Phospholipids Chiral at Phosphorus. Dramatic Effects of Phosphorus Chirality on the Deuterium NMR Properties of the Choline Head Group of Phospholipids in the Liquid Crystalline Phase†

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ABSTRACT: To probe the motional and conformational properties of the choline head group of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine (DPPsC), the R_p, S_p, and $R_p + S_p$ isomers of [α-D_2]DPPsC, [β-D_2]DPPsC, and [δ-D_9]DPPsC in the subgel, gel, and liquid crystalline phases were investigated with deuterium NMR, and the results were compared with those of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) labeled at the same positions. In the subgel phase (5 °C) all isomers of [α-D_2]DPPsC and [β-D_2]DPPsC displayed amorphous line shapes characteristic of a restricted and disordered motional environment, whereas [δ-D_9]DPPsC showed narrower and symmetric line shapes indicating substantial motions. For all three labeled positions the apparent line width of the R_p isomer is larger than those of S_p and $R_p + S_p$ isomers, and the amorphous line shape of the R_p isomer also persists at 25 and 35 °C, which confirm the previous observation that the R_p isomer is unusually stable in the subgel phase and suggest that the R_p isomer is more rigid than the other isomers in the choline head group. In the gel phase (25 and 35 °C) narrower and symmetric line shapes were observed for S_p and $R_p + S_p$ isomers, and the apparent line widths were comparable to those of DPPC. In the liquid crystalline phase there are dramatic differences between the spectra of DPPC and different isomers of DPPsC. For [α-D_2]DPPsC, two quadrupolar splittings with very different $\Delta\nu_Q$ values (6.5–7.1 and 1–2 kHz) were observed for both R_p and S_p isomers, which suggest significant differences in the average orientation of the two C_α–D bonds. For [β-D_2]DPPsC, four sets of quadrupolar splittings were observed for the R_p isomer and two sets for the S_p isomer, with very different $\Delta\nu_Q$ values. These were interpreted to suggest that the β-CD_2 segment of the R_p isomer exists in two slowly exchanging conformational states, and the two C_β-D bonds have different average orientation in both conformational states. The S_p isomer, on the other hand, exists in only one conformational state with the two C_β-D bonds in different average orientations. The shorter T_1 values (longer τ_c) for DPPsC relative to DPPC also suggest that the motions of the C_α–C_β segment are slower in DPPsC than in DPPC. These results indicate that the motional and conformational properties of the C_α–C_β segment of DPPsC is very sensitive to the configuration at phosphorus. Structurally, this provides strong support for noncovalent interactions between the quaternary ammonium group of choline and the phosphate group of a neighboring molecule in the bilayers of phosphatidylcholine and suggests that such interactions are important to the motion of the choline chain.

In the preferred conformation of phosphatidylcholine (PC)[1] bilayers the phosphorylcholine group aligns approximately parallel to the bilayer surface, as reviewed by Yeagle (1978). In this model the quaternary ammonium group is likely to interact noncovalently with the phosphate group of a neighboring molecule (possibly an ionic interaction). This has been supported by the following studies: (i) NOE between the *N*-methyl protons and the ^{31}P nucleus has been observed by ^{31}P NMR (Yeagle et al., 1975, 1976, 1977). (ii) T_1 relaxation studies suggested that the flexibility of the head group of DPPC lies between two extremes of a flexible propyl head group (for which no such interaction is possible) and a rigid ethanolamine head group (which could form a hydrogen bonding instead of a noncovalent interaction) (Browning, 1981). (iii) Using the R_p and S_p isomers of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine (DPPsC) (Figure 1), we have shown that the POS triad of the phosphorothioate group exists predominantly in the mesomeric form, which supports an ionic interaction between the quaternary ammonium group and the phosphorothioate group (Chang et al., 1986). In the absence of such an ionic interaction the negative charge of phosphorothioates is more likely to be localized at sulfur (Iyengar et al., 1984).

In this paper we add stereochemical evidence supporting such an interaction and demonstrate that such an interaction is very important to the conformational and motional properties of the C_α–C_β segment (see Figure 1 for labeling) of the choline chain. The rationale of the stereochemical probe using chiral thiophospholipids is as follows. Since DPPsC is a chemical analogue of DPPC, differences between the properties of DPPsC and DPPC reflect the effect of a chemical modification at the phosphate group. While this is useful and desirable information, the key point in the stereochemical approach is that, since the phospholipid bilayer is a chiral matrix and

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[1] Abbreviations: AQ, aquisition; $\Delta\nu_Q$, quadrupolar splitting; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPsC, 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine; DSC, differential scanning calorimetry; E_a, activation energy; FT-IR, Fourier-transform infrared; PC, phosphatidylcholine; T_1, spin–lattice relaxation time; TLC, thin-layer chromatography; T_m, main (gel to liquid crystalline) transition temperature; VD, variable delay.

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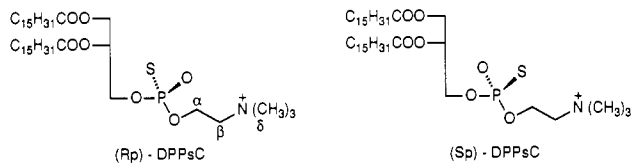

FIGURE 1: Structure of (R_p)- and (S_p)-DPPsC.

DPPsC molecules are chiral at phosphorus, interactions involving the phosphate group will be different between the two isomers. If this interaction is important to a particular structural or functional property, then the differences between R_p and S_p isomers of DPPsC can be manifested in the particular property. Thus, whenever a large difference between R_p and S_p isomers of DPPsC has been observed, it can be safely concluded that the phosphate group of DPPC plays an important role in that particular property or function.

This stereochemical approach has led us to demonstrate that the phosphate group of phospholipids is involved in the interaction with phospholipase A_2 even though it is not directly involved in the reaction (Bruzik et al., 1983; Tsai et al., 1985) and that the same interaction is absent in a functionally related enzyme lecithin-cholesterol acyltransferase (Rosario-Jansen et al., 1990). Applying this approach to the biophysical properties of lipid bilayers, we have shown that the R_p isomer of DPPsC is unusually stable in the subgel phase on the basis of DSC (Wisner et al., 1986), X-ray diffraction, ^{31}P NMR, and FT-IR studies (Sarvis et al., 1988). The FT-IR results also reveal that a key structural feature that could be related to the stable subgel phase of (R_p)-DPPsC is that its C=O stretching band is asymmetric. In the liquid crystalline phase, however, the behavior of (R_p)-, (S_p)-, and ($R_p + S_p$)-DPPsC is almost identical or only slightly different on the basis of X-ray diffraction, ^{31}P NMR, ^{14}N NMR, and FT-IR studies.

The above results seem to have covered different structural segments of the molecules, and the results taken together seem to suggest that except for a localized perturbation in the subgel phase, the structural and conformational properties of (R_p)-, (S_p)-, and ($R_p + S_p$)-DPPsC are essentially the same. However, to our great surprise, deuterium NMR studies of DPPsC labeled at the choline head group (α-D_2, β-D_2, and δ-D_9) indicated that the conformational properties of these isomers in the α–β segment of the choline chain are very sensitive to the configuration at phosphorus.

Materials and Methods

Synthesis of ^{2}H-Labeled DPPsC. The synthesis involves three major steps: (a) synthesis of specifically deuterated choline; (b) synthesis of DPPsC; (c) separation of DPPsC into R_p and S_p isomers. The specifically deuterated cholines were obtained as follows: [2,2-D_2]choline iodide (precursor of [α-D_2]DPPsC) from *N,N*-dimethylglycine ethyl ester according to Dauben and Gee (1952), [1,1-D_2]choline iodide (precursor of [β-D_2]DPPsC) from cyanoethyl benzoate according to Douglas and Burditt (1955), and [$(CD_3)_3$]choline chloride from MSD Isotopes (99 atom % enrichment). These choline halides were converted to their respective tosylate salts by using silver tosylate. Silver tosylate was synthesized by a modified procedure of Dauben and Gee (1952). In a 100-mL round-bottom flask, 1.9 g (0.012 mol) of *p*-toluenesulfonic acid monohydrate was dissolved in 8 mL of water. A solution containing 1.7 g (0.01 mol) of silver nitrate in 6 mL of water was added dropwise to the vigorously stirring *p*-toluenesulfonic acid solution. The reaction mixture was cooled to 0 °C and the white crystals of silver tosylate (>98% yield) were filtered, washed with cold acetone, and dried under high vacuum in the absence of light. The product was characterized by ^1H NMR at 200 MHz in $CDCl_3$: δ 2.26 ppm (s, 3 H, CH_3–phenyl); 7.10 ppm (d, 2 H, J = 7.4 Hz, *m*-phenyl); 7.61 ppm (d, 2 H, J = 7.4 Hz, *o*-phenyl). Aqueous solutions containing equimolar amount of the choline halide and silver tosylate were then mixed. The silver salts were filtered off and the choline tosylate was dried, recrystallized from hot acetone, and stored in vacuo. Proton NMR analysis of the choline tosylates showed that the deuterium enrichments were quantitative within the limit of detection (>97%).

The respective choline tosylates were used to synthesize the respective deuterated DPPsC by the procedure of Bruzik et al. (1986), which uses a key phosphorylating agent ClP(OMe)N(*i*-Pr)$_2$. Separation of DPPsC into R_p and S_p isomers was performed as described earlier (Bruzik et al., 1983; Bruzik & Tsai, 1991). The isomeric purity was >98% in all cases as judged by ^{31}P NMR. The samples were repetitively purified by precipitating from acetone/ethanol (10/1) until their DSC traces are identical with those described by Wisner et al. (1986).

Sample Preparation for ^2H NMR. Samples for the ^2H NMR experiments typically consisted of between 50 and 100 mg of lipid dispersed in an equal weight of ^2H-depleted water (Aldrich), which were sealed in 5-mm-o.d. glass tubes. The lipids were assured of full hydration by first heating the sample to 60 °C, mixing vigorously with a Vortex stirrer, and then cooling to room temperature. This process was repeated from five to nine times until the lipid was fully hydrated as judged by the complete dispersion of the of dry lipid in water. The lipid samples were then annealed at 0–4 °C for at least 10 days.

Upon completion of NMR experiments, samples were checked for chemical purity by TLC. No detectable hydrolysis was observed.

Deuterium NMR Methods. The deuterium NMR experiments (46.07 MHz) were performed on a Bruker MSL-300 spectrometer. ^2H NMR spectra were obtained by using a two-pulse quadrupole echo sequence, $90°_x$–τ_1–$90°_y$–τ_2–AQ (Davis et al., 1976), with a 90° pulse length of 4.5 μs and τ_1 and τ_2 of 25 and 28 μs, respectively. The recovery time between sequences was >500 ms. For spectra below T_m a typical sweep width of 625 kHz was used and between 50000 and 100000 transients were collected (depending upon the sample size). Above T_m the sweep width was typically 62.5 kHz and between 1000 and 10000 transients were collected. T_1 experiments were performed by using a modified inversion recovery pulse sequence. The recovery of magnetization after a single 180° pulse was detected by using a quadrupole echo sequence, 180°–VD–$90°_x$–τ_1–$90°_y$–τ_2–AQ. The quadrupole echo parameters were the same as described above. The value of τ_2 was different for each value of the variable delay, in order to assure a maximum echo. Because the spin–lattice relaxation rate of PC in the liquid crystalline phase is independent of orientation (Brown & Davis, 1981), the intensity of the perpendicular edge was used to provide a measure of the recovery rate. The T_1 value was obtained by a least-squares fit of the amplitude data. Phase cycling and quadrature detection were used in all NMR experiments.

Results and Discussion

[α-D_2]DPPsC below T_m. The ^2H NMR spectra of [α-D_2]DPPsC below its main transition temperature are shown in Figure 2. Although these spectra are all featureless, we list their apparent half-widths ($W_{1/2}$) in Table I for the purpose of comparison. Since all samples have been annealed at 0–4 °C for 10 days, the 5 °C spectra should represent those in the

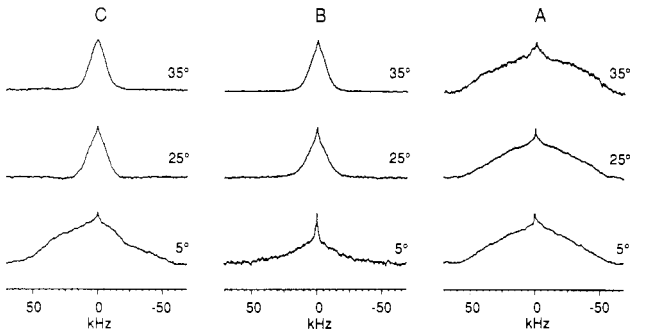

FIGURE 2: Deuterium NMR spectra of [α-D_2]DPPsC below T_m. (A) R_p isomer; (B) S_p isomer; (C) $R_p + S_p$ mixture. Specific temperatures are indicated in the figure.

Table I: Apparent Half-Widths ($W_{1/2}$, in kHz) of the ^2H NMR Spectra of DPPsC below T_m

deuteron	lipid	subgel phase 5 °C	gel phase 25 °C	gel phase 35 °C
α-CD_2	(R_p)-DPPsC	74	69	85
	(S_p)-DPPsC[a]		16	13
	($R_p + S_p$)-DPPsC	59	15	14
β-CD_2	(R_p)-DPPsC	69	73	73
	(S_p)-DPPsC	38	13	8
	($R_p + S_p$)-DPPsC	37	15	12
δ-CD_3	(R_p)-DPPsC	6.2	6.2	6.2
	(S_p)-DPPsC	3.1	2.2	0.8
	($R_p + S_p$)-DPPsC	4.2	2.2	1.9

[a] The $W_{1/2}$ of the spectrum of this sample at 5 °C cannot be determined accurately.

subgel (L_ϵ) phase (Wisner et al., 1986; Sarvis et al., 1988). The spectra at 5 °C show the amorphous line shapes characteristic of a restricted motional environment, and they are also clearly different from that expected for pure solid state. Thus in the subgel phase the choline side chain undergoes slight, restricted, and possibly disordered motions.

At 25 °C the line shapes of ($R_p + S_p$)- and (S_p)-DPPsC narrow considerably, indicating a higher degree of mobility experienced by the C–D bonds. Such narrowing is clearly a result of transition from the subgel phase to the gel phase, since the subtransition temperature of both ($R_p + S_p$)- and (S_p)-DPPsC is 22 °C (Wisner et al., 1986). The R_p isomer, on the other hand, did not show a change from 5 to 25 °C, which agrees with the previous finding that the R_p isomer does not undergo a subtransition until it reaches the main transition

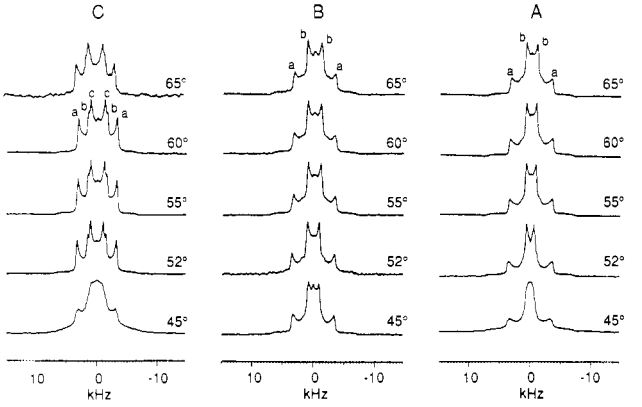

FIGURE 3: Deuterium NMR spectra of [α-D_2]DPPsC above T_m. (A) R_p isomer; (B) S_p isomer; (C) $R_p + S_p$ mixture. Specific temperatures are indicated in the figure.

temperature (Wisner et al., 1986; Sarvis et al., 1988). The spectra at 35 °C are essentially the same as those at 25 °C, except that the half-widths for ($R_p + S_p$)- and (S_p)-DPPsC are slightly reduced.

The ^2H NMR spectra of [α-D_2]DPPC below the T_m have been reported earlier by Gally et al. (1975) and recently by Ruocco et al. (1985). The line shape and $W_{1/2}$ of the spectrum of (R_p)-DPPsC at 5 °C are similar to those of [α-D_2]DPPC at −23 °C (without prior annealing) reported by Ruocco et al. (1985). The line shapes and $W_{1/2}$ for both ($R_p + S_p$)- and (S_p)-DPPsC at 25 °C agree very well with the results of [α-D_2]DPPC at 28 and 16 °C (nonannealed sample) (Ruocco et al., 1985).

[α-D_2]DPPsC above T_m. The deuterium NMR spectra of [α-D_2]DPPsC diastereomers near and above T_m (45.9, 45.0, and 44.8 °C for R_p, S_p, and $R_p + S_p$, respectively; Wisner et al., 1986) are shown in Figure 3. At 45 °C the line shapes reflect the fact that the lipids are in transition. From 52 to 65 °C all spectra assume the fast-limit powder pattern indicative of axially symmetric motions. It is important to note that the spectra of both (R_p)- and (S_p)-DPPsC show two sets of quadrupolar splittings, with $\Delta\nu_Q$ in the range 6–7 kHz for one (splitting a) and 1–2 kHz for the other (splitting b). The specific values of $\Delta\nu_Q$ are listed in Table II. The spectra of ($R_p + S_p$)-DPPsC (Figure 3C) are not exactly the superposition of the corresponding spectra of (R_p)- and (S_p)-DPPsC. Careful examination suggests that the larger splitting (a) is likely to be the superposition of the two isomers since only one

Table II: Summary of $\Delta\nu_Q$ Values (kHz) in the L_α Phase

deuteron	lipid	splitting	49 °C	52 °C	55 °C	60 °C	65 °C
α-CD_2	(R_p)-DPPsC	a	6.9	7.1	6.9	6.8	6.6
		b		1.0	1.5	1.6	1.8
	(S_p)-DPPsC	a	6.5	6.5	6.5	6.6	6.4
		b	1.7	1.9	1.9	2.2	2.2
	($R_p + S_p$)-DPPsC	a	6.4	6.4	6.4	6.3	6.2
		b	3.0	3.0	3.1	3.1	3.1
		c	2.1	2.1	2.2	2.3	2.4
β-CD_2	(R_p)-DPPsC	a	7.1	7.0	7.0	7.0	7.0
		b	1.2	1.6	1.6	1.7	1.9
		c		0.92	0.85	0.70	0.52
		d	0.55	0.31	0.19		
	(S_p)-DPPsC	a	1.5	0.81	0.70	0.55	0.44
		b		0.72	0.30	0.15	
	($R_p + S_p$)-DPPsC	a	6.5	5.7	5.8	5.6	5.6
		b	2.8	2.8	2.8	2.9	2.9
		c	1.8	1.8	1.8	2.0	2.1
		d				1.1	0.76
		e				0.43	0.32
δ-CD_3	(R_p)-DPPsC		0.44	0.44	0.41	0.33	0.22
	(S_p)-DPPsC		0.37	0.33	0.26	0.18	0.11
	($R_p + S_p$)-DPPsC		0.37	0.33	0.30	0.18	0.11

Table III: Summary of T_1 Values and Activation Energies (E_a) in the L_α Phase[a]

deuteron	lipid	splitting	T_1 (ms) 49 °C	52 °C	55 °C	60 °C	65 °C	E_a (kJ/mol)
α-CD_2	(R_p)-DPPsC	a		17.4	19.6	22.1	26.6	24.5
		b		18.2	20.1	23.9	28.4	26.7
	(S_p)-DPPsC	a	16.0	15.8	19.5	26.6	26.4	33
		b	16.2	17.7	22.1	23.8	30.8	35
	(R_p + S_p)-DPPsC	a	17.6	19.6	22.7	27.8	32.7	31.4
		b	17.4	18.3	22.4	25.9	31.7	30.9
		c	18.2	18.9	22.5	26.3	30.9	27.6
	DPPC (at 47 °C)[b]		26					30.1
β-CD_2	(R_p)-DPPsC	d		17.1	17.7	21.3		
	(S_p)-DPPsC	b		21.2	32.5	42.1		
	DPPC (at 47 °C)[b]		39					27.1
δ-CD_3	(R_p)-DPPsC			57.7	67.1	78.4	87.1	23.8
	(S_p)-DPPsC		61.2	73.2	77.7	90.5	98.8	25.5
	(R_p + S_p)-DPPsC		50.4	56.8	62.3	68.9	72.5	19.5
	DPPC (at 47 °C)[b]		91					16.7

[a] The correlation coefficients are typically >0.99 for T_1 data and >0.97 for activation energies. [b] From Browning (1981).

outer splitting is observed and its intensity is relatively high compared to that of the outer splittings in the R_p and S_p isomers. On the other hand, two inner splittings (b and c) can be observed in the spectra of (R_p + S_p)-DPPsC, with the $\Delta\nu_Q$ values larger than the corresponding values for R_p and S_p isomers (see data in Table II).

The $\Delta\nu_Q$ mentioned above is actually the splitting between the perpendicular edges of the powder pattern, and it is generally interpreted in terms of the order parameter S_{CD}, which is an indication of the "average orientation" of the C–D bond with respect to the bilayer normal (Seelig, 1977; Oldfield et al., 1978):

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD} \quad (1)$$

where $S_{CD} = \langle(3\cos^2\theta - 1)/2\rangle$ and $e^2qQ/h \approx 167$ kHz for an inert C–D bond. Since the focus of this paper is in the comparison between DPPC and isomers of DPPsC, the $\Delta\nu_Q$ values are discussed directly without conversion to S_{CD} values.

Possible Interpretations for the Inequivalence in $\Delta\nu_Q$. A possible interpretation for the existence of two quadrupolar splittings is the presence of two slow-exchanging conformational states. We believe this is not the case for two reasons: (a) If there are two stable conformational states, the motions should be quite restricted and the two deuterons are likely to have different average orientations in each conformational state. This should result in four sets of splittings instead of two. This is indeed the case for (R_p)-[β-D_2]DPPsC as described in a later section. (b) If the two sets of quadrupolar splittings are due to two sets of conformers, they are likely to converge to each other with increasing temperature unless the rate of exchange is very slow. The spectra in Figure 3 indicate that for both R_p and S_p isomers splitting a is insensitive to temperature, while splitting b increases with increasing temperature.

Thus it is more likely that there is only one conformational state (which could be an average of many rapidly exchanging conformations). According to eq 1, the two sets of quadrupolar splittings should then arise from different "average orientation" of the two C–D bonds. Such a phenomenon has been termed "motional inequivalence" of the two deuterons by Seelig and co-workers (Wohlgemuth et al., 1980; Browning & Seelig, 1981) and has also been observed for [α-D_2]DPPC, though the difference in $\Delta\nu_Q$ is only 0.3 kHz (Browning & Seelig, 1980). The $\Delta\nu_Q$ values of both splittings of [α-D_2]DPPC are ca. 6 kHz, which correspond to the larger $\Delta\nu_Q$ of [α-D_2]-DPPsC. The splittings of [α-D_2]DPPC above T_m are also temperature-independent (Gally et al., 1975), which agrees with splitting a of [α-D_2]DPPsC.

The difference between DPPsC and DPPC is undoubtedly related to the existence of a chiral phosphorus center in DPPsC. However, it is important to note that chirality itself is insufficient to induce the such inequivalence. Otherwise the inequivalence should diminish in the β-CD_2 segment, which is farther away from the chiral center. As described later the two β-deuterons are even more different than the two α-deuterons. Thus we think that *the effect of chirality is manifested by (possibly stereospecific) interactions involving the phosphate group*. This interpretation will be further elaborated in the last section.

[α-D_2]DPPsC above T_m, Spin–Lattice Relaxation Times. The magnitude of $\Delta\nu_Q$ is related to the *order* or the *average orientation*, not the *rate* of motions. the latter is related to spin–lattice relaxation time (T_1). In view of the large difference in the magnitudes of the $\Delta\nu_Q$ values for the two deuterons in DPPsC, it would be interesting to compare the T_1 values between the two deuterons of DPPsC and between DPPsC and DPPC. Table III lists the T_1 data for the liquid crystalline phase of [α-D_2]DPPsC at temperatures from 49 to 65 °C. Since of the T_1 values increase with increasing temperature, τ_c should be in the fast-time regime ($\omega_o\tau_c < 1$). The apparent activation energies (E_a) have also been calculated from the T_1 data by using the Arrhenius equation and the results are also listed in Table III.

The data in Table III indicate that for both (R_p)- and (S_p)-[α-D_2]DPPsC there are no significant differences in either T_1 values or activation energies between the two α-deuterons. On the other hand, the T_1 values at 49 and 52 °C are in the range 16–19 ms, which is smaller than the corresponding value of 26 ms for [α-D_2]DPPC at 47 °C (Browning, 1981). This suggests that the motions in the α-CD_2 segment are slower in DPPsC than in DPPC (the data of DPPC at 47 °C is used for comparison with DPPsC at 49–52 °C since the T_m of DPPC is lower than that of DPPsC by 3–4 deg). There are also small differences in E_a between isomers of [α-D_2]DPPsC and [α-D_2]DPPC, but the significance of such small differences in E_a is unclear.

[β-D_2]DPPsC below T_m. The ^2H NMR spectra of the diastereomers of [β-D_2]DPPsC below their main transition temperatures are shown in Figure 4. The spectral patterns are essentially the same as those of [α-D_2]DPPsC, except that the apparent half-widths are somewhat smaller in the spectra of [β-D_2]DPPsC. Qualitatively, these results reaffirm the phase transition properties of the isomers of DPPsC and suggest that the motions of the C_β–D bonds are slightly less

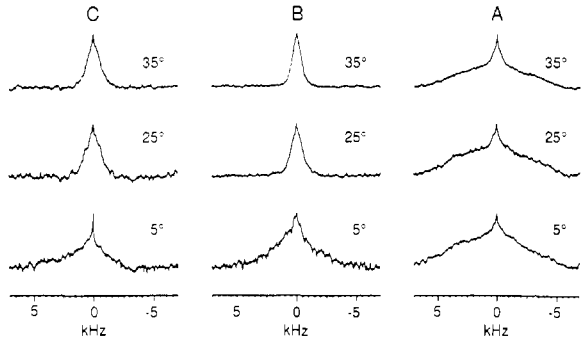

FIGURE 4: Deuterium NMR spectra of [β-D_2]DPPsC below T_m. (A) R_p isomer; (B) S_p isomer; (C) $R_p + S_p$ mixture. Specific temperatures are indicated in the figure.

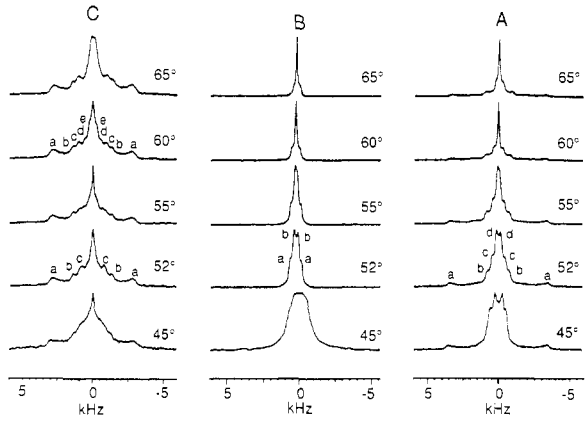

FIGURE 5: Deuterium NMR spectra of [β-D_2]DPPsC above T_m. (A) R_p isomer; (B) S_p isomer; (C) $R_p + S_p$ mixture. Specific temperatures are indicated in the figure.

restricted than the motions of the C_α–D bonds in gel and subgel phases. This does not appear to be the case in the liquid crystalline phase as discussed in the next section.

According to Gally et al. (1975), the ^2H NMR spectrum of [β-D_2]DPPC shows measurable quadrupolar splittings of 8 kHz at 25 °C, which is somewhat different from the property DPPsC.

[β-D_2]DPPsC above T_m. The ^2H NMR spectra of the diastereomers of [β-D_2]DPPsC near and above their main transition temperatures are shown in Figure 5. Again, the spectra at 45 °C are in transition and will not be interpreted. The spectral patterns at higher temperatures (52–65 °C) are dramatically different from those of [α-D_2]DPPsC, and the spectra of R_p, S_p, and $R_p + S_p$ isomers are also dramatically different from each other.

The least complicated system is the S_p isomer, which shows two sets of splittings (a and b, see Figure 5B). The $\Delta\nu_Q$ values of both splittings are all very small (<1.5 kHz) and decrease with increasing temperature, as shown in Table II. At 65 °C the inner splitting collapses into an isotropic component. Although both are temperature-dependent, they should not arise from two conformations since the two splittings are not converging into one with increasing temperature. Instead, the two sets of splittings are likely to be due to different average orientations of the two deuterons as in the case of [α-D_2]-DPPsC.

The spectra of the R_p isomer of [β-D_2]DPPsC consist of *four* sets of quadrupolar splittings (a–d), which are clearly resolved at 52 and 55 °C (Figure 5A). Since there are only two deuterons, there must be at least two different (long-lived) conformational states. Each conformational state could be either a single, rigid conformation or a time-averaged set of conformations. The two inner splittings (c and d) behave

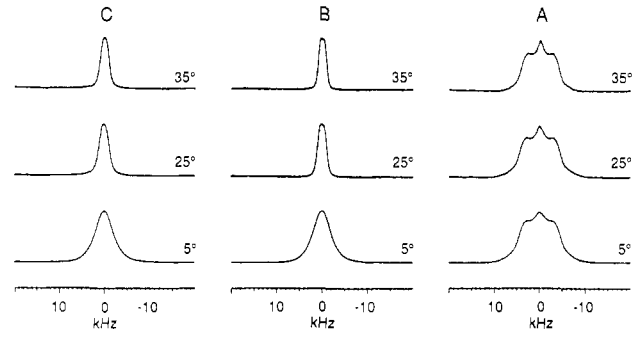

FIGURE 6: Deuterium NMR spectra of [δ-D_9]DPPsC below T_m. (A) R_p isomer; (B) S_p isomer; (C) $R_p + S_p$ mixture. Specific temperatures are indicated in the figure.

similarly to the two splittings of the S_p isomer in the magnitudes of $\Delta\nu_Q$ and their temperature dependence. Thus these two inner splittings possibly arise from one conformational state similar to that of the S_p isomer. The two outer splittings (labeled a and b) could then arise from the two deuterons of the other conformation. Since the two conformational states are not converging upon increasing temperature, they appear to be in slow exchange on the deuterium NMR time scale in the entire temperature range.

The spectra of ($R_p + S_p$)-[β-D_2]DPPsC (Figure 5C) look like the superposition of the spectra of the R_p and S_p isomers, but the magnitudes of splittings are not exactly the same. This is also the case in the ^2H NMR of [α-D_2]DPPsC as mentioned earlier and in the ^{31}P chemical shift anisotropy and the ^{14}N quadrupolar splitting of DPPsC bilayers (Tsai et al., 1983; Sarvis et al., 1988). In the spectrum at 60 °C, for example, five splittings (a–e) and an isotropic component can be identified. The $\Delta\nu_Q$ of the splittings that can be resolved are also listed in Table II.

[β-D_2]DPPC shows only one set of quadrupolar splitting above T_m, with the $\Delta\nu_Q$ value decreasing from 5.5 kHz at 42 °C to 3.8 kHz at 70 °C (Gally et al., 1975). The magnitude of the $\Delta\nu_Q$ of DPPC is similar to that of the outer splitting of (R_p)-[β-D_2]DPPsC, but the latter is temperature-independent.

[β-D_2]DPPsC above T_m, Spin–Lattice Relaxation Times. Due to the complex nature of the spectra the T_1 of [β-D_2]-DPPsC could be determined accurately only for the innermost splitting of the R_p and S_p isomers. The data are listed in Table III. The values are again smaller than the T_1 of [β-D_2]DPPC. Thus the motions in the β-CD_2 segment are also slower in DPPsC than in DPPC. In addition, the T_1 values of the R_p isomer are smaller than those of the S_p isomer, which suggests that the motions in the β-CD_2 segment are slower in the R_p isomer than in the S_p isomer.

[δ-D_9]DPPsC below T_m. The ^2H NMR spectra of the diastereomers of [δ-D_9]DPPsC below their main transition temperatures are shown in Figure 6, and their apparent half-widths are also listed in Table I. In the subgel phase (5 °C), the spectral patterns suggest that there are substantial motions in the CD_3 groups and that the motions of the CD_3 groups are more restricted in the R_p isomer. This is also the case for the α-CD_2 and the β-CD_2 segments as described earlier.

The NCD_3 group of DPPC shows a quadrupolar splitting of ca. 2 kHz from 15 to 38 °C (Gally et al., 1975), which is similar to the apparent half-widths of 2.2 kHz for (S_p)- and ($R_p + S_p$)-[δ-D_9]DPPsC at 25 °C.

[δ-D_9]DPPsC above T_m. The ^2H NMR spectra of the diastereomers of [δ-D_9]DPPsC above their main-transition temperatures are shown in Figure 7. The $\Delta\nu_Q$ values are quite

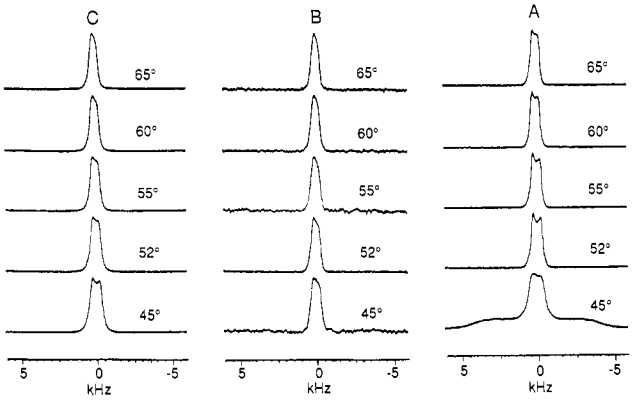

FIGURE 7: Deuterium NMR spectra of [δ-D_9]DPPsC above T_m. (A) R_p isomer; (B) S_p isomer; (C) $R_p + S_p$ mixture. Specific temperatures are indicated in the figure.

small and decrease with increasing temperatures (see Table II). There are no significant differences between the two diastereomers, except that the $\Delta\nu_Q$ values are slightly larger for the R_p isomer. The $\Delta\nu_Q$ values for the NCD_3 group of DPPC (ca. 1 kHz) are larger than those of DPPsC and decrease only slightly with increasing temperature (Gally et al., 1975).

The T_1 values and activation energies are listed in Table III. The T_1 values at ca. 50 °C are in the range 50–60 ms, which is smaller than the corresponding value of 91 ms for DPPC (Browning, 1981) and thus supports that the motions in the δ-CD_3 segment are also slower in DPPsC than in DPPC.

Implications on the Structure of PC Bilayers. In this section we summarize the results and discuss their implications on the structure of PC bilayers. In the subgel (L_ϵ) phase motions of the choline chain are more restricted in (R_p)-DPPsC than in (S_p)-DPPsC on the basis of apparent half-widths. This could be related to the unusual stability of the subgel phase of the R_p isomer. In the liquid crystalline (L_α) phase there are two significant findings: (a) There are two quadrupolar splittings with large differences in $\Delta\nu_Q$ for (R_p)- and (S_p)-[α-D_2]DPPsC and (S_p)-[β-D_2]DPPsC and four quadrupolar splittings for (R_p)-[β-D_2]DPPsC. (b) The T_1 data suggest that the motions related to T_1 relaxation are generally slower in DPPsC than in DPPC (i.e., τ_cs are larger for DPPC) and that such motions are particularly slow in the β-CH_2 segment of (R_p)-DPPsC. We think both findings support interactions between the phosphate group and the ammonium group as discussed below.

The fact that the deuterium NMR properties of α-CD_2 and β-CD_2 are different between R_p and S_p isomers of DPPsC and DPPC suggests that the effects are related to the phosphate group. In other words, the chirality at phosphorus is manifested through stereospecific interactions to produce the dramatic effects on the motional and conformational properties of the C_α–C_β segment of the choline side chain of DPPsC. Since the effects are larger on the β-CD_2 segment than the α-CD_2 segment, they are likely to be induced by molecular (and most likely stereospecific) interactions involving both the phosphate group and the ammonium group, not simply by the chirality at phosphorus (otherwise the effects should diminish at the β-position). Thus, although phospholipid molecules are undergoing rapid lateral motions, the transient interactions between the ammonium ion and the phosphorothioate group of a neighboring molecule could dictate the conformation of the choline group.

Such interactions should also occur in the natural phospholipid bilayers. Since the two nonbridging oxygen atoms are diastereotopic, interaction of the ammonium group with the *pro-R* and the *pro-S* oxygen should be different intrinsically. However, in natural DPPC bilayers the two types of interactions are likely to be in rapid exchange, or the interaction could be fully delocalized between the two nonbridging oxygens. When an oxygen atom is substituted by a sulfur atom, the R_p and S_p isomers of DPPsC are nonexchangable, thus the differences between the two stereospecific interactions can be detected. Replacement of a nonbridging oxygen by a sulfur atom could also hinder the rapid exchange of the quaternary ammonium ion between the two nonbridging oxygens (now oxygen and sulfur), slow down the motion of the choline side chain, and result in detectable differences in average orientations and detectable multiple conformations for DPPsC.

According to the results in this paper, the motions of the C_α–C_β segment appear to be sensitive to such interactions, but such interactions may not necessarily cause differences in the motional properties of the phosphate group and the ammonium group. The ^2H NMR property of the δ-CD_3 groups is quite insensitive to the configuration at phosphorus. The chemical shift anisotropy ($\Delta\sigma$) in ^{31}P NMR is indicative of the motional averaging in the phosphate group, and $\Delta\sigma$ values of 36, 39, and 31 ppm have been reported for the L_α phase of (R_p)-, (S_p)-, and ($R_p + S_p$)-DPPsC, respectively (Sarvis et al., 1988). For ^{14}N NMR, the quadrupolar splittings of R_p and S_p isomers are only slightly different (7.5 and 7.9 kHz, respectively).

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Interaction of a Macrocyclic Bisacridine with DNA†

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ABSTRACT: The binding of the macrocycle SDM to DNA was investigated by visible spectroscopy, stopped-flow kinetics, and NMR spectroscopy. SDM is composed of two 9-aminoacridines linked via the amino groups by a spermine side chain and via the 4-positions by a *N,N'*-[(methylthio)ethyl]succinamide side chain [Zimmerman, S. C., Lamberson, C. R., Cory, M., & Fairley, T. A. (1989) *J. Am. Chem. Soc. 111*, 6805-6809]. The visible spectrum of SDM bound to poly[d(A-T)]$_2$ or poly[d(G-C)]$_2$ is red-shifted relative to the spectrum of SDM alone and displays considerable hypochromicity. Results from titrations of SDM with polymer indicate a binding site size of three base pairs per macrocycle. The dissociation constant for SDM bound to either poly[d(A-T)]$_2$ or poly[d(G-C)]$_2$ is an order of magnitude lower than that for a similar bisacridine linked only by a spermine side chain. In addition, the dependence of the dissociation constant on ionic strength is significantly reduced. NMR studies of SDM complexes with poly[d(A-T)]$_2$ or a tetramer, d(CGCG)$_2$, show that intercalation is the mode of binding. The magnitudes of the chemical shift differences for SDM aromatic protons in the free and bound states support intercalation with the acridine ring systems essentially parallel to the long axis of the base pairs. Cross peaks from NOESY spectra of the SDM complex with d(CGCG)$_2$ further support this mode of binding and provide information on the structure of the complex. The results are analyzed for consistency with each of three binding models: (i) bisintercalation with the two side chains in the same groove; (ii) bisintercalation according to the neighbor-exclusion principle with the two side chains in opposite grooves; and (iii) bisintercalation with two side chains in opposite grooves but with violation of the neighbor-exclusion principle. Model i is found to be unlikely on the basis of all evidence obtained, including preliminary modeling studies. Both models ii and iii can be reconciled with the experimental evidence and from a modeling standpoint are energetically feasible.

The classical view of DNA as a static B-form structure has been modified by high-resolution structural studies which have shown that the double helix geometry is sensitive to both local environment and DNA sequence (Saenger, 1984; Wells & Harvey, 1987; Blackburn & Gait, 1990). Similar studies of reversible DNA complexes with aromatic molecules have recently illustrated an even wider array of polymorphic behavior (Scott et al., 1988; Gao & Patel, 1989; Pelton & Wemmer, 1990; Wilson, 1990; Wilson & Li, 1990). The classical paradigms for intercalation and minor groove complexes are being substantially expanded by the discovery of interactions which show significant variations with ligand structure and DNA sequence.

Many unique interactions occur in DNA complexes with bisintercalators, and the development of additional bisintercalators and polyintercalators is of interest for several reasons. These compounds can bind to DNA with high affinity and be very specific in their base pair recognition (Wakelin, 1986; Laugaa et al., 1987; Hurley & Boyd, 1987; Gao & Patel, 1989). Some natural and synthetic bisintercalators have substantial anticancer activity (Wakelin, 1986; Delbarre et al., 1987; Hurley & Boyd, 1987; Jaycox et al., 1987; Gao & Patel, 1989). In addition, they can provide probes for large-amplitude DNA dynamics and may also serve as models for some types of protein-DNA interactions (Suzuki, 1990). In ways not possible for simple monointercalators, bisintercalators can induce substantial changes in DNA conformation and base pairing such as Watson-Crick to Hoogsteen base pairing in their complexes (Wakelin, 1986; Hurley & Boyd, 1987; Gao & Patel, 1988, 1989). Bisintercalators have also been reported to bind in violation of the phenomenological neighbor-exclusion principle (Atwell et al., 1985; Wakelin, 1986).

When the aromatic rings of bisintercalators are connected by more than one linking chain, they form macrocyclic systems which may bind to DNA by bisintercalation only if extensive base pair disruption occurs. The macrocyclic bisacridine SDM (Figure 1) has recently been designed and synthesized by Zimmerman et al. (1989). Preliminary physical studies using hydrodynamic, spectroscopic, and thermal-melting methods indicated that the macrocycle binds to DNA in a manner similar to a bisacridine connected by a single spermine chain, SPDA (Figure 1), which is an established bisintercalator

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