Fluorine-19 Nuclear Magnetic Resonance Studies of Lipid Fatty Acyl Chain Order and Dynamics in *Acholeplasma laidlawii* B Membranes. A Direct Comparison of the Effects of Cis and Trans Cyclopropane Ring and Double-Bond Substituents on Orientational Order†

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Received December 5, 1984

ABSTRACT: The hydrocarbon chain orientational order parameters of membranes of *Acholeplasma laidlawii* B, enriched with large quantities of fatty acids containing either a cis or a trans cyclopropane ring or a cis or trans double bond, plus small quantities of one of an isomeric series of monofluoropalmitic acids, were determined via fluorine-19 nuclear magnetic resonance spectroscopy over a range of temperatures spanning the corresponding gel to liquid-crystalline phase transitions (determined via differential scanning calorimetry). Membrane orientational order profiles in the liquid-crystalline state were generally similar, regardless of the particular fatty acid structure present, showing a region of relatively constant order preceding a region of progressively decreasing order toward the methyl terminus of the acyl chain. In the gel state, the order profiles in the presence of either a trans cyclopropane ring or a trans double-bond substituent were similar and were characterized by a pronounced head to tail gradient of order at temperatures just below the lipid phase transition, while at temperatures far below the lipid phase transition this gradient was less pronounced, all chain positions showing a more uniformly high degree of orientational ordering. In the gel state, the order profiles in the presence of either a cis cyclopropane ring or a cis double-bond substituent were also similar but were highly unusual in that order first increased and only then subsequently decreased toward the acyl chain methyl terminus. In addition, the substituents in the cis configuration, whether a cyclopropane ring or a double bond, were overall more disordered in the gel state than the corresponding substituents in the trans configuration. Thus, at a constant value of reduced temperature below the lipid phase transition, overall order decreased in the progression 19:0cp,tΔ9 ~ 18:1tΔ9 >> 19:0cp,cΔ9 ~ 18:1cΔ9. Since this same relationship describes the temperatures of the membrane lipid gel to liquid-crystalline transition in the presence of these structures, these results suggest that specific fatty acyl structural substituents lower the lipid phase transition temperature by disrupting the gel phase fatty acyl chain packing.

Cyclopropane fatty acids are common membrane lipid constituents in many Gram-negative and a few Gram-positive bacteria (Christie, 1970, 1973). Biosynthesis of the cyclopropane ring occurs by the introduction, via *S*-adenosylmethionine, of a methylene bridge across the double bond of a cis-unsaturated homologue, and cyclopropane fatty acids have been postulated as more stable replacements for unsaturated fatty acids (Christie, 1970; Cronan & Vagelos, 1972). Generally, in those organisms in which they are found, the proportion of cyclopropane fatty acids increases during later stages of growth.

The physical properties of cis-cyclopropane and cis-unsaturated substituted lipids are very similar if not identical. The molecular packing for lipids containing cyclopropane fatty acids and for those containing monounsaturated fatty acids is alike (Cullen et al., 1971) as is their thermotropic phase behavior (Cronan et al., 1979; Silvius & McElhaney, 1979). However, recent deuterium (^{2}H) nuclear magnetic resonance (NMR)[1] studies indicate that the cyclopropane ring constitutes a barrier to propagation of motion along the acyl chain and is, at least in this sense, distinct in its behavior from that of the cis double bond (Dufourc et al., 1983, 1984; Jarrell et al., 1983). These results support the hypothesis that cyclopropane fatty acids are associated with increased organizational stability in the membrane, while simultaneously permitting a degree of "fluidity" consistent with proper membrane functioning.

Recently, ^{19}F NMR studies using monofluoropalmitic acids (MFPA's) as membrane nuclear spin probes, in combination with differential scanning calorimetric (DSC) studies, have begun to elucidate the effects of various fatty acyl structural substituents on membrane lipid orientational order in both the liquid-crystalline state and the gel state (Macdonald et al., 1983, 1984, 1985a–c). In the present study, these investigations are extended to a direct comparison of the effects of cyclopropane and monounsaturated substituents, both cis and trans, on the orientational order of MFPA's in membranes of the organism *Acholeplasma laidlawii* B. ^{19}F NMR offers several distinct advantages for the study of membrane lipid physical properties. The monofluorinated fatty acyl probes are relatively nonperturbing, as has been demonstrated by using a number of biological, biochemical, and biophysical criteria (McDonough et al., 1983), and report a picture of the conformational state of membrane lipid acyl chains which is both qualitatively (Macdonald et al., 1983) and quantitatively (Macdonald et al., 1984) similar to that provided by use of ^{2}H NMR techniques. The sensitivity of the fluorine nucleus in the NMR experiment permits a single series of isomeric

† This work was supported by operating and major equipment grants from the Medical Research Council of Canada (to R.N.M.) and by the MRC Group in Protein Structure and Function (to B.D.S.). P.M.M. was supported by a studentship from the Alberta Heritage Foundation for Medical Research.

[1] Abbreviations: NMR, nuclear magnetic resonance; PC, phosphatidylcholine; DSC, differential scanning calorimetry; FID, free induction decay; MFPA, monofluoropalmitic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

0006-2960/85/0424-4651$01.50/0

MFPA's to be used in small amounts to survey the effects of a wide range of fatty acyl structural substituents upon membrane lipid orientational order. The ^{19}F NMR spectrum apparently reflects axially symmetric motions of the membrane lipids in both the liquid-crystalline *and* gel states, so that the orientational order of the MFPA's may be described in terms of a single order parameter in both phases (Macdonald et al., 1984).

Acholeplasma laidlawii B is capable of biosynthetically incorporating a variety of exogenously supplied fatty acids into its membrane glyco- and phospholipids (McElhaney, 1974), and when de novo fatty acid biosynthesis is inhibited with the biotin-binding protein avidin, these membranes can be made virtually homogeneous with respect to many exogenously supplied fatty acids (Silvius & McElhaney, 1978). Therefore, the membrane fatty acid composition and physical properties can be readily manipulated almost at will. In addition, this cell wall less organism possesses only a single membranous structure, the plasma membrane, so that acquiring relatively homogeneous membrane preparations is particularly facile (Razin, 1975). Thus, a membranous venue for a comparison of the effects of monounsaturation and cyclopropane ring substitution can be readily obtained. Finally, previous ^{19}F NMR studies in *A. laidlawii* B will provide a basis for a comparison of the effects of these substituents with those of a variety of alternative fatty acyl structures.

Materials and Methods

Materials. The synthesis of the various isomeric monofluoropalmitic acids has been described previously (McDonough et al., 1983). *cis*-9,10-Octadecenoic acid (18:1cΔ9) and *trans*-9,10-octadecenoic acid (18:1tΔ9) were purchased from Nu Check Prep Co. (Elysian, MN), while *cis*-9,10-methyleneoctadecanoic acid (19:0cp,cΔ9) and *trans*-9,10-methyleneoctadecanoic acid (19:0cp,tΔ9) were purchased from Analabs Co. (North Haven, CT). All fatty acids were greater than 99.9% pure as judged from analytical thin-layer and gas chromatographic results. All other chemicals and biochemicals were of the highest quality available.

Cell Culture, Membrane Isolation, Lipid Analysis, and Differential Scanning Calorimetry. The growth medium and conditions used for culturing *A. laidlawii* B with fatty acids plus avidin have been described in detail previously (Silvius & McElhaney, 1978). All cultures were supplemented with 20 mol % MFPA plus 80 mol % of the particular fatty acid of interest. Cultures supplemented with either 18:1tΔ9 or 19:0cp,tΔ9 were inoculated from regular maintenance cultures of *A. laidlawii* B and grown at 37 °C. Cultures supplemented with either 18:1cΔ9 or 19:0cp,cΔ9 were inoculated from cultures previously adapted to the presence of 18:1cΔ9 and were grown at 32 °C. This protocol, in conjunction with the presence of 20 mol % of the higher melting MFPA, circumvented the otherwise deleterious effects on the growth of *A. laidlawii* B in the presence of avidin of the low-melting fatty acids 18:1cΔ9 and 19:0cp,cΔ9.

Membranes were prepared from late log-phase cultures of *A. laidlawii* B essentially as previously described (Silvius et al., 1977). The extraction and purification of total membrane polar lipids, the preparation of methyl esters, and the analysis of these methyl esters by gas–liquid chromatography have been detailed elsewhere (Saito & McElhaney, 1977). Differential scanning calorimetry (DSC) on total membrane polar lipids was performed as described previously (Macdonald et al., 1984).

Nuclear Magnetic Resonance. A. laidlawii B membranes were suspended in buffer (0.154 M NaCl, 0.05 M Tris-HCl, and 20 mM β-mercaptoethanol, pH 7.4) which had been diluted 20-fold with 95% deuterium oxide or, for low-temperature studies, in the above solution made 1:1 (v/v) with ethylene glycol to prevent sample freezing. ^{19}F NMR spectra were collected at 254.025 MHz on a Bruker HXS-270 spectrometer equipped with a ^{2}H lock, operating in the Fourier-transform mode and using quadrature detection, at a spectral width of ±50 000 Hz. Bessel filters with a filter width of ±100 000 Hz were employed. Cooling was achieved by passing an air flow over the cooling coil of a Cryocool unit and subsequently over the probe and sample. For very low temperatures, the air stream was passed additionally through a stainless-steel coil immersed in liquid nitrogen. Temperature control was achieved by balancing the cooling of the air flow with an electric heating coil to maintain the desired temperature to within ±1 °C. Membrane samples were equilibrated at a particular temperature for 30 min prior to data acquisition. Fluorine nuclei were subjected to a 15-μs (~75°) pulse followed by a 10-μs delay and a 20-ms acquisition time. The recycle time was 200 ms. Typically, 20K scans were accumulated for samples in which the acquisition temperature was above the main lipid phase transition and 100K scans for samples at lower temperatures. The distortion of the first three points of the free induction decay (FID), which is associated with the receiver dead time, was corrected by a smooth extrapolation of the FID back to time zero such that the signal intensity of the early portion of the FID closely approximated a t^2 time dependency for the in-phase channel and a t dependency for the out-of-phase channel (Bloom et al., 1978). The signal to noise ratio was enhanced with an exponential multiplication which corresponded to a line broadening of 100 Hz, and the FID was Fourier transformed to 2K data points in the real domain. The integrity of the spectrum and the flatness of the base line were taken as indicating that the extrapolation procedure had been performed correctly.

Results and Discussion

Fatty Acid Composition of Acholeplasma laidlawii B Membrane Polar Lipids. Table I lists the fatty acid composition for each case of enrichment with either 18:1cΔ9, 18:1tΔ9, 19:0cp,cΔ9, or 19:0cp,tΔ9 plus a particular isomeric MFPA. In all cases, the fatty acids provided exogenously accounted for greater than approximately 97% of the membrane lipid fatty acids. The products of de novo fatty acid biosynthesis in *A. laidlawii* B (12:0, 14:0, 16:0, and 18:0; Saito et al., 1977a) contributed minimally to the overall membrane lipid fatty acid composition, as expected for cells grown in the presence of avidin. In general, the ratio of the two fatty acids provided in the supplement was faithfully reflected in the membrane lipid fatty acid composition. However, some preference for the higher melting MFPA's may have been manifest in those cultures supplemented with the low-melting fatty acids 18:1cΔ9 and 19:0cp,cΔ9. This may be an attempt by the organism to compensate for the "hyperfluidizing" effect of these fatty acids on the membrane lipids.

Thermotropic Behavior of A. laidlawii B Membrane Polar Lipids. Previous ^{19}F NMR studies of *A. laidlawii* B have indicated that the membrane lipid gel to liquid-crystalline phase transition is the single greatest effector of overall membrane lipid orientational order, so that any comparison of orientational order between different fatty acyl structures must necessarily consider the effects of those structures on the thermotropic properties of the lipid bilayer [see, for example, Macdonald et al. (1985a)]. Phase transition endotherms of *A. laidlawii* B membrane polar lipids were obtained by DSC for each case of enrichment with a particular isomeric MFPA

Table I: Fatty Acid Composition of *A. laidlawii* B Membrane Lipids Enriched with Various Isomeric MFPA's plus 18:1cΔ9, 18:1tΔ9, 19:0cp,cΔ9, or 19:0cp,tΔ9.

	fatty acid compositon (mol %)							
supplement (0.12 mM total)	12:0	14:0	16:0	18:1cΔ9	18:1tΔ9	19:0cp,cΔ9	19:0cp,tΔ9	*x*F16:0
80% 18:1cΔ9 + 20% 6F16:0			1.9	72.8				25.3
80% 18:1cΔ9 + 20% 8F16:0			2.8	73.2				24.0
80% 18:1cΔ9 20% 10F16:0			2.2	75.9				21.9
80% 18:1cΔ9 + 20% 12F16:0			1.9	71.3				26.8
80% 18:1cΔ9 + 20% 14F16:0			2.0	73.7				24.3
80% 18:1tΔ9 + 20% 6F16:0	0.7	0.2	2.3		78.9			17.9
80% 18:1tΔ9 + 20% 8F16:0	0.1	0.3	1.3		76.9			21.4
80% 18:1tΔ9 + 20% 10F16:0	0.2	0.2	1.9		78.3			19.4
80% 18:1tΔ9 + 20% 12F16:0	0.6	0.5	2.9		78.1			17.9
80% 18:1tΔ9 + 20% 14F16:0	0.8	0.5	1.7		77.2			29.8
80% 19:0cp,cΔ9 + 20% 6F16:0	0.7	0.4	3.7			71.3		24.9
80% 19:0cp,cΔ9 + 20% 8F16:0	1.0	0.3	2.8			73.4		22.5
80% 19:0cp,cΔ9 + 20% 10F16:0	0.1	0.9	2.9			71.6		24.5
80% 19:0cp,cΔ9 + 20% 12F16:0	1.1	1.2	1.8			74.3		21.6
80% 19:0cp,cΔ9 + 20% 14F16:0	0.5	1.3	2.9			75.2		21.1
80% 19:0cp,tΔ9 + 20% 6F16:0	0.1	0.2	0.4				78.7	20.6
80% 19:0cp,tΔ9 + 20% 8F16:0	0.3	0.1	0.2				76.9	22.5
80% 19:0cp,tΔ9 + 20% 10F16:0	0.1	0.1	0.4				78.3	21.1
80% 19:0cp,tΔ9 + 20% 12F16:0	0.1	0.3	0.3				77.7	21.6
80% 19:0cp,cΔ9 + 20% 14F16:0	0.2	0.1	0.3				79.1	20.3

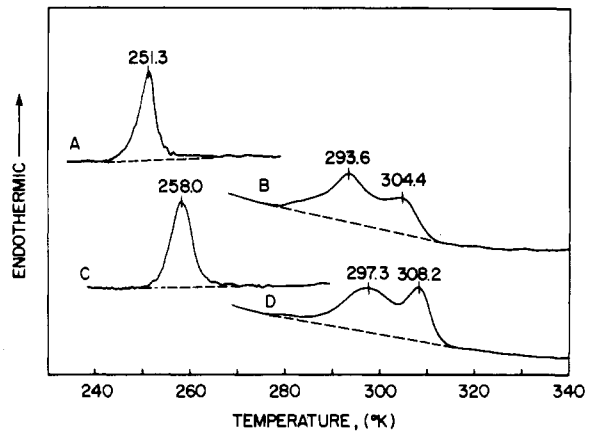

FIGURE 1: Lipid phase transition endotherms obtained by differential scanning calorimetry of the membrane polar lipid fraction from *A. laidlawii* B grown in the presence of 20 mol % 6F16:0 plus 80 mol % (A) 18:1cΔ9, (B) 18:1tΔ9, (C) 19:0cp,cΔ9, and (D) 19:0cp,tΔ9. The scan rate was 5 °C/min. The dashed line corresponds to the interpolated base line.

plus either 18:1cΔ9, 18:1tΔ9, 19:0cp,cΔ9, or 19:0cp,tΔ9. Figure 1 illustrates endotherms obtained in the case of enrichment with 6F16:0 plus one of the particular fatty acids of interest. These endotherms are typical in that, for cases of enrichment with other isomeric MFPA's, the overall shapes of the endotherms were similar and the T_m (or T_m's) varied by less than ±1 °C, about the average for that particular case. In the case of membrane lipids enriched with either 18:1cΔ9 or 19:0cp,cΔ9, a single, broad, somewhat asymmetric transition endotherm was observed. Most fatty acids, when incorporated into membranes of *A. laidlawii* B, manifest this type of lipid phase transition endotherm (McElhaney, 1974). In contrast, the transition endotherms obtained with those membrane lipids enriched with either 18:1tΔ9 or 19:0cp,tΔ9 were composed of two partially resolved transitions. Such phase separations have been observed previously in *A. laidlawii* B membrane lipids enriched with 18:1tΔ9 and seem to be attributable to lipid head-group immiscibility (Macdonald et al., 1985b). Clearly the trans configuration of either the monounsaturated substituent or the cyclopropane ring substituent is associated with more complex phase behavior in *A. laidlawii* B membrane lipids, since both structures lead to observable phase separations. Generally, the miscibility of the components of a mixture of lipids decreases as the difference between their phase transition temperatures increases (Mabrey & Sturtevant, 1978). In *A. laidlawii*, there exists the additional complication that lipid head-group composition is altered under conditions of supplementation with different fatty acyl species (Silvius et al., 1980). Were it not for the fact that membrane lipids enriched with a very low-melting fatty acid (e.g., 18:1cΔ9 or 19:0cp,cΔ9) plus a relatively high-melting fatty acid (e.g., MFPA) showed no disposition toward phase separation, one might be tempted to invoke fatty acyl chain immiscibility as leading to the observed phase separation in membrane lipids enriched with 18:1tΔ9 or 19:0cp,tΔ9 plus MFPA. Since this explanation is untenable, and since similar phase separations are observed in membrane lipids which are 99.9% enriched with 18:1tΔ9 (Macdonald et al., 1985b), it can only be suggested that the lipid head-group composition of *A. laidlawii* B alters under conditions of enrichment with these trans fatty acids in such a fashion as to lead to the calorimetrically observed phase separations. More detailed investigation into the interactions among the glyco- and phospholipid species of *A. laidlawii* will be required before this point can be clarified.

Table II summarizes the calorimetric data for these various cases of enrichment. Briefly, the midpoint of the gel to liquid-crystalline phase transition (T_m) was always lowest when the structural substituent, either a monounsaturated compound or the cyclopropane ring, assumed the cis configuration. The T_m's of the two lipids with cis structural substituents (18:1cΔ9 and 19:0cp,cΔ9) were very similar as were the T_m's of the two lipids with trans structural substituents (18:1tΔ9 and 19:0cp,tΔ9) although the lipids containing the cyclopropane ring substituent (either cis or trans) always melted several degrees higher than the lipids containing the monounsaturated substituents (either cis or trans). The quantity ΔT_{10-90}, corresponding to the temperature range over which the phase transition passes from 10% to 90% of completion, has been used as a measure of the cooperativity of the lipid phase transition in biological membranes such as those of *A. laidlawii* B (Silvius et al., 1980). Values of ΔT_{10-90} were in the range 5–10 °C for 18:1cΔ9 and 19:0cp,cΔ9 and of the order 15–20 °C for 18:1tΔ9 and 19:0cp,tΔ9, in good agreement with values of ΔT_{10-90} previously reported from this laboratory for *A. laidlawii* B membrane lipids [e.g., see Macdonald et al. (1984, 1985a–c)].

The preceding calorimetric data were obtained with mem-

Table II: Calorimetrically Determined Gel to Liquid-Crystalline Phase Transition Parameters for *A. laidlawii* B Membrane Polar Lipids Enriched with Various Isomeric MFPA's plus One of either 18:1tΔ9, 18:1cΔ9, 19:0cp,tΔ9, or 19:0cp,cΔ9

	phase transition parameters (K)				
supplement (0.12 mM total)	T_1[a]	T_2[b]	$\Delta T_1 - T_2$[c]	T_m[d]	ΔT_{10-90}[e]
80% 18:1tΔ9 + 20% 6F16:0	293.6	304.4	10.8	295.9	18.6
80% 18:1tΔ9 + 20% 8F16:0	293.1	301.2	8.1	295.4	17.6
80% 18:1tΔ9 + 20% 10F16:0	295.0	302.4	7.4	296.9	16.8
80% 18:1tΔ9 + 20% 12F16:0	293.9	302.1	8.2	296.0	16.4
80% 18:1tΔ9 + 20% 14F16:0	295.9	305.1	9.2	297.6	17.4
80% 19:0cp,tΔ9 + 20% 6F16:0	297.3	308.2	10.9	300.8	17.7
80% 19:0cp,tΔ9 + 20% 8F16:0	296.4	307.3	10.9	300.4	17.8
80% 19:0cp,tΔ9 + 20% 10F16:0	297.9	307.2	9.3	302.8	16.8
80% 19:0cp,tΔ9 + 20% 12F16:0	299.1	309.6	10.5	302.4	16.7
80% 19:0cp,tΔ9 + 20% 14F16:0	300.5	310.3	9.8	302.8	17.3
80% 18:1cΔ9 + 20% 6F16:0				251.3	5.6
80% 18:1cΔ9 + 20% 8F16:0				253.6	4.8
80% 18:1cΔ9 + 20% 10F16:0				253.4	4.4
80% 18:1cΔ9 + 20% 12F16:0				253.3	4.3
80% 18:1cΔ9 + 20% 14F16:0				252.6	4.0
80% 19:0cp,cΔ9 + 20% 6F16:0				258.9	6.0
80% 19:0cp,cΔ9 + 20% 8F16:0				257.9	5.8
80% 19:0cp,cΔ9 + 20% 10F16:0				257.7	6.1
80% 19:0cp,cΔ9 + 20% 12F16:0				259.3	5.3
80% 19:0cp,cΔ9 + 20% 14F16:0				258.4	5.7

[a] T_1 corresponds to the maximum of the lower temperature transition. [b] T_2 corresponds to the maximum of the higher temperature transition. [c] $\Delta T_1 - T_2$ corresponds to the temperature separation of T_1 and T_2. [d] T_m corresponds to the temperature at which the transition was 50% complete. [e] ΔT_{10-90} corresponds to the temperature range over which the transition passes from 10% to 90% of completion.

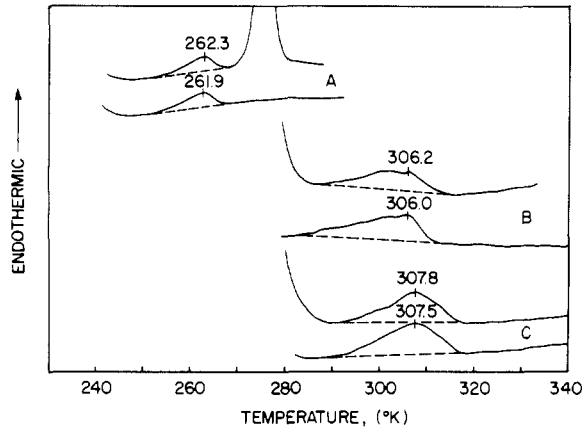

FIGURE 2: Lipid phase transition endotherms obtained by differential scanning calorimetry of the membrane polar lipid fraction from *A. laidlawii* B enriched with (A) 18:1cΔ9, (B) 18:1tΔ9, and (C) 16:0. The lower endotherms were obtained in the presence of 50% ethylene glycol. The scan rate was 5 °/min. The dashed line corresponds to the interpolated base line.

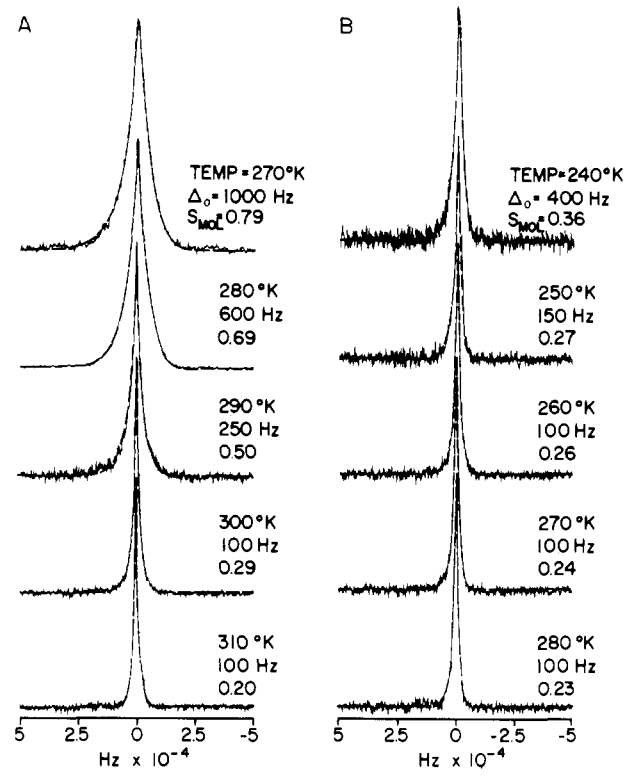

FIGURE 3: Experimental and simulated ^{19}F NMR spectra of membranes of *A. laidlawii* B grown in the presence of 20 mol % 6F16:0 plus 80 mol % (A) 19:0cp,tΔ9 and (B) 19:0cp,cΔ9. The simulated spectra are indicated by dashed lines superimposed upon the experimental spectra. The values of the computer input parameter Δ_0 (interchain dipolar broadening) and S_{mol} (molecular order parameter) are indicated for the simulated spectra.

brane polar lipid samples resuspended in ethylene glycol/water (1:1 v/v). While this has the advantage of eliminating the excess water transition which might otherwise obscure the lipid phase transition endotherm, it is possible that the presence of ethylene glycol might alter the thermotropic properties of the *A. laidlawii* membrane lipids. For example, it has been known for some time that ethylene glycol can raise the observed T_m of phosphatidylcholines (PC's) containing unsaturated fatty acids but has little effect on the T_m of PC's containing saturated fatty acids (Van Dijk et al., 1976). Figure 2 compares the DSC endotherms of *A. laidlawii* B membrane lipids enriched with either 18:1cΔ9, 18:1tΔ9, or 16:0 (grown in the absence of avidin) and resuspended in either the presence or the absence of ethylene glycol. Clearly, there are few differences in the thermotropic behavior of these lipids attributable to ethylene glycol and certainly no systematic increase in T_m at lower phase transition temperatures in the presence of ethylene glycol.

^{19}F NMR Analysis of A. laidlawii B Membranes. The ^{19}F NMR line shape is influenced by both chemical shift anisotropic and dipole–dipole interactions which are averaged by the anisotropic motions peculiar to a lipid molecule embedded in a bilayer membrane (Gent & Ho, 1978). The superposition quality of these two interactions, their relative magnitudes, and the axially symmetric molecular motions in the lipid bilayer lead to the super-Lorentzian fluorine line shapes shown in Figure 3. There is, then, no one simple spectral parameter which is unequivocally related to the orientational order of the

bilayer lipids, and we have therefore resorted to spectral simulations to extract this information.

First, the maximum chemical shift anisotropy and the maximum F–H interchain dipolar interaction were estimated for a monofluoropalmitate-containing lipid whose fatty acyl chains had assumed an all-trans configuration aligned perpendicular to the plane of the bilayer and which was experiencing rapid (on the ^{19}F NMR time scale) rotations about the lipid long axis. The decrease in the observed chemical shift anisotropy and intrachain dipolar interaction from their estimated maximum was quantitated by the introduction of an orientational order parameter, S_{mol}. The hydrocarbon chain orientational order parameter, S_{mol}, quantitates the amplitude of the time-averaged angular excursions of the fatty acyl chains away from an axis perpendicular to the plane of the bilayer. Tilting of the whole molecule with respect to the bilayer normal, as well as trans–gauche isomerization at individual methylene segments, could contribute to the deviation of S_{mol} from a value of unity, indicative of acyl chains assuming a fully extended all-trans configuration and aligned parallel to the bilayer normal, toward a value of zero, at which individual chain segments would experience essentially free isotropic motion. Seelig (1977) defined the molecular order parameter such that

$$S_{mol} = (1/2)\langle \cos^2 \theta - 1 \rangle$$

Here, θ is the angle between the segment direction and the bilayer normal where the segment direction is defined as the normal to the plane formed by the H–C–H atoms of the methylene segment. The broken brackets represent an average over time.

The mathematical model employed to generate the spectral simulations has been described in detail elsewhere (Macdonald et al., 1983, 1984). The validity of the model and, hence, of the extracted values of S_{mol} rests upon the accuracy of the estimated values of the maximum chemical shift anisotropy and intrachain dipolar interaction (Δ_1) as well as the assumption of effectively axially symmetric motions of the lipid molecules.

The value of the maximum chemical shift anisotropy was estimated from a consideration of the fluorine chemical shift tensor elements reported for Teflon (Mehring, 1971) and was found to be 82.2 ppm (Macdonald et al., 1983). If this value were an over- or underestimate, the extracted values of S_{mol} would be correspondingly under- or overestimated. In the absence of a direct measurement of the chemical shift tensor elements of a monofluoro-type compound, we have consistently used the above estimated value as the maximum. Although it is possible that the values of the fluorine chemical shift tensor elements or the orientation of the tensor principal axis system might alter under different experimental conditions, it is most likely that these quantities are insensitive to the changes in temperature, label position, etc. to which we have subjected them. Thus, the alterations in the ^{19}F spectra have been attributed entirely to changes in the value of S_{mol}.

The maximum F–H intrachain dipolar interaction (Δ_1) was determined to be approximately 20 000 Hz from a study of the magnetic field strength dependence of the ^{19}F spectrum of a monofluoropalmitate incorporated into a lipid bilayer (Macdonald et al., 1983), and this value was in good agreement with that estimated theoretically from a consideration of the geometry of a monofluoromethylene segment within an acyl chain (Macdonald, 1984). A third line-shape parameter, the interchain dipolar interaction (Δ_0), becomes of importance only in the gel state where increasingly larger values (in hertz) are required to simulate the fluorine line shape.

Of paramount importance to our spectral simulations is the assumption of effectively axially symmetric motions of the bilayer lipid molecules. In the absence of long-axis rotational motions of the whole lipid or torsional motions of the fatty acyl chains of sufficient rapidity on the ^{19}F NMR time scale to provide effective axial symmetry, it would no longer be possible to completely describe the orientational order of the monofluoromethylene segment in terms of a single order parameter. It is clear that the ^{2}H NMR spectrum reflects axially symmetric lipid motions in the liquid-crystalline state but not in the gel state [e.g., see Seelig (1977)]. On the other hand, ^{13}C NMR spectra are characteristic of axial symmetry even to temperatures 40 °C below the lipid phase transition [e.g., see Wittebort et al. (1981)], and this difference between the gel-state ^{2}H and ^{13}C spectra can be attributed to the approximately 1 order of magnitude difference in the time scales relevant to the two procedures (Davis, 1983). The time scale relevant to the ^{19}F spectrum more closely corresponds to that of ^{13}C (Macdonald et al., 1984) so that on theoretical grounds one would expect effective axial symmetry to be manifest in both the gel- and liquid-crystalline-state ^{19}F spectra. The ^{19}F spectrum in the absence of axial symmetry has a broad Gaussian line shape in contrast to the super-Lorentzian obtained at temperatures below the lipid phase transition shown in Figure 3 (Macdonald et al., 1984). Furthermore, it is apparent that the spectral simulations, which explicity assume axial symmetry, more than adequately reproduce the experimental ^{19}F line shapes in both the gel and the liquid-crystalline phases. Although these various points do not prove that on the ^{19}F NMR time scale axial symmetry exists in the gel phase, and it has not been demonstrated directly that the ^{19}F spectrum has an asymmetry parameter of zero (i.e., effective axial symmetry), the weight of the present evidence, including the theoretical considerations, the super-Lorentzian character of the gel-state ^{19}F spectra, and the successful simulations assuming an asymmetry parameter of zero, certainly suggests that the motions of the lipid molecules in the gel state are of a nature and of sufficient rate to validate the assumption of axial symmetry on the ^{19}F NMR time scale.

Examples of experimentally obtained ^{19}F NMR spectra of *A. laidlawii* B membrane samples as a function of temperature and the corresponding computer-generated simulated spectra are illustrated in Figure 3 for the cases of enrichment with 15 mol % 6F16:0 plus 85 mol % either 19:0cp,tΔ9 or 19:0cp,cΔ9. At a temperature above the particular lipid phase transition, the ^{19}F NMR spectrum consisted of a single, narrow, somewhat asymmetric resonance line reflecting the extent and rapidity of the fatty acyl chain motions in the liquid-crystalline state. As the temperature was decreased and the lipid phase state altered, the fluorine spectrum progressively broadened in response to the restriction of fatty acyl chain motions which accompanies the transition to the gel state. At all temperatures employed, an apparently super-Lorentzian line shape was observed, suggesting the presence of considerable motional averaging on the ^{19}F NMR time scale even in the gel state. In the region of the lipid phase transition, where other NMR techniques indicate that the spectrum should be a superposition of spectral components from slowly exchanging gel-state and liquid-crystalline-state domains [e.g., see Wittebort et al. (1981) and Davis (1983)], the fluorine spectrum does not obviously consist of separate gel and fluid components. However, since the fluorine spectrum in the coexistence region can be simulated by the addition of 100% fluid spectra and 100% gel spectra in the proper proportion (Macdonald, 1984), it can be considered a superposition of

gel- and liquid-crystalline-state spectral components.

As indicated in Figure 3, in the presence of 19:0cp,tΔ9, over the range of temperatures tested, the value of S_{mol} increased from approximately 0.20 at 310 K to 0.79 at 270 K. The value of Δ_0 remained constant at 100 Hz until the lipid phase transition was encountered and thereafter rapidly increased, reaching a value of 1000 Hz at 270 K. This is the same dependence of S_{mol} and Δ_0 upon temperature and membrane lipid phase state which is observed with most fatty acid enriched *A. laidlawii* B membranes. In marked contrast to this situation was the dependence of S_{mol} on temperature and membrane lipid phase state in membranes of *A. laidlawii* B enriched with 19:0cp,cΔ9. Here, S_{mol} increased from approximately 0.23 at 280 K to only 0.36 at 240 K, a temperature almost 20 °C below the corresponding membrane lipid phase transition. Over the same temperature range, Δ_0, the intermolecular interaction indicator, increased from 100 to only 400 Hz. It does not in fact require a quantitative analysis of the ^{19}F NMR spectra to discern that the spectra obtained in the gel state in the presence of 19:0cp,cΔ9 reflect considerably greater motional averaging than the corresponding gel-state spectra obtained in the presence of 19:0cp,tΔ9. This point may be ascertained qualitatively by a visual comparison of the spectra.

Since the ^{19}F spectra in the region of the lipid phase transition can be considered a superposition of gel-state and liquid-crystalline-state components and since the ^{19}F spectrum in the coexistence region can apparently be adequately described in terms of a single order parameter (see Figure 3), the value of S_{mol} obtained at a temperature within the lipid phase transition must be considered an average of the orientational order of the fluid and gel components. This average value will be a function of the relative proportions of the two components as well as of the temperature dependence of the orientational order within any one gel or fluid domain over the temperature range of the phase transition.

^{19}F NMR Order Profiles. The variation of S_{mol} with the position of the monofluoro substituent provides an order profile for the fatty acyl chain in a given experimental situation. Figure 4 depicts the ^{19}F NMR order profiles of *A. laidlawii* B membranes enriched with either 18:1cΔ9, 18:1tΔ9, 19:0cp,cΔ9, or 19:0cp,tΔ9 obtained over a range of temperatures spanning the gel to liquid-crystalline phase transitions. In the liquid-crystalline state, in all cases of enrichment, the order profiles were very similar, showing a region of approximately constant orientational order preceding a decline in orientational order toward the methyl terminus of the acyl chain. Similar results are obtained by using ^2H NMR techniques in both model (Seelig, 1977; Seelig & Seelig, 1980) and biological (Stockton et al., 1977; Gally et al., 1979) membranes, and this gradient of orientational order has come to be recognized as the "signature" of a lipid bilayer. Since the presence of a cyclopropane ring or a double bond on an acyl chain adjacent to the MPFA had little if any effect on the orientational ordering of the MFPA, it must be concluded that in the liquid-crystalline state the two fatty acyl chains experience a degree of independence sufficient to negate any influence of structural substitutents in one acyl chain upon the orientational order of the other. Previous ^{19}F NMR results have demonstrated that the orientational order profiles of the MFPA chains in membranes of *A. laidlawii* B are highly similar in the liquid-crystalline state whether those membranes were enriched with straight-chain saturated fatty acids such as 15:0 (Macdonald et al., 1983, 1985c) or 16:0 (Macdonald et al., 1984), with any of an isomeric series of *cis*-octadecenoic

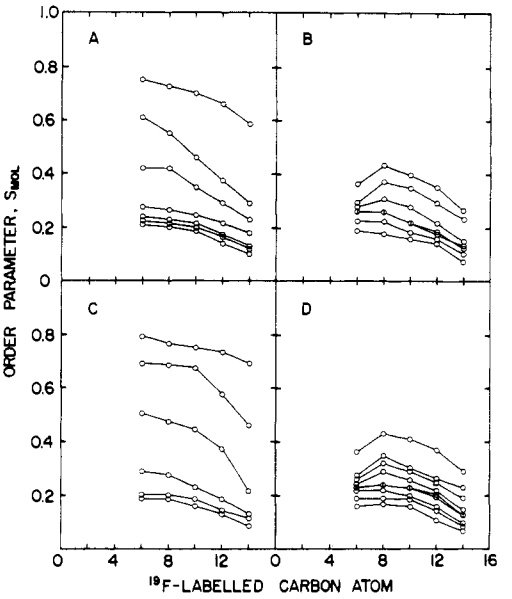

FIGURE 4: ^{19}F NMR orientational order profiles of membranes of *A. laidlawii* B grown in the presence of 20 mol % various monofluoropalmitic acids plus 80 mol % (A) 18:1tΔ9 (279, 284, 289, 294, 299, 304, and 309 K), (B) 18:1cΔ9 (240*, 250*, 266*, 279*, 279, 289, and 310 K), (C) 19:0cp,tΔ9 (270, 280, 290, 300, 310, and 320 K), or (D) 19:0cp,cΔ9 (240*, 250*, 260*, 270*, 280*, 280, 290, 300, and 310 K). The numbers in parentheses represent the temperatures at which the order profiles, from top to bottom, were acquired. Asterisks represent the presence of 50% ethylene glycol.

acids (Macdonald et al., 1985a) or *trans*-octadecenoic acids (Macdonald et al., 1985b), or with methyl-branched fatty acids such as 16:0i and 16:0ai (Macdonald et al., 1983, 1985c). ^2H NMR results also indicate that the order profile is relatively invariant to alterations in fatty acyl structure in the liquid-crystalline state. The orientational order profiles of PC's containing cis-unsaturated fatty acids were very similar to those obtained with trans-unsaturated fatty acids after correction for geometric considerations, and these were in turn very similar to the order profiles of straight-chain saturated fatty acids (Seelig & Waespe-Šarčević, 1978). These results were confirmed by ^2H NMR studies in *Escherichia coli* (Gally et al., 1979) and *A. laidlawii* B (Stockton et al., 1977; Rance et al., 1980). The one exception to this generalization appears to be the cyclopropane ring where, even after correction for geometric considerations, the ^2H NMR order profiles in both model (Dufourc et al., 1983) and biological membranes (Jarrell et al., 1983) indicate that the cyclopropyl substituent experiences a far greater degree of orientational ordering than any other portion of the fatty acyl chain. Nevertheless, when a deuterated palmitic acyl chain adjacent to a cyclopropane ring containing acyl chain was monitored by ^2H NMR, the observed order profile gave little if any indication that the neighboring cyclopropyl substituent had influenced the ordering of the palmitate chain (Dufourc et al., 1984). It would appear, then, from both ^{19}F and ^2H NMR results that the resilience of the fatty acyl chains in the liquid-crystalline state permits them to accommodate the presence of structural substituents without unduly altering the character of the orientational order profile.

With decreasing temperature, ^{19}F orientational order parameters increased slowly, and the character of the order profiles remained relatively constant until the lipid phase transition was encountered. As the proportion of gel-state lipid increased, overall orientational order increased markedly, and the order profiles in the presence of different fatty acyl chain structures became increasingly dissimilar.

Consider first the cases of 18:1tΔ9 and 19:0cp,tΔ9. At a temperature of approximately 290 K, in both these enrichment situations, the membrane lipids have all but completely assumed the gel state. Reflecting this condition, the values of S_{mol} at fluorine positions closer to the fatty acyl chain carbonyl head group indicated a pronounced increase in orientational ordering. Nevertheless, at fluorine positions proximal to the fatty acyl methyl terminus, the increase in the value of S_{mol} was only relatively marginal. This disparity between the manner in which orientational order near the carbonyl head group and near the methyl terminus altered with the increased proportion of gel-state lipid was then manifested as a pronounced head to tail gradient of orientational order in the presence of 18:1tΔ9 or 19:0cp,tΔ9. At temperatures sufficiently below the lipid gel to liquid-crystalline phase transition, the degree of orientational ordering was at all positions uniformly high, although a residual ordering gradient could be discerned. Consequently, the configuration of the MFPA chain in the gel state in the presence of either 18:1tΔ9 or 19:0cp,tΔ9 may be described as overall highly ordered, approaching but not achieving an all-trans configuration with a residual head to tail gradient of orientational ordering. To put these observations in perspective, it should be noted that in the presence of straight-chain saturated fatty acids such as 15:0 (Macdonald et al., 1985c) or 16:0 (Macdonald et al., 1984), the orientational order of the MFPA's increases approximately uniformly at all fluorine positions with increasing proportions of gel-state lipid and their remains little, if any, head to tail gradient of order in the gel state. It would appear that the trans cyclopropane ring or the trans-monounsaturated structural substituents were able to disrupt the gel-state packing of the fatty acyl chains, constituting a barrier to the assumption of a more highly ordered acyl chain configuration, which in turn is reflected in an order gradient in the gel state.

Turning next to the cases of 18:1cΔ9 and 19:0cp,cΔ9, it can be seen that these structural substituents lead to highly unusual gel-state orientational order profiles. The orientational order at the 6-position in the presence of these substituents in the gel state was always less than that observed at the 8-position, suggesting that the MFPA chain is forced to bend around the bulky cis cyclopropane ring or cis double bond in the closely packed gel state or that these substituents are unable to pack closely in the gel state, thus permitting the MFPA's a greater degree of motional freedom in their immediate locale. Since the MFPA chain should preferentially distribute to the *sn*-1 position of the glycerolipid backbone in the presence of 18:1cΔ9 or 190:cp,cΔ9 (by analogy with 16:0; Saito et al., 1977b), and given the physical inequivalence of the fatty acyl chains esterified to the *sn*-1 and *sn*-2 positions, it is not surprising that the greatest effect of a structural substituent located at the Δ9 position on the *sn*-2 chain should be manifest at the C-6 position on the *sn*-1 chain. Previous ^{19}F NMR results have indicated that a cis double bond located near the methyl terminus of the fatty acyl chain can locally disrupt gel-state chain packing (as do methyl iso- and anteiso-branched structures) but that gel-state chain packing densities are sufficient to overcome any local disruptive effect of a cis double bond located near the carbonyl head group of the fatty acyl chain (Macdonald et al., 1985a). The present results would indicate that structural substituents located near the center of the acyl chain are also capable of disrupting gel-state chain packing, and indeed, when the overall ordering achieved in the presence of such structures is considered, this would appear to be the most efficient position from which to influence gel-state ordering. Overall, the degree of ordering achieved in the presence of 18:1cΔ9 or 19:0cp,cΔ9 was much lower than that obtained in the presence of 18:1tΔ9 or 19:0cp,tΔ9. Thus, the configuration of the MFPA chain in the gel state in the presence of 18:1cΔ9 or 19:0cp,cΔ9 may be described as highly disordered, apparently reflecting both local and overall disruption of the density of gel-state fatty acyl chain packing.

The ^{19}F NMR spectra of *A. laidlawii* B membranes enriched with either 18:1cΔ9 or 19:0cp,cΔ9 were acquired in the presence of 50% ethylene glycol at temperatures of 270 K or lower in order to prevent sample freezing. It has already been noted that the presence of 50% ethylene glycol had little if any effect on the thermotropic properties of *A. laidlawii* B membrane lipids whether those lipids were enriched with 18:1cΔ9, 18:1tΔ9, or 16:0. It seems unlikely then that the presence of ethylene glycol unduly influenced the position of the membrane lipid phase transition during acquisition of the ^{19}F NMR spectra at temperatures below 270 K. The ^{19}F NMR spectra of *A. laidlawii* B membranes enriched with 20% 6F16:0 plus 80% 18:1tΔ9 at 279 K were identical whether acquired in the presence or absence of 50% ethylene glycol, so that ethylene glycol itself did not prevent the membrane lipid fatty acyl chains from assuming a highly ordered, nearly all-trans configuration (data not shown). The ^{19}F NMR order profiles of *A. laidlawii* B membranes enriched with either 18:1cΔ9 or 19:0cp,cΔ9 were obtained at 279 or 280 K, respectively, in the presence or absence of 50% ethylene glycol. As shown in Figure 4, the order profiles at this temperature were essentially identical whether obtained with or without ethylene glycol. These various observations strongly suggest that the highly disordered gel state assumed in the presence of 18:1cΔ9 or 19:0cp,cΔ9 is unrelated to any effect of ethylene glycol but rather is characteristic of the influence of the cis cyclopropane ring and the cis double bond upon the motional freedom of the MFPA probe in the gel state.

Normalization with Respect to the Lipid Phase Transition. Further insights into the consequences of the inclusion of cis or trans cyclopropyl ring or monounsaturated substituents can be gained by a direct comparison of the orientational order of the MFPA probes at a particular reduced temperature in the presence of a particular structural substituent. The introduction of a reduced temperature, T_R, is intended to eliminate or at least minimize effects attributable to differences in lipid phase transition temperatures, where

$$T_R = (T - T_m)/T_m$$

Here, T is the measuring temperature, and T_m is the lipid phase transition temperature for a particular case of enrichment with one fatty acid, both in degrees kelvin (Seelig & Browning, 1978). Therefore, measurements made at equal values of T_R should sample equal and coinciding physical states with respect to the lipid phase transition, regardless of the absolute measuring temperature.

Figure 5 depicts the chain-average order parameter as a function of the reduced temperature, T_R, for each case of enrichment with either 18:1cΔ9, 19:0cp,cΔ9, 18:1tΔ9, or 19:0cp,tΔ9. The chain-average order is simply the numerical average of the five values of S_{mol} obtained at any one temperature for any one case of enrichment. While such a data reduction will obscure differences in the characters of the order profiles, it does provide a convenient measure of overall orientational order in a given situation. In the presence of the two fatty acids containing structural substituents in the trans configuration, 18:1tΔ9 and 19:0cp,tΔ9, the overall order of the MFPA's showed a profound increase at the temperature of the lipid phase transition, indicating the preeminence of that phase change as an effector of overall orientational order. Both

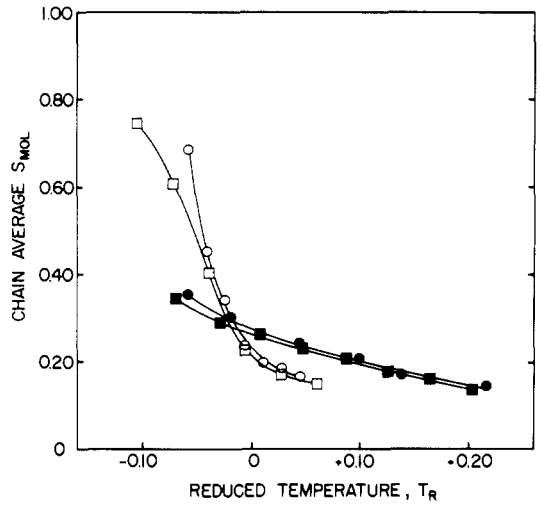

FIGURE 5: Chain-average orientational order vs. reduced temperature. The chain-average order and T_R were calculated as described in the text. Open circles, 18:1tΔ9; open squares, 19:0cp,tΔ9; closed circles, 18:1cΔ9; closed squares, 19:0cp,cΔ9.

fatty acids behaved more or less similarly with respect to T_R, although significant differences in the overall order between the membranes enriched in 18:1tΔ9 and 19:0cp,tΔ9 were evident at values of $T_R < 0.5$. Whether these disparities reflect real differences in the response of the trans cyclopropane ring and the trans double bond to the gel state, or whether they result from differences in the distribution of the MFPA's between the two calorimetrically observed phase transitions in the presence of these trans structures, cannot be unequivocally stated. However, in a previous ^{19}F NMR study of various isomeric *trans*-octadecenoic acids, values of the chain-average order in the presence of 18:1tΔ6 or 18:1tΔ11 approached 0.75 at a T_R value of −0.10, indicating that the differences between 19:0cp,tΔ9 and 18:1tΔ9 in the gel state may be more apparent than real (Macdonald et al., 1985b).

It is clear from Figure 5 that, although the MFPA's behaved similarly in the presence of the fatty acids 18:1cΔ9 and 19:0cp,tΔ9, the sharp increase in overall orientational order at the phase transition, which was observed in the presence of 18:1tΔ9 and 19:0cp,tΔ9, was absent or nearly so when the cis fatty acids were present. It is further evident that the overall order achieved in the gel state in the presence of the cis fatty acids is indicative of a highly disordered state, at values of T_R where in the presence of the trans fatty acids the MFPA's were approaching an all-trans configuration. Evidently, these structural substituents when in the cis configuration were capable of profoundly disrupting gel-state acyl chain packing in both a local and an overall sense, effectively preventing the ready assumption of an all-trans configuration of the acyl chains. If the temperature of the membrane lipid gel to liquid-crystalline phase transition is viewed as a measure of the stability of the gel state, then the decreased phase transition temperatures observed in the presence of 18:1cΔ9 or 19:0cp,cΔ9 are a consequence of their disruption of gel-state chain packing as was observed via ^{19}F NMR. This same relationship between gel-state disordering and decreased lipid phase transition temperature accounts for the relative overall gel-state orientational order of straight-chain saturated vs. methyl iso- and anteiso-branched fatty acids and their respective membrane lipid phase transition temperatures (Macdonald et al., 1985c).

It is further evident in Figure 5 that in the liquid-crystalline state (i.e., $T_R > 0$), at comparable values of T_R, those membranes enriched with the lower melting cis fatty acids were more highly ordered that those enriched with the higher melting trans fatty acids. This inverse relationship between the temperature of the lipid phase transition and the overall orientational order at equal values of $T_R > 0$ has been observed previously by using ^{19}F NMR in *A. laidlawii* B membranes enriched with a multitude of various fatty acyl structures (Macdonald et al., 1983, 1984, 1985a–c). Liquid-crystalline-state chain-packing considerations seem insufficient to explain this relationship, since monolayer studies indicate that most fatty acyl structural types occupy approximately equal cross-sectional areas in the liquid-expanded state [see, for example, Kannenburg et al. (1983)]. Since in the absence of a phase change orientational order increased in an approximately linear fashion, and since at 310 K the orientational order was approximately equal in all four cases of enrichment (but see below), the greater ordering of the lower melting membranes at comparable values of T_R is purely a consequence of their remaining in the liquid-crystalline state at lower temperatures.

Finally, we would note that at 310 K the overall order in the presence of 18:1tΔ9 or 19:0cp,tΔ9 ($T - T_m \simeq 10$ °C) was approximately 20% higher than that measured in the presence of 18:1cΔ9 or 19:0cp,cΔ9 ($T - T_m \simeq 50$ °C). We have previously observed that, for any one acquisition temperature at which all fatty acyl chains are in the liquid-crystalline state, overall order is relatively independent of specific acyl chain structure and can be shown to be an approximately inverse linear function of $T - T_m$ (i.e., order decreases as proximity to the lipid phase transition decreases, or as $T - T_m$ increases) (Macdonald et al., 1985a). It seems probable that some minimal degree of hydrocarbon chain ordering should be required to maintain a properly functional lipid bilayer. Although a precise hyperfluid point remains to be defined, it is reasonable to suppose that proximity to the particular phase transition temperature will be an important determinant of which fatty acyl structural types are considered to be hyperfluidizing under given circumstances.

CONCLUSIONS

From the perspective provided by the MFPA nuclear spin probes, the cyclopropane ring and double-bond fatty acyl chain substituents influence the ordering properties of the hydrocarbon chains of membrane lipids in a remarkably similar fashion. In physical terms, these two structures may be considered to represent approximately equivalent solutions to the problem of modulating the phase state of membrane lipids through structural variations in the fatty acyl components. The results obtained in this study further support the contention that specific fatty acyl structural substituents are able to destabilize gel-state lipid packing and, hence, decrease the temperature of the membrane lipid gel to liquid-crystalline phase transition. However, these studies do not address the question of why cyclopropane fatty acids are substituted for double-bond-containing fatty acids or the hypothesis that cyclopropane fatty acids are associated with increased organizational stability of the membrane lipids. Obtaining such insights will require further direct study of the cyclopropane fatty acids themselves.

Registry No. 18:1tΔ9, 112-79-8; 18:1cΔ9, 112-80-1; 19:0cp,cΔ9, 4675-61-0; 19:0cp,tΔ9, 29203-99-4.

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

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Received October 23, 1984

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

† Issued as NRCC Publication No. 24656.

0006-2960/85/0424-4659$01.50/0