19F-Nuclear Magnetic Resonance Study of Glycerolipid Fatty Acyl Chain Order in Acholeplasma laidlawii B Membranes

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The technique of 19F-nuclear magnetic resonance (19F-NMR) spectroscopy offers a number of advantages for studies of lipid fatty acyl chain orientation and dynamics in biomembranes. However, the geminal difluoromethylene fatty acid probes usually employed in such studies appreciably perturb the organization of lipid bilayers. We have thus synthesized a series of specifically monofluorinated palmitic acids and carried out biophysical, biochemical, and physiological studies establishing their suitability as relatively non-perturbing probes of lipid hydrocarbon chain organization. These 19F-NMR probes were then used to determine the fatty acyl chain order profiles of Acholeplasma laidlawii B membranes highly enriched in a variety of different exogenous fatty acids, particularly those containing a methyl branch or a trans-double bond.

INTRODUCTION

The technique of 19F-nuclear magnetic resonance (19F-NMR) spectroscopy offers unique advantages in studies of the orientation and motional rates of lipid fatty acyl groups in model and biological membranes [1]. The 19F nucleus is unique among those nuclei (1H, 2H, 13C, and 19F) usually used in NMR studies of membrane lipid hydrocarbon chains in having both a high sensitivity and a zero natural abundance in the membrane. Since the fluorine atom or atoms may be selectively placed at specific positions in the fatty acyl chain, the order and dynamics of individual chain segments can be obtained. Also, the relatively similar sizes and physical properties of the 19F and 1H atoms, and the relatively similar geometries and reactivities of C-F and C-H bonds, suggest that the presence of fluorine atoms should not greatly perturb the hydrocarbon chain organization of lipid bilayers [2]. Moreover, the high sensitivity of 19F in the NMR experiments permits the use of relatively low amounts of 19F-fatty acid probes.

A number of 19F-NMR studies of hydrocarbon chain organization in model and biological membranes have been published in recent years (see [3] for review). In almost all of these studies fatty acids containing two fluorine atoms attached to a single carbon atom were used as probes. However, recent DSC [2] and 2H-NMR [4] studies have shown that the geminal difluoromethylene group appreciably alters fatty acyl group organization in model membranes. Moreover, the biosynthetic incorporation of difluoro fatty acids into Escherichia coli membrane lipids results in a
substantial inhibition of cell growth and transport activity and an altered morphology [5]. Thus the suitability of geminal difluoro fatty acids as relatively non-perturbing probes of membrane lipid structure is questionable.

We have synthesized a series of specifically monofluorinated palmitic acids, since these $^19$F-NMR probes would be expected to be less perturbing than their difluoro fatty acid analogues. These fatty acids were then evaluated by biophysical, biochemical, and physiological methods in both model and biological membranes, and their suitability as relatively non-perturbing probes was established. These specifically monofluorinated fatty acids were then utilized as low-level probes of the lipid hydrocarbon chain segmental orientation in membranes of Acholeplasma laidlawii B highly enriched in linear saturated, methyl iso-branched, methyl antelso-branched, and $\text{trans}$-monounsaturated fatty acids. All previous NMR studies of the order parameter profiles of lipid hydrocarbon chains in biological membranes have been done on membranes containing predominantly linear saturated or $\text{cis}$-monounsaturated fatty acyl groups. However, since methyl-branched fatty acids occur widely in the membrane lipids of procaryotic microorganisms, and since various single branched-chain and $\text{trans}$-monounsaturated fatty acids can support the growth of several intensively studied bacterial and mycoplasma fatty acid auxotrophs (for a review, see [6]), physical studies of membranes containing these fatty acyl groups are desirable.

Acholeplasma laidlawii B belongs to the fermentative class of that diverse group of cell wall-less procaryotes, the mycoplasmas. The mycoplasmas are the smallest and simplest microorganisms capable of autonomous replication and they thus provide useful models for the study of a number of problems in cell biology in general and membrane biology in particular. A. laidlawii B, being procaryotic and lacking a cell wall, has only a single membrane system, the limiting or plasma membrane, which contains essentially all the cellular lipid and a considerable proportion of the total cellular protein as well. The absence of a rigid cell wall permits A. laidlawii B membranes to be easily and quickly isolated in pure form by gentle osmotic lysis and washing procedures, a practical advantage not offered by other procaryotic microbes. Moreover, the fatty acid composition and cholesterol content of the membrane of this organism can be markedly yet controllably manipulated and some variation in polar headgroup composition is also possible. The reader should consult McElhaney [7] for a recent review of the structure and function of the intensively studied A. laidlawii B plasma membrane.

MATERIALS AND METHODS

All the materials and methods used in this study are described in two previous publications [3,8] and in the references cited therein.

$^19$F-NMR spectra of Acholeplasma laidlawii B membranes containing approximately 10 percent of a monofluoropalmitic acid plus 90 percent of the fatty acid of interest were acquired at 254.025 MHz on a Bruker HXS-270 NMR spectrometer operating in the Fourier transform mode, and the resulting free-induction delays were transformed using a Nicolet-1180 data system to the final spectra, as described elsewhere [3,8]. Orientational order parameters were obtained by fitting an experimentally acquired spectrum with a computer-simulated spectrum which had been generated using equations designed to accommodate terms representing the maximum $^19$F chemical shift anisotropy, both intramolecular and intermolecular dipole interactions, and the order parameter, $S_{\text{mol}},$ as applicable to the case of fatty acyl chain motions in a lipid bilayer [8,9].
RESULTS AND DISCUSSION

The specifically monofluorinated palmitic acids synthesized were used to produce the corresponding di-monofluoropalmitoyl phosphatidylcholines, and the thermotropic phase behavior of aqueous, multilamellar dispersions of these lipids was studied by high-sensitivity differential scanning calorimetry (data not presented). Although the cooperativity of the gel to liquid-crystalline phase transition was found to be substantially reduced in the di-monofluoropalmitoyl phosphatidylcholines as compared to dipalmitoyl phosphatidylcholine, only small reductions in the transition temperatures and enthalpies were observed. The largest perturbations were noted when the fluorine atom was located near the carbonyl function of the palmitoyl group and the smallest when the fluorine was present near the methyl terminus. Most important, all di-monofluoropalmitoyl phosphatidylcholines studied exhibited nearly ideal mixing in all proportions with dipalmitoyl phosphatidylcholine. In addition, the thermotropic phase behavior of A. laidlawii B membranes enriched in one of several monofluoropalmitic acids was identical to that of membranes comparably enriched in palmitic acid. These results demonstrate that, in contrast to geminal difluoro fatty acids, monofluoropalmitic acids are physically relatively non-perturbing probes of membrane lipid structure [3].

The biochemical effects of the biosynthetic incorporation of monofluoropalmitic acids into the membrane lipids of A. laidlawii B were also investigated (data not presented). The quantitative distribution of the membrane glyco- and phospholipids of this organism, and the lipid/protein ratio of the membrane, were affected only slightly by monofluoropalmitic acid incorporation. Moreover, A. laidlawii B utilized the various exogenous monofluoropalmitic acids for glycerol-lipid biosynthesis almost as well as exogenous palmitic acid, and the fluoropalmitoyl groups were incorporated relatively evenly into the various lipid classes present. In addition, the fluoropalmitate residues were esterified primarily at the 1-position of the glycerol backbone, just as is palmitate, when compared to branched-chain and unsaturated fatty acids, which are located primarily at position 2. Thus these monofluoropalmitic acids appear to be good biochemical analogues of palmitic acid [3], which is not the case for difluoro fatty acids, at least in E. coli [5].

The effects of the biosynthetic incorporation of monofluoropalmitic acids on the growth and morphology of A. laidlawii B was also studied (data not presented). Even at high levels of incorporation (up to 60–80 mole % of the total esterified fatty acid), no alteration in growth rates or yields were observed, and cell morphology remained normal. Thus these monofluoropalmitic acids appear to support normal membrane function, at least at low levels of incorporation [3].

The $^{19}$F-NMR orientational order profiles obtained at 37°C for pentadecanoic (15:0)-, isopalmitic (16:0i)-, anteisopalmitic (16:0ai)-, and palmitelaidic acid (16:1tΔ9)-enriched membranes, isolated from cells cultured in the presence of avidin, a potent inhibitor of de novo fatty acid biosynthesis [10], are compared in Fig. 1. These membranes contained about 90 mole % of the fatty acid of interest and 10 mole % of one of a series of monofluoropalmitic acid isomers. The three fatty acids, 15:0, 16:0i, and 16:0ai, are each fifteen carbons in length with 16:0i and 16:0ai having methyl branches at the 14- and 13-positions, respectively; the 16:1tΔ9 is slightly longer. At 37°C the overall orientational order through the bilayer decreased in the progression 15:0 > 16:0i > 16:0ai > 16:1tΔ9. The thermal transition midpoints (Tm) of membranes enriched with these fatty acids also decreased in the order 15:0 > 16:0i > 16:1tΔ9 > 16:0ai [11]. Thus the overall membrane order, at a constant temperature, varied with the position of the gel to liquid-crystalline phase transition.
temperature. Order parameters generally increased with increasing proximity to the gel to liquid-crystalline phase transition. Therefore, the decrease in overall membrane order resulting from the addition of a methyl branch substituent proximal to the methyl terminus of the fatty acid, or from the addition of a trans-double bond, can be correlated with changes in the thermotropic phase transition temperature of the particular membrane. Clearly the veracity of this relationship may not necessarily hold when two species of fatty acids having very similar thermotropic properties but markedly dissimilar order profiles are compared. This point is relevant to the case of 16:0ai and 16:1Δ9 where the Tm's are similar (Tm = 4.1°C and 6.7°C, respectively [11]), but the dissimilarity of the two order profiles masks the small effect, in this case, of relative proximity to the phase transition.

The character of the order profile itself was altered by the addition of either methyl branch substituents or by the addition of a trans-double bond. Membranes enriched with straight-chain saturated fatty acids yielded order profiles very similar to those obtained using ¹H-NMR [12], with an initial order plateau region extending out to approximately carbon number eight or ten of the acyl chain and a subsequent rapid decrease toward the methyl terminus. The presence of a methyl branch substituent extended the plateau region of the order profile to the twelve position of the fatty acyl chain in the case of the anteiso-branched fatty acid (16:0ai), while the rate of the decrease of the order parameters in the post-plateau region of the order profile was markedly reduced in the case of the iso-branched fatty acid (16:0i). It can be concluded then that the presence of a methyl branch substituent tends to increase the orientational order of neighboring fatty acids in the immediate vicinity of that substituent. The rather more clear-cut effect of the anteiso-methyl branch upon the order profile, when compared to the iso-methyl branch, can be related to the different depths of penetration of these two groups into the hydrocarbon milieu of the membrane. The ability of the anteiso-methyl branch substituent to cause a more
pronounced disruption is evident as well when the temperatures of the major phase transitions of membranes enriched with 15:0, 16:0i, or 16:0ai are compared (Tm = 36.7°C, 21.8°C, and 4.1°C, respectively, see [11]).

The effect of the trans-double bond upon the character of the orientational order profile was to decrease the length of the order plateau relative to that observed with straight-chain saturated fatty acids. In particular, the values of the order parameters at positions 8 and 10 showed a significant decrease. Thus, in contrast to studies in which the cis-double bond was found to locally increase the orientational order of a neighboring saturated acyl chain [13], our results indicate that the trans-double bond affects a local decrease in the order of the probe molecule.

In order to eliminate, or at least to minimize, the contribution to the absolute overall order of the relative proximity to the gel to liquid-crystalline phase transition, the order parameter profiles of the above membranes were acquired at a constant temperature of 15°C above their respective phase transition temperatures. The resulting order parameter profiles are illustrated in Fig. 2. Under these conditions the effects of methyl branch and trans-double bond structural substituents upon the character of the order profiles which were observed at 37°C were again evident. The relative ordering effect of a methyl branch substituent of either an iso- or anteiso-configuration, or the relative disordering effect of a trans-double bond, appear then to be independent of the acquisition temperature, provided the phase state of the membrane lipids remains constant. This observation is consistent with the findings of other investigators, who have noted that the character of the order profile is not altered as the temperature is changed but, rather, the values of the order parameter increase or decrease in a similar fashion across the width of the bilayer with decreasing or increasing temperature (see, for example, [13]).

The situation with regard to the relative overall orientational order through the bilayer of the various enriched membranes, when compared at 15°C above each particular Tm, was found to be almost completely reversed from that observed at 37°C. Under conditions in which the various membrane fatty acids could be considered to

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be in a constant thermodynamic state relative to the thermotropic phase transition, the overall orientational order decreased in the progression 16:0ai > 16:1Δ9 > 16:0i > 15:0. Therefore, in addition to the local ordering effect of the iso- or anteiso-methyl branch substituent, or the local disordering effect of the trans-double bond, these structural substituents appear to mediate as well an overall increase in orientational order throughout the width of the bilayer. This observation is not without precedent. Seelig and Seelig [13] observed that when the order profiles of specifically deuterated DPPCs were compared with those of DOPC, the oleyl acyl chains were more ordered than the palmitoyl chains when compared at 19°C above the respective Tm’s, but the reverse was true when these two model membrane systems were compared at the same absolute temperature.

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