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Acyl Chain Dynamics of Phosphatidylethanolamines Containing Oleic Acid and Dihydrosterculic Acid: ^{2}H NMR Relaxation Studies†

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ABSTRACT: The dynamical behavior of the acyl chains of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine, and 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphoethanolamine has been investigated by using ^{2}H T_1 and T_{2_e} relaxation times. Lipids were labeled at the 5-, 9-, 10-, and 16-positions of the *sn*-2 acyl chain. The profile of deuterium spin–lattice relaxation rate (T_1^{-1}) vs. chain position is characterized in all systems by a marked discontinuity at the positions of the carbon–carbon double bond and the cyclopropane ring; the deuterons at these positions have relaxation rates which are greater than at any other labeled position of the *sn*-2 chain. For both types of *sn*-2 acyl chain, assuming a single-exponential correlation time and that the motion is within the rapid regime, the phosphatidylcholine lipid systems are less mobile than their phosphatidylethanolamine analogues. Systems containing an oleoyl chain are more dynamic than their analogues containing a dihydrosterculoyl chain. The rates of motion of the *sn*-2 acyl chains of phosphatidylethanolamine in a bilayer structure are slower than those of the lipid in an inverted hexagonal structure. In the hexagonal phase, the motional rates of a dihydrosterculoyl chain are slower than those of the corresponding positions of an oleoyl chain.

Several recent studies have examined the ramifications on membrane structure and dynamics of replacing an olefinic group with a cyclopropane ring (Silvius et al., 1979; Dufourc et al., 1983; Dufourc, 1983; Jarrell et al., 1983; Perly et al., 1985) and with branched methyl groups (Silvius et al., 1980; Wieslander et al., 1982). Interest in such systems arose because of the paucity of information on the properties of membranes composed of fatty acids containing a cyclopropane

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ring or branched methyl groups, even though the occurrence of such acyl chains is not uncommon (Christie, 1969; Kaneda, 1979). Earlier ^2H NMR studies on aqueous dispersions of phosphatidylcholines (Dufourc et al., 1983; Dufourc, 1983) and *Acholeplasma laidlawii* membranes (Jarrell et al., 1983) established that the replacement of the carbon–carbon double bond of oleic acid by a cyclopropane ring had several effects on membrane properties. First, the angular fluctuations of the acyl chain at the position of the cyclopropane ring were significantly reduced, leading to a much larger segmental order parameter relative to that of the corresponding position of oleic acid. Second, the presence of the cyclopropane ring resulted in a tilting of the C-9–C-10 bond relative to the director of motion (assumed to be the bilayer normal) which was greater than that reported for the corresponding segment of the oleoyl chain. Finally, the rates of the rapid acyl chain motions, as monitored by spin–lattice relaxation times, were slower for the dihydrosterculoyl chains than for the oleoyl chains in analogous lipid systems. Interestingly, the presence of the relatively bulky cyclopropane ring did not lead to a large shift in the temperature of the gel to liquid-crystalline phase transition (T_c) relative to that of the corresponding unsaturated lipid (Dufourc et al., 1983; Jarrell et al., 1983).

Phosphatidylethanolamine (PE) has been closely associated with lipid polymorphism under a variety of conditions (Cullis & de Kruijff, 1979), and the polymorphic behavior of PE has been proposed to be intimately related to the shape of the lipid molecule (Weislander et al., 1980; Israelachvili et al., 1980). Since, biosynthetically, "cyclopropane" fatty acyl chains appear (at least initially) in the phosphatidylethanolamine fraction of the lipids of natural membranes (Christie, 1969; Cronan & Vagelos, 1972), we thought it of interest to examine the effect of replacing an olefinic group with a cyclopropane ring on the structure and dynamics of aqueous dispersions of PE. In particular, we sought to examine the influence of chain substitution on the polymorphic behavior of PE. In a preceding study, we reported the structural effects (Perly et al., 1985). We now report a comparison of the acyl chain dynamics of 1-palmitoyl-2-oleoyl- and 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphoethanolamines as monitored by ^2H NMR relaxation. The acyl chain dynamics are compared with those of the corresponding phosphatidylcholines.

Materials and Methods

[5-$^2H_2$]-, [9,10-$^2H_2$]-, and [16-$^2H_2$]oleic acid were a generous gift of Dr. A. P. Tulloch. [5-$^2H_2$]-, [9,10-$^2H_2$]-, and [16-$^2H_2$]dihydrosterculic acid were prepared as described previously (Jarrell et al., 1983). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine were prepared by acylation of 1-palmitoyl-*sn*-glycero-3-phosphocholine with the corresponding deuterium-labeled fatty acid anhydride (Perly et al., 1984). Phosphatidylethanolamines were prepared from the corresponding phosphatidylcholines by transphosphatidylation with phospholipase D in the presence of ethanolamine (Perly et al., 1984). The lipids were homogeneous to thin-layer chromatography (TLC) [$CHCl_3$–MeOH–H_2O, 65:25:4 (v/v)] using iodine, phosphate, and ninhydrin detection (Kates, 1972). The amount of *sn*-1 to *sn*-2 chain scrambling during the lipid synthesis was determined to be ≤5% (Perly et al., 1984).

The NMR samples consisted of ca. 100 mg of lipid hydrated with deuterium-depleted water (Aldrich Chemical Co., Milwaukee, WI) in a weight ratio of 1:1. The phosphatidylcholine (PC) samples in sealed glass tubes were cyclically incubated at 25 °C and freeze–thawed until homogeneous. The PE samples in sealed glass tubes (10-mm o.d.) were incubated at 70 °C for 1 h and then treated in a similar manner to that of the PC samples.

^2H NMR spectra were obtained at 46 MHz on a Bruker CXP-300 spectrometer using a home-built probe with a 10-mm solenoid coil. NMR spectra were acquired by using the quadrupolar echo technique (Davis et al., 1976) with full-phase cycling of the radio-frequency pulses. The spacing between the two $\pi/2$ pulses of the basic echo sequence was 50–60 μs, with a $\pi/2$ pulse length of 4.5–5.0 μs. The recycle delay was adjusted to be greater than 5 times the longest T_1 value. The frequency of the spectrometer was carefully adjusted to be at the center of the symmetrical powder pattern to avoid off-resonance amplitude modulation (Boden et al., 1978). Spectra were acquired with quadrature detection but, unless indicated otherwise, were folded so that the two halves were superimposed. In all cases, the folded and nonfolded spectra were compared to ensure that no spectral distortions were introduced by the folding procedure.

The NMR data were transferred from the Bruker Aspect 2000 computer via an RS-232 line to a Nicolet 1280 data processor where spectral dePaking calculations were done as described elsewhere (Rance et al., 1983).

Spin–lattice relaxation times (T_1) were measured by using the inversion–recovery technique modified to include the quadrupolar echo pulse sequence ($180°_x$–τ_1–$90°_x$–τ_2–$90°_y$–τ_3–Acq) and with a recycle time ca. 5 times the largest T_1 value. Specific regions of the Fourier-transformed spectra were integrated to examine the orientational dependence of T_1 (Rance, 1981). Integrated intensities were fitted to the equation (MacDonald, 1980; Rance, 1981):

$$M(\tau_1) = M_0[1 - A \exp(-\tau_1/T_1)]$$

where M_0 is the equilibrium magnetization and A is a parameter to account for imperfect 180° pulses. In cases where more than one axially symmetric powder spectrum was present, oriented ($\theta = 90°$) spectra (dePaked) were calculated for the entire series of partially relaxed spectra and the T_1 values of the individual components determined (Dufourc et al., 1984).

Quadrupolar echo decay times (T_{2_e}; Rance, 1981) were determined by fitting the integrated spectral intensity of various regions of the spectra as a function of the spacing between the two pulses (τ) of the quadrupolar echo sequence to the equation:

$$M(2\tau) = A \exp(-2\tau/T_{2_e})$$

where A is a normalization constant. The angular dependence of T_{2_e} across the powder spectrum was determined by calculating T_{2_e} for regions of the spectrum corresponding to director orientations (θ) around 0° (edge), 90° (peak), and 54.7° (center). Spectral simulations of quadrupolar echo spectra were performed in the time domain (Rance et al., 1983) on a Nicolet 1280 computer. The values of $\Delta\nu_Q$ were determined as described elsewhere (Perly et al., 1985). Spectra moments were calculated for experimental and simulated spectra as described previously (Jarrell et al., 1981). Estimates of the errors in M_2 values were obtained by calculating M_2 starting at five different points and determining the standard error.

Results and Discussion

Spin–Lattice Relaxation Times. Interpretation of ^2H relaxation data obtained for lipid acyl chains in terms of various motional modes and rates is at present incompletely developed (Brown et al., 1979; Brown, 1979, 1982, 1984; Pace & Chan, 1982). Since the purpose of the present study is to compare the overall acyl chain dynamics of PE containing oleoyl or

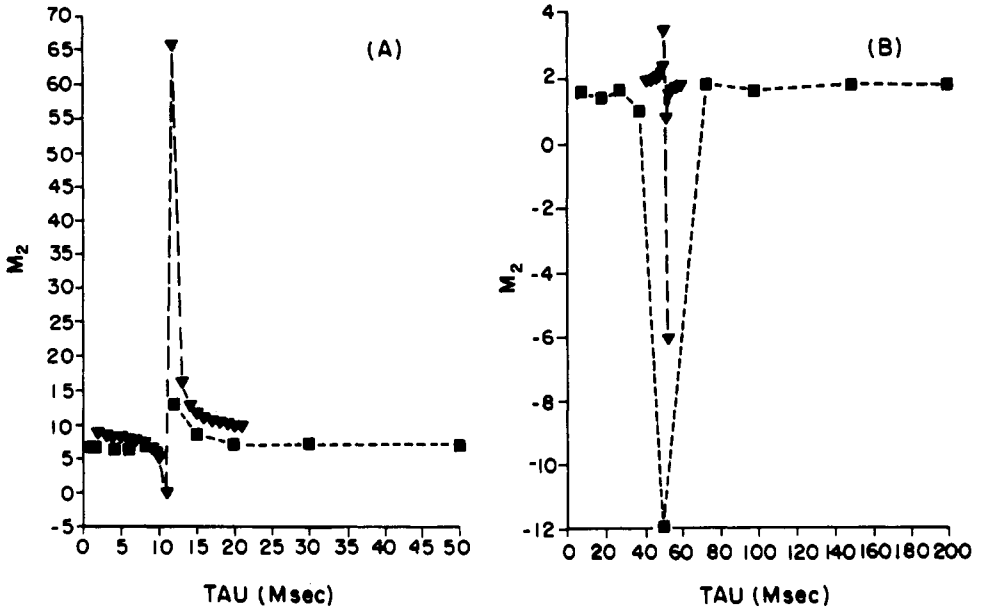

FIGURE 1: Second moments of partially T_1 relaxed spectra from inversion–recovery experiments: (■) experimental values at 46 MHz; (▼) theoretical values; spectral simulations were performed as described under Materials and Methods using the T_1 values measured for each of the labeled lipids indicated in (A) and (B). (A) PDSPE labeled at position 5 of the *sn*-2 acyl chain, 30 °C; (B) PDSPE labeled at position 16 of the *sn*-2 acyl chain, 55 °C. Estimates of the experimental error are given by the size of the symbols except for the large singularities. For these points, standard errors of 4% and 30% were estimated for the [5-^{2}H]PDSPE and [16-$^2H_2$]PDSPE systems, respectively.

dihydrosterculoyl chains rather than to investigate the actual details of the motion, the spin–lattice relaxation data are interpreted in terms of a single effective correlation time. If the relevant motions are in the short correlation time regime ($\omega_0^2\tau^2 << 1$), the relaxation rate is given by (Brown et al., 1979; Brown, 1979)

$$T_1^{-1} = (3/8) \times (e^2qQ/h)^2\{[1 - P_2(\cos\theta)]S_{CD} - [1 - P_2(\cos\theta)]S_{CD}^2\}\tau_c \quad (1)$$

where e^2qQ/h is the quadrupolar coupling constant, θ is the angle between the director and the external magnetic field, and S_{CD} is the C–D bond order parameter. Because of the $P_2(\cos\theta)$ term in eq 1, a variation in T_1 values across the ^{2}H powder spectrum is expected. An examination of the temperature dependence of the T_1 values (Table I) reveals that for these systems T_1 increases with temperature, indicating that $\omega_0^2\tau^2 << 1$. To assess the angular dependence of T_1, parts of the ^{2}H spectra were integrated for θ values around 0° (edge), 54.7° (center), and 90° (peak) and the relaxation times calculated. For all labeled positions and all temperatures studied, no unambiguous angular dependence in the T_1 values across the powder spectrum could be discerned. A similar observation for hydrated dipalmitoylphosphatidylcholine (Brown & Davis, 1981) was ascribed to rapid lateral diffusion in the liquid-crystalline phase which averages eq 1 over all orientations (θ) and leads to an observed relaxation rate given by

$$T_1^{-1} = (3/8)(e^2qQ/h)^2(1 - S_{CD}^2)\tau_c \quad (2)$$

If T_1 is independent of the director orientation, the ^{2}H spectrum should have the same shape for all values of τ in the inversion–recovery experiment (Davis & Brown, 1981). This may be tested by use of the second moments of the spectrum, M_2, where

$$M_2 = A^{-1}\int_{-\infty}^{\infty} g(\omega)(\omega - \omega_0)^2\, d\omega \quad (3)$$

and A is the area of the spectrum, ω_0 is the Larmor frequency, and $g(\omega)$ is the line shape. If T_1 is angular independent, then M_2 should be constant for all values of τ in the inversion–recovery experiment. In several cases, substantial changes in the value of M_2 as a function of τ were noted at values of τ near the null points. Spectral simulations of the inversion–recovery series for several labeled positions were performed by using the angular dependence in T_1 as defined by eq 1, and the values of M_2 were calculated (Figure 1). The predicted changes in M_2 are similar to those observed. However, the large changes in M_2 occur over a small interval of τ values (2–4 ms), and therefore, the large change in M_2 may be easily missed in a normal inversion–recovery experiment. Further-

Table I: T_1 Values as a Function of Labeled Position and Temperature for POPC, POPE, and PDSPE Aqueous Dispersions

lipid	label	temp (°C)	T_1[a,b] (ms)
POPC	[5-$^2H_2$]18:1	25	19.3 ± 0.5
		36	24.7 ± 0.5
	[9,10-$^2H_2$]18:1	25	16.5 ± 0.3, 13.7 ± 0.1[c]
		36	20.9 ± 0.4, 18.1 ± 0.4
	[16-$^2H_2$]18:1	25	56.0 ± 0.8
		36	70.1 ± 0.8
POPE	[5-$^2H_2$]18:1	30	19.7 ± 0.1
		55	31.0 ± 0.2
		75	38.4 ± 0.6
	[9,10-$^2H_2$]18:1	30	19.5 ± 0.2, 16.2 ± 0.1
		55	29.2 ± 0.4, 27.8 ± 0.3
		75	39.4 ± 0.5, 40.9 ± 0.4
	[16-$^2H_2$]18:1	30	67 ± 0.4
		55	105 ± 2.0
		75	160 ± 4.0
PDSPE	[5-$^2H_2$]19cp[d]	30	18.0 ± 0.1
		37	20.3 ± 0.1
		55	24.0 ± 0.1
	[9,10-$^2H_2$]19cp	30	10.0 ± 0.1, 9.4 ± 0.1
		37	11.7 ± 0.1, 11.0 ± 0.2
		55	15.5 ± 0.1, 15.5 ± 0.2
	16-$^2H_2$	30	51.1 ± 1.0
		37	63.2 ± 0.5
		55	75.9 ± 0.7

[a] T_1 values are for θ = 90° (peak). [b] Errors are those obtained from the fitting of inversion–recovery data to an exponential decay (Materials and Methods). [c] Separate values measured for positions 9 (left) and 10 (right). [d] 19cp indicates dihydrosterculic acid.

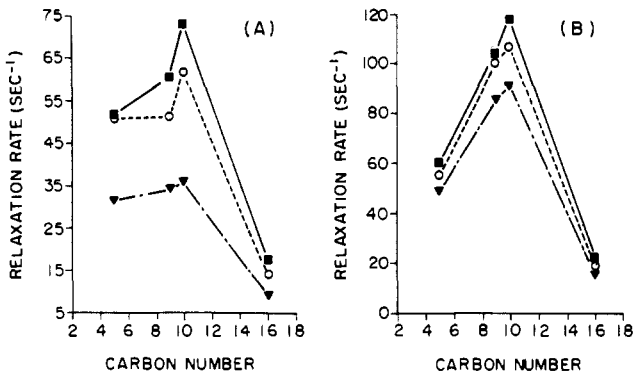

FIGURE 2: Positional dependence of relaxation rate (T_1^{-1}) at 46 MHz: (A) (■) POPC, 25 °C; (○) POPE, 30 °C; (▼) POPE, 55 °C; (B) (■) PDSPC, 25 °C (Dufourc et al., 1984); (○) PDSPE, 30 °C; (▼) PDSPE, 37 °C.

more, the large change in M_2 occurs at τ values where the ^{2}H spectrum is near the null point and hence where the value of M_2 is least reliable. These results suggest that the detection of an angular-dependent T_1 for these systems may be very difficult, as suggested previously (Pace & Chan, 1982). For the present systems, the data suggest that lateral diffusion is insufficient to average completely the value of T_1 for all orientations of the director with respect to the magnetic field.

The rates of motion of the acyl chain were monitored by examining the spin–lattice relaxation rates of deuterons at C-5, C-9, C-10, and C-16 of the oleoyl and dihydrosterculoyl chains; essentially the entire chain is monitored with these labeled positions. The dependences of the relaxation rates on position in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) at 25 °C and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) at 30 °C are shown in Figure 2A, and the corresponding T_1 values are given in Table I. Except for the C-5 position, the relaxation rates of the acyl chain positions of POPC are greater than those of the corresponding positions of POPE. The differences appear to be relatively small, on the order of 10%. However, the POPC system is ca. 30 °C above the temperature of its gel–liquid-crystal phase transition, −5 °C (Seelig & Waespe-Šarčevič, 1978), whereas the POPE system is only 3–4 °C above its T_c of 26–27 °C (Gosh & Seelig, 1982; Perly et al., 1985). The relaxation rates of the various labeled positions of the POPE system at 55 °C are shown in Figure 2A. At approximately the same reduced temperature (55 °C, ~28 °C above T_c), the relaxation rates of the acyl chain positions of POPE are strikingly different from those of the corresponding labeled positions of POPC. These results suggest that the *sn*-2 chain of POPE is significantly more mobile than that of POPC. According to eq 1, the relaxation rate is dependent on both the amplitudes and rates of the acyl chain motion (Brown, 1979, 1982). A previous study of the orientational order of these lipid systems (Perly et al., 1985) demonstrated that, at the same reduced temperatures, the orientational order of the PE systems was greater than that of the corresponding PC systems. At the same reduced temperature, POPE has slightly larger (~10%) S_{CD} values than POPC, for all the positions except C-10 (Table II). The relatively large increase in S_{CD} at the C-10 position of POPE may be the result of a slight change in the angle of tilt of the C-9–C-10 bond with respect to the axis of motion (Perly et al., 1985).

Similar results are obtained when 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine (PDSPC) (Dufourc et al., 1984) and the corresponding phosphatidylethanolamine (PDSPE) are compared (Figure 2B). The relaxation rates for all the labeled positions of PDSPC ($T_c \simeq -15$ °C) at 25 °C are greater than those of the corresponding PDSPE ($T_c \simeq$ 10–15 °C) at 30 °C. At similar reduced temperatures, the differences in the relaxation rates are again greater, with the relaxation rates of PDSPC being larger (Figure 2B). A comparison of the S_{CD} values at similar reduced temperatures (Dufourc et al., 1984; Perly et al., 1985) reveals that the PE system is slightly more ordered. A similar small increase of 10–20% in the orientational order of the PE systems with respect to the corresponding PC systems at the same reduced temperature has been reported for myristic acid containing phosphatidylcholine and phosphatidylethanolamine systems (Marsh et al., 1983). Equations 1 and 2 suggest that the spin–lattice relaxation rate for these systems has only a weak dependence on the order parameter S_{CD}. In addition, for the lipid systems being discussed, the S_{CD} values are small (Table II) so that the effect of order on the relaxation rate may be expected to be small or negligible. The differences in the relaxation rate–position profiles (Figure 3), therefore, reflect that the rates of *sn*-2 acyl chain motion, as detected by T_1, are greater in PE than those present in PC.

Table II: Correlation Times and S_{CD} Values as a Function of Position and Temperature for Aqueous Dispersions of POPC, POPE, PDSPC, and PDSPE in Bilayer Structures

lipid	label	$\lvert S_{CD}\rvert$	τ_c ($\times 10^{-10}$ s)	temp (°C)
POPC	[5-^{2}H$_2$]18:1	0.20	1.31	25
		0.18	1.0	36
	[9,10-^{2}H$_2$]18:1	0.10, 0.020[b]	1.36, 1.61[b]	25
		0.099, 0.018	1.1, 1.2	36
	[16-^{2}H$_2$]18:1	0.093	0.42	25
		0.083	0.35	36
POPE	[5-^{2}H$_2$]18:1	0.25	1.27	30
		0.22	0.79	55
	[9,10-^{2}H$_2$]18:1	0.13, 0.048	1.15, 1.36	30
		0.11, 0.035	0.76, 0.79	55
	[16-^{2}H$_2$]18:1	0.15	0.36	30
		0.10	0.22	55
PDSPC[a]	[5-^{2}H$_2$]19cp	0.22	1.5	25
	[9,10-^{2}H$_2$]19cp	0.13, 0.09	2.1, 2.4	25
	[16-^{2}H$_2$]19cp	0.11	0.53	25
PDSPE	[5-^{2}H$_2$]19cp	0.25	1.39	30
		0.23	1.22	37
	[9,10-^{2}H$_2$]19cp	0.16, 0.12	2.07, 2.18	30
		0.15, 0.11	1.76, 1.86	37
	[16-^{2}H$_2$]19cp	0.15	0.47	30
		0.13	0.38	37

[a] Data from Dufourc et al. (1984). [b] Separate values measured for positions 9 (left) and 10 (right).

τ_c values (Table II) may be calculated from the relaxation data in Table I by using eq 2 as a first approximation relating relaxation rates to effective correlation times. The data indicate that at the same reduced temperature, both PE systems have shorter correlation times than the corresponding PC systems and are therefore more mobile. In another study of PDSPC and POPC acyl chain dynamics, the dihydrosterculoyl chain was found to be the less mobile, particularly in the region of the cyclopropane ring (Dufourc et al., 1984). A comparison of the relaxation rates (panels A and B of Figure 2) and calculated correlation times (Table II) reveals that at all temperatures studied the relaxation rates of the positions in the dihydrosterculoyl chain and the correlation times are greater than those of the oleoyl chains. The latter results indicate that the acyl chain motions in PDSPE are less rapid than those of POPE, as found in the phosphatidylcholine systems (Dufourc et al., 1984).

The transition from bilayer to hexagonal structures was demonstrated to occur between 37 and 55 °C for PDSPE and between 55 and 75 °C for POPE (Perly et al., 1985). The

Table III: T_{2e} Values as a Function of Labeled Position and Temperature for POPC, POPE, and PDSPE Aqueous Dispersions

lipid	label	temp (°C)	T_{2e}[a,b] (μs) edge	center	peak
PDSPE	[5-$^2H_2$]19cp	30	268 ± 11	282 ± 4	485 ± 13
		37	253 ± 24	264 ± 5	427 ± 10
		55	433 ± 33	270 ± 9	347 ± 7
	[9,10-$^2H_2$]19cp	30		415 ± 15	538 ± 20, 538 ± 21[c]
		37	415 ± 20	419 ± 17	533 ± 22, 533 ± 22
		55	718 ± 85	650 ± 34	718 ± 10, 853 ± 20
	[16-$^2H_2$]19cp	30	366 ± 25	318 ± 12	653 ± 18
		37	435 ± 40	274 ± 16	650 ± 18
		55	1105 ± 132	694 ± 27	1053 ± 44
POPE	[5-$^2H_2$]18:1	30	429 ± 9	571 ± 14	728 ± 22
		55	414 ± 14	429 ± 10	656 ± 22
		75	644 ± 38	717 ± 47	842 ± 33
	[9,10-$^2H_2$]18:1	30	432 ± 18	845 ± 37	824 ± 17, 974 ± 34
		55			791 ± 28, 969 ± 25
		75			1000 ± 20, 1862 ± 95
	[16-$^2H_2$]18:1	30	401 ± 17	417 ± 15	770 ± 15
		55	740 ± 73	593 ± 30	1145 ± 31
		75	1368 ± 60	1630 ± 38	1788 ± 52
POPC	[5-$^2H_2$]18:1	30	291 ± 18	253 ± 8	373 ± 8
	[9,10-$^2H_2$]18:1	30		674 ± 17	652 ± 32, 763 ± 10
	[16-$^2H_2$]18:1	30	625 ± 55	464 ± 9	1030 ± 4
PDSPC	[5-$^2H_2$]19cp	25	290 ± 58	250 ± 25	470 ± 47
	[9,10-$^2H_2$]19cp	25			580 ± 58, 590 ± 58
	[16-$^2H_2$]19cp	25	310 ± 62	530 ± 53	700 ± 53

[a] Edge, center, and peak refer to the parts of the powder spectrum. [b] Errors are those obtained from the fitting of experimental data to an exponential decay (Materials and Methods). [c] Separate values measured for positions 9 (left) and 10 (right).

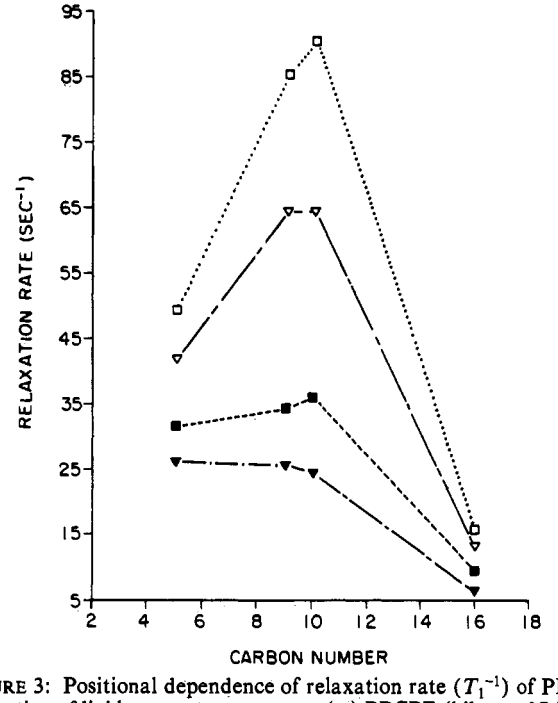

FIGURE 3: Positional dependence of relaxation rate (T_1^{-1}) of PE as a function of lipid aggregate structure: (□) PDSPE (bilayer, 37 °C); (▽) PDSPE (hexagonal, 55 °C); (■) POPE (bilayer, 55 °C); (▼) POPE (hexagonal, 75 °C).

relaxation rates (T_1^{-1}) for the labeled positions of the lipids just before and after the bilayer–hexagonal transition are shown in Figure 3. It is clear that the relaxation rates of the hexagonal phase lipids are lower, for all positions, than those of the lipid in the bilayer phase. In the hexagonal phase, the lipid molecules diffuse about the long axis of the inverted cylinders sufficiently fast to give rise to a reduction, by at least a factor of 2, of the residual quadrupolar interaction for both the POPE and PDSPE systems (Perly et al., 1985). If this motion has an effect on the relaxation rate, it may be expected to be similar for both POPE and PDSPE. Inspection of Figure 3 reveals that for the lipid in the hexagonal phase, the relaxation rates for all positions of the PDSPE system are much greater than those of the POPE system. The results indicate that in the hexagonal phase, as in the bilayer structure, the rapid motions of the cyclopropane ring containing systems are slower than those of the corresponding unsaturated system.

Relaxation studies on aqueous dispersions of phosphatidylcholine containing dihydrosterculic acid revealed that the cyclopropane ring at the C-9–C-10 position of the *sn*-2 acyl chain moderates the rates of motion of the entire chain (Dufourc et al., 1984). Comparison of the T_1 data for the PDSPE and POPE systems (Table I) reveals that the presence of the cyclopropane ring on the *sn*-2 acyl chain moderates the rates of acyl chain motion relative to those of the corresponding oleoyl chain. The replacement of a choline head group with the smaller ethanolamine head group results in an increase in the rates of acyl chain motion (at the same reduced temperature) while the amplitudes of motion (reflected by S_{CD}) may in fact be reduced (Table II).

Transverse Relaxation (T_{2e}). The quadrupolar echo amplitude decays with a time constant, T_{2e}, which is sensitive to slow motions (Jeffrey, 1981). To assess, on a relative basis, the slow motions of the PDSPE and POPE model lipid systems, T_{2e} values were measured as a function of temperature and label position (Table III). The T_{2e} spectral set, obtained as described under Materials and Methods, was integrated by regions, namely, those corresponding to director orientations (θ) around 0° (edge), 90° (peak), and 54.7° (center). The spectral integrations were fitted to the expression $M(2\tau) = A\exp(-2\tau/T_{2e})$ where $M(2\tau)$ is the transverse magnetization at time 2τ and A is a normalization constant. Figure 4 shows a representative series of partially T_{2e} relaxed spectra. In almost all cases the decay of the echo amplitude for various regions (values of θ) of the frequency spectrum was described by a single-exponential decay so that quadrupolar relaxation is apparently dominating (Davis, 1983). Inspection of Table III and Figure 4 reveals that the value of T_{2e} is dependent on θ, with $T_{2e}(0°) < T_{2e}(90°)$.

Table III reveals that at all temperatures and for all labeled positions the T_{2e} values of PDSPE are shorter than the cor-

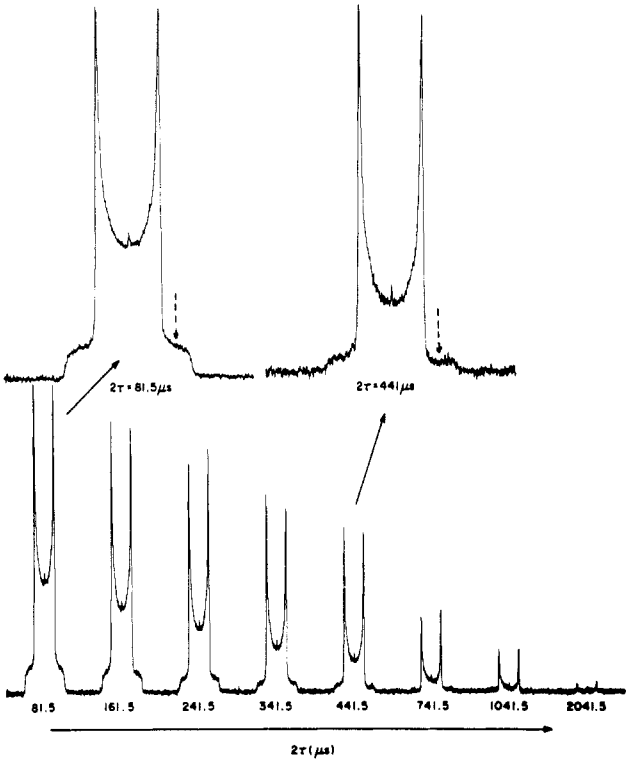

FIGURE 4: Partially T_{2_e} relaxed spectra at 46 MHz for PDSPE labeled at the 5-position of the *sn*-2 acyl chain, 30 °C. Spectra are not folded about the Larmor frequency.

responding values for the POPE system. In addition, the T_{2_e} values obtained for labeled POPC at 30 °C (~35 °C above T_c) are smaller than the corresponding values for POPE at 55 °C (~28 °C above T_c). The PDSPE and PDSPC lipid systems give rise to T_{2_e} values which are similar for each of the corresponding labeled positions whereas the POPC system exhibits shorter T_{2_e} values than those of the POPE system (Table III). These results suggest that, for the lipids containing a cyclopropane ring on the *sn*-2 acyl chain, the densities of slow motions giving rise to transverse relaxation, as measured by T_{2_e}, are similar for both types of head groups. For the unsaturated systems, either the rates of slow motion are less or the types of slow motion differ in the hydrophobic core for the larger head group; that is, the PC system is less mobile than the PE analogue. It seems reasonable to expect that the types of motions undergone by the acyl chains in PDSPE and POPE are similar so that the shorter T_{2_e} values observed for the PDSPE system reflect a larger contribution of slow motions to the transverse relaxation. Qualitatively, the T_{2_e} data extend the conclusions derived from T_1 data, that the *sn*-2 acyl chains of the PDSPE membrane system are less dynamic than the corresponding unsaturated phosphatidylethanolamine in both the bilayer and hexagonal phases.

Examination of Figure 4 reveals an interesting feature (dashed arrows) when 2τ is 441.5 μs. The shape of the shoulders deviates from that of a typical powder spectrum (Figure 4, 2τ = 81.5 μs). Similar angular-dependent T_{2_e} values have been observed with lipids from the membranes of *A. laidlawii* (Rance, 1981) and have been predicted to occur when lateral diffusion having a suitable correlation time, on the order of 10^{-4} s, is occurring (Rance, 1981; Sillescu, 1971; Campbell et al., 1979). In addition, if lateral diffusion is contributing to transverse relaxation, use of more than one refocusing of the quadrupolar echo in a period τ has been predicted (Woessner et al., 1969) to give a larger echo amplitude than that obtained with a single refocusing in the same period τ. Preliminary results of PDSPE labeled at C-5 indicate that multiple refocusing of the quadrupolar interaction does lead to much longer $T_{2_e}(\theta)$ values (B. Perly, I. C. P. Smith, and H. C. Jarrell, unpublished results). More detailed studies using multiple echos are being pursued and will be reported elsewhere.

CONCLUSIONS

Previous studies on phosphatidylcholines (Dufourc et al., 1983, 1984) concluded that replacement of a cis-olefinic function with a cyclopropane ring leads to a more ordered and less dynamic system. Replacement of the phosphocholine head group with the smaller phosphoethanolamine head group leads to a higher orientational ordering of the *sn*-2 acyl chain and at elevated temperatures the reorganization of the bilayer structure into a hexagonal structure (Perly et al., 1985).

The present study demonstrates that while the orientational ordering is greater for PE relative to that of PC, the acyl chains as monitored by spin–lattice relaxation times (T_1) are more mobile in PE. The data indicate that the PC and PE systems having dihydrosterculic acid at the *sn*-2 position have similar rates and types of slow motion. The unsaturated lipids appear to have different rates or types of slow acyl chain motion with PE being the more mobile. Previous studies on phosphatidylcholines (Dufourc et al., 1984) indicate that replacement of a cis-olefinic group by a cyclopropane ring leads to a significant reduction in the rates and amplitudes of acyl chain motion. The present study demonstrates that a similar modification of PE results in a corresponding attenuation in the rates and amplitudes of acyl chain motion with the largest reduction occurring at the C-9 and C-10 positions of the *sn*-2 chain.

The predisposition of lipid to aggregate structural transitions (e.g., bilayer to hexagonal structure) is associated with the dynamic shape of the lipid molecule (Israelachvili et al., 1980; Wieslander et al., 1980). The presence of a cyclopropane ring reduces the dynamic properties of the hydrophobic core but also decreases the temperature of the bilayer–hexagonal transition relative to that of the unsaturated analogue. One might speculate that regions of a natural membrane enriched in PE containing a cyclopropane ring (and perhaps branched methyl groups) would exhibit the interesting property of being more ordered and less dynamic than a corresponding region containing an unsaturated PE, but with the potential to reorganize into nonbilayer structures.

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

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Deglycosylation of Asparagine-Linked Glycans by Peptide:*N*-Glycosidase F†

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ABSTRACT: Endo-β-*N*-acetylglucosaminidase F (Endo F) and peptide:*N*-glycosidase F (PNGase F) were purified from cultures of *Flavobacterium meningosepticum* by ammonium sulfate precipitation followed by gel filtration on TSK HW-55(S). This system separated the two enzymes and provided PNGase F in a high state of purity, but the basis for the resolution appeared to be hydrophobic interaction and not molecular size. Studies using purified Endo F and PNGase F with defined glycopeptides demonstrated that Endo F was somewhat similar to Endo H in that it hydrolyzed many, but not all, high-mannose and hybrid oligosaccharides, as well as complex biantennary oligosaccharides. PNGase F, in contrast, hydrolyzed all classes of asparagine-linked glycans examined, provided both the α-amino and carboxyl groups of the asparagine residue were in peptide linkage. Deglycosylation studies with PNGase F revealed that many proteins in their native conformation were susceptible to this enzyme but that prior denaturation in sodium dodecyl sulfate greatly decreased the amount of enzyme required for complete carbohydrate removal.

Recently (Plummer et al., 1984), we reported that cultures of *Flavobacterium meningosepticum* contain two oligosaccharide chain cleaving enzymes active on asparagine-linked glycans: an endo-β-*N*-acetylglucosaminidase F (Endo F),[1] first reported by Elder & Alexander (1982), and a new peptide:*N*-glycosidase F, designated PNGase F (Plummer et al., 1984) in accordance with our previous nomenclature (Plummer & Tarentino, 1981). Endo F cleaves the oligosaccharide chain between the di-*N*-acetylchitobiose moiety of some asparagine-linked glycans, primarily those of the high-mannose type. PNGase F, a potent enzyme of broader substrate specificity, hydrolyzes at the glycosylamine linkage and generates a carbohydrate-free peptide and an intact oligosaccharide with the di-*N*-acetylchitobiose unit at the reducing end.

Partially purified preparations of Endo F, including the commercially available material, have enjoyed widespread

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[1] Abbreviations: Endo F, endo-β-*N*-acetylglucosaminidase F; PNGase F, peptide:*N*-glycosidase F; Dns, 5-(dimethylamino)naphthalene-1-sulfonyl; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; RNase B, ribonuclease B; HTF, human transferrin; α_1GP, α_1-acid glycoprotein; OVB, ovalbumin; IgM, immunoglobulin M; Fet, fetuin; PGP, pentaglycopeptide; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; HPLC, high-pressure liquid chromatography; Con A, concanavalin A.

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