Role of Cyclopropane Moieties in the Lipid Properties of Biological Membranes: A ^2H NMR Structural and Dynamical Approach†

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ABSTRACT: The organization and dynamics of specifically ^2H-enriched 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine have been investigated by ^2H NMR. The presence of a cyclopropane ring in the *sn*-2 chain, which gives rise to a significant increase in the segmental order parameter at the position of the cyclopropane ring, leads to no corresponding discontinuity in the segmental order profile of the *sn*-1 chain but does lead to increased order parameters with respect to the corresponding position in 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine at the same reduced temperature. The order–position profile of the *sn*-1 chain is very similar to that reported for lipid systems containing two palmitoyl chains. The quadrupolar splittings associated with the labeled positions of the *sn*-1 palmitoyl chains are more sensitive to temperature changes than are the corresponding positions of the dihydrosterculoyl (DS) chains. At low temperatures, the ^2H NMR spectra of the *sn*-1 chain differ markedly in shape and width from those of the corresponding positions of the *sn*-2 chain. The results suggest that between the bilayer surface and the C-9, C-10 positions, the DS chain possesses larger motional amplitudes than do the corresponding positions of the *sn*-1 saturated acyl chain, as the lipid system enters the gel phase. The deuterium spin–lattice relaxation rates (T_{1z}^{-1}) associated with the *sn*-1 and *sn*-2 chain positions may be interpreted in terms similar to those used for the segmental order–position profiles. The relaxation rate–position profile is characterized by a marked discontinuity at the positions of the cyclopropane ring, indicating that the deuterons at these positions have relaxation rates which are greater than those at any other labeled position of the *sn*-2 chain. The *sn*-1 chain does not exhibit a discontinuity, for positions up to C-10, when T_{1z}^{-1} is monitored as a function of label position. The observed positional dependence of both the rates and amplitudes of the C–^2H bond motions indicates that the effect of the cyclopropane ring is mainly localized on the *sn*-2 chain and does not affect the *sn*-1 chain significantly. The values of both the segmental order parameter (S_α) and the ^2H relaxation rates associated with positions on the cyclopropane ring suggest that this group in effect is a barrier to propagation of motion along the acyl chain. Consequently, the membrane characteristics are relatively insensitive to large thermal variations, maintaining the liquid-crystalline or "fluid" state at physiological temperatures.

In a recent study (Dufourc et al., 1983), the behavior of 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine (PDSPC)[1] as aqueous lamellar dispersions was monitored by deuterium (^2H) NMR of specifically enriched PDSPC molecules. This was undertaken in order to try to explain why monoenoic fatty acids are converted in the late log phase of growth, through the energy-expensive addition of a CH_2 group, to cyclopropane fatty acids in some bacteria (Hofmann et al., 1959; Christie, 1969). It led to the conclusion that the PDSPC model membranes exhibit properties quite different, especially in their temperature behavior and their order parameter profile in the liquid-crystalline phase, from those of other phospholipid–water mixtures in general, and from those of POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) model membranes (Seelig & Waespe-Šarčevič, 1978) in particular. The most dramatic effect of the cyclopropane ring is a large perturbation, at its own level, in the molecular order profile: the molecular order parameter of the cyclopropane ring is 40% higher than that of the plateau region (from C-9, C-10 toward the glycerol backbone). The present study was undertaken to determine if the perturbation is also present in the *sn*-1 palmitoyl chain and to monitor the dynamics of both acyl chains through the deuterium relaxation times T_1 and T_2.

Materials and Methods

1-Palmitoyl-2-dideuteriodihydrosterculoyl-*sn*-glycero-3-phosphocholine (*sn*-2-deuterated PDSPC) was prepared as already reported (Dufourc et al., 1983). 1-Dideuteriopalmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine (*sn*-1-deuterated PDSPC) was obtained by direct *sn*-1 acylation, with specifically deuterated palmitic acid anhydride, of the cadmium chloride complex of *sn*-glycero-3-phosphocholine (GPC·$CdCl_2$) (Sigma, St. Louis, MO) and subsequent *sn*-2 acylation with undeuterated dihydrosterculic acid anhydride (Dufourc, 1983). The selectivity of chain labeling was monitored as described elsewhere (Perly et al., 1983).

Membrane preparation, nuclear magnetic resonance spectroscopy, and data treatment were accomplished as described by Dufourc et al. (1983).

Results and Discussion

Organization of the sn-1 Palmitoyl Chain in PDSPC Model Membranes. (A) Theoretical Background. For lamellar liquid-crystalline phases of phospholipids, the ^2H NMR spectra of C–^2H fragments often have the shape corresponding to axially symmetric motion. In this case, the quadrupolar splitting, $\Delta\nu_Q$, between the peaks of the powder spectrum is related to the orientational order parameter, S_{C-^2H}, through the equation (Seelig, 1977)

$$\Delta\nu_Q = \tfrac{3}{4}A_Q S_{C-^2H} \quad (1)$$

where A_Q is the quadrupolar coupling constant (e^2qQ/h). Assuming axially symmetric motions of a given subunit of the

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[1] Abbreviations: DS, dihydrosterculoyl (*cis*-9,10-methyleneoctadecanoyl); PDSPC, 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphocholine; PDSPE, 1-palmitoyl-2-dihydrosterculoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine.

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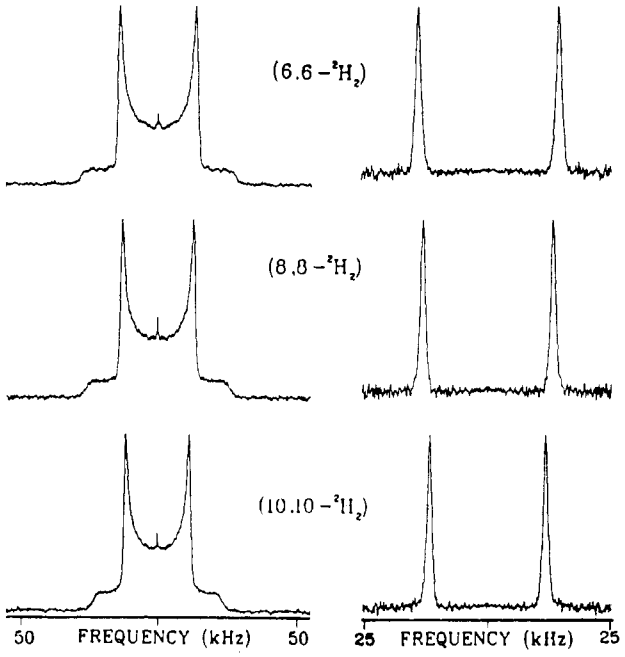

FIGURE 1: ^2H NMR spectra (left) and dePaked spectra (right) of PDSPC model membranes at 25 °C. The palmitoyl chains are deuterated at the positions indicated. Typical experimental parameters: $\pi/2$ pulse length of 5 μs; pulse spacing of 60 μs; recycle time of 0.1–0.2 s; 250-kHz spectral window; 20 000 accumulations.

lipid acyl chains (Seelig & Waespe-Šarčevič, 1978), e.g., methylene group, double bond, cyclopropane group, the S_{C-^2H} order parameter can be split into (Seelig, 1977; Dufourc et al., 1983)

$$S_{C-^2H} = S_\alpha S_\gamma \quad (2)$$

where S_α monitors the angular fluctuations of the axis of motion of the subunit, with respect to the main axis of motion (usually the bilayer normal), and S_γ relates the average orientation of a given C–^{2}H bond of the subunit, with respect to the axis of subunit motion. The segmental order parameter S_α is often denoted S_{mol}.

It has been widely verified that the two deuterons of fatty acyl methylene segments of DMPC and DPPC are equivalent and make an average angle of 90° with respect to the axis of segmental motion (Seelig & Seelig, 1974). In such a case, $S_\gamma = -0.5$ and S_{C-^2H} thus gives a direct measure of the segmental chain fluctuations. However, the above assertion is not true for deuterons linked to the carbon α to the *sn*-2 carboxyl function, for deuterons at the 9′- and 10′-positions in POPC molecules (Seelig & Waespe-Šarčevič, 1978), and for deuterons at the 8′- and 9′-positions and 10′-, 19′-, and 11′-positions in PDSPC molecules (Dufourc et al., 1983). In the latter cases, the S_γ term has to be calculated from the average orientation of the C–^{2}H bond with respect to the axis of segmental motion.

(*B*) *Liquid-Crystalline Phase.* The deuterium spectra of six different samples ([3-$^2H_2$]-, [6-$^2H_2$]-, [8-$^2H_2$]-, [10-$^2H_2$]-, [13-$^2H_2$]-, and [15-$^2H_2$]PDSPC) were recorded at 25 °C.[2] Figure 1 shows some of these spectra. All samples gave rise to a single quadrupolar powder pattern whose shape is characteristic of axially symmetric motions of the lipids in a liquid-crystalline phase (Seelig, 1977). In order to obtain the "true" quadrupolar splitting, $\Delta\nu_Q$, all spectra were "dePaked" (Bloom et al., 1981) according to the procedure described in Dufourc et al. (1983). Some dePaked spectra are also shown

[2] Positions on the *sn*-1 acyl chain are given as unprimed numbers, and those on the *sn*-2 chain as primed numbers.

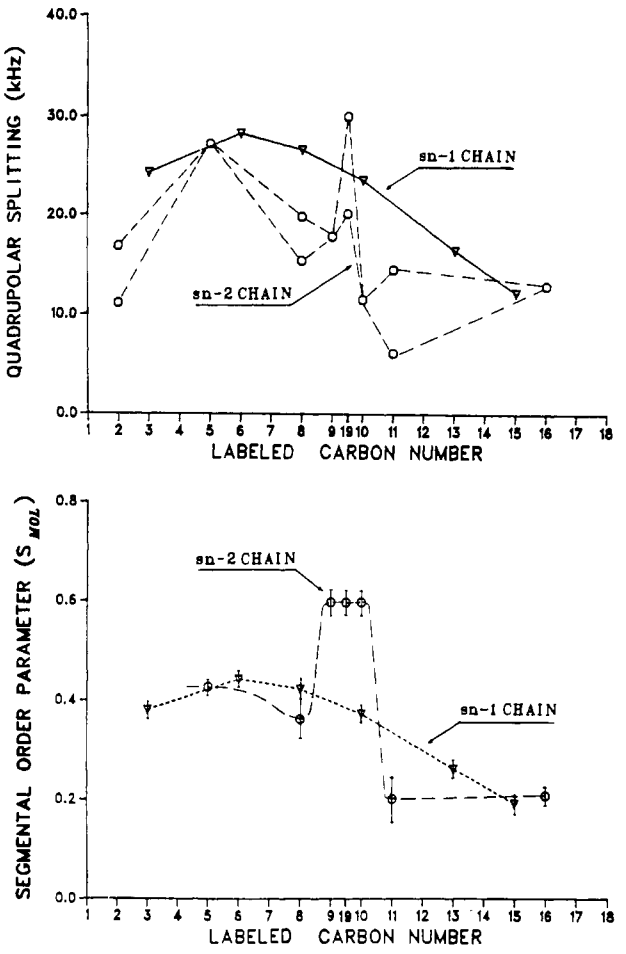

FIGURE 2: (Top panel) Variation of the quadrupolar splitting, $\Delta\nu_Q$, with the position of labeling at 25 °C. (○) Dihydrosterculoyl *sn*-2 chain; (▽) palmitoyl *sn*-1 chain. (Bottom panel) Variation of the segmental order parameter, S_{mol}, with the position of labeling for PDSPC model membranes at 25 °C. Same notations as for the top panel in this figure. The bars give an estimate of the error.

in Figure 1; they show that the two deuterons at any given position in the palmitoyl chain are equivalent; i.e., they have the same average spatial orientation with respect to the instantaneous chain orientation. The positional dependence of the quadrupolar splittings for the *sn*-1 chain and, for comparison, the *sn*-2 chain (Dufourc et al., 1983) is shown in the upper panel of Figure 2. Dramatic differences are apparent. The *sn*-1 palmitoyl chain has a smooth order–position profile whereas that of the *sn*-2 DS chain shows the perturbation induced by the cyclopropane ring. The complex S_{C-^2H} profile of the *sn*-2 chain can be simplified by calculating the S_α (or S_{mol}) order parameter (Dufourc et al., 1983). The lower panel of Figure 2 shows this "geometry-corrected" segmental order profile for the *sn*-2 DS chain. The values reported for the segmental order parameter of the *sn*-1 chain in Figure 2 are taken to be $-2S_{C-^2H}$; in doing so, we assumed that the individual C–^{2}H bonds of this chain make an average angle of 90° with respect to the axis of segmental motion. There is no reason a priori for such a choice. It is possible that the cyclopropane ring of the *sn*-2 chain perturbs the *sn*-1 chain in such a way that the deuterons at positions 8 or 10 are still equivalent; i.e., they make the same average angle with respect to the axis of segmental motion, but this angle has a value of 80° or 70°. In this case, S_γ would be less than −0.5, and consequently S_α would be greater than $-2S_{C-^2H}$. However, such a molecular order profile has already been encountered in DMPC or DPPC bilayers: indeed, it was observed that the plateau region (up to C-10) was progressively broken when

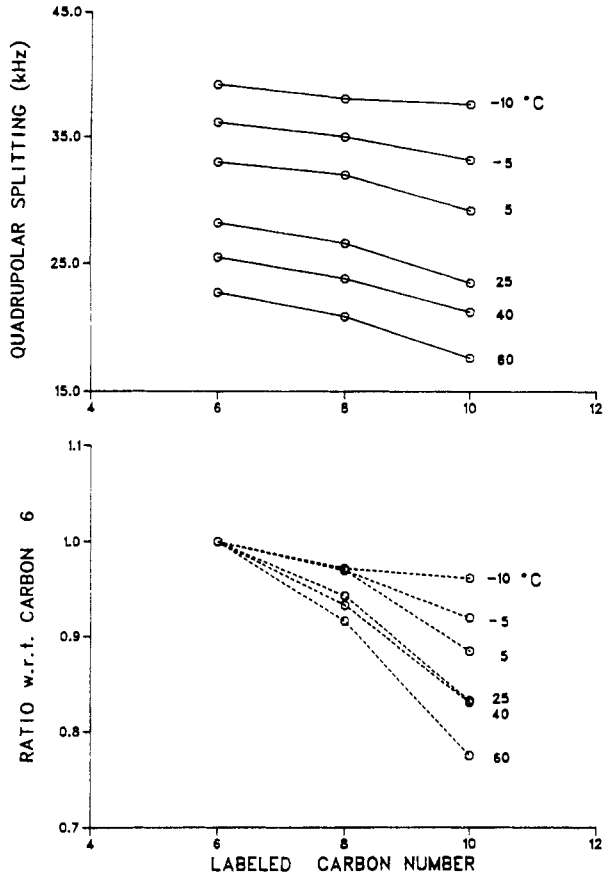

FIGURE 3: Temperature dependence of quadrupolar splittings for specifically deuterium enriched PDSPC model membranes (palmitoyl *sn*-1 chain). (Top panel) Quadrupolar splittings as a function of the labeled positions at the indicated temperatures. (Bottom panel) Quadrupolar splitting ratios as a function of the labeled positions. The ratio is made with respect to the quadrupolar splitting at C-6 at a given temperature (see text). The symbols give an estimate of the error.

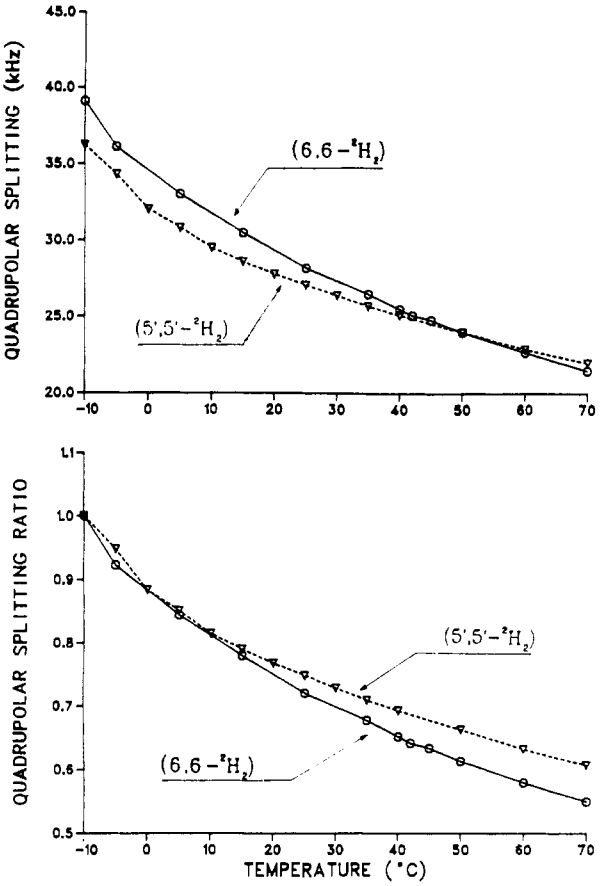

FIGURE 4: Temperature dependence of the quadrupolar splittings of specifically deuterium enriched PDSPC molecules. (Top panel) Quadrupolar splittings at the indicated labeled positions. (Bottom panel) Quadrupolar splitting ratios (see text). The symbols give an estimate of the error.

the temperature was raised above that of the phase transition, T_c (Oldfield et al., 1978; Seelig & Seelig, 1974). For PDSPC bilayers, the measuring temperature of Figure 2 is 35 °C above the temperature of the phase transition (−10 to −15 °C). It is therefore not surprising to find such a profile, with almost no plateau, for the *sn*-1 palmitoyl chain.

(C) Plateau Region of the sn-1 Palmitoyl Chain. Figure 3 represents the temperature behavior of deuterons at positions 6, 8, and 10 (half of the plateau region). The upper panel shows the absolute response of the quadrupolar splittings to increasing temperature, whereas the lower panel of Figure 3 exhibits the changes relative to carbon 6. The latter shows clearly the progressive loss of plateau character from −10 to 60 °C. At −10 °C, just above the transition temperature, the 6-, 8-, and 10-positions have almost equivalent $\Delta\nu_Q$ values, very similar to those observed for DPPC bilayers at 41 °C (Seelig & Seelig, 1974). Therefore, the assumption that $S_\gamma = -0.5$ made in the preceding section seems to be justified. A more detailed analysis of the lower panel of Figure 3 reveals that the loss of "plateau character" proceeds in two steps: from −10 to 5 °C, only position 10 shows a decrease in its quadrupolar splitting, relative to positions 6 and 8, whereas above 5 °C, the plateau character is completely lost.

(D) Relative Responses to Temperature of the sn-1 and sn-2 Chains. To gain further insight into the interchain interactions, spectra of [6-$^2H_2$]PDSPC were recorded between −10 and 70 °C and compared with those obtained for the [5′-$^2H_2$]PDSPC system by Dufourc et al. (1983). All spectra had axially symmetric shapes and were dePaked; the corresponding quadrupolar splittings are reported in Figure 4 (top panel). The bottom panel of Figure 4 shows, for both labeled positions, the quadrupolar splitting ratio $\Delta\nu^k_{Q,T_j}/\Delta\nu^k_{Q,T_{-10}}$, where $\Delta\nu^k_{Q,T_j}$ and $\Delta\nu^k_{Q,T_{-10}}$ are the quadrupolar splittings of the kth position at temperature T_j and at $T = -10$ °C, respectively. Figure 4 reveals that the temperature dependences of the two chains are different: above 10 °C, the *sn*-2 chain at position 5′ is less sensitive to temperature changes than is the *sn*-1 chain at position 6. At low temperatures, the palmitoyl chain exhibits larger quadrupolar splittings than the DS chain. Figure 4 suggests that just above T_c both positions have an identical rate of decrease in their $\Delta\nu_Q$ values, whereas at temperatures which exceed T_c by 20 °C or more, the palmitoyl chain at C-6 exhibits a stronger relative decrease (Figure 4, bottom panel) than the DS 5′-position. It has been shown (Dufourc et al., 1983) that the temperature response of the upper half of the *sn*-2 chain (from the cyclopropane ring to the glycerol backbone) was very similar for all C–^{2}H bonds. Thus, one can conclude that the response of ordering to increasing temperature is greater for the *sn*-1 palmitoyl chains than for the *sn*-2 DS chain.

It is well-known that in phospholipids the two acyl chains are not physically equivalent (Seelig & Seelig, 1975; Davis, 1979); the CH_2 segments of the *sn*-1 chain are closer to the center of the bilayer than are the corresponding segments of the *sn*-2 chain. From the inequivalence of the two deuterons at position 2′, it appears that this is also the case in PDSPC model membranes (Dufourc et al., 1983). It is therefore somewhat surprising that the palmitoyl chain at position 6 has

Table I: Quadrupolar Splittings at Absolute and Relative Temperatures[a]

model membrane system	T_c (°C)	$\Delta\nu_Q$ (kHz), temp (°C)	$\Delta\nu_Q$ (kHz), temp (°C)	ref
PDSPC, *sn*-1 6-$^2H_2$	−10, −15	39.2, −10	25.0, 42	
PDSPC, *sn*-2 5′-$^2H_2$	−10, −15	36.2, −10	25.2, 40	*b*
DPPC, *sn*-1 5-$^2H_2$	41	29.5, 41	29.5, 41	*c*
DPPC, *sn*-2 5′-$^2H_2$	41	29.5, 41	29.5, 41	*c*
POPC, *sn*-1 5-$^2H_2$	−5	33.8, −1	24.5, 42	*d*
POPC, *sn*-1 6-$^2H_2$	−5	35.1, −1	24.9, 42	*d*
POPC, *sn*-2 5′-$^2H_2$	−5, 0	31.2, 0		*e*

[a] Comparison of quadrupolar splittings for different systems at similar relative and absolute temperatures. Data from Dufourc et al. (1983) and B. Perly et al. (unpublished results) are from dePaked spectra. $\Delta\nu_Q$ from Seelig & Seelig (1977) was calculated from the value of S_{mol}, taking A_Q = 170 kHz. [b] Dufourc et al. (1983). [c] Seelig & Seelig (1974). [d] Seelig & Seelig (1977). [e] B. Perly, I. C. P. Smith, and H. C. Jarrell (unpublished results).

a higher $\Delta\nu_Q$ than the DS chain at position 5′ (see Table I). It is instructive to compare the PDSPC system with the POPC and DPPC systems under conditions where they are in the same physical state, that is, subjected to the same average molecular forces. In theories of phase transitions, this is often assumed to occur when systems are at the same temperatures relative to their individual phase transition temperatures (T_c); temperature on this scale is known as reduced temperature (Stanley, 1971). Consequently, the quadrupolar splittings of positions 5 and/or 6 for both chains of the three model membranes are reported in Table I at temperatures just above the respective phase transition of each of the systems. These data indicate that in this region of the bilayer there is no difference in $\Delta\nu_Q$ between the two chains in DPPC, whereas the marked difference in quadrupolar splitting observed between the palmitoyl and dihydrosterculoyl chains in PDSPC is also present between the palmitoyl and oleoyl chains of POPC. Furthermore, at the same reduced temperature, one can classify the three systems on a scale of increasing order, at the plateau positions, as DPPC < POPC < PDSPC. However, for all systems at 41 °C, the converse is true (Table I). In order to account for the increased order of the *sn*-1 chain of POPC relative to that of DPPC, at the same reduced temperature, Seelig & Seelig (1977) suggested that the C=C bond of the oleoyl chain was inducing a stiffening in the plateau region of the palmitoyl chain in POPC. Our results are consistent with this hypothesis. The cyclopropane ring would thus, by its ordered structure [it has been demonstrated that the ring has a higher S_{mol} than all other *sn*-2 positions (Dufourc et al., 1983)], increase the segmental order parameter of both chains and preserve the plateau region of the palmitoyl chain at temperatures well above the transition temperature T_c. Figure 4 shows that this stiffening effect operates at position 6 up to 40 °C (50 °C above T_c).

If the cyclopropane ring reduces the angular fluctuations of the *sn*-1 chain as does the double bond, it is still unclear why the plateau region of its own chain is less ordered, at low temperatures, than the corresponding region of the palmitoyl chain (Figure 4). A possible answer might be found in the calculated average orientation of the cyclopropane. The C-9′–C-10′ bond was shown to make an average angle of 90° with respect to the instantaneous chain orientation, i.e., the axis of segmental motion of the cyclopropane ring (Dufourc et al., 1983). For clarity, the average spatial orientation, extracted from these calculations, is roughly sketched in Figure 5 for S_{mol} = 1. The aim of Figure 5 is not to represent the exact structures of the chains but rather to suggest the cylinders of influences (assuming axially symmetric motion) for each chain subunit. The cyclopropane ring has a larger radius of influence than the other segments of the chains, so that at low temperatures this region of the *sn*-2 acyl chain would not be readily accommodated into the more ordered gel phase but rather form a defect in the ordered packing of the lipid matrix. The residual angular fluctuations observed for the 5′-methylene segment may result from the accommodation of the defect in the gel state. For the order–position profile of the *sn*-1 acyl chain, the interchain effects of the cyclopropane ring are not dramatic, so that in the gel phase the putative defect is not transmitted to the *sn*-1 chain. According to this model, a lower local ordering of the DS chain for the plateau positions would be expected relative to that for the corresponding segments of the palmitoyl chain.

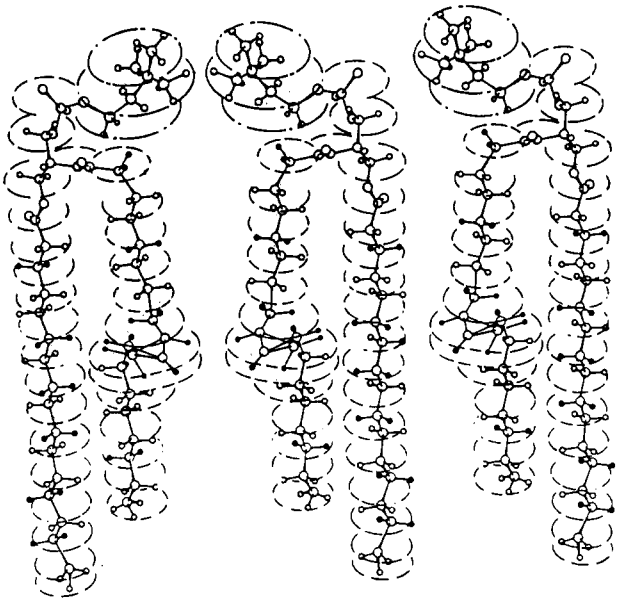

FIGURE 5: Hypothetical (average) configuration of PDSPC molecules embedded in a model membrane, where S_{mol} = 1. The filled circles represent the labeled positions. The dashed circles show the cylinder of influence of each subunit (see text).

(*E*) *Phase Transition.* By monitoring the spectral response at the 5′-$^2H_2$-position to decreasing temperature, it was shown that the phase transition of PDSPC model membranes occurred between −10 and −15 °C (Dufourc et al., 1983). A similar study was carried out for position 6-$^2H_2$ on the *sn*-1 chain. The corresponding spectra are shown in Figure 6, as well as the spectrum of [5′-$^2H_2$]PDSPC at −20 °C. Spectra at −20 °C were recorded by using a 500-kHz spectral width, a $\pi/2$ pulse width of 6 μs, and a 35-μs spacing between the $\pi/2$ pulses of the echo sequence. Figure 6 shows that the spectra for position 6 begin to lose their axially symmetric shape at −10 °C and that at −20 °C the gel phase is clearly present. One notices also that at −20 °C the liquid-crystalline component still contributes to the spectrum. However, most striking is the marked difference in spectral features between the *sn*-1 and *sn*-2 positions at −20 °C. The maximum width observed for the spectrum of [6-$^2H_2$]PDSPC is ca. 125 kHz whereas the [5′-$^2H_2$]PDSPC spectrum has a maximum width of ca. 100 kHz. The dramatic differences in the spectral shapes due to positions 6 and 5′, at −20 °C, could arise from several sources; a possible explanation follows. The 125-kHz broad feature in the *sn*-1 spectrum indicates the cessation of angular fluctuations of that chain, a situation which has already been encountered for straight-chain saturated lipids in the gel phase (Smith et al., 1979; Davis, 1982). The 100-kHz maximum width appearing in the spectrum of the *sn*-2 chain

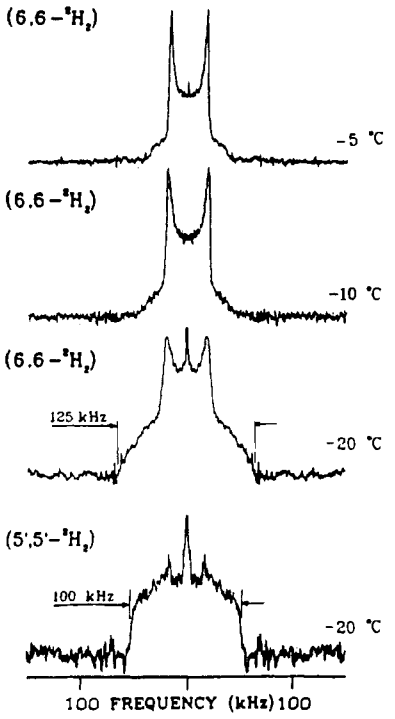

FIGURE 6: Spectral shapes of [6-$^2H_2$]- and [5'-$^2H_2$]PDSPC model membranes at low temperatures. Same experimental parameters as in Figure 1 except for spectra recorded at −20 °C where the spectral window was 500 kHz.

at position 5' may be explained in at least two ways: either there are residual angular fluctuations [hence, $S_\alpha \sim 0.8$ ($S_\gamma = -0.5$)] or there are no angular fluctuations ($S_\alpha = 1$) but the C–^{2}H bonds at position 5' are tilted with respect to the axis of residual motion, giving rise to $S_\gamma = -0.4$. Such a value of S_γ would imply a 15° tilt of these C–^{2}H bonds with respect to the bilayer normal, at −20 °C. Although the present data do not allow distinction between these two hypotheses, the latter hypothesis raises the possibility that this supposed tilt would be present in the axially symmetric liquid-crystalline phase which would also explain why the *sn*-1 chain is more ordered in the plateau region than is the *sn*-2 chain. The hypothesis of a tilt at positon 5' in PDSPC should then also hold for [5'-$^2H_2$]POPC since the same spectral differences are observed between the *sn*-1 and *sn*-2 positions for both systems (see Table I). However, it seems improbable that, in the liquid-crystalline phase, either the double bond or the cyclopropane moiety induces a tilt of a C–^{2}H bond four segments removed. Furthermore, spectra of [5'-$^2H_2$]POPC at temperatures below the phase transition gave a maximum width of ca. 125 kHz (B. Perly, unpublished results). It appears more likely, therefore, that the 100-kHz width observed for the [5'-$^2H_2$]PDSPC sample at −20 °C is characteristic of that position in the gel phase and is due to residual angular fluctuations.

This study of the organization of the *sn*-1 palmitoyl chain in model membranes of PDSPC brings new details in the understanding of the system. It has been observed that the plateau region of the *sn*-1 chain is preserved at temperatures considerably above that of the phase transition. At low temperatures, the *sn*-1 plateau is more ordered than the corresponding *sn*-2 positions. However, the ordering of the *sn*-1 chain appears to be more sensitive to temperature changes than is that of the *sn*-2 chain. From these observations, the hypothesis that the cyclopropane ring possesses a cylinder of influence which induces an ordering of the *sn*-1 plateau region and additional flexibility for the upper half of the *sn*-2 chain has been postulated. Finally, one can classify the DPPC, POPC, and PDSPC model membrane systems in terms of increasing degree of order of the upper regions of the *sn*-1 and *sn*-2 chains, at similar temperatures relative to their respective T_c values, as follows: DPPC < POPC < PDSPC. At the same absolute temperature, e.g., ~41 °C, this classification is effectively reversed: POPC ~ PDSPC < DPPC.

Dynamics of the sn-1 and sn-2 Chains in PDSPC Model Membranes. The deuterium quadrupolar splitting $\Delta\nu_Q$ reflects structural information on the average orientation of a particular C–^{2}H bond and on the amplitude of the angular fluctuations of that segment. Deuterium NMR relaxtion time measurements yield complementary information about the dynamics of the lipid molecules. Although a larger number of ^{2}H NMR structural studies have appeared in the scientific literature, there have been few ^{2}H relaxation time measurements. Relaxation measurements are time consuming, and their theoretical analysis is just under development. However, some aspects of relaxation of spin 1 systems in anisotropic media have recently been elucidated by Jeffrey (1981) and Brown (1982).

(*A*) *Theoretical Background.* Brown (1982) has made the most recent attempt to formulate relaxation rates in terms of acyl chain motions. Assuming a general model for anisotropic rotational diffusion to describe the segmental or molecular reorientations in lipid bilayers, he derived the rates of spin–lattice relaxation as a function of the limited amplitude fluctuations of the C–^{2}H bond vectors with respect to the axis of motion. He extended his model to account for slow fluctuations of the local director with respect to the macroscopic bilayer normal.

In the case where spin–lattice relaxation is only due to fast motions, assuming that the autocorrelation function for C–^{2}H bond fluctuations (which gives rise to the T_{1z} relaxation) can be described by a single exponential decay of the time constant, τ_c, and that these motions fall into the short correlation limit, i.e., $\omega_0^2\tau_c^2 << 1$, Brown derived the following expression for the spin–lattice relaxation rate:

$$\frac{1}{T_{1z}} = \tfrac{3}{8}(2\pi)^2 A_Q^2 (1 - S_{C\text{-}^2H}^2)\tau_c \qquad (3)$$

A similar equation had been derived by Brown et al. in 1979, predicting that T_{1z} would vary across the powder pattern. It has been shown (Brown & Davis, 1981) that this was not indeed the case for DPPC; our T_{1z} data for model membranes of PDSPC also demonstrate essentially an independence of orientation (vide infra).

(*B*) *^{2}H Spin–Lattice Relaxation Measurements on sn-1 and sn-2 Chains of the PDSPC Model Membrane System.* The spin–lattice relaxation rates were measured for each of the ^{2}H-labeled positions along both the dihydrosterculoyl and palmitoyl chains at 25 °C. The relaxation data were obtained by the inversion–recovery technique (Dufourc, 1983). In all cases, the spin–lattice relaxation process was characterized by a single exponential. T_{1z} values were extracted by fitting the equation $M(\tau_1) = M(0)[1 - A\exp(-\tau_1/T_{1z})]$ to the integrated areas of certain regions of the spectral shape, namely, "windows" around the $\theta' = 0°$, 90°, and 54.7° orientations (θ' represents the angle between the direction of motional averaging and the magnetic field), where $M(\tau_1)$ and $M(0)$ represent the longitudinal magnetization at times τ_1 and $\tau_1 = 0$, respectively. The adjustable parameter A was used to account for the imperfections of the 180° inverting pulse. A typical fit of the exponential decay is shown in Figure 7 (top panel). The results are summarized in Table II. Among all spectra recorded, those of [16'-$^2H_2$]PDSPC show the most apparent

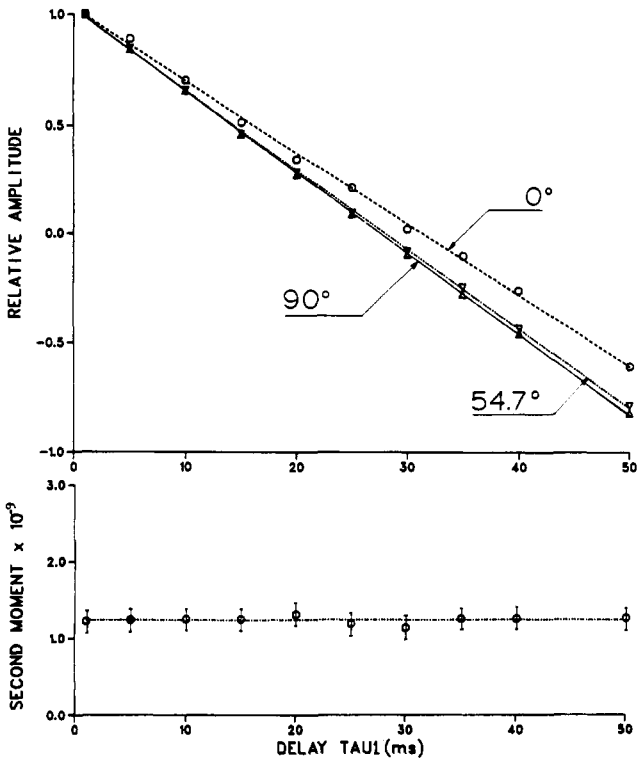

FIGURE 7: (Top panel) Typical T_{1z} measurement for macroscopic orientations (angles $\theta' = 0°$, 90°, and 54.7°), in this case for [5-$^2H_2$]PDSPC at 25 °C. The relative amplitude represents the value $\ln\{[1 - M(\tau_1)]/M_0\}/\ln A$ (see text). The data are normalized to the value at the smallest τ_1. (Bottom panel) Variation of the second moment of spectra yielding the above data as a function of the delay τ_1.

Table II: Relaxation Times of PDSPC, POPC,[a] and Model Membranes at 46.1 MHz

model membrane system	T (°C)	$\lvert S_{C-^2H}\rvert$	T_{1z}[c] (ms)	τ_c[d] (s)
PDSPC, *sn*-1				
3-$^2H_2$	25	0.190	16.6	1.5×10^{-10}
6-$^2H_2$	25	0.221	20.1	1.2
8-$^2H_2$	25	0.209	20.7	1.2
10-$^2H_2$	25	0.184	21.8	1.1
15-$^2H_2$	25	0.096	72.0	0.33
PDSPC, *sn*-2				
5′-$^2H_2$	25	0.216	16.5	1.5
8′-$^2H_2$	25	0.123	13.8	1.7
8′-$^2H_2$	25	0.158	14.0	1.7
9′-$^2H_2$[b]	25	0.130	9.6	2.1
19′-$^2H_2$	25	0.146	8.4	2.5
19′-$^2H_2$	25	0.219	9.4	2.3
10′-$^2H_2$[b]	25	0.084	8.5	2.4
11′-$^2H_2$	25	0.049	14.1	1.7
11′-$^2H_2$	25	0.115	14.2	1.7
16′-$^2H_2$	25	0.103	44.9	0.53
POPC, *sn*-2				
5′-$^2H_2$	36	0.183	24.1	1.0
9′-$^2H_2$	36	0.099	20.9	1.1
10′-$^2H_2$	36	0.017_7	17.6	1.2
16′-$^2H_2$	36	0.083_4	67.8	0.35

[a] From B. Perly, I. C. P. Smith, and H. C. Jarrell (unpublished results). [b] The assignment of these positions is arbitrary (and could be inverted). [c] The accuracy of T_{1z} is about ±5%. The values reported herein are measured for the peaks ($\theta' = 90°$). [d] From eq 3 (see text), with $A_Q = 175$ and 183 kHz for C-^{2}H segments on double bonds (Seelig, 1977) and on cyclopropane (Dufourc et al., 1983), respectively, and 170 kHz (Seelig, 1977) otherwise.

angular dependence in T_{1z} (Figure 7). The second moment, M_2, also shown in Figure 7, is independent of τ_1 within experimental error. This shows that there is no systematic change in the shape of the spectra for τ_1 values between 1 and 50 ms. The angular dependence described by Brown et al. (1979) would affect the second moment by at least 1 order of magnitude for τ_1 values near the "null" point, i.e., spectra in the τ_1 domain for which the intensity is near zero (H. C. Jarrell et al., unpublished results). The apparent angular dependence in Figure 7 (top panel) is most likely due to the inaccuracy involved in measuring areas near the 0° orientation. In cases where more than one powder spectrum is present, an inaccurate determination of the T_{1z} of each component could result from a direct analysis of the superimposed spectra. In such cases, the entire set of partially relaxed spectra were dePaked, and the calculated oriented spectra were analyzed as described above to obtain the T_{1z} values of the individual components. Figure 8 shows a series of partially T_{1z} relaxed powder and corresponding dePaked spectra for the [19-$^2H_2$]PDSPC system.

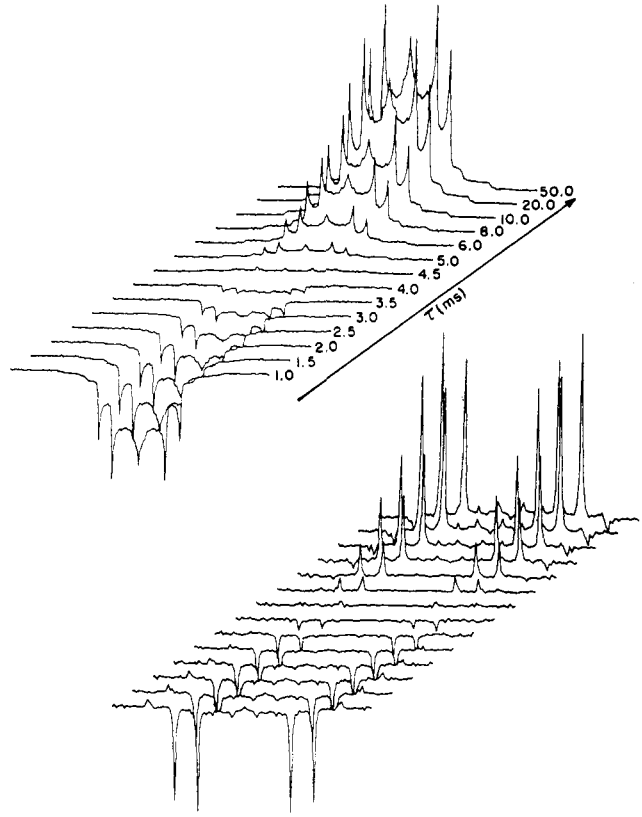

FIGURE 8: Stacked plot of spectra (top) and dePaked analogues (bottom) resulting from a T_{1z} experiment. The data are for [19′-$^2H_2$]PDSPC at 10 °C. The τ values are shown in milliseconds.

The positional dependence of relaxation rates of both *sn*-1 and *sn*-2 chain labels is reported in Figure 9. This is the first case where the relaxation rate profiles of the *sn*-1 and *sn*-2 chains of a lipid in a model membrane can be compared. The comparison is dramatic. Whereas the *sn*-2 DS chain shows a considerable increase in the relaxation rate at the level of the cyclopropane moiety, the *sn*-1 palmitoyl chain exhibits a plateau at the corresponding positions. An almost identical picture was found with segmental order parameter (S_{mol}) profiles (see Organization of the *sn*-1 Palmitoyl Chain in PDSPC Model Membranes). Correlation times corrected for the influence of local ordering can be calculated by using eq 3 (Table II). The positional dependences are very similar to those for relaxation rates in Figure 9. It can therefore be concluded that the motions of the cyclopropane unit are slow and rather strongly correlated. Furthermore, the discontinuity

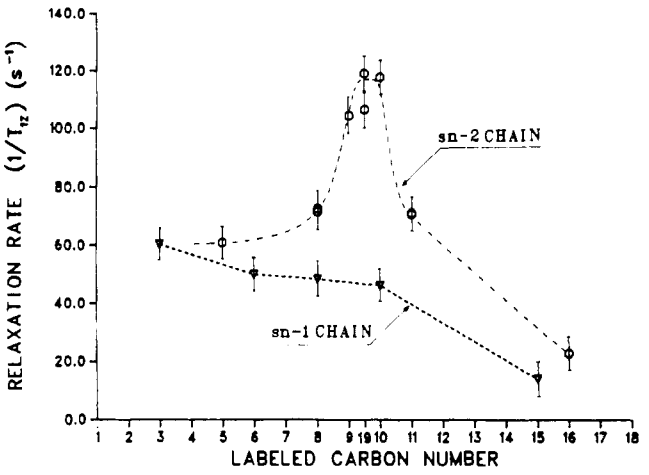

FIGURE 9: Comparison of the relaxation rates of both *sn*-1 and *sn*-2 chains of PDSPC as a function of labeled positions at 25 °C.

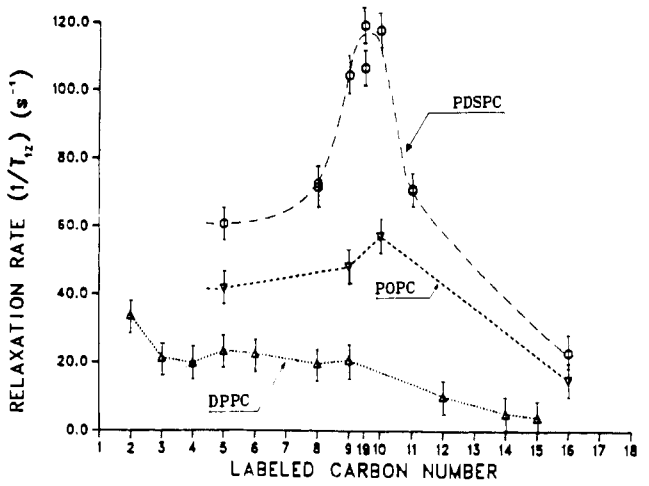

FIGURE 10: Comparison of the relaxation rate profiles for several model membrane systems: *sn*-2 PDSPC at 25 °C and 46.1 MHz (○); *sn*-2 POPC at 36 °C and 46.1 MHz (B. Perly et al., unpublished results) (▽); DPPC at 80 °C and 54.4 MHz (Brown et al., 1979) (△).

in the relaxation rate profile observed for the *sn*-2 chain is not seen for the *sn*-1 chain. It appears, therefore, that the motions are more correlated through bonds than through space. Although the shape of the *sn*-1 relaxation rate profile is not perturbed by the presence of the cyclopropane moiety on the *sn*-2 chain, the correlation times of all *sn*-1 positions are longer than the corresponding correlation times of DPPC, at the same reduced temperature (Brown et al., 1979). The relaxation rate at C-3 is the highest among all *sn*-1-labeled positions examined. This reflects the attachment of the chains to the glyceryl moiety, which has been found to have a long correlation time in DPPC, at least twice that of the plateau positions (Brown et al., 1979). The relaxation rates for all *sn*-2 positions are higher than those of the corresponding *sn*-1 position, indicating that the cyclopropyl unit is more efficient at attenuating the rates of motion of its own chain than it is for those of the neighboring chain. Furthermore, one notices in Figure 9 that positions adjacent to the ring (namely, C-8′ and C-11′) are more sensitive to this reduction than are positions far away from it (C-5′ and C-16′). This is not surprising in view of the structural studies (Dufourc et al., 1983) which showed that the cyclopropyl moiety perturbs positions 8′ and 11′ (by inducing orientational inequivalences of the deuterons bound to these carbons) but not positions 5′ and 16′. The generally slow motions of the *sn*-2 positions could also be correlated with the fact that the degree of ordering of this chain was found to be less sensitive to temperature variations than that of the *sn*-1 chain (vide supra).

Carbon–deuterium bonds attached to the same carbon atom or to a rigid unit like cyclopropane are expected to have the same motional correlation time, providing a basis to test the validity of eq 3. From Table II, it appears that this equation gives the same correlation time for deuterons at C-8′ and C-11′, whereas there is a discrepancy of 10–20% among the deuterons of the ring. In order to account for this fact, on might consider that given the slow motions of the cyclopropane moiety, movements other than molecular rotations, torsional oscillations, and bond stretching and bending could contribute to the spin–lattice relaxation. The present data could thus be reanalyzed by adding to eq 3 terms accounting for noncollective or collective modes of low-frequency motions (lateral diffusion, whole lipid bilayer reorientation, etc.) as described by Brown (1982). According to eq 3, differences in relaxation rates can arise if there are differences in the static quadrupolar coupling constants (e^2qQ/h). However, the differences in the relaxation rates for deuterons at the 19′-position (Table II) must be due to motional properties since the A_Q term in eq 3 is expected to be identical for the two deuterons. Similarly, the differences in the relaxation rates of deuterons at the 9′- and 10′-positions must have a motional origin since again the A_Q value should be identical for the two deuterons.

Spin–lattice relaxation times were measured for [19′-$^2H_2$]PDSPC from −5 to 45 °C. A marked increase in the relaxation time was observed on raising the temperature, implying that the motions dominating the relaxation process have correlation times such that $\omega_0^2\tau_c^2 << 1$. Assuming that τ_c has an Arrhenius dependence on temperature, one can determine from the temperature dependence of ln T_{1z} the magnitude of E_a, the activation energy for molecular motions. The activation energy estimated from the [19′-$^2H_2$]PDSPC data is 12.3 ± 1.0 kJ·mol^{-1}, which lies between the 14.6 ± 1.3 and the 10.5 kJ·mol^{-1} found in bilayers and in an isotropic solution of DPPC, respectively (Brown et al., 1979).

(C) Comparison of Relaxation Rate Profiles between Several Model Membrane Systems. As mentioned earlier, model membrane systems may be compared when subjected to the same average molecular forces. This is believed to occur at equal temperatures relative to that of the phase transition. Although the available deuterium relaxation data are not extensive, we compare in Figure 10 the relaxation rates as a function of the labeled carbon position of *sn*-2 PDSPC at 25 °C, *sn*-2 POPC at 36 °C (B. Perly et al., unpublished results), and DPPC at 80 °C and 54.4 MHz (Brown et al., 1979). All relaxation rates were therefore monitored at 35–40 °C above the gel to liquid-crystalline phase transition of each of the three systems. Again, the behavior of these model membranes is significantly different. The most obvious general comment is that the rates of relaxation increase from the fully saturated to the cyclopropane-containing lipids. On the basis of eq 3, at the same relative temperature one can therefore classify the three systems on a scale of increasing correlation time of the chain motions as follows: DPPC < POPC < PDSPC. The above classification for the *rates* of motions is similar to that found for the *amplitudes* of the motions, at the same reduced temperature. Figure 10 also shows that, at the level of the functionality, i.e., the double bond or the cyclopropane ring, there is an increase in the rate of relaxation with respect to the positions preceding the functional group. Therefore, one can conclude that the double bond of POPC impedes chain motions, as does the cyclopropane ring, but to a lesser degree. In PDSPC, the increase in correlation time at the level of the cyclopropane ring can be correlated with an increase in the

Table III: Transverse Relaxation Times for *sn*-2 PDSPC

labeled carbon	$\lvert S_{C-^2H}\rvert$	T (°C)	T_2 (μS)[a] 0°	90°	54.7°	$1/[\pi T_2(90°)]$ (Hz)	deP(90°)[b] (Hz)	$T_2(90°)/T_2(0°)$[c]
5′-$^2H_2$	0.216	25	290	470	250	680	900	1.62
8′-$^2H_2$	0.158	25	340	810		390	650	2.38
8′-$^2H_2$	0.123	25		790		400	650	
9′-$^2H_2$	0.130	25	430	580		550	550	1.35
10′-$^2H_2$	0.084	25		590		540	500	
19′-$^2H_2$	0.219	25	470	750		420	700	1.60
19′-$^2H_2$	0.146	25	510	900		350	550	1.76
11′-$^2H_2$	0.115	25	580	1000		320	550	1.72
11′-$^2H_2$	0.049	25		1030		310	650	
16′-$^2H_2$	0.154	−5	310	700	530	460	900	2.26
16′-$^2H_2$	0.113	10	500	840	420	380	730	1.68
16′-$^2H_2$	0.103	25	650	1070	540	300	480	1.65
16′-$^2H_2$	0.092	35	430	560	320	570	640	1.30

[a] Accuracy of T_2 measurements ~10% for the 90° and 54.7° orientations and ~20% for the 0° orientation. [b] Line width estimated by fitting the dePaked spectrum with Lorentzian subcomponents; the fit accuracy is estimated to be 10%. [c] Ratio of T_2 values for the 90° and 0° orientations.

segmental order parameter (decrease in the amplitude of the C–^{2}H bond fluctuations) (see previous section). Seelig & Waespe-Šarčevič (1978) corrected the C-9′, C-10′ S_{C-^2H} order parameters for the geometrical orientation of the double bond with respect to the segmental axis of motion and found that the S_{mol} of the double bond was at the plateau level. However, they were not able to do so when they used only deuterium NMR data (as was done for the geometrical correction of the cyclopropane S_{C-^2H} values). These authors obtained the missing observable from infrared linear dichroism measurements of the C=C stretching frequency at room temperature for egg yolk lecithin (Fringeli, 1977), which contains about 70 wt % POPC. When separating the geometric from the segmental order contributions in the cyclopropane S_{C-^2H}, Dufourc et al. (1983) used two different methods. One of them gives the unique solution (S_{mol}) and is only applicable when one has the number of observables (S_{C-^2H}) consistent with the symmetry of the molecule and when these observables are different enough in magnitude to allow accurate calculations. The other method is less specific and does not give a unique solution but can be used as long as one has two observables for the same rigid unit. Although the first method is not applicable with only the ^{2}H NMR observables of the 9′,10′ double bond, the second was tried for POPC data at 36 °C (see Table II, positions 9′ and 10′). Keeping the axis system defined by Seelig & Waespe-Šarčevič (1978) for the double bond, we found the following two main solutions: $S_{mol} = 0.36 \pm 0.03$ and $S_{mol} = 0.47 \pm 0.03$. The resultant orientations of the double bond were slightly different from that calculated previously: instead of a 7–8° one-dimensional tilt found by the above authors, a small two-dimensional tilt was observed; i.e., the axis of motion was not exactly in the double-bond plane. Although discrimination between these two solutions is not possible, one notices that one of them would lead to a S_{mol} value for the double bond distinctively above the plateau of segmental order parameters for positions anterior to it, analogous to the increase in relaxation rate observed in Figure 10, at the double-bond level. The present data thus confirm that the relaxation rate in ordered lipid systems is strongly dependent on the *segmental* order parameter (S_α or S_{mol}) as well as on the *rate* of motions.

Comparison of relaxation rates between the *sn*-1 palmitoyl and *sn*-2 dihydrosterculoyl deuterium-labeled chains of PDSPC model membranes showed that the cyclopropyl moiety perturbs the correlation time–position profile of the *sn*-2 chain by inducing a considerable slowing down of the motions at the C-9, C-10, and C-19 positions, and at neighboring positions. The *sn*-1 chain, although exhibiting slower motions than in DPPC at the same relative temperature, did not manifest the huge perturbation seen in the *sn*-2 chain profile; instead, a plateau of correlation times up to C-10 was observed. This demonstrates a strong intrachain, but a weak interchain, correlation of motional rates. In addition, the perturbation induced by the ring on the *sn*-2 chain diminishes with distance from the ring. The cyclopropane ring thus anchors the motions at its own level and preserves the order–position plateau of the neighboring palmitoyl chains. The net effect is a stronger correlation of the motions of the entire system, leading to a lesser sensitivity to temperature changes.

Since the positional dependences of the relaxation rates for *sn*-1 and *sn*-2 chains were nearly identical with those found for the segmental order parameters, we conclude that the relaxation rate is *strongly dependent* on the *local ordering* of the system.

Comparison of *sn*-2 chain relaxation rate profiles for PDSPC, POPC, and DPPC at the same relative temperature, and similar magnetic field strengths, showed that these systems could be classified on a scale of increasing correlation time as DPPC < POPC < PDSPC. Because of the small inflection in the relaxation rate–position profile at the C-10 position of the *sn*-2 chain of POPC, an earlier calculation of S_{mol} for the double-bond segment was reexamined. The result suggested that the segmental order parameter of the double bond could be larger than that of the plateau region (C-2–C-8) of the oleoyl chain.

(*D*) *Transverse Relaxation Time Measurements for the sn-2 PDSPC Positions.* The transverse relaxation times, T_2, were estimated for the seven samples labeled on the *sn*-2 chain of PDSPC (Table III). The T_2 spectral set was integrated by windows, namely, macroscopic orientations around $\theta' = 0°$, 90°, and 54.7°, as described for T_{1z}, and the areas were fitted to the expression $M(2\tau) = M(2\tau_{min}) \exp(-2\tau/T_2)$ where $M(2\tau)$ and $M(2\tau_{min})$ are the transverse magnetizations at times 2τ and $2\tau_{min}$, respectively, and where τ_{min} represents the shortest pulse spacing between the two $\pi/2$ pulses required to form the spin-echo. Figure 11 shows an example of a T_2 data set as well as an example of the fit of the above equation for various orientations θ'. One sees clearly that there is a marked angular dependence of T_2. The data for the *sn*-2 positons (Table III) show different values of T_2 at angles of 0°, 90°, and 54.7°. The ratio between T_2 at 90° and at 0° indicates that the angular dependence is not $P_2(\cos\theta')$, since this ratio should equal 2. The discrepancy between the line width estimated from dePaking ($\theta' = 90°$) and the line width calculated from

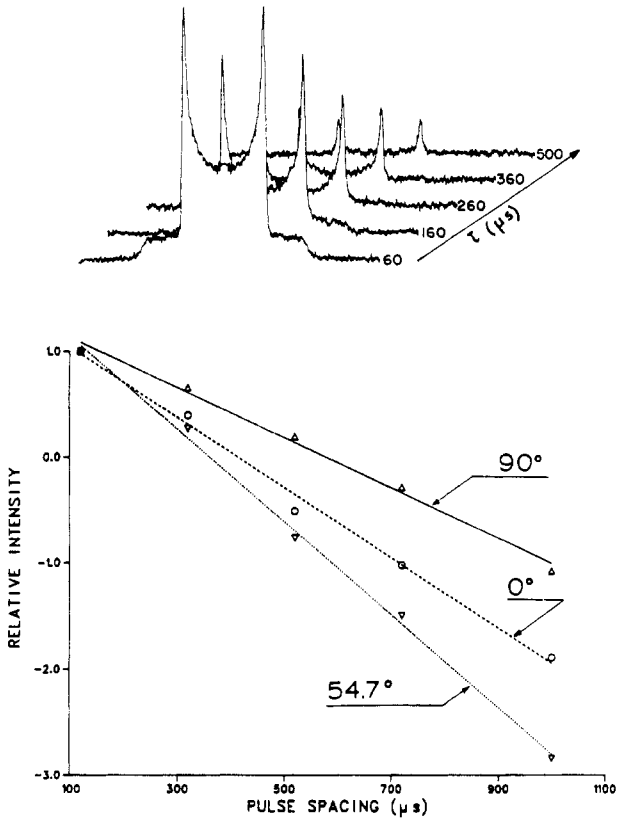

FIGURE 11: (Top) Example of stacked plot of spectra for measuring T_2; τ values are shown in microseconds. The data are from [5'-$^2H_2$]PDSPC at 25 °C. (Bottom) Example of T_2 measurement at several regions on the powder pattern. The relative intensity represents $\ln [M(2\tau)/M(2\tau_{min})]$. The data, from the top, are normalized to the value at the smallest τ, τ_{min}.

the T_2 at $\theta' = 90°$ (assuming a Lorentzian line shape) suggests also that the $P_2(\cos \theta')$ dependence is violated (the dePaking algorithm *assumes* a $P_2(\cos \theta')$ angular dependence of the individual lines within the powder pattern). The line width that one can obtain from dePaking is therefore not the "true" line width at $\theta' = 90°$, for our set of spectra. One also notices that the T_2 at the magic angle, $\theta' = 54.7°$, is the shortest in the powder pattern at 25 °C; transverse relaxation is most efficient when the motional averaging axis is oriented at 54.7° with respect to the static magnetic field direction. The absence of a $P_2(\cos \theta')$ type angular dependence might be due to collective or noncollective modes of motion (e.g., lateral diffusion of the lipid molecule in the bilayer) which provide more efficient relaxation near the magic angle. The type of angular dependence appears also to vary with temperature (see Table III, position 16'-$^2H_2$). The deviation from a $P_2(\cos \theta')$ dependence in the T_2 relaxation process has already been reported in ^2H NMR studies of *Acholeplasma laidlawii* membranes (Rance, 1981).

Conclusions

The present study has revealed many important consequences of the presence of cyclopropane groups in membrane lipids. To put these in perspective, we compared the behavior of a fully saturated lipid, DPPC, with that of a cyclopropane-containing lipid, PDSPC. A necessary property for lipid in membranes is that it be in a "fluid" state in the biologically significant temperature range, T_L (35–45 °C). The fully saturated lipids can pack so easily that their T_c is high and increases with chain length. The palmitoyl chain induces T_c to be very close to T_L in DPPC-like lipids. In contrast, the cyclopropane moiety, whose bulkiness prevents an easy packing of the chains around its *sn*-2 plateau positions, induces T_c to be far from T_L. At the same low absolute temperature, the PDSPC-like systems possess more motional amplitude (more disorder) than the DPPC-like model membranes. One might think that if the cyclopropane ring perturbs membrane packing, it would lead to a facile disruption of the membrane at high temperatures. This is not the case; indeed, it has been demonstrated herein that the cyclopropane moiety induces a high correlation of the motions and that the flexibility of the chains does not change appreciably for 20–30 °C above T_c. The best example is the preservation of the *sn*-1 plateau (up to the eighth carbon position) some 20 °C above T_c. Over the same temperature interval, fully saturated lipids undergo rather important structural and dynamical modifications, whereas the cyclopropane-containing membranes do not. The ring, by its high local order and by the long correlation time of its motions, acts therefore as a barrier preventing the propagation of the motions. One can thus imagine that changing the temperature by 10 or 20 °C about T_L will not affect the structural and dynamical properties of cyclopropane-containing membranes. The cyclopropyl moiety has a *regulatory role* with respect to the amplitude and the rates of the motions involved in membrane stability. However, although some understanding of the behavior of PDSPC-like membranes around T_L has been gained, their packing arrangement at lower temperatures requires further investigation. ^2H NMR studies of PDSPE model membranes (which possess a head group significantly smaller than that of PDSPC and hence allow closer intermolecular spacings) at low temperatures might help elucidate this packing.

With regard to the biological synthesis of the cyclopropyl moiety from a cis double bond, one can imagine, from the profiles shown in Figure 10, that the unsaturated lipids are precursors of membrane stabilization by the above process. The unsaturated systems possess the properties of their cyclopropane analogues, but to a lesser extent. The final developmental stage in membrane growth would occur when the double bonds have been converted to cyclopropyl groups, the membrane thus reaching its highest stability with respect to external perturbations.

Registry No. PDSPC, 26853-31-6.

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DNase I Sensitive Domain of the Gene Coding for the Glycolytic Enzyme Glyceraldehyde-3-phosphate Dehydrogenase†

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ABSTRACT: We have cloned a 36-kilobase segment of chicken DNA containing the gene coding for glyceraldehyde-3-phosphate dehydrogenase [GAPDH (EC 1.2.1.12)], a glycolytic enzyme which is expressed constitutively in all cell types. Using defined segments of this cloned DNA as probes, we have determined the DNase I sensitive domain of the GAPDH natural gene in the hen oviduct. When nuclei isolated from hen oviduct were treated with DNase I under conditions known to preferentially degrade actively transcribed genes (i.e., 15–20% of the DNA rendered perchloric acid soluble), a region of approximately 12 kilobase(s) (kb) containing the GAPDH coding sequences and flanking DNA was found to be highly susceptible to digestion by DNase I. Approximately 4 kb downstream from the end of the coding sequences, there was an abrupt transition from the DNase I sensitive or "open" configuration to the resistant or "closed" configuration. The chromatin then remained in a closed conformation for at least 10 kb further downstream. On the 5′ side of the gene, the transition from a sensitive to a resistant configuration was located about 4 kb upstream from the gene. In addition, we have localized two repeated sequences in the area of DNA that was cloned. One of these is of the CR1 family of middle repetitive elements. It is located about 18 kb 3′ to the gene and as such lies well outside of the DNase I sensitive region which encompasses GAPDH. The other repetitive element is of an uncharacterized family. It is located upstream from the gene and appears to be within a region of transition from the DNase I sensitive to resistant states.

It is well documented that actively transcribed genes are maintained in chromatin in a configuration such that they are rendered relatively sensitive to degradation by DNase I [see Weisbrod (1982) for a review]. However, virtually all of the genes studied to date represent special cases in the sense that they either have been of viral origin (Panet & Cedar, 1977; Flint & Weintraub, 1977; Groudine et al., 1978), have been involved in highly specialized functions (Weintraub & Groudine, 1976; Garel & Axel, 1976; Miller et al., 1978; Wu et al., 1979; Bellard et al., 1980; Gerber-Huber et al., 1981), or have been present in multiple copies (Stalder et al., 1978; Samal et al., 1981). Furthermore, only in the case of the ovalbumin multigene family has an entire DNase I sensitive domain been defined (Lawson et al., 1980, 1982). Therefore, in order to gain further insight into the relationship between DNase I sensitivity and gene expression, we set out to clone a constitutive, "housekeeping" gene and compare its DNase I sensitive domain to that of ovalbumin, a hormonally regulated gene switched on in the chicken oviduct during differentiation of this tissue. Thus, we hoped to find common elements of gene expression even among two genes that presumably are regulated in a very different manner. For this purpose, we decided to clone the natural gene coding for the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme expressed in all tissues. We chose this gene because its cDNA had already been cloned (Arnold et al., 1982; Dugaiczyk et al., 1983); thus, a probe was readily available.

The ovalbumin gene family DNase I sensitive domain has been defined by using solution hybridization as an assay to measure the concentration of specific DNA sequences remaining after extensive digestion of nuclei with DNase I (Lawson et al., 1980, 1982). It has been reported that this domain remains in the DNase I sensitive or "open" configuration for approximately 100 kilobase(s) (kb), encompasses the X and Y genes in addition to ovalbumin, and then makes a gradual transition to the resistant or "closed" conformation on each end (Lawson et al., 1980, 1982). In addition, several middle repetitive elements have been mapped within this domain (Stumph et al., 1981, 1982). Finally, since expression of the ovalbumin gene family is limited to the oviduct, these genes are found in a DNase I sensitive state in this tissue only (Garel & Axel, 1976; Lawson et al., 1980).

While the ovalbumin gene family exists in oviduct chromatin as a rather large DNase I sensitive domain with gradual transitions from sensitive to resistant states, other genes may exist in smaller, more compact domains. For example, it has been reported that in a cell line containing integrated adenovirus genes, the transition region from the open to the closed configuration lies in relatively close proximity to the ends of the transcriptional unit (Flint & Weintraub, 1977). Fur-

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