids (Koyama et al., 1977).

The above results represent a major difference between  $(R_p + S_p)$ -DPPsC and DPPC. In the case of DPPC, the factor group splitting was observed in the gel phase and became more prominent as the temperature was lowered (Cameron et al., 1980a, b; Mantsch et al., 1980) but disappeared upon transformation of the sample to the subgel phase (Cameron & Mantsch, 1982). According to the results in Figures 8 and 9A, the factor group splitting is present in the low-temperature region ( $< 20$  °C) of nonannealed  $(R_p + S_p)$ -DPPsC, which is consistent with the behavior of DPPC. However, the splitting is greatly enhanced in the annealed sample below  $T_s$ , in con-



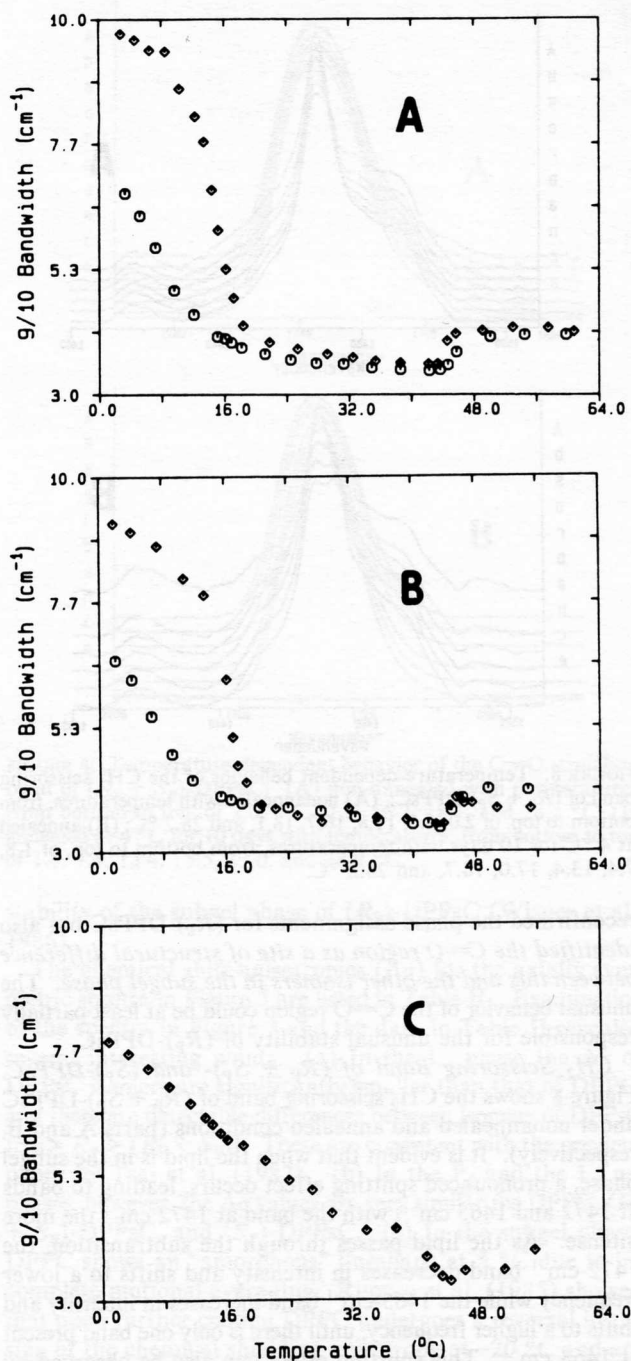


FIGURE 9: Plots of the bandwidth at  $9/_{10}$  height of the  $\text{CH}_2$  scissoring band of  $(R_p + S_p)$ -DPPsC (A),  $(S_p)$ -DPPsC (B), and  $(R_p)$ -DPPsC (C) as a function of temperature. The diamond represents the annealed lipid, and the circle represents the nonannealed sample.

trast to the result of DPPC.

As in the case of the  $\text{C}=\text{O}$  stretching band, the behavior of the  $\text{CH}_2$  scissoring band of  $(S_p)$ -DPPsC is similar to that of  $(R_p + S_p)$ -DPPsC; that is, the factor group splitting effect is also enhanced in the subgel phase. The plot of the bandwidth (at  $9/_{10}$  height) shows that when the lipid is in the subgel phase, the bandwidth increases and the subtransition occurs at  $16^\circ\text{C}$  (Figure 9B).

**$\text{CH}_2$  Scissoring Band of  $(R_p)$ -DPPsC.** The temperature-dependent behavior of the  $\text{CH}_2$  scissoring band of the  $R_p$  isomer is noticeably different from that of  $(S_p)$ - and  $(R_p + S_p)$ -DPPsC. The plot of bandwidth at  $9/_{10}$  height versus temperature (Figure 9C) shows that there is a gradual decrease in the bandwidth until the transition temperature is reached. The result indicates that the factor group splitting effect is

present in the  $R_p$  isomer (though it gradually decreases) until the lipid passes through the transition temperature at  $45^\circ\text{C}$ . The results suggest that the  $R_p$  isomer exists in the subgel phase and packs in the orthorhombic subcell throughout  $0$ – $45^\circ\text{C}$ .

#### DISCUSSION

**Structural Differences between DPPC and  $(S_p)$ - and  $(R_p + S_p)$ -DPPsC.** Results from X-ray diffraction,  $^{31}\text{P}$  NMR, and FT-IR studies suggest that  $(S_p)$ - and  $(R_p + S_p)$ -DPPsC behave similarly to DPPC and support the previous assignments (Wisner et al., 1986) of the subtransition, pretransition, and main transition temperatures of these isomers analogous to the phase transition temperatures of DPPC. There are, however, distinct structural differences between DPPC and the  $S_p$  and  $R_p + S_p$  isomers of DPPsC. X-ray diffraction data showed that the chain spacings in the subgel phase are different between DPPC and DPPsC.  $^{31}\text{P}$  NMR results suggest that DPPsC isomers are motionally very rigid in the subgel phase relative to DPPC at the same temperature ( $5^\circ\text{C}$ ). These results suggest that DPPsC may be packed more tightly in the subgel phase.

In the FT-IR studies, the  $\text{CH}_2$  scissoring bands of DPPsC undergo a pronounced factor group splitting effect in the subgel phase. The splitting effect has been observed with  $n$ -alkanes and fatty acids when the acyl chains are packed in an orthorhombic subcell (Snyder, 1979; Koyama, 1977). This result is different from what was observed with the subgel phase of DPPC, where the factor group splitting disappeared (Cameron & Mantsch, 1982). Thus, DPPC and DPPsC may be packed in different subcells in the subgel phase, which may also explain the differences in the chain spacings listed in Table I. Therefore, the introduction of the sulfur atom into the phosphate group has affected the manner in which the lipid packs in the subgel phase. This splitting effect of the  $\text{CH}_2$  scissoring band in the subgel phase has also been observed in dipalmitoylphosphatidylsulfocholines, where the trimethylammonium group of DPPC is replaced with a dimethylsulfonium group (Mantsch et al., 1982). Thus, the structures of the phosphate group and the choline group seem to play a role in determining the acyl chain packing in the subgel phase.

**Comparison between  $(R_p)$ -DPPsC and Other Isomers.** The results of X-ray diffraction,  $^{31}\text{P}$  NMR, and FT-IR studies show that  $(R_p)$ -DPPsC exists in the subgel phase until it "melts" into the liquid-crystalline phase at  $\sim 46^\circ\text{C}$ . Furthermore, the  $\text{C}=\text{O}$  stretching band of this isomer is quite different from that of  $(S_p)$ - and  $(R_p + S_p)$ -DPPsC and DPPC. The frequency ( $1738\text{ cm}^{-1}$ ) is higher than what was observed for  $(S_p)$ - and  $(R_p + S_p)$ -DPPsC when they are in the subgel phase ( $1734\text{ cm}^{-1}$ ). This effect could be due to a specific interaction involving one or both of the carbonyl groups. Such an interaction may exclude more water from the headgroup region and result in a greater extent of dehydration. This is consistent with the smaller bilayer spacing ( $56$ – $57\text{ \AA}$ ) of the subgel phase of  $(R_p)$ -DPPsC relative to DPPC and other isomers, as shown in Table I. When the temperature reaches above the main transition, this interaction is broken and the band becomes similar in appearance to the other isomers and DPPC. It is noted from Figure 7 that the transition of  $46^\circ\text{C}$  is very sharp and involves a  $>4\text{-cm}^{-1}$  decrease in the peak frequency and a  $>10\text{-cm}^{-1}$  increase in the bandwidth. This is suggestive of a hydration effect at the transition. In the liquid-crystalline phase X-ray and FT-IR data show the same behavior for isomers of DPPsC, but the  $^{31}\text{P}$  NMR data indicate different  $\Delta\sigma$  values for different isomers of DPPsC.

At this stage we are still unable to fully describe the structural nature of the differences between (*R<sub>p</sub>*)-DPPsC and other isomers. For future studies, labeling of the *sn*-2 carbonyl group with <sup>13</sup>C will induce separation of the *sn*-2 component from the *sn*-1 component (Green et al., 1987) and allow more quantitative examination of these bands. Furthermore, <sup>2</sup>H NMR studies will allow examination of the structural property at specific sites of the molecule. These studies are currently in progress. Since some phosphatidylethanolamines also undergo direct subgel → liquid-crystalline transition (Chang & Epanand, 1983; Seddon et al., 1983; Wilkinson & Nagle, 1984), the C=O stretching bands of these phosphatidylethanolamines and their phosphorothioate analogues should be examined.

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