Takano, T., Trus, B. L., Mandel, N., Mandel, G., Kallai, O. B., Swanson, R., & Dickerson, R. E. (1977) *J. Biol. Chem. 252*, 776.
Urbanski, G. J., & Margoliash, E. (1977a) *Immunochemistry of Enzymes and Their Antibodies* (Salton, M. R. H., Ed.) p 204, Wiley, New York.
Urbanski, G. J., & Margoliash, E. (1977b) *J. Immunol. 118*, 1170.
White, T. J., Ibrahimi, I. M., & Wilson, A. C. (1978) *Nature (London) 274*, 92.
Zimmerman, S. S., & Scheraga, H. A. (1978a) *Biopolymers 17*, 1849.
Zimmerman, S. S., & Scheraga, H. A. (1978b) *Biopolymers 17*, 1871.
Zimmerman, S. S., Pottle, M. S., Nemethy, G., & Scheraga, H. A. (1977) *Macromolecules 10*, 1.

Effects of the Replacement of a Double Bond by a Cyclopropane Ring in Phosphatidylethanolamines: A ^2H NMR Study of Phase Transitions and Molecular Organization†

Bruno Perly,‡ Ian C. P. Smith,§ and Harold C. Jarrell*,§

Département de Physico-chimie, CEN de Saclay, 91191 Gif-sur-Yvette Cédex, France, and Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6
Received July 3, 1984

ABSTRACT: The thermotropic behavior and molecular properties of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphoethanolamine (PDSPE) have been investigated by ^2H NMR spectroscopy using samples selectively labeled at the 5′-, 9′-, 10′-, and 16′-positions of the *sn*-2 chains. Comparison with the corresponding phosphocholine analogues (POPC and PDSPC), obtained as intermediate synthetic products, was used to monitor the role of the polar head group. Replacement of the choline moiety by ethanolamine increased the gel to liquid-crystal transition temperature by 10–32 °C and led to a significantly higher ordering of the fatty acyl chains in the liquid-crystalline bilayer state. The lateral compression effect, due to the smaller area per polar head group in PE, results in a bilayer to hexagonal phase transition at elevated temperatures. The effects on both PC and PE due to replacement of the olefinic group by a cyclopropane unit are similar. A decrease in the temperature of the gel to liquid-crystal phase transition, T_c, is observed upon introduction of a cyclopropane ring; it goes from 26 °C in POPE to ≈10 °C in PDSPE. In addition, a very significant broadening of the transition profile is observed. These observations are consistent with the poor packing ability of mixed saturated and cyclopropane-containing chains due to the bulky substituent effect. The temperature of the bilayer–hexagonal phase transition of PE samples was decreased by 15–20 °C on replacement of oleoyl chains by dihydrosterculoyl chains at the *sn*-2 position. These effects of dihydrosterculic acid have been tentatively correlated with the observation that most of the olefinic chains are replaced by the cyclopropane analogue when certain microorganisms enter the stationary phase of their growth cycle or respond to differing environmental conditions. According to what is observed in biologically relevant model compounds, it may be assumed that this transformation will prevent the lipid molecules from entering the nonviable gel state as well as allow the membrane to contain small proportions of lipid molecules in nonbilayer structures. It also confers a lesser temperature sensitivity of the acyl chain order parameter.

Fatty acids containing a cyclopropane unit are found in a large variety of microorganisms and protozoa where they may occur as permanent constituents of the lipids, such as in *Lactobacillus plantarum* and *Salmonella typhimurium* (Christie, 1969), or, more frequently, appear at a given stage of the growth cycle. For example, in *Escherichia coli*, most double bonds of the fatty acid chains are converted into the cyclopropane-containing analogue when the bacteria enter the stationary phase (Goldfine, 1972). The biological reasons for this energy-consuming transformation are not understood, and very little research has been devoted to this class of fatty acids. The first attempt to define the role of these structures on membrane organization was performed by Silvius & McElhaney (1979) using model phosphatidylcholines (PC) containing two identical fatty acid chains (either olefinic or containing a cyclopropane ring). The cyclopropane moiety was found to raise the gel to liquid-crystal transition temperature (T_c) by some 15 °C for cis systems. However, since naturally occurring phospholipids generally contain mixed acyl chains, one saturated (located mainly at the *sn*-1 position) and one unsaturated, we have investigated model systems with this type of structure. A ^2H NMR investigation of phosphocholine (PC) bearing a cyclopropane-containing fatty acyl chain (Dufourc et al., 1983) established that the effects of replacing an olefinic group with a cyclopropane ring in a mixed-chain system are significantly different from what had been previously observed on PC containing a homogeneous acyl chain composition (Silvius & McElhaney, 1979).

In the present paper, we shall now consider phosphatidylethanolamines (PE) since they represent a ubiquitous class of

† Issued as NRCC Publication No. 23738.
‡ CEN de Saclay.
§ National Research Council of Canada.

0006-2960/85/0424-1055$01.50/0

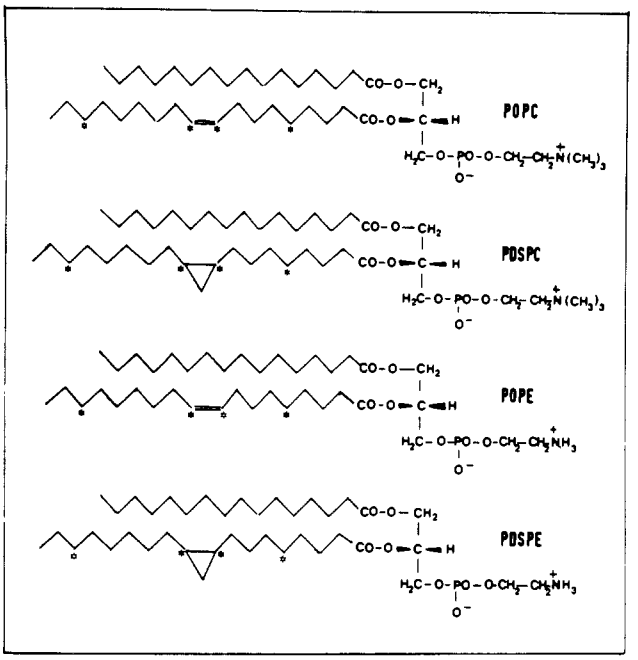

FIGURE 1: Chemical structures of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine (PDSPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), and 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphoethanolamine (PDSPE).

lipids, including some 80% of the total membrane lipids in *E. coli* (Gally et al., 1980). This is true as well as for many viral and mammalian cells (Rothman & Lennard, 1977). In addition, cyclopropane-containing fatty acyl chains appear in the PE fraction of membrane lipids (Christie, 1969). For this purpose, we have synthesized the four following phospholipids, the structures of which are shown in Figure 1: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine (PDSPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), and 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphoethanolamine (PDSPE). The corresponding PC derivatives have already been studied extensively (Dufourc et al., 1983), and the data provide the basis for useful comparisons. Deuterium NMR (^{2}H NMR) has been demonstrated to be an excellent tool to investigate both phase transitions and molecular organization in model systems (Davis, 1983) as well as in natural membranes (Smith, 1984) and will be used throughout this study. To probe the lipid organization at all depths in the bilayer, four carbon atoms of the lipid *sn*-2 chains were selectively labeled, i.e., the 5′-, 9′-, 10′-, and 16′-positions; with these four positions, the properties of the various regions of the acyl chain are probed.

Materials and Methods

Synthesis of Phospholipids. Detailed procedures for the improved synthesis of labeled PC and PE have been reported elsewhere (Perly et al., 1984) and will only be outlined here. Selectively deuterated oleic acid samples were a generous gift from Dr. A. P. Tulloch. Dihydrosterculic acid (*cis*-9,10-methyleneoctadecanoic acid) and the deuterated analogues were prepared from the corresponding oleic acids by using known procedures (Jarrell et al., 1983). 1-Palmitoyl-*sn*-glycero-3-phosphocholine (lyso-PC) was purchased from Calbiochem (San Diego, CA) and was found to be at least 98% pure by thin-layer chromatography (TLC) and ^{1}H NMR. Reacylation of the lyso-PC with fatty acid anhydrides derived from labeled oleic or dihydrosterculic acids was performed in chloroform using *N,N*-dimethyl-4-aminopyridine as catalyst. Yields of >90% of pure PC were consistently obtained after purification on silica gel (Bio-Sil A from Bio-Rad). Conversion of PC to PE was achieved by using head-group exchange catalyzed by phospholipase D, the latter obtained as a crude preparation from savoy cabbage according to standard procedures (Davidson & Long, 1958). After extensive purification, the pure phospholipids were freeze-dried from dilute suspensions in water and stored as powders under nitrogen at −20 °C.

Calorimetry was performed on a Microcal MC-1 differential scanning calorimeter (DSC) with a temperature scanning rate of 1.0 °C/min. The gel to liquid-crystal transition of fully hydrated dipalmitoylphosphatidylcholine was used to calibrate the temperature scale. Samples weighed approximately 2 mg and were dispersed in 1.5 mL of water.

Sample Preparation. A solution of the lipid in chloroform–methanol (2:1 v/v) was concentrated to dryness under a stream of dry nitrogen at room temperature, leaving a film which was then hydrated with a 5-fold excess of deuterium-depleted water (Aldrich, Milwaukee, WI). A lipid dispersion was obtained by extensive vortexing followed by three freeze–thaw cycles. Water and residual traces of organic solvents were removed by freeze-drying. The resulting fluffy powder was finally hydrated with a known amount of deuterium-depleted water, and the sample was sealed under vacuum. In the case of PE samples, heating at 70 °C for 1 h is required to obtain reproducible results. This treatment did not affect the integrity of pure PE, as shown by TLC analysis. In all cases, the weight ratio of water to lipid was 1:1 and 3:1 for PE and PC, respectively.

Deuterium NMR Experiments. ^{2}H NMR spectra were obtained at 46.06 MHz on a Bruker CXP 300 spectrometer with a home-built variable-temperature probe with a $\pi/2$ pulse length of 4–5 μs. Spectra were acquired by using the quadrupolar echo sequence (Davis et al., 1976) with extensive phase cycling of the radio-frequency pulses to remove extraneous signals and to compensate for pulse imperfections (Griffin, 1981). Spectra were acquired with quadrature detection, the radio-frequency pulse being applied exactly on resonance so that subsequent folding of the symmetrical powder pattern about its center increased the signal to noise ratio by $\sqrt{2}$. Folded and unfolded spectra were compared to ensure that no artifacts were introduced by the folding procedure. Delay times in the quadrupolar echo were selected to ensure proper definition of the echo maximum (typically 50–60 μs between the two $\pi/2$ pulses). A spectral width of 250 kHz was used (1 MHz for gel-state lipids), 2048 data points being collected in the free introduction decay (FID). The recycle delay was 0.1 s for the 5′- and 9′-,10′-labeled positions and 0.3 s for the 16′-deuterons. In all cases, separate T_1 measurements were performed and showed that the recycle times were long enough to ensure complete longitudinal relaxation. The raw data were transferred to a Nicolet 1280 computer for further processing and calculations. In most cases, an oriented-sample spectrum (θ = 90° dePaked spectrum) was extracted from the powder pattern by using the procedure introduced by Bloom and coworkers (Bloom et al., 1981). The calculation was performed on 300–600 data points of the Fourier-transformed spectrum. Line-width and frequency measurements were performed on the "dePaked" spectrum by using the curve-fitting routine provided by the Nicolet software package.

^{2}H NMR Background Definitions. Since the theory and application of deuterium NMR to this type of system are well documented (Jarrell & Smith, 1983; Davis, 1983; Seelig,

1977), only a few definitions and comments will be outlined here. In bilayer systems, in which the lipid molecules in the liquid-crystalline phase undergo rapid axially symmetric motion about the director of motion, usually assumed to be the bilayer normal, the observed quadrupolar splitting, $\Delta\nu$, is

$$\Delta\nu = \frac{3}{2}A_Q\frac{3\cos^2\theta - 1}{2}S_{CD} \quad (1)$$

where θ is the angle between the external magnetic field direction and the director. A_Q, the static quadrupolar coupling constant, e^2qQ/h, is known to be 170 kHz for a normal, sp^3, C–^{2}H bond (Burnett & Muller, 1971), 175 kHz for an olefinic group (Kowalewski et al., 1976), and 183 kHz for the cyclopropane unit (Dufourc et al., 1983). In unoriented samples, there is a random distribution of director orientations relative to the external magnetic field direction, yielding a powder pattern having two distinct sharp peaks corresponding to θ = 90°. The observed splitting between these peaks, $\Delta\nu_Q$, is

$$\Delta\nu_Q = \frac{3}{4}\frac{e^2Qq}{h}S_{CD} \quad (2)$$

In relations 1 and 2, the bond order parameter, S_{CD}, is defined as

$$S_{CD} = \left\langle\frac{3\cos^2\beta - 1}{2}\right\rangle \quad (3)$$

where β is the angle between the director axis and the C–^{2}H bond. The angular brackets denote an average over periods of time which are short relative to the quadrupolar interaction. The bond order parameter, S_{CD}, may be given by (Dufourc et al., 1983; Oldfield et al., 1978)

$$S_{CD} = \left\langle\frac{3\cos^2\alpha - 1}{2}\right\rangle\left\langle\frac{3\cos^2\gamma - 1}{2}\right\rangle \quad (4)$$

where γ represents the angle between the C–^{2}H bond direction and the instantaneous segmental chain orientation and α is the angle between the instantaneous segmental chain orientation and the director of motion (often the bilayer normal). The term $\langle 3\cos^2\alpha - 1\rangle/2$ is frequently referred to as the molecular order parameter, S_{mol}.

Results and Discussion

Gel to Liquid-Crystal Transition Temperatures (T_c). Deuterium NMR and differential scanning calorimetry (DSC) were used to monitor the gel to liquid-crystal transition temperatures of the aqueous phospholipid suspensions. Gel-state lipids are not easily visualized by ^{2}H NMR since they frequently give rise to very broad, poorly resolved spectra, rather than the sharp axially symmetric powder patterns observed for lipids in the liquid-crystalline state. Figure 2 shows a typical temperature profile for POPE and PDSPE.

The transition from the liquid-crystalline phase to the gel phase is conveniently monitored by the second moments, M_2, of the ^{2}H NMR spectra. For the symmetric spectra, M_2 is given by

$$M_2 = \frac{\int_0^\infty \omega^2 F(\omega)\,d\omega}{\int_0^\infty F(\omega)\,d\omega}$$

where $F(\omega)$ is the line-shape function and ω is the frequency relative to the Larmor frequency (Davis, 1983). The appearance of gel phase lipid is characterized by a sharp increase in M_2. The values of M_2 for the series of spectra shown in Figure 2 are presented in the inset of Figure 2.

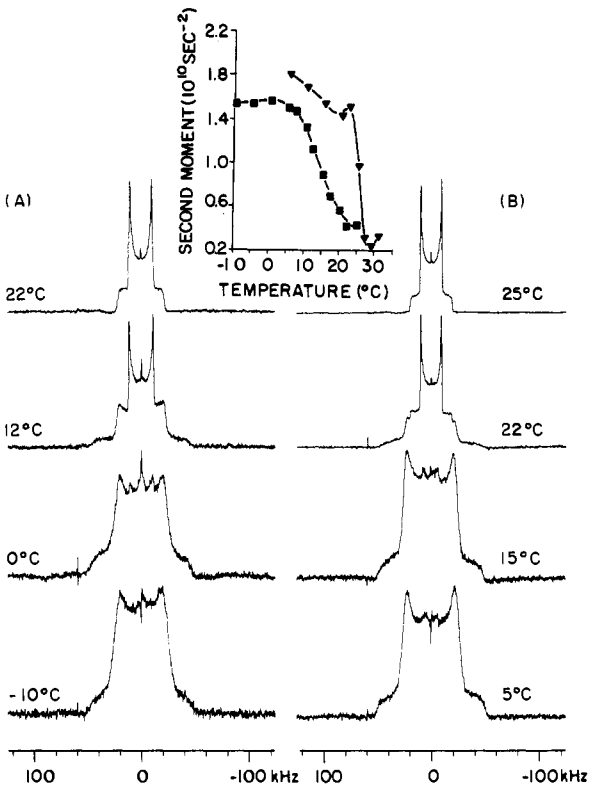

FIGURE 2: Gel to liquid-crystal transition of (A) [16-$^2H_2$]PDSPE and (B) [16-$^2H_2$]POPE, as monitored by ^{2}H NMR spectroscopy at 46 MHz. The inset shows the second moments of spectra in (A) (■) and in (B) (▼). The spectra were not folded about the Larmor frequency.

Table I: Gel to Liquid-Crystal Transition Temperatures (T_c) of Fully Hydrated Lipids As Determined by ^{2}H NMR

lipid	T_c (°C) obsd	T_c (°C) lit.	app width (°C)
POPC	−5	−5[a]	2[a]
PDSPC		−10[b]	≃30[b]
POPE	26	27[c]	3[c]
PDSPE	10		≃25

[a] Seelig & Waespe-Sărčevič (1978). [b] Dufourc et al. (1983). [c] Ghosh & Seelig (1982).

The temperatures of the gel to liquid-crystalline phase transitions for the relevant PE and PC species are given in Table I. The first observation to be made deals with the effect of the polar head group. For systems with identical fatty acyl chain composition, the transition from gel to liquid-crystalline state occurs at significantly lower temperatures in PC as compared to PE. For example, T_c is 26 °C for POPE but −5 °C for POPC. Similar trends are observed for dihydrosterculic acid containing lipids, although a precise determination of the transition midpoint is made very difficult by the excessive width of the transition (vide infra). This behavior is consistent with literature data [see, for example, Van Dijck et al. (1976) and Dekker et al. (1983)] and is clearly due to the smaller area per polar head group in PE as compared to PC. The smaller head group leads to a larger lateral compression of the lipid molecules and can even be observed on the CH_2 groups of the polar heads in the gel state (Seelig & Gally, 1976).

Another striking difference (Table I and Figure 2) concerns the respective effects of the oleoyl and dihydrosterculoyl moieties on the width of the transition. In oleic acid-containing lipids (PE and PC), very sharp transitions are observed, the

apparent width being of the order of 2–3 °C as measured by DSC and 7–10 °C by ^2H NMR (Figure 2). However, in lipids containing the dihydrosterculoyl unit, very broad profiles are observed spreading over 25 °C or more. A less cooperative transition seems to take place, and a progressive change from the liquid-crystalline state toward the gel phase seems likely to occur in these systems. This behavior has been carefully monitored in PC samples (Dufourc et al., 1983). In PC with two identical cyclopropane-containing fatty acids, Silvius & McElhaney (1979) also reported a significant broadening of the transition relative to that of the olefinic analogues, but the observed width was only about 10 °C. In our case, even using very slow cooling rates, much broader transitions were observed. In terms of transition temperatures, introduction of the cyclopropane units decreases T_c by about 17 °C in PE and only 5–10 °C in PC relative to the corresponding oleoyl analogue. This contrasts with PC containing two identical chains, where replacement of the oleoyl moieties by their cyclopropane-containing analogue *increased* the transition temperature by ca. 15 °C for cis systems. We observe the converse in the mixed-chain systems, which resemble more closely the naturally occurring membrane lipids. One may speculate that since the methylene positions of the *sn*-1 and *sn*-2 chains are probably not at equivalent depths in the bilayer (by analogy with dipalmitoylphosphatidylcholine) (Büldt et al., 1979), the disruptive effect of the bulky ring structure is not cumulative. The individual effects may be self-compensating, leading to improved packing of the acyl chains relative to the mixed acyl chain systems. In the case of PDSPC, the palmitoyl chain properties (ordering) are relatively insensitive to the presence of the cyclopropane ring, suggesting that there is no special conformation change required to accommodate the cyclopropane ring on the neighboring acyl chain (Dufourc et al., 1984).

Inspection of Figure 2 reveals that at −10 °C for PDSPE and 5 °C for POPE labeled at C-16 of the *sn*-2 chain, the gel phase lipid gives rise to axially symmetric spectra having $\Delta\nu_Q$ values of 46.6 and 50.4 kHz, respectively. At the same temperatures, the corresponding lipids labeled at C-5 give rise to a superposition of spectra which are characteristic of lipid in the liquid-crystalline phase (minor) and in the gel state (Figure 3). The spectra of gel phase lipid are not characteristic of axially symmetric motion. If the acyl chains were in the all-trans conformation and were undergoing rapid diffusion about the molecular long axis, a $\Delta\nu_Q$ value of ca. 63 kHz would be expected. It appears that in the gel state the acyl chain at the C-16 methylene position is undergoing axially symmetric motion in both PE systems with angular fluctuations such that the quadrupolar interaction is averaged further to give a value less than 63 kHz; although a tilting of the C–^2H bonds with respect to the segmental axis of motion cannot be disproved, it seems rather fortuitous that both PE systems would have similar tilt angles. It is interesting to note that while only lipid in one motional environment is observed for the C-16 methylene group at least two environments are detected at the C-5 position. The difference in behavior of the two segments of the acyl chain reflects the fact that the phase transition is less cooperative than that of DPPC (for example) and that different regions of an acyl chain may have drastically different dynamical properties.

Bilayer to Hexagonal Transition. The transition from a lamellar structure to an inverted hexagonal structure (H_{II}) is not observed for PC but is well documented for PE and some other lipids (Cullis & de Kruijff, 1979). In the hexagonal structure, the lipid molecules diffuse rapidly about the long

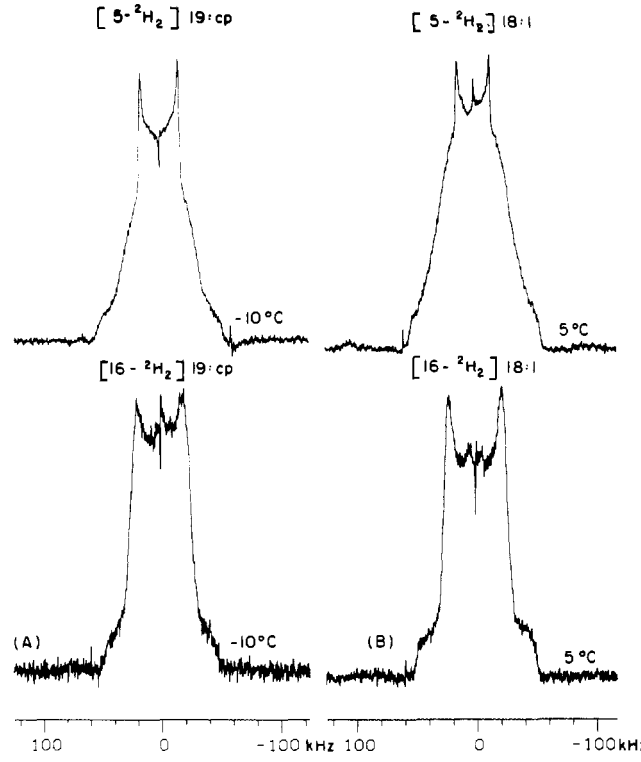

FIGURE 3: ^2H NMR spectra at 46 MHz: (A) PDSPE at −10 °C; (B) POPE at 5 °C. Spectra were acquired as described under Materials and Methods but are not folded about the Larmor frequency.

axis of the hexagonal cylinders which, if all other structural and motional parameters remain invariant, leads to a reduction by a factor of 2 in the value of the quadrupolar splitting (Seelig, 1977). The onset of the transition from a lamellar structure to the inverted hexagonal structure is reflected by the superposition of two axially symmetric spectra with the ratio of corresponding quadrupolar splittings being roughly equal to 2; this, of course, assumes that exchange of lipid between the two coexisting structures is occurring at a rate which is less than the reciprocal of the differences in the respective quadrupolar splittings. The large expected difference in the value of $\Delta\nu_Q$ for the two structures facilitates the detection of small fractions of lipid in the hexagonal phase. Furthermore, dePaking of the composite powder spectrum allows quantitation of the proportions of the coexisting phases, assuming that relaxation effects are negligible. Since the acyl chain is being monitored in these experiments, the assignment of the macroscopic structure of the lipid aggregate by ^2H NMR should be less ambiguous than using ^{31}P NMR (Thayer & Kohler, 1981). However, X-ray diffraction as well as ^{31}P NMR spectroscopy has been used to monitor the aggregate structure of these systems and will be reported elsewhere (B. Perly et al., unpublished results).

Figure 4 shows typical ^2H NMR spectra associated with the bilayer to hexagonal structural transition for [9′,10′-$^2H_2$]PDSPE; other positions have been monitored as well and yield identical results (spectra not shown). In all cases, for a given lipid (POPE or PDSPE), identical transition temperatures (T_{BH}) were obtained, whatever the site labeled. POPE undergoes a bilayer to hexagonal structural transition between 55 and 75 °C with a midpoint of 65 °C while the corresponding transition of PDSPE occurs between 38 and 58 °C and is centered at 48 °C. In addition, a sample hydrated with a 10-fold increase in the amount of water or with tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl) buffer (pH 7.4) (data not shown) gave results identical with

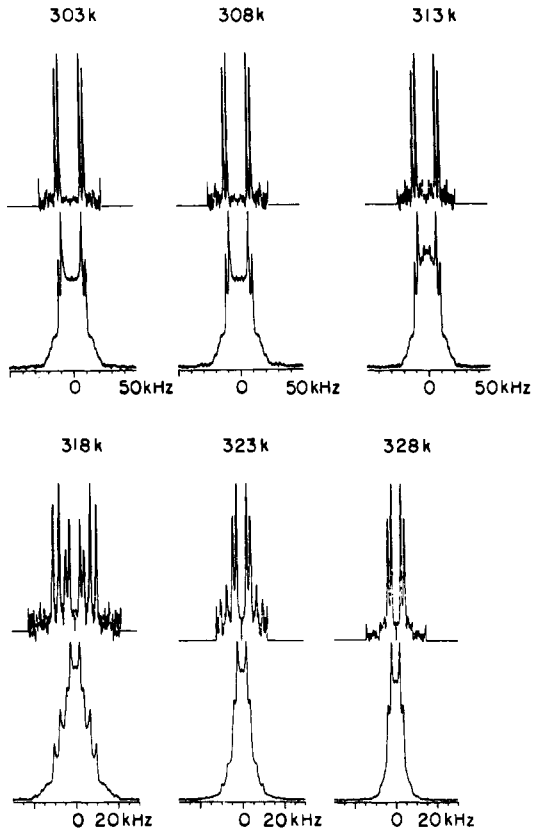

FIGURE 4: Bilayer to hexagonal phase transition, as monitored by ^2H NMR spectroscopy of [9′,10′-$^2H_2$]PDSPE (heating cycle). Spectra were acquired as described under Materials and Methods. For each temperature, upper and lower traces correspond to the dePaked and powder spectra, respectively.

those obtained with samples hydrated with an equal amount, by weight, of water.

In Figure 4, "oriented" spectra are shown with the associated powder spectra. Figure 4 illustrates that such oriented spectra greatly facilitate quantitation of the relative lipid populations in both states, as well as the measurement of individual quadrupolar splittings and line widths associated with each phase. The respective populations of lipid molecules in bilayer and hexagonal phases are shown in Figure 5 as a function of temperature. These values have been obtained from the respective areas of the peaks in the dePaked spectra. In order that the spectral areas reflect the real population ratios, no significant differences in the transverse relaxation rate of deuterons in each phase can be present; spin–lattice relaxation measurements (B. Perly and H. C. Jarrell, unpublished results) reveal that the systems are fully relaxed with respect to T_1. The total spectral intensity as a function of temperature is shown in Figure 5 for [5-$^2H_2$]POPC and [9,10-$^2H_2$]PDSPE. The total intensity does not vary with temperature by more than ±10%, reflecting that no significant transverse relaxation effects are present. Figure 5 reveals a linear dependence of the populations on temperature for PDSPE; similar results were obtained for POPE (data not shown). No thermal hysteresis could be detected by using cooling or heating rates not exceeding 1 °C min^{-1}. Furthermore, no significant change in the phase composition of [5-$^2H_2$]PDSPE at 45 °C, where bilayer and hexagonal structures coexist in a 1:1 ratio, could be detected after equilibrating the sample for 12 h. The latter result indicates that the lipid system is at thermal equilibrium.

For both lipid structures to be detected by ^2H NMR, lipid molecules in each of the two must be exchanging slowly compared to the inverse of the difference in quadrupolar in-

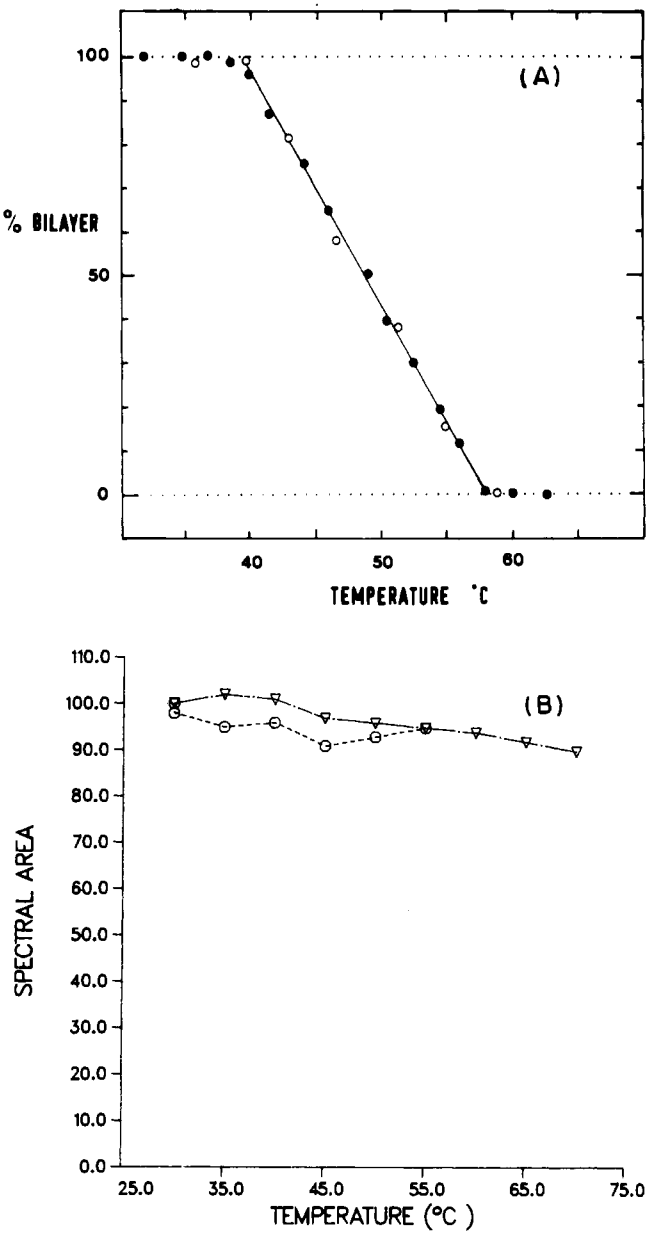

FIGURE 5: (A) Proportion of the bilayer phase, as determined from the areas of peaks in the dePaked spectra, in [5′-$^2H_2$]PDSPE as a function of temperature: (●) heating cycle; (○) cooling cycle. (B) Total spectral intensity as a function of temperature: (○) [9,10-$^2H_2$]PDSPE; (▽) [5-$^2H_2$]POPE.

teractions associated with each phase. However, this does not indicate that exchange of lipid molecules between the two phases is not occurring. Figure 6 shows a plot of the apparent line width of the peaks corresponding to $\theta = 90°$ in eq 1 (obtained from dePaked spectra) associated with the bilayer and hexagonal phase lipids throughout the structural transition. Similar plots have been obtained for all PE species studied here, that is, with labeled acyl chains. The apparent line width for a given phase increases dramatically when the other phase is present in excess. These data indicate that the lipid molecules exchange between the two phases in a time longer than the reciprocal of the difference between the respective quadrupolar splittings (10^{-3} s).

The introduction of a methylene group for the cis double bond of oleic acid does not change the total width of the transition, which remains ca. 15–18 °C, but induces a shift of about 17 °C in the value of T_{BH} toward lower temperatures. In PDSPE, the transition starts at ca. 38 °C, which is in the

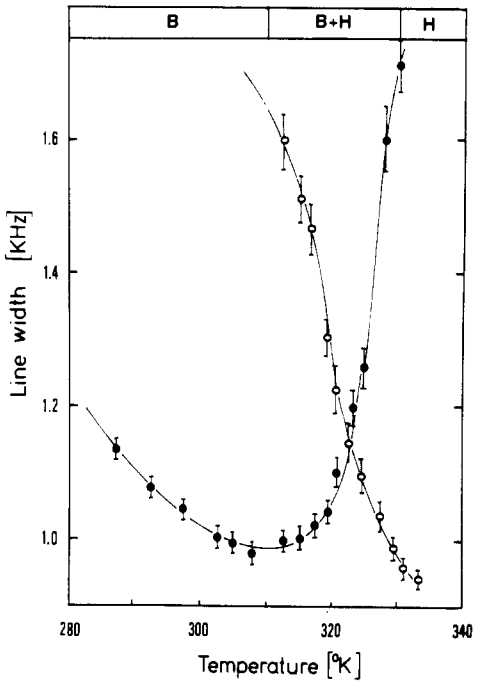

FIGURE 6: Plot of the apparent ^2H line width (derived from spectra dePaked to $\theta = 90°$) for [5′-$^2H_2$]PDSPE in bilayer and hexagonal phases.

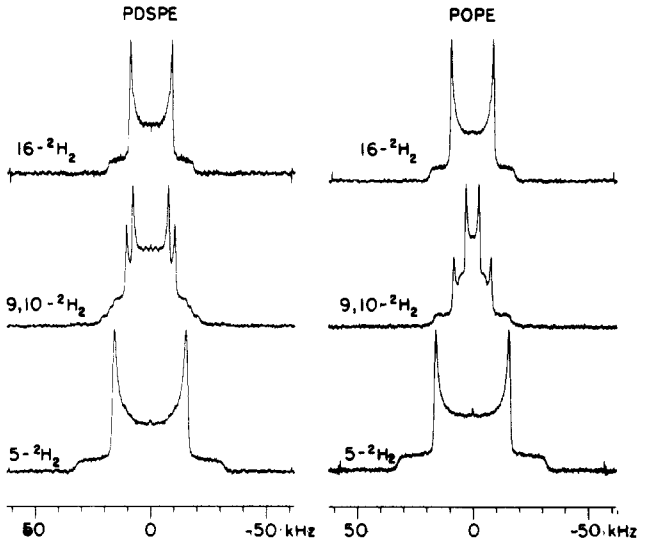

FIGURE 7: ^2H NMR spectra at 46 MHz of selectively labeled POPE and PDSPE at 30 °C. Under these conditions, all systems are in the liquid-crystalline phase. Corresponding spectra for PDSPC have been published elsewhere (Dufourc et al., 1983).

range of optimal growth conditions for many microorganisms. The same behavior is observed for all samples, and the thermal cycle could be repeated several times without any change in the values of T_{BH}.

Molecular Order of Fatty Acid Chains in Bilayer and Hexagonal Phases. ^2H NMR spectra have been recorded for all samples as a function of temperature for bilayer and hexagonal phases. Representative spectra of the six PE samples at 30 °C are shown in Figure 7. In single-phase systems, all samples labeled at either the 5′- or the 16′-carbon atoms of the *sn*-2 chains exhibited a single powder pattern. This means that in both cases the two deuterons are equivalent. Conversely, simultaneously labeling at the 9′,10′-positions led to two superimposed quadrupolar powder patterns since the 9′- and 10′-deuterons are not equivalent. Assignments of the two observed quadrupolar splittings was done according to the

Table II: Quadrupolar Splittings and Order Parameters, S_{CD}, for Hydrated Lipids

lipid	labeled carbon	quadrupolar splitting (kHz) $\Delta\nu_{QB}$[d]	$\Delta\nu_{QH}$[d]	temp (°C)	S_{CD}[a]	ν_{QH}:ν_{QB}
POPC	5	24.7		30	0.19	
	9	13.2		30	0.10	
	10	2.4		30	0.02	
	16	11.2		30	0.08	
PDSPC[b]	5	26.4		30	0.21	
	9	17.6		30	0.13	
	10	11.4		30	0.08	
	16	11.5		30	0.09	
POPE	5	32.5		30	0.26	
		26.2	11.3	65	0.21	0.43
	9	16.8		30	0.13	
		14.3	6.0	65	0.11	0.41
	10	6.2		30	0.047	
		4.3	*c*	65	0.033	*c*
	16	18.9		30	0.15	
		11.8	3.9	65	0.09	0.33
PDSPE	5	31.8		30	0.25	
		28.0	12.4	48	0.22	0.44
	9	22.1		30	0.16	
		20.0	8.3	48	0.15	0.41
	10	16.7		30	0.12	
		13.8	4.8	48	0.10	0.34
	16	18.7		30	0.15	
		14.3	4.6	48	0.11	0.32

[a] S_{CD} values are for lipid in the lamellar phase and have been calculated by using the following static quadrupolar coupling constants: 170 kHz for C-5 and C-16 positions, 175.3 kHz for C-9 and C-10 positions of oleic acid, and 183 kHz for C-9 and C-10 positions of dihydrosterculic acid. [b] Data from Dufourc et al. (1983). [c] The value of $\Delta\nu_{QH}$ was too small to be estimated, and the ratio $\Delta\nu_{QH}$:$\Delta\nu_{QB}$ could not be calculated. [d] Quadrupolar splittings $\Delta\nu_{QB}$ and $\Delta\nu_{QH}$ refer to lipid in bilayer and hexagonal structures, respectively.

data published on PC samples (Seelig & Waespe-Šarčevič, 1978; Dufourc et al., 1983). The quadrupolar splittings, measured from dePaked spectra, are summarized in Table II, as are pertinent data from the literature.

Inspection of Table II reveals that at a given absolute or reduced temperature the orientational order of the entire acyl chain, as reflected by the C-5 and C-16 positions, is increased for PE relative to that of the corresponding PC. A similar conclusion has been reported for saturated lipid systems (Marsh et al., 1983). Although the relative increase in order is not identical at these positions, the general trend is that reducing the size of the polar head group limits the angular fluctuations of the molecular subunit with respect to the director. This behavior is consistent with the increase in the temperature of the gel to liquid-crystal transition for PE relative to that of the corresponding PC and reflects the stronger lateral compression effect in PE due to the smaller head group.

Effect of the Cyclopropane Unit on Molecular Order. According to eq 4, $\Delta\nu_Q$ is dependent upon the angular fluctuations of the C–^2H bond with respect to the instantaneous segmental chain orientation (S_γ) and/or to the angular fluctuations of the instantaneous segmental chain orientation with respect to the director ($S_\alpha = S_{mol}$). In the case of the cyclopropane ring and the carbon–carbon double bond, S_γ is no longer time averaged so that each C–^2H bond has a fixed geometry with respect to the rigid subunit and has the same molecular order parameter (S_{mol}). For the C-9 and C-10 positions of PDSPE and POPE, different quadrupolar splittings (or S_{CD} values) are observed, indicating that the two C–^2H bond vectors are oriented at different angles with respect to the director of motional averaging (the bilayer normal).

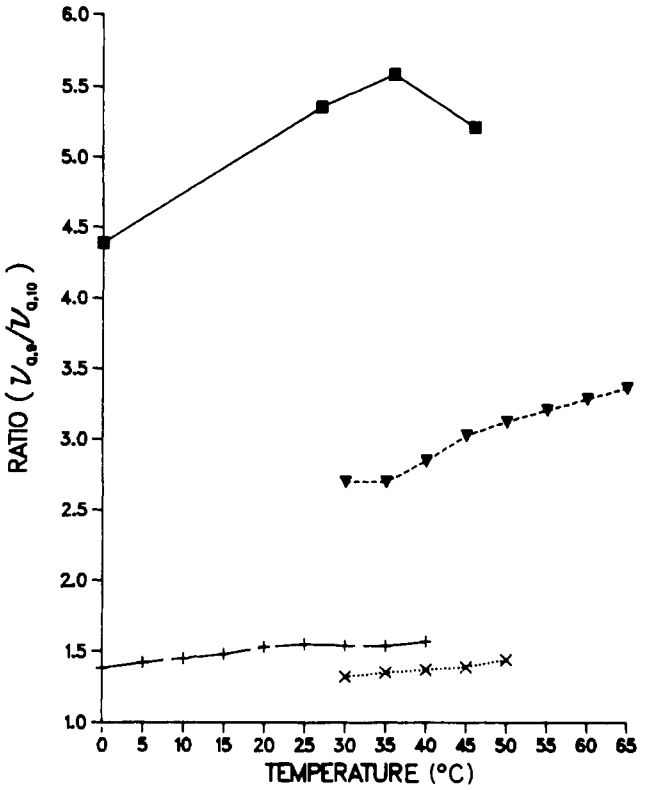

FIGURE 8: $\Delta\nu_Q(9):\Delta\nu_Q(10)$ ratio as a function of temperature: (■) POPC; (▼) POPE; (+) PDSPC; (×) PDSPE.

Similar results have been obtained for PDSPC (Dufourc et al., 1983) and POPC (Seelig & Waespe-Šarčevič, 1978) dispersions. If at least three independent S_{CD} values are available, one can calculate the value of S_{mol} and the orientation of the molecular subunit with respect to the director. The C-9–C-10 bond in PDSPC (*sn*-2 chain) was calculated to be oriented at almost 90° with respect to the bilayer normal (Dufourc et al., 1983) while the same bond was calculated to be tilted by only ca. 7–8° in the POPC system (Seelig & Waespe-Šarčevič, 1978). In the present cases, there are insufficient data to calculate unambiguously the molecular ordering and geometrical factors for the PDSPE and POPE dispersions. However, according to eq 4, if the geometrical factors were the same in the phosphatidylcholine and phosphatidylethanolaine systems, the ratio

$$\frac{\nu_Q(9)}{\nu_Q(10)} = \frac{S_\gamma(9)}{S_\gamma(10)} \tag{5}$$

would be very similar. Values for these ratios are plotted in Figure 8 for the POPC, POPE, PDSPC, and PDSPE systems as a function of temperature. In the case of the unsaturated systems, the ratios are considerably different, differing by a factor of ca. 2. Therefore, the double bond must be oriented at different angles with respect to the director in POPC and POPE. However, both the PDSPC and PDSPE systems exhibit similar ratios, suggesting that the C-9–C-10 bond has similar orientations with respect to the director in the two lipid systems. Assuming that the geometrical factors for the PDSPC and PDSPE systems are nearly identical, as Figure 8 suggests, then at 30 °C a value for S_{mol} of 0.66 ± 0.05 is calculated according to eq 4 (Dufourc et al., 1983) which is greater than that of PDSPC (S_{mol} of 0.58 ± 0.05) at 30 °C, a result consistent with the trends observed for the C-5 and C-16 positions. However, at similar reduced temperatures, PDSPE is less ordered (S_{mol} of 0.46 ± 0.03, 37 °C) while PDSPC has a value for S_{mol} of 0.62 ± 0.04 at 20 °C [calculated from the data reported in Dufourc et al. (1983)]. Due to the approximate nature of the calculations (assumed geometrical factors), the results should only be interpreted as qualitative rather than quantitative. Similar calculations are not possible with the unsaturated systems since the geometrical factors may be very different for the two systems. Interestingly, the orientation of the C-9–C-10 bond with respect to the director is not constant; it must be temperature dependent since the value of the ratio, $\Delta\nu_Q(9)/\Delta\nu_Q(10)$, changes with temperature (Figure 8). The temperature dependence of the C-9:C-10 ratio is greater for the unsaturated lipids than for the corresponding cyclopropane ring containing lipids. The *molecular ordering* for PDSPE and POPE is essentially the same at each of the C-5′ and C-16′ positions at the same absolute temperature.

Effect of the Bilayer–Hexagonal Transition on the Molecular Order of the Fatty Acyl Chains. As mentioned previously, rapid diffusion of the lipid molecules about the long axis of the hexagonal cylinders should decrease $\Delta\nu_Q$ by a factor of 2 relative to that associated with lipids in the lamellar phase, if all other geometrical and motional parameters remain

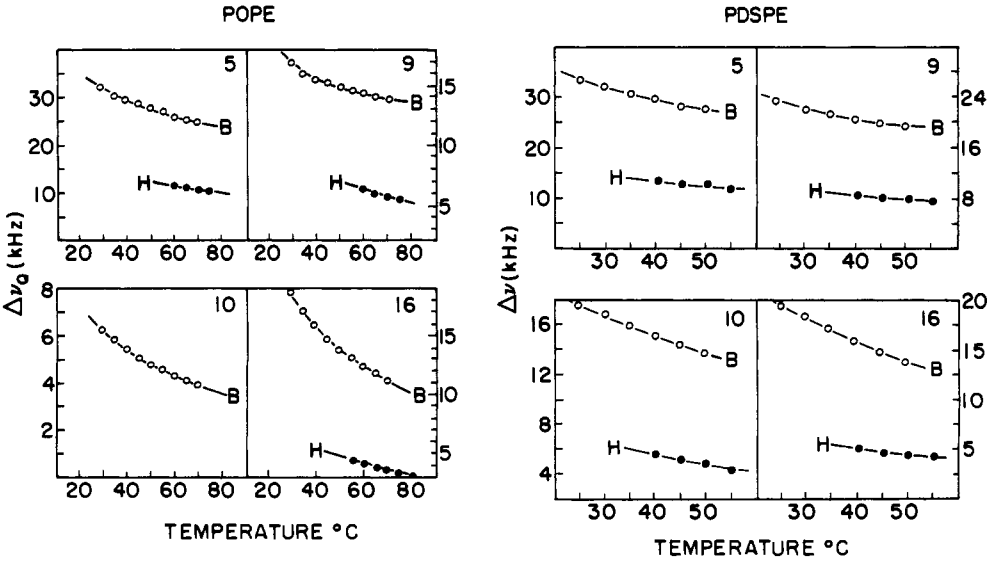

FIGURE 9: Observed quadrupolar splittings (derived from dePaked spectra) for labeled PE in (○) bilayer and (●) hexagonal phases as a function of temperature.

constant. Figure 9 shows plots of the observed quadrupolar splittings as a function of temperature for all samples under study. The data, corresponding to temperatures where both phases are present in equal amounts, are collected in Table II. The ratio of quadrupolar splittings in both phases ($\Delta\nu_{QH}/\Delta\nu_{QB}$) is always less than 0.5, and moreover, this ratio decreases toward the end of the acyl chain. A ratio less than 0.5 indicates that the transition from a lamellar to a hexagonal structure corresponds to a significant increase in the allowed angular fluctuations at any acyl chain segment, the increase being larger toward the end of the chain. This behavior is expected if one considers that the available volume per segment will be very similar in both phases in the vicinity of the polar head group but will, in an inverted hexagonal structure, increase rapidly toward the acyl chain methyl group. Fourier-transform infrared spectroscopic studies of phosphatidyletholamines (Mantsch et al., 1981) and ^{2}H NMR studies of dielaidoylphosphatidylethanolamine (DEPE) (Gally et al., 1980) have also concluded that a transition to inverted hexagonal phase is accompanied by additional conformational disordering of the acyl chains.

In the hexagonal phase, the $\nu_Q(9):\nu_Q(10)$ ratio of PDSPE increases from approximately 1.4 to 1.7, suggesting that the orientation of the C-9–C-10 bond with respect to the long axis of the acyl chain has changed relative to that present in lipid in the lamellar phase at the same temperature (48 °C, Table II). The same trend is observed for the POPE system at 65 °C.

In the self-assembly theory of lipid aggregates (Israelachvili et al., 1976, 1980), the structure assumed by aqueous dispersions of lipids can be associated with a geometric parameter (P) defined by

$$P = \frac{V}{al_c}$$

where V is the hydrocarbon chain volume, a is the hydrocarbon–water interfacial area associated with the lipid, and l_c is the hydrocarbon chain length. The following results were obtained for the various structures assumed by lipid dispersions: $^1/_2 < P \leq 1$, bilayer structure; $P > 1$, inverted hexagonal structure. For phosphatidylethanolamines, the value of a is expected to be very similar for the various acyl chains that are found in membranes. Substitution of cis-unsaturated fatty acids for saturated ones will reduce the value of l_c and give rise to a corresponding increase in P. The present results as well as those reported for PDSPC (Dufourc et al., 1983) indicate that replacing an olefinic function by a cyclopropane ring leads to orientation of the C-9–C-10 bond at almost 90° to the bilayer normal, and as a result the acyl chain would be shortened by about one carbon–carbon bond relative to the cis-unsaturated system. Hence, if only this effect is considered, the value of l_c of the unsaturated system will be the greater. An additional contribution to l_c that must be considered is its temperature dependence; the populations of trans–gauche isomers, as well as kinks, in the hydrocarbon chains will vary with temperature (Wieslander et al., 1980). In general, an increase in the amount of chain rotational isomerization leads to a decrease in S_{mol} and a corresponding decrease in l_c (Seelig & Seelig, 1974). Inspection of Table II reveals that at the midpoint of the bilayer to hexagonal phase transition the S_{mol} values ($-2S_{CD}$) of the C-5 and C-16 methylene units of PDSPE are greater than the corresponding values for POPE; that is, the S_{mol} values would imply that l_c for PDSPE is greater than that for POPE. However, since the T_{BH} temperature of PDSPE is some 17 °C lower than that of POPE, the structural defect introduced by the cyclopropane ring appears to be the determining factor in the value of l_c (and P) relative to POPE, facilitating the bilayer–hexagonal transition for PDSPE.

Conclusions

Replacement of the choline head group of POPC with the smaller ethanolamine group, to give POPE, results in a higher temperature (T_c) for the gel to liquid-crystalline transition of the latter lipid system. Similarly, T_c of PDSPE is greater than that of PDSPC. The replacement of a cis-olefinic function (POPC) with a cyclopropane ring (PDSPC) has been shown previously to result in a decrease in the value of T_c by 5–10 °C (Dufourc et al., 1983). A similar transformation in the corresponding phosphatidylethanolamine systems results in a decrease in T_c by ca. 17 °C. The larger effect of the cyclopropane ring on T_c in phosphatidylethanolamine is presumably due to the smaller intermolecular separation, making it more sensitive to perturbation by bulky groups.

As in the case of phosphatidylcholines, the presence of the cyclopropane ring in phosphatidylethanolamine gives rise to a more ordered (greater S_{mol}) hydrophobic core than that of the corresponding unsaturated systems in a liquid-crystalline bilayer structure at the same reduced temperature. In addition, the response of the segmental ordering to temperature changes is less than that of the cis-unsaturated lipid (see Figure 9). The latter results suggest that the unsaturated lipid system is the more dynamic; ^{2}H NMR relaxation studies also suggest that the acyl chain motions of the olefinic systems are faster than those of the corresponding cyclopropane ring containing systems (B. Perly, I. C. P. Smith, and H. C. Jarrell, unpublished results; Dufourc et al., 1984). At the same absolute temperature, both PDSPE and POPE exhibit similar ordering at the C-5 and C-16 methylene units (Table II and Figure 9).

Another major effect of replacing a cis-unsaturated fatty acid with the corresponding cyclopropane-containing fatty acid in phosphatidylethanolamine is a significant reduction in the temperature at which inverted hexagonal structures appear. In the present systems, replacement of an olefinic function by a cyclopropane ring reduced T_{BH} (onset) from 55 to 38 °C. Thus, in a biological membrane, a similar transformation may change the system from one in which, at typical growth temperatures, nonbilayer structures are not present to one in which small but significant quantities of lipid molecules can exist in nonbilayer phases within a lamellar framework. Although similar effects could be achieved by increasing the unsaturated or polyunsaturated acyl chain content, such modifications may be more slowly achieved or be more energy expensive than adding a methylene group to the acyl chain of an intact lipid. In addition, such changes may lead to additional significant undesirable changes in the characteristics of the hydrocarbon core.

The formation of cyclopropane-containing fatty acids is induced by changes in the cell growth cycle (Christie, 1969), as well as by changes in osmotic pressure or temperature (Hara et al., 1980). With such changes, membrane constituents may vary, in particular the relative composition of the lipid headgroup classes (Hara et al., 1980). The transformation of acyl chains from unsaturated to those containing a cyclopropane ring gives rise to a lipid matrix which can accommodate nonbilayer structures and enters the gel state with more difficulty, and whose "fluidity" (ordering and acyl chain dynamics) is less responsive to perturbations (such as temperature) than the precursor lipid matrix. The presence of proteins, and heterogeneity of lipid head groups and acyl chains, in natural membranes containing high amounts of cyclopropane-bearing fatty acids apparently does not alter the membrane properties significantly from those of PDSPC and

PDSPE model systems (Jarrell et al., 1983; Dufourc et al., 1983). It still remains to be established that the differences in the properties of the lipid matrix observed in these model systems are correlated with those present when, as a response to cell cycle or environmental changes, an organism replaces much of its unsaturated fatty acid content with cyclopropane-containing analogues. It is indeed intriguing that fatty acids containing cyclopropane or branched methyl groups can be major constituents of natural membranes.

ACKNOWLEDGMENTS

We thank Dr. K. Butler for the DSC measurements and Dr. J. H. Davis for making his dePaking program available.

Registry No. POPC, 26853-31-6; POSPC, 81004-53-7; POPE, 26662-94-2; POSPE, 85506-89-4.

REFERENCES

Bloom, M., Davis, J. H., & McKay, A. L. (1981) *Chem. Phys. Lett. 80*, 198.

Büldt, G., Gally, H. U., Seelig, J., & Zaccai, G. (1979) *J. Mol. Biol. 134*, 673.

Burnett, L. J., & Muller, B. H. (1971) *J. Chem. Phys. 55*, 5829.

Christie, W. W. (1969) *Lipid Chem. 1*, 1.

Cullis, P. R., & de Kruijff, B. (1979) *Biochim. Biophys. Acta 559*, 399.

Davidson, F. M., & Long, C. (1958) *Biochem. J. 9*, 458.

Davis, J. H. (1983) *Biochim. Biophys. Acta 737*, 117.

Davis, J. H., Jeffrey, K. R., Bloom, M., Valic, M. I., & Higgs, T. P. (1976) *Chem. Phys. Lett. 42*, 390.

Dekker, C. J., Geurts Van Kessel, W. S. M., Klomp, J. P. G., Pieters, J., & de Kruijff, B. (1983) *Chem. Phys. Lipids 33*, 93.

Dufourc, E. J., Smith, I. C. P., & Jarrell, H. C. (1983) *Chem. Phys. Lipids 33*, 153.

Gally, H. U., Pluschke, G., Overath, P., & Seelig, J. (1980) *Biochemistry 19*, 1638.

Ghosh, K., & Seelig, J. (1982) *Biochim. Biophys. Acta 691*, 151.

Goldfine, H. (1972) *Adv. Microb. Physiol. 8*, 1.

Griffin, R. G. (1981) *Methods Enzymol. 72*, 108.

Hara, H., Hyono, A., Kuriyama, S., Yano, Z., & Masui, M. (1980) *J. Biochem. (Tokyo) 88*, 1275.

Israelachvili, J. N., Mitchell, D. J., & Ninham, B. W. (1976) *J. Chem. Soc., Faraday Trans. 2, 72*, 1525.

Israelachvili, J. N., Marčelja, S., & Horn, R. G. (1980) *Q. Rev. Biophys. 13*, 121.

Jarrell, H. C., & Smith, I. C. P. (1983) in *NATO ASI Series C103* (Lambert, J. B., & Riddell, F. G., Eds.) p 151, D. Reidel Publishing Co., Dordrecht, The Netherlands.

Jarrell, H. C., Tulloch, A. P., & Smith, I. C. P. (1983) *Biochemistry 22*, 5611.

Kowalewski, J., Lindblom, T., Vestin, R., & Drakenberg, T. (1976) *Mol. Phys. 31*, 1669.

Mantsch, H. H., Martin, A., & Cameron, D. G. (1981) *Biochemistry 20*, 3138.

Marsh, D., Watts, A., & Smith, I. C. P. (1983) *Biochemistry 22*, 3023.

Perly, B., Dufourc, E. J., & Jarrell, H. C. (1984) *J. Labelled Compds. Radiopharm. 21*, 1.

Rothman, J. E., & Lennard, J. (1977) *Science (Washington, D.C.) 195*, 741.

Seelig, A., & Seelig, J. (1974) *Biochemistry 13*, 4839.

Seelig, J. (1977) *Q. Rev. Biophys. 3*, 353.

Seelig, J., & Neiderberger, W. (1974) *J. Am. Chem. Soc. 96*, 2069.

Seelig, J., & Gally, H. U. (1976) *Biochemistry 15*, 5199.

Seelig, J., & Waespe-Šarčevič, N. (1978) *Biochemistry 17*, 3310.

Silvius, J. R., & McElhaney, R. N. (1979) *Chem. Phys. Lipids 25*, 125.

Smith, I. C. P. (1984) *Biomembranes 12*, 133.

Thayer, A. M., & Kohler, S. J. (1981) *Biochemistry 20*, 6831.

Van Dijck, P. W. M., de Kruijff, B., Van Deenene, L. L. M., de Gier, J., & Demel, R. A. (1976) *Biochim. Biophys. Acta 455*, 576.

Wieslander, A., Christiansson, A., Rilfors, L., & Lindblom, G. (1980) *Biochemistry 19*, 3650.