^{19}F NMR Studies of Lipid Fatty Acyl Chain Order and Dynamics in *Acholeplasma laidlawii* B Membranes. ^{19}F NMR Line Shape and Orientational Order in the Gel State†

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ABSTRACT: The orientational order parameters, S_{mol}, of whole cells, membranes, and membrane polar lipids of *Acholeplasma laidlawii* B enriched with palmitic acid plus small amounts of one of a series of positional isomers of monofluoropalmitic acid have been determined via fluorine-19 nuclear magnetic resonance spectroscopy. It is demonstrated that a theoretical description of the ^{19}F NMR line shape, which assumes that long-axis rotational reorientations of the whole lipid molecule are fast compared to the ^{19}F NMR time scale of measurement, adequately describes the ^{19}F NMR spectrum obtained in either the liquid-crystalline or the gel lipid phase state. At a temperature above the calorimetrically determined main lipid phase transition, overall orientational order was low while the characteristic dependence of order upon chain segment position was observed, with a region of relatively constant order preceding a gradient of order diminishing toward the acyl chain methyl terminus. At a temperature just below the main lipid phase transition, considerable orientational disorder was still in evidence while the gradient of order along the length of the acyl chain was less pronounced. Far below the phase transition orientational order approached the theoretical maximum equally, or nearly so, at all chain positions. At all temperatures orientational order was nearly identical in whole cells, membranes, and membrane lipids, indicating that membrane-associated proteins have little effect on the range of conformations available to membrane lipid fatty acyl chains in either the liquid-crystalline or the gel state.

Nuclear magnetic resonance (NMR)[1] techniques can provide detailed insights into both the molecular dynamics and the configurations of the hydrocarbon chains within a lipid bilayer. Each of the various commonly studied nuclei possess inherent advantages and disadvantages in this regard. The ^1H NMR spectrum of phospholipid–water dispersions can be interpreted in terms of overall hydrocarbon chain motion [see, for example, MacKay (1981)], but the resonance lines associated with individual chain segments cannot be resolved. Specifically deuterated or ^{13}C-enriched fatty acids are commonly employed to obtain orientational and/or dynamical information at discrete chain positions (Davis, 1983; Griffin, 1981), but the requisite synthetic procedures are neither facile nor inexpensive, and the relatively low sensitivity of these nuclei dictates that for each individual fatty acid structure of interest an entire set of specifically enriched nuclear spin probes must be synthesized. Furthermore, analysis of the ^2H NMR spectra of deuterated fatty acyl chains esterified within a lipid bilayer in terms of segmental orientational order parameters—the most generally employed and definitive source of such information—is restricted to the liquid-crystalline lipid phase state because of slow motion effects manifest in the gel-state ^2H NMR spectrum (Davis, 1983).

Specifically fluorinated monofluoropalmitic acids (MFPA) have proven to be versatile and veracious monitors of membrane lipid orientational order. These nuclear spin probes are simple and inexpensive to synthesize and have been shown to be relatively nonperturbing on the basis of a number of biological, biochemical, and biophysical criteria (McDonough et al., 1983). The ^{19}F NMR spectra of MFPA's biosynthetically incorporated into a biological membrane can be interpreted in terms of an orientational order parameter, S_{mol}, and the variation of S_{mol} with the location of the fluorine substituent provides a picture of the bilayer order gradient which is essentially identical with that obtained via ^2H NMR (Macdonald et al., 1983). Furthermore, the sensitivity of the fluorine nucleus in the NMR experiment permits a single series of MFPA's to be used in small quantities to report the effects of a variety of fatty acid structures (present in much larger quantities) upon the bilayer orientational order gradient (Macdonald et al., 1983). In addition, the wide range of chemical shifts undergone by fluorine permits the simultaneous and independent monitoring of the physical state of both membrane proteins and lipids when fluorine-labeled protein and lipid are incorporated together into a lipid bilayer (Dettman et al., 1982, 1984).

We report here our analysis of the ^{19}F NMR spectra of whole cells, membranes, and membrane polar lipids of the organism *Acholeplasma laidlawii* B enriched with palmitic acid plus small quantities of various MFPA's. This organism readily incorporates a variety of exogenously supplied fatty acids into its membrane glycerolipids to high levels of enrichment (McElhaney, 1984). Moreover, this cell-wall-less organism possesses but a single membranous structure, the plasma membrane, so that the isolation of "pure" membranes highly enriched in a particular fatty acid is remarkably straightforward (Razin, 1975).

Our results indicate that the theoretical description of the ^{19}F NMR line shape previously applied to the liquid-crystalline phase state (Macdonald et al., 1983) can be successfully applied to the gel state, that the values of S_{mol} obtained at temperatures below the main lipid phase transition approach the theoretical maximum, and that the gradient of orientational order characteristic of the liquid-crystalline state is absent or

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[1] Abbreviations: NMR, nuclear magnetic resonance; MFPA, monofluoropalmitic acid; DSC, differential scanning calorimetry; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; Tris, tris(hydroxymethyl)-aminomethane.

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Table I: Fatty Acid Composition of *A. laidlawii* Membrane Polar Lipids Isolated from Cells Supplemented with Palmitic Acid plus Various Monofluoropalmitic Acids

supplement (0.12 mM total)	12:0	14:0	16:0	MF16:0	18:0	exogenous % incorpn[a]	ratio MF16:0/ 16:0
20% 6F-16:0 + 80% 16:0	4.54	18.94	64.75	10.43	1.44	75.18	0.161
20% 8F-16:0 + 80% 16:0	0.91	16.51	69.81	12.46	0.31	82.27	0.178
20% 10F-16:0 + 80% 16:0	2.84	18.78	67.17	10.35	0.86	77.47	0.154
20% 12F-16:0 + 80% 16:0	2.97	16.82	72.23	7.82	0.16	80.05	0.108
20% 14F-16:0 + 80% 16:0	3.77	18.80	64.00	11.63	1.80	75.63	0.185

[a] Sum of mole percent 16:0 plus MF16:0.

nearly so in the gel state. Furthermore, values of S_{mol} were nearly identical at all temperatures whether obtained with whole cells, isolated membranes, or membrane polar lipids, indicating that, on the NMR time scale, membrane-associated proteins, whether peripheral or intrinsic, do not particularly influence the orientational order of membrane lipids in either the liquid-crystalline or the gel state.

Experimental Procedures

Acholeplasma laidlawii B was cultured at 37 °C in a lipid-extracted growth medium supplemented with 0.096 mM palmitic acid plus 0.024 mM of a particular MFPA, and the cells were harvested as previously described (Silvius & McElhaney, 1978). Whole cells from 1.0 L of culture medium (containing approximately 4 mg of fluorinated and 28 mg of nonfluorinated lipid) were washed once, resuspended in β buffer (0.154 M NaCl, 0.05 M Tris-HCl, and 20 mM β-mercaptoethanol, pH 7.4) containing 90% deuterium oxide and the ^{19}F NMR spectra acquired as described below. Cell viability was then checked microscopically and by reculturing in growth medium. Cells were 99% intact following data acquisition, and all cultures grew successfully.

Plasma membranes were isolated from the whole cells as described elsewhere (Silvius et al., 1977), suspended in β buffer which had been diluted 20-fold with 95% deuterium oxide, and the ^{19}F NMR spectra again were acquired.

Membrane polar lipids were subsequently extracted and purified as previously described (Saito & McElhaney, 1977). The polar lipids were rehydrated in β buffer (diluted 20-fold with 95% deuterium oxide) by gentle heating and mild vortexing, and once more the ^{19}F NMR spectra were acquired.

The polar lipid samples were then taken for thermal analysis by differential scanning calorimetry as described below and for fatty acid analysis by gas–liquid chromatography as described elsewhere (Saito & McElhaney, 1977).

^{19}F NMR spectra were collected at 254.025 MHz on a Bruker HXS-270 NMR 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. The probe was maintained at the specified temperature to within ±1 °C. Samples were equilibrated at a particular temperature for 30 min prior to data acquisition. ^{19}F nuclei were subjected to a 15-μs (~75°) pulse followed by a 10-μs delay and 20-ms acquisition time. The recycle time was 200 ms. Typically, 15–20K scans were accumulated for samples at which the 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) was corrected by a smooth extrapolation of the FID back to time zero such that the early portion of the FID closely approximated a t^2 time dependency (Bloom et al., 1978). The distortion is associated with the dead time of the receiver. The signal-to-noise ratio was enhanced with an exponential multiplication which corresponded to a line broadening of 50 Hz, and the FID was Fourier transformed to 2K data points in the real domain.

Thermal transition endotherms were collected on a Perkin-Elmer DSC-2 scanning calorimeter equipped with a subambient temperature accessory and a thermal analysis data station. Polar lipid samples were lyophilized and rehydrated in water by gently heating and vortexing, and aliquots were scanned at least twice at a heating rate of 5 °C/min. The midpoint (T_m), ΔT_{10-90}, and percent transition were calculated by using the partial area computer program provided with the Perkin-Elmer thermal analysis data station.

The various monofluoropalmitic acids were synthesized as described previously (McDonough et al., 1983). Palmitic acid was purchased from Nu-Chek Prep Co., Elysian, MN. All other chemicals and reagents used were of reagent grade or better.

Results

Fatty Acid Composition of A. laidlawii B Membrane Lipids. Many fatty acids will, by themselves, support the growth of *A. laidlawii* B in the presence of an inhibitor of de novo fatty acid biosynthesis such as avidin, often leading to the production of membranes containing up to 99% of a single fatty acid species, but palmitic acid is not one of these (Silvius & McElhaney, 1978). The fatty acid compositions of the membrane polar lipids from cells grown in the absence of avidin on media supplemented with 80 mol % palmitic acid plus 20 mol % sample of a particular MFPA are shown in Table I. The fatty acid compositions were similar regardless of the particular positional isomer of MFPA present. In addition, the products of de novo fatty acid biosynthesis in *A. laidlawii* B (12:0, 14:0, 16:0, and 18:0) contributed significantly to the overall composition.

The percent enrichment with exogenously supplied fatty acids (the sum of palmitic acid plus MFPA) was between 75% and 82%, but this may be an overestimate due to the contribution of de novo biosynthesis of palmitic acid. Hence, the fact that the ratio of MFPA to palmitate present in the membrane did not correspond to that provided in supplement was most probably not due to a disproportionate uptake of either of the supplementary fatty acids—MFPA's are proportionately incorporated in all cases tested to date (McDonough et al., 1983; Macdonald et al., 1983)—but rather may be attributed to the obscuring effect of de novo biosynthesis. Regardless, the levels of MFPA present were sufficient for the practical purpose of obtaining ^{19}F NMR spectra.

Thermotropic Behavior of Membrane Polar Lipids. Figure 1 shows the membrane polar lipid phase transition endotherms obtained by using differential scanning calorimetry (DSC) for each case of coenrichment with a particular isomeric MFPA. The transition endotherms were typically somewhat asymmetric and similar to those of *A. laidlawii* membranes and lipids previously reported from this laboratory [see, for ex-

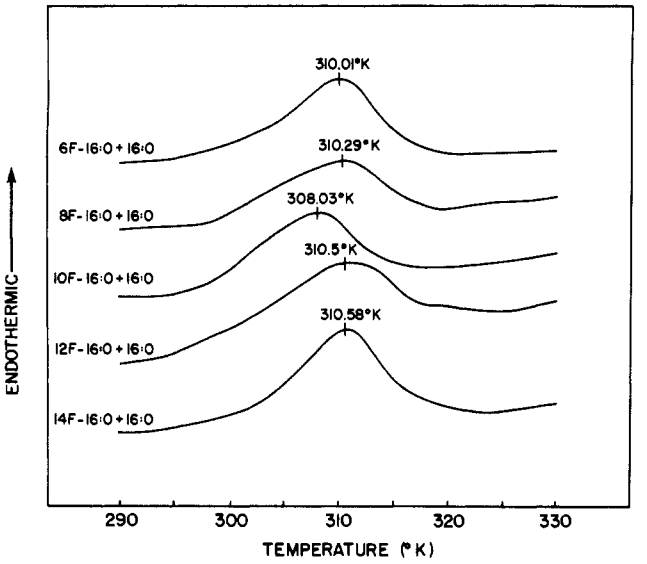

FIGURE 1: Main lipid phase transition endotherms obtained via DSC of isolated membrane polar lipids for each case of supplementation with a particular MFPA.

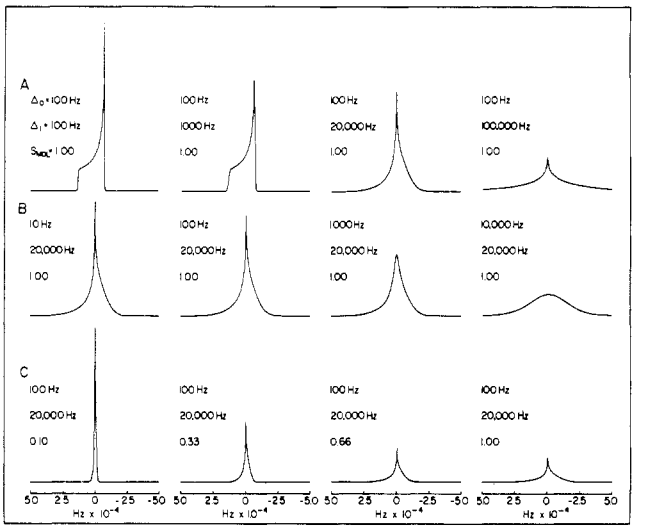

FIGURE 2: Effect of varying the line-shape parameters on the simulated ^{19}F NMR line shape. (A) Variation of the intramolecular dipole parameter Δ_1; (B) variation of the intermolecular dipole parameter Δ_0; (C) variation of the orientational order parameter S_{mol}.

ample, McDonough et al. (1983)].

When the thermotropic transition obtained in the case of coenrichment with 10F-16:0 was excluded, the midpoints of the phase transitions (T_m) varied over a range of approximately 1.0 °C about the average T_m of 308.9 K. It is not clear why the particular case of 10F-16:0 exhibited a T_m approximately 2.0 °C lower than average. The phase transition temperatures reported here for enrichment with 80 mol % palmitic acid plus 20 mol % MFPA are somewhat lower than those which have previously been reported for enrichment of *A. laidlawii* membranes with palmitic acid alone (DeKruyff et al., 1973; McDonough et al., 1983). However, levels of enrichment being somewhat variable and de novo biosynthesis making up any shortfall lead to the situation apparent in Table I, where myristic acid makes up nearly 20 mol % of the membrane fatty acids and, hence, lowers the T_m.

The parameter ΔT_{10-90}, corresponding to the temperature range over which the phase transition passes from 10% to 90% completion, can be used as a measure of the cooperativity of the phase transition in biological membranes. Values of ΔT_{10-90} were of the order of 11–12 °C, in good agreement with those previously reported from this laboratory (Silvius et al., 1980).

^{19}F NMR Line Shape in the Liquid-Crystalline State and in the Gel State. We have previously demonstrated that the ^{19}F NMR line shape obtained with a MFPA incorporated into a lipid bilayer can be described by a mathematical model which assumes a rapid rotation about the long axis of the lipid molecule and, consequently, an axially symmetric line shape (Macdonald et al., 1983). The pertinent interactions, which include the residual chemical shift anisotropy ($\sigma_\parallel - \sigma_\perp$), the interchain proton–fluorine dipole interaction (Δ_0) and the intrachain proton–fluorine dipole interaction (Δ_1), are treated essentially as described in Niederberger & Seelig (1976). The orientation-dependent terms, $\sigma_\parallel - \sigma_\perp$ and Δ_1, are further modulated by the orientational order parameter S_{mol}. S_{mol} is a quantitative measure of the time-averaged angular excursions of particular methylene segments about the long molecular axis. When for each methylene segment S_{mol} equals unity, the acyl chain will have assumed an all-trans configuration aligned perpendicular to the plane of the bilayer. Tilting of the entire acyl chain and/or trans–gauche isomerization at individual methylene segments will decrease the value of S_{mol}.

Figure 2 illustrates the manner in which the values of these various parameters alter the ^{19}F NMR line shape within the context of our theoretical model. The simulated spectra in Figure 2A demonstrate the consequences of altering the value of Δ_1. When Δ_1 is small, the anisotropic chemical shift line shape, characteristic of rapid long-axis rotation of the entire lipid molecule, is obtained. The sharp perpendicular edges of this spectrum correspond to the quantities $\sigma_\parallel$ and $\sigma_\perp$. The value of $\sigma_\parallel - \sigma_\perp$ has been estimated to be 82.2 ppm for a MFPA experiencing rapid long-axis rotation (Macdonald et al., 1983) from a consideration of the chemical shift tensor elements of the model compound Teflon (Mehring et al., 1971). As the value of Δ_1 increases, the anisotropic chemical shift spectrum first broadens and then, when Δ_1 equals approximately 15 000 Hz, assumes the dipolar or "super-Lorentzian" line shape which resembles that of experimentally obtained ^{19}F NMR spectra (see below). We have previously estimated the value of Δ_1 to be in the range of 20 000 Hz from a study of the dependence of the experimental ^{19}F NMR line width on the field strength (Macdonald et al., 1983). Increasing Δ_1 by a further order of magnitude extensively broadens the simulated spectra, particularly in the wings.

The spectral simulations in Figure 2B illustrate the effect of increasing the value of Δ_0. Here Δ_1 was set to 20 000 Hz so that the overall line shapes would correspond to an experimental situation. Increasing the value of Δ_0 when Δ_1 is small causes broadening of the entire spectrum without affecting an alteration equivalent to increasing Δ_1 (data not shown). The same broadening over the entire width of the spectra occurs when Δ_1 is set to 20 000 Hz, and Δ_0 is progressively increased. The broadening of the simulated spectra which occurs upon increasing Δ_0 corresponds to an increase in the efficacy of interchain dipole interactions such as would result from, for example, a transition from the liquid-crystalline to the gel state. We have found experimentally that values of Δ_0 generally fall in the range from 100 to 1000 Hz (see below).

The manner in which S_{mol} modulates the model spectra is shown in Figure 2C. As S_{mol} decreases, the line shape progressively narrows, approaching the isotropic limit at very small values.

The correspondence of experimentally acquired ^{19}F NMR spectra and "best-fit" model spectra is illustrated in Figure

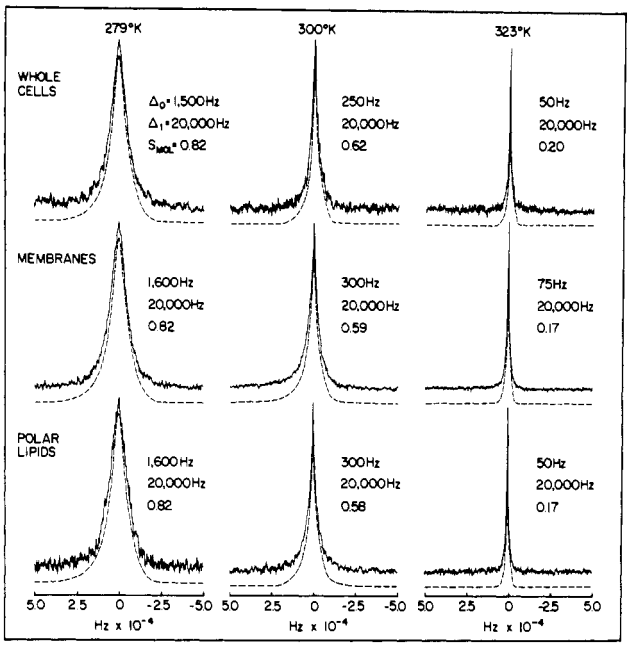

FIGURE 3: Correspondence of experimental ^{19}F NMR spectra (whole cells, membranes, and lipids of *A. laidlawii* enriched with 80 mol % 16:0 plus 20 mol % 8F-16:0) and best-fit simulated spectra (---). Experimental spectra were acquired with 100K, 50K, and 25K acquisitions at 279, 300, and 323 K, respectively. The corresponding values of the line-shape parameters, Δ_0, Δ_1, and S_{mol}, used to generate the best-fit simulated spectra are indicated.

3 for the case of cells supplemented with 20 mol % 8F-16:0 plus 80 mol % 16:0. The spectra were acquired at temperatures at which the membrane lipids were just above (323 K), just below (300 K) and far below (279 K) their gel to liquid-crystalline phase transition. Whether obtained with whole cells, isolated membranes, or membrane polar lipids, the ^{19}F NMR spectra were remarkably similar, exhibiting identical changes with alterations in temperature and lipid phase state. At all temperatures and regardless of the lipid phase state, the ^{19}F NMR spectra were adequately simulated by using the model which assumed an axially symmetric line shape. The values of S_{mol} increased from approximately 0.20 to 0.82 while Δ_0 increased from about 100 to 1600 Hz as the acquisition temperature was lowered from 323 to 279 K. The ^{19}F NMR spectrum obtained at an intermediate temperature was apparently a simple superposition of gel phase and liquid-crystalline phase spectra in that, by adding together in the proper proportion spectra obtained at the extremes of temperature, the intermediate spectrum could be reproduced (P. M. Macdonald, B. D. Sykes, R. N. McElhaney, unpublished results).

Figure 4 compares the ^{19}F NMR spectrum of a solid-crystalline powder of 12F-16:0 with that of membranes of *A. laidlawii* enriched with 20% 12F-16:0 plus 80% 16:0 in the gel state. The two spectra were obtained under identical instrumental conditions. It is expected that in the absence of rapid motions acting to average the chemical shift and dipolar interactions the spectrum should broaden in proportion to the values of the unaveraged chemical shift tensor elements (estimated to be $\sigma_{11} = -80$ ppm, $\sigma_{22} = 21$ ppm, $\sigma_{33} = 59$ ppm, by comparison with the model compound Teflon; Mehring et al., 1971). Further broadening due to fluorine–proton dipolar interactions would effectively obscure the inflections corresponding to the values of the individual chemical shift tensor elements resulting in the approximately Gaussian line shape of the solid 12F-16:0 in Figure 4. In contrast the spectrum of gel state *A. laidlawii* membrane lipids is considerably narrowed, indicating that significant motional averaging occurs

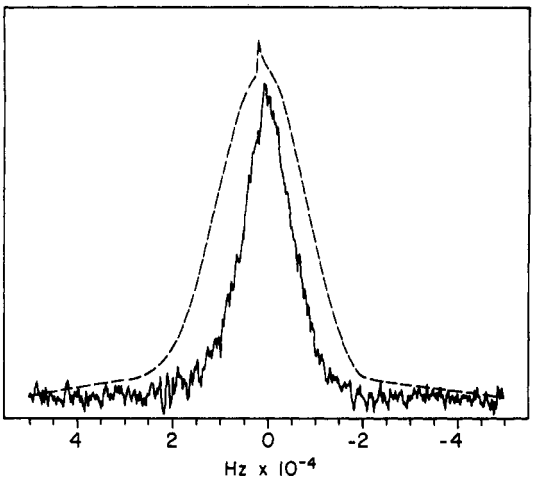

FIGURE 4: ^{19}F NMR spectra of solid 12F-16:0 (dashed line, 1000 scans) and *A. laidlawii* membranes enriched with 20% 12F-16:0 plus 80% 16:0 at 279 K (solid line, 100 000 scans; $S_{mol} = 0.82$, $\Delta_0 = 1500$ Hz, and $\Delta_1 = 20\,000$ Hz).

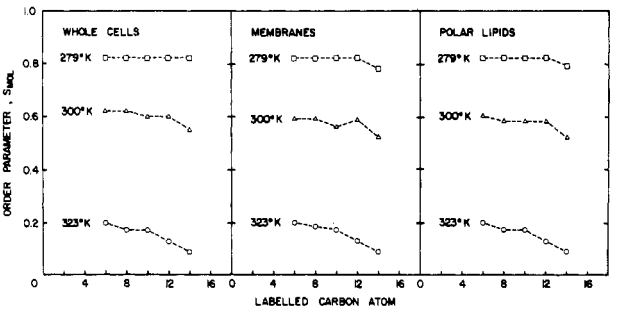

FIGURE 5: ^{19}F NMR orientational order profiles for whole cells, membranes, and membrane polar lipids of *A. laidlawii* enriched with palmitic acid.

on the time scale of the ^{19}F NMR experiment. Furthermore, the gel-state spectral line shape is super-Lorentzian, an indication that the motions which average the chemical shift and dipolar interactions are axially symmetrical in nature.

^{19}F NMR Order Profiles. The variation of S_{mol} with the position of the fluorine substituent provides an orientational order profile for a particular membrane. The ^{19}F NMR order profiles of *A. laidlawii* whole cells, isolated membranes, and extracted polar lipids are shown in Figure 5. At 323 K, the values of the order parameters were relatively constant out to approximately carbon atom 10 and thereafter decreased as the nuclear spin probe was relocated further toward the methyl terminus of the acyl chain. Similar order profiles have been reported by using ^{2}H NMR techniques in both model (Seelig, 1977) and biological (Stockton et al., 1977) membranes, although some questions remain concerning the absolute values of order parameters obtained by ^{19}F NMR techniques relative to those obtained via ^{2}H NMR (Macdonald et al., 1983). The disorder gradient across the width of the membrane, which is characteristic of the orientational order profile, has come to be regarded as the "signature" of a lipid bilayer.

With decreasing temperature, orientational order increased. At 300 K, where the calorimetric data indicate that the phase transition is at least 90% complete, the average overall chain order was approximately 0.6, indicating that, despite the overwhelming preponderance of gel-state lipid, a substantial degree of disorder remained. The order profile itself indicated that, although a gradient of disorder was still present, the "incline" of the gradient was much less pronounced than that observed with entirely liquid-crystalline lipid at 323 K.

When the temperature was decreased further to 279 K, the average order parameter increased to approximately 0.8, indicative of a high degree of acyl chain ordering. In addition, all chain positions displayed an equal degree of ordering with the exception of the 14-position. Therefore, although some degree of disorder may exist near the methyl terminus, at temperatures well below the lipid phase transition the bulk of the acyl chain experiences an equally high degree of orientational order.

It is interesting to note that few if any differences in orientational order can be discerned between live cells, membranes, and polar lipids. Studies of orientational order in isolated biological membranes would, therefore, appear to be directly applicable to the situation in whole cells. It is equally significant that the absence of integral membrane proteins had no effect upon S_{mol}. While it has been clear for some time that values of S_{mol} obtained by ^2H NMR techniques are nearly identical whether obtained in model or biological membranes in the liquid-crystalline state (Seelig & Seelig, 1980), this point had not been demonstrated for ^{19}F NMR techniques.

Discussion

^{19}F NMR Line Shape. The validity of the spectral simulations reported here, and hence of the extracted values of S_{mol}, requires that the implicit assumption of an axially symmetric line shape should pertain at all temperatures employed. The assumption of axial symmetry requires that the rate of long-axis rotation of the entire lipid molecule be faster than the time scale of the interaction being measured, τ, where for chemical shift anisotropy (Davis, 1983)

$$\tau = 1/(\gamma H_0 \Delta\sigma)$$

An axially symmetric line shape is not characteristic of the gel-state component of the ^2H NMR spectra of DPPC containing deuterium-labeled fatty acyl chains, where $\tau(^2H) \simeq 1.3 \times 10^{-6}$ s (Davis, 1983). In contrast, the line shape obtained with ^{13}C NMR of DPPC, which was ^{13}C enriched at the C1 position of the *sn*-2 chain, was characteristic of axial symmetry even at a temperature approximately 40 °C below the phase transition (Wittebort et al., 1981), where the time scale of measurement was $\tau(^{13}C) \simeq 2.0 \times 10^{-5}$ s (Davis, 1983). The rotational correlation time of the lipid itself at temperatures immediately below the phase transition must then lie somewhere between $\tau(^2H)$ and $\tau(^{13}C)$.

The time scale of measurement for ^{19}F, assuming a maximum chemical shift anisotropy of 82.2 ppm (Macdonald et al., 1983) and at 254.025 MHz, would be $\tau(^{19}F_{csa}) \simeq 7.6 \times 10^{-6}$ s. Thus, it is to be expected that the assumption of axial symmetry should hold for our ^{19}F NMR spectra in the gel state at temperatures lower than for ^2H NMR but not so low as for ^{13}C NMR. The ^{19}F NMR spectra of MFPA's in the gel state exhibit none of the spectral characteristics of axial asymmetry that are observed at extremely low temperatures when ^{13}C NMR (Wittebort et al., 1981) or ^{31}P NMR (Campbell et al., 1979) lipid spectra are investigated and can apparently be adequately simulated by using an axially symmetric model. The difficulty of proton–fluorine decoupling has so far prevented a direct demonstration of the occurrence of axial symmetry in the ^{19}F NMR spectra of MFPA's in the gel state. However, the ^{19}F NMR spectra of 2-[$^2H_6$,8,8-$^{19}F_2$]DMPC reported by Engelsberg et al. (1982), in which the effects of axial symmetry upon the line shape are more readily apparent due to the reduced intrachain dipole interactions in the presence of the deuterons, retain characteristics of axial symmetry at temperatures below the phase transition. Here the time scale of measurement was $\tau(^{19}F_2) \simeq 7 \times 10^{-6}$ s (assuming $\Delta\sigma > 58$ ppm and $\nu_0 = 282.4$ MHz), in close proximity to the time scale of our measurements. These various points strongly suggest that the assumption of axial symmetry is valid for the case of ^{19}F NMR spectra of MFPA's in the gel state.

For the case of heteronuclear dipole–dipole interactions, which also contribute to the line shape of the MFPA ^{19}F NMR spectrum, long-axis molecular reorientations must occur on a time scale that is short compared to the inverse of the static interaction frequency $\tau(^{19}F_{dd})$ and

$$\tau(^{19}F_{dd}) \simeq [\gamma_H \gamma_F \hbar / (2\pi r_{H\text{-}F}^3)]^{-1}$$

where γ_H and γ_F are the magnetogyric ratios of the proton and fluorine, respectively, and $r_{H\text{-}F}$ is the proton–fluorine internuclear distance (Farrar & Becker, 1971). Assuming that the strongest dipole interaction in a MFPA is between fluorine and the geminal proton, with C–C, C–H, and C–F distances of 1.541, 1.094, and 1.32 Å and tetrahedral angles, then

$$\gamma_H \gamma_F \hbar / (2\pi r_{H\text{-}F}^3) \simeq 14\,600 \text{ Hz}$$

Therefore, $\tau(^{19}F_{dd}) \simeq 6.8 \times 10^{-5}$ s, indicating that the assumption of rapid long-axis rotational reorientation should pertain to the case of intramolecular fluorine–proton dipole interactions at temperatures below the lipid phase transition at least equal to those where an axially symmetric line shape is obtained with ^{13}C NMR.

When a calculation of the fluorine–proton dipole interaction is extended to include all dipole pairs with an internuclear distance of less than 4 Å, the total fluorine–proton intramolecular dipole interaction is estimated to be

$$r_j \sum_{<4\text{Å}} \gamma_H \gamma_F \hbar / (2\pi r_{jHF}^3) \simeq 47\,000 \text{ Hz}$$

Averaging about the long molecular axis due to rapid rotational motion would reduce the value of the vicinal fluorine–proton dipole interaction by a factor of $^1/_2(3\cos^2\theta - 1)$ equal to a 50% reduction from the rigid lattice value, where θ is the angle between the dipole interaction vector and the axis of motional averaging. The strength of the dipolar couplings to protons located on carbons α and β to the monofluoromethylene segment would remain relatively unchanged by this particular type of motional averaging. When it is further noted that the value of the intra-methylene proton residual second moment for the methylene chains of dipalmitoylphosphatidylcholine is reduced by approximately 50% over the temperature range −10 to 30 °C (MacKay, 1981), then the value of 20 000 Hz which we have previously estimated from experiment to correspond to the extent of the total fluorine–proton dipole interactions appears reasonable (Macdonald et al., 1983).

A further test of the validity of these spectral simulations is to compare the information obtained with values previously reported in the literature by using comparable techniques. Stockton et al. (1977) have reported the values of S_{CD} obtained in membranes of *A. laidlawii* highly enriched with specifically deuterated palmitic acids where, at 42 °C (just above the main lipid phase transition), in the plateau region $S_{CD} \simeq 0.22$. Values of S_{mol} obtained with ^{19}F NMR in *A. laidlawii* membranes highly enriched with palmitic acid at a temperature just above the lipid phase transition were approximately equal to 0.27 (Macdonald et al., 1983). Petersen & Chan (1977) have derived formulas designed to provide a basis for the comparison of order parameters obtained using different techniques where the common factor is an evaluation of the probability of finding a trans vs. a gauche plus or gauche minus isomer at a particular chain position. These are

$$S_{CD} = -\tfrac{1}{2}p_t$$

$$S_{mol} = {}^{9}/_{8}p_t - {}^{1}/_{8}$$

where p_t is the probability of being in the trans configuration. As noted by Petersen & Chan (1977), the common transformation $S_{mol} = -2S_{CD}$ is valid only as p_t approaches a value of 1. Thus, when $S_{CD} = 0.22$, p_t can be calculated to be 0.44. In turn a value of p_t equal to 0.44 should give S_{mol} equal to 0.325. Thus, our value of $S_{mol} \simeq 0.27$ is 87% of that expected by comparison with ^{2}H NMR results, and therefore, our spectral simulations appear valid.

Orientational Order in the Liquid-Crystalline and Gel State. The configurational profiles reported here represent the first instance in which the orientational order of a membrane fatty acyl ester has been characterized in both the liquid-crystalline and the gel state. At temperatures above the main lipid phase transition overall orientational order is low. The characteristic dependence of order upon chain segment position is observed with a region of relatively constant order preceding a gradient of order diminishing toward the acyl chain methyl terminus. With decreasing temperature and altered lipid phase state overall orientational order increases while the gradient of order along the length of the acyl chain becomes less pronounced.

Allegrini et al. (1983) have studied the gel-state ^{2}H NMR spectra of specifically deuterated palmitic acids mixed in equimolar amounts with 1-palmitoyllyso-PC. These compounds together assume a bilayer structure, most probably via the formation of a functional dimer of lysophospholipid and free fatty acid. The free deuterated palmitic acid experiences a greater degree of rotational freedom in the gel state than would DPPC, which permits the characterization of its configuration in the gel state in terms of orientational order parameters. The values of S_{mol} obtained in the gel state nearly approached the theoretical maximum of unity for positions C2 to C13 but thereafter decreased toward the acyl chain methyl terminus. These results substantiate our observations of a highly ordered state with a minimal number of gauche conformers in the gel phase. However, the maximum value of S_{mol} reported by Allegrini et al. (1983) was 0.94, which is some 15% higher than the maximum of 0.82 that we have obtained. Although it is possible to question the applicability of results obtained with a mixture of lysophospholipid and free fatty acid to the situation in a diacyl phospholipid membrane, this difference in maximum ordering is most probably attributable to the considerable fatty acyl chain heterogeneity of the *A. laidlawii* membrane lipids in contrast to the absolute homogeneity of the model membrane system. It is possible to demonstrate, for example, that the presence of shorter carbon chains markedly reduces orientational order in lyotropic nematic phases (Covello et al., 1983). Moreover, values of S_{mol} obtained via ^{19}F NMR approach 0.9 in the gel state when *A. laidlawii* membranes made homogeneous with respect to a particular fatty acid are analyzed (unpublished results).

It would be premature, however, to exclude the possibility that lipid head-group heterogeneity makes some additional contribution to gel-state disorder in *A. laidlawii*. As discussed by Seelig & Browning (1978), when the ^{2}H NMR order parameters of acyl chains esterified to a variety of lipid head-group classes are compared in the liquid-crystalline state at a temperature normalized relative to their respective phase transitions, the values are all very similar [but see Marsh et al. (1983)]. Again in the liquid-crystalline state, Rance et al. (1983), in a ^{2}H NMR study, could discern little dependence of conformational order on head-group class among *A. laidlawii* glycerolipids. Such generalizations may not apply in the gel state where the densities of acyl chain packing and head-group packing become more pronounced and interdependent. Indeed, without even considering the respective gel-state stabilities of the individual classes of glycerolipids present in *A. laidlawii*, their heterogeneity alone could constitute a barrier to complete orientational ordering.

The 60-kHz splitting that appears in the gel-state ^{2}H NMR spectrum of *A. laidlawii* enriched with deuterated fatty acids has been interpreted as indicating that the acyl chains still retain considerable motional freedom at low temperatures (Smith et al., 1979). These authors concluded that at temperatures just below the lipid phase transition, the acyl chains have assumed an all-trans configuration and that only "rotational disorder" resulting from rapid rotation about the long molecular axis, and not trans–gauche isomerizations, reduces the observed quadrupolar splitting from the theoretical maximum of 126 kHz to the observed 60 kHz. The interpretation has been challenged by Pink & Zuckermann (1980), who point out that the results of Raman scattering studies indicate that there are a nonnegligible number of gauche bonds excited in the gel state of pure PC's. From consideration of a theoretical model of a biological membrane, these authors concluded that the gel-state ^{2}H NMR spectra of *A. laidlawii* could be interpreted as indicating the occurrence of trans–gauche isomerizations in addition to rotational disorder in the gel state.

NMR-derived orientational order parameters are modulated by whole chain tilt as well as by trans–gauche isomerizations at individual chain segments (Petersen & Chan, 1980). A straight-chain, saturated fatty acid in the all-trans configuration, experiencing only rotational disorder and/or fluctuations in the orientation of the long molecular axis, should not manifest a gradient of disorder along its length since tilting of the entire acyl chain should decrease S_{mol} equally at all positions. The ^{19}F NMR order profiles observed at 300 K indicate that at temperatures just below the lipid phase transition a gradient of disorder is still present, implying that the acyl chain has not assumed an all-trans configuration regardless of the contribution of whole chain fluctuations to the average order parameter. While the observation of an order gradient precludes the existence of an all-trans configuration, the converse, that the absence of an order gradient implies the all-trans state, does not hold. A population of gauche isomers distributed with equal probability or diffusing rapidly along the acyl chain could diminish S_{mol} homogeneously as observed at 279 K in *A. laidlawii* via ^{19}F NMR.

Jarrell et al. (1982) investigated the ^{2}H NMR spectra of *A. laidlawii* whole cells, membranes, and lipids enriched with deuterated myristic acid, while Kang et al. (1981) compared ^{2}H NMR splittings in *A. laidlawii* membranes and extracted lipids enriched with deurated myristic acids. These authors were able to discern little difference in the quadrupolar splittings obtained in cells, membranes, or lipids. These results are in agreement with our conclusions concerning the applicability of studies in isolated lipids and membranes to the membrane lipid organization in intact cells. Furthermore, both ^{2}H NMR and ^{19}F NMR results agree that the presence of membrane proteins does little to alter the range of conformations available to membrane fatty acyl chains in the liquid-crystalline state. This apparent lack of influence on lipid order by membrane proteins may be the result of the relatively low protein/lipid ratio in *A. laidlawii* compared to the ratio in model systems where some effects are discernible (Kang et al., 1981). The lateral segregation of intrinsic membrane proteins into protein-rich domains, which can be observed by electron microscopy in *A. laidlawii* membranes cooled below the lipid phase transition temperature (Silvius & McElhaney,

1980), further suggests that, in the gel state, bulk membrane lipid conformations should not be, and as concluded from ^{19}F NMR data, are in fact not, particularly influenced by membrane proteins.

Registry No. 16:0, 57-10-3; 6F-16:0, 58763-54-5; 8F-16:0, 86569-21-3; 10F-16:0, 90866-62-9; 12F-16:0, 90866-63-0; 14F-16:0, 86569-22-4; 12:0, 143-07-7; 14:0, 544-63-8; 18:0, 57-11-4.

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