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^{19}F NMR of monofluorostearic acids in lecithin vesicles

N.J.M. BIRDSALL, A.G. LEE, Y.K. LEVINE and J.C. METCALFE

Medical Research Council, Molecular Pharmacology Unit, Medical School, Hills Road, Cambridge CB2 3EF (Great Britain)

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SUMMARY

1. The linewidths of monofluorostearic acids in lecithin vesicles have been measured as a function of ^{19}F substituent position and temperature.

2. The linewidths decrease steeply towards the terminal methyl group of the alkyl chain. The change can be attributed to the molecular motion of the chain in the bilayer. The results are consistent with similar spin label experiments by W.L. Hubbell and H.M. McConnell (*J. Am. Chem. Soc.*, 93 (1971) 914).

The nuclear magnetic resonance (NMR) spectra from lipids in vesicles can be simplified by isotopic substitution (*e.g.* ^{19}F or ^{13}C) in each part of the lipid structure. This eliminates the problem of overlap of resonances from the alkyl chains and allows structural information in the spectra to be extracted. In this first report we demonstrate the use of specific ^{19}F substitution in lipids using three monofluorostearic acid derivatives $CH_3(CH_2)_mCHF(CH_2)_{n-2}COOH$ where $n = 4$, 7 and 12. The ^{19}F resonances are readily observed when the fatty acids are incorporated into lecithin bilayers and correspond to single ^{19}F nuclei at known positions in the alkyl chains. The relaxation rates of the ^{19}F nuclei have been estimated from linewidth measurements for each derivative as a function of temperature. The data are compared with similar spin label experiments by Hubbell and McConnell[1] for nitroxide labelled ($>N^{\cdot}-O$) stearic acid derivatives.

The preparation of the fluorinated stearic acid derivatives is described elsewhere[2]. Hen egg lecithin was prepared by the method of Dawson[3] and gave a single spot on thin layer chromatography in two solvent systems (chloroform–methanol–7 M NH_4OH (690:270:45, by vol.); and light petroleum–ether–glacial acetic acid (60:40:1, by vol.)). Lecithin and [^{19}F] monofluorostearic acids were dissolved in chloroform and dried in a rotary evaporator under nitrogen at 30°. The solvent free mixture was agitated under nitrogen in a ^2H_2O buffer (45 mM NaCl–30 mM sodium acetate –5 mM sodium phosphates, p^2H 7.8) to yield a final suspension of

100 mM lecithin (mol.wt. taken as 800) and 20 mM [^{19}F] monofluorostearic acid, so that the molar ratio of lecithin to [^{19}F] monofluorostearic acid was 5:1. The samples (3 ml) were sonicated in glass vials at 30° under nitrogen until the sample was translucent and the residual light scattering was minimised. The sonicated samples were transferred under nitrogen to 12-mm NMR tubes containing a coaxial capillary of C_6F_6 used as an external reference and homogeneity control.

Spectra were obtained on a Varian XL100-15 NMR spectrometer locked on solvent deuterium and operating at 94.1 MHz. The ^{19}F spectra were proton noise decoupled with a Gyrocode covering a bandwidth of 1000 Hz at a power level which produced the minimum ^{19}F linewidth for the monofluorostearic acids in [^{2}H] chloroform. These fully decoupled spectra had linewidths ($\Delta\nu_{1/2}$) of approx. 4.0 Hz compared with approx. 100 Hz for proton coupled spectra. The linewidth of the C_6F_6 external reference under the same conditions was 0.8 Hz. The spectra from the monofluorostearic acids in lecithin bilayers were generally accumulated for 25 scans at each temperature in a C1024 computer averaging transients and operating at a radiofrequency power level which caused no significant saturation broadening (< 5%). In spite of the tendency of a small part of the lecithin sample to coagulate and collect at the surface over several hours, reproducible linewidths (± 10%) could be obtained over the same period either at a fixed temperature or on repeated cycling of the temperature between 25 and 50°. In this respect the ^{19}F spectra are comparable in stability with the ^{1}H NMR spectra of the same samples. Relaxation rates were calculated from $(1/T_2) = \pi \Delta \nu_{1/2}$ where $\Delta\nu_{1/2}$ is the linewidth in Hz at half-height. Instrumental and accumulation broadening were negligible (< 2 Hz) under the experimental conditions, and in all experiments the ^{19}F resonances were symmetrical. Chemical shifts were measured with a radiofrequency counter.

The results of the main experiment are plotted in Fig. 1a which shows the relaxation rates of the [^{19}F] monofluorostearic acids substituted at n = 4, 7 and 12 as a function of temperature. The linewidths decrease steeply with increasing temperature and as n increases. The data also suggest that the decrease in $(1/T_2)$ with increasing n is approximately linear (Fig. 1b), but the linewidths below 40° for n = 4 and n = 7 are not sufficiently accurate to allow a firm conclusion. The temperature curves for the three derivatives are similar in form and the fractional decrease in linewidth between 27 and 49° is 45, 51 and 67% for n = 4, 7 and 12, respectively.

The chemical shifts in Hz of the three [^{19}F] monofluorostearic acids in [^{2}H] chloroform relative to a C_6F_6 external standard were 1509 (n = 4); 1259 (n = 7); 1221 (n = 12). The same differences in chemical shifts between the derivatives were observed in lecithin vesicles within an experimental error of ± 20 Hz for the broadened resonances. This strongly suggests that the ^{19}F nuclei in each derivative experience a similar "solvent" environment of lecithin within the bilayer, since the chemical shifts are not significantly affected by the localisation of the ^{19}F nuclei with respect to the polar interface.

To examine the perturbation of the lecithin bilayer by the [^{19}F] monofluorostearic acids, the ESR spectra of two nitroxide derivatives of methyl stearate[4]

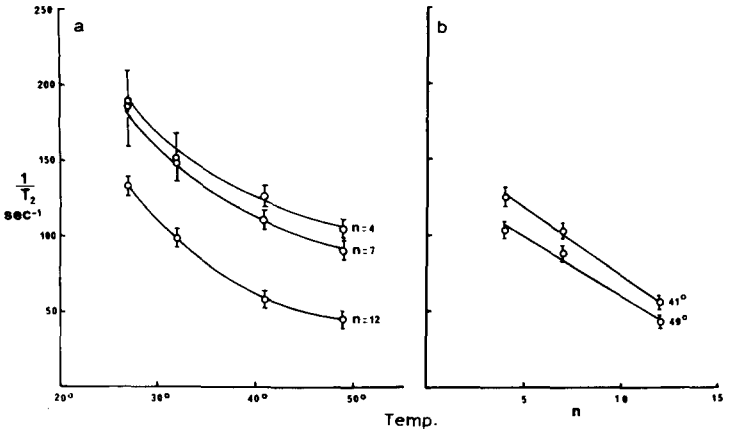
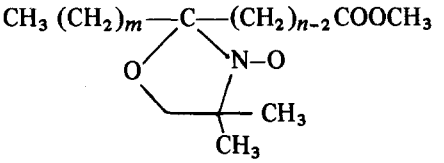


Fig.1. Relaxation rates of ^{19}F nuclei in monofluorostearic acids as a function of (a) temperature and (b) ^{19}F substituent position (see text).



where n = 7 and 12, were compared in lecithin vesicles alone and in the samples containing each [^{19}F] monofluorostearic acid. Any changes in the ESR spectra of the spin labels due to the presence of [^{19}F] monofluorostearic acid in the bilayer were at the limit of experimental accuracy. The line space shapes and positions of the resonances were almost unaltered between control and sample when compared between 27 and 49°, and no differences were detected between the [^{19}F] monofluorostearic acids.

The simplest explanation of the sharp decrease in ^{19}F relaxation rate as n increases is that the relaxation is dominated by the motion of the chain segment carrying the ^{19}F nucleus. This implies that there is a progressive and approximately linear increase in motional freedom towards the centre of the bilayer. The spin label experiments above confirm that the basic bilayer structure of the lecithin vesicles is not significantly perturbed by the presence of the [^{19}F] monofluorostearic acids under the present experimental conditions. The relaxation rate at 27° decreases by approximately 40% from n = 4 to n = 12, and by linear extrapolation would correspond to a linewidth of about 7 Hz at the terminal methyl (n = 18). The ^{19}F linewidths observed are of the same order as those in ^{1}H NMR spectra under comparable conditions[5].

Quantitative analysis of chain motion from these linewidth measurements is not possible because there may be a small contribution to the observed linewidths from exchange broadening. It is possible *a priori* that an ^{19}F nucleus localised near the polar interface region would experience a greater range of chemical shifts (for example by oscillations perpendicular to the plane of the bilayer) than an ^{19}F nucleus buried further in the interior of the bilayer. Part of the observed variation in linewidth with n could then be attributed to a decrease in exchange broadening as n increases. We can

rule this out as the dominant relaxation change along the chain, however, since the relative chemical shifts of the ^{19}F nuclei are similar in the bilayer and in [^{2}H] chloroform, whereas a change in the relative chemical shifts in the two systems of at least 100 Hz between $n = 4$ and $n = 12$ would be necessary to attribute the observed linewidth changes in the bilayer entirely to exchange broadening. We conclude that the increase in motional freedom with increase in n is the dominant relaxation mechanism. For a quantitative analysis we either require direct $(1/T_1)$ relaxation measurements or we need to show that the linewidths are independent of field strength, which would imply the absence of any significant exchange broadening. Full relaxation data for an extended series of ^{19}F-labelled lecithin derivatives will be reported shortly.

An important feature of these results is that they are consistent with similar spin labelling data of Hubbell and McConnell[1] which they interpreted as showing an increase in motional freedom as n increased. An obvious objection to the use of nitroxide spin labels for probing steric interactions in membranes is the perturbation the nitroxide group introduces into the structure. It is therefore important to test the spin label conclusions by independent techniques in which the structural perturbation is reduced (^{19}F substitution) or non-existent (^{13}C substitution). The quantitative relationship between the NMR relaxation rate $(1/T_1)$ and the order parameter from the spin label experiments should enable any perturbation introduced by the nitroxide group to be estimated.

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