DEUTERON RESONANCE: A NOVEL APPROACH TO THE STUDY OF HYDROCARBON CHAIN MOBILITY IN MEMBRANE SYSTEMS

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1. Introduction

Probe methods, for example spin [1] and fluorescence [2] labelling, have recently become popular tools with which to study the structure of both natural and artificial membrane systems. However, implicit in the use of these methods is the uncertainty of the degree of environmental perturbation caused by the probe. It has been suggested that many spin labels have the same mobility in stearic acid 10° below its melting point as they have 10° above it [3]. Nuclear magnetic resonance appears to have the greatest potential for studying these systems, at least at the level of molecular mobility and diffusion.

The principal difficulty encountered in the use of ^{1}H NMR lies in the extreme difficulty of resolving the resultant multicomponent spectra into assignable resonances [4, 5], especially when these individual resonances occur over a wide frequency range (typically several kHz), and overlap each other. ^{1}H time-domain (either conventional pulse or Fourier transform) techniques offer few advantages since the broadening in these systems has now been shown to be of a homogeneous dipolar nature (in the range 10–60 MHz) [6, 7] and $T_2^+ \ll T_1$ [8]. The use of ^{13}C NMR however, would appear to be more promising, since chemical shifts are characteristically very large [9]. In an effort to further characterise the motions of particular groups in membranes, we are using deuteron magnetic resonance (DMR) to study the mobility of the hydrocarbon chains, in isotopically enriched molecules. As a simple model, we have chosen to study the modulation, by cholesterol, of the quadrupole splitting in a synthetic di(perdeuterio)myristoyl-Lα-lecithin, as a function if temperature.

2. Experimental

Myristic acid (puriss. > 99.5%) was purchased from Fluka, Büchs, and was "perdeuterated" using the method of Stenhagen and Dinh-Nguyen [10]. The product contained 95 ± 2% deuterium (NMR and mass spectrometry of the methyl ester), and the methyl ester (prepared by diazomethane methylation) was 99% pure by GLC. Perdeuterio myristoylchloride was prepared from the acid by refluxing with oxalyl chloride in benzene, using pyridine as catalyst.

Egg lecithin was extracted from *Gallus domesticus* eggs according to Singleton et al. [11], and purified on Woelm Alumina, Grade V. The lecithin was then converted into glyceryl phosphoryl choline using the method of Dawson [12], and its cadmium chloride adduct prepared according to Tattrie and McArthur [13]. This was acylated with the perdeuterated myristoyl chloride, according to Baer and Buchnea [14]. The product was purified on Mallinckrodt SilicAR CC-7 (100–200 mesh), eluting with chloroform–methanol 2:1, and was chromatographically pure on Merck Kieselgel F and Aluminium oxide F plates (in $CHCl_3$–MeOH–7 M NH_4OH 230:90:15). The gel → liquid crystal phase transition temperature

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of the lecithin, as a 10 wt% dispersion in water, was 23°, and was measured on a Perkin-Elmer DSC-1B differential scanning calorimeter.

Cholesterol was purchased from BDH, Poole, Dorset, and was recrystallised twice from absolute ethanol.

Lecithin–cholesterol samples were prepared by dissolving 1:1 mole ratios in chloroform, which was then removed under reduced pressure. Any solvent residues were removed under high vacuum overnight. Water was then added and the mixture (5 wt% lipid) was hand dispersed.

DMR spectra were run in 10 mm Bruker NMR tubes at 8 MHz, on a Varian wide-line spectrometer.

3. Results and discussion

In the gel phase, at 10°, the lipid hydrocarbon chains are relatively rigid, and the DMR spectrum shows only a very broad, ill-defined resonance (fig. 1a).

On heating, structure begins to appear in the spectrum upon the transition to the liquid crystalline phase (fig. 1b), and at 30° a quadrupole splitting is well defined (fig. 1c). The 30° spectrum has a shape characteristic of a powder spectrum of a spin $I = 1$ nucleus with a very low (0–0.2) asymmetry parameter [15]. The observed splitting is

$$(\Delta\nu)_{max} = \tfrac{3}{4}\ (e^2qQ)/h\ \langle(3\cos^2\theta - 1)\rangle$$

where e^2qQ/h is the deuteron quadrupole coupling constant, applicable to deuterons in static C–D bonds, and is approximately 180 kHz. θ is the angle between the applied magnetic field and the direction of the principal component of the electric field gradient tensor, at the deuteron, and $\langle(3\cos^2\theta - 1)\rangle$ is the time averaged value of $(3\cos^2\theta - 1)$ over any motion occurring that is rapid compared with 180 kHz.

If the methylene deuterons were static, the powder spectrum would consist of 2 pairs of peaks separated by 270 kHz and 135 kHz. The observed spectrum at 10° does not show these distinctive features, and the evident reduction in linewidth from these values implies that some motion is occurring. The lack of observable structure implies that deuterons at different sites are subject to different rates or types of motion, and this gives rise to different *effective* quadrupole coupling constant.

At 23.5° the splitting $(\Delta\nu)_{max}$ is 29.8 ± 1 kHz, and at 30°, 27 ± 1 kHz. This represents a significant time-averaging of the field gradient tensor at each deuteron in this liquid crystalline state.

A central line of peak to peak width 4.2 ± 0.5 kHz, is also seen quite clearly on the 30° spectrum. This may represent the relatively narrow ^1H signal seen by Chan, Feigenson and Seiter [6], when using a pulse Fourier transform system with the equivalent of a long dead-time, and ascribed by them to relatively mobile terminal methyl groups, together with some terminal methylene groups. These authors suggest a rather sharp discontinuity in the correlation time distribution down the chain, a view supported by Charvolin and Rigny [16], in a smectic potassium laurate–D_2O mesophase.

A similar narrow component is seen in the 10° gel spectrum, and this may arise from the same source.

On addition of cholesterol, at 1:1 mole ratio, the splitting at 30° increases from 27 kHz to 49.4 ± 1.5 kHz (fig. 1d). This value of $(\Delta\nu)_{max}$ indicates that $\langle(3\cos^2\theta - 1)\rangle$ is much larger than in the pure liquid

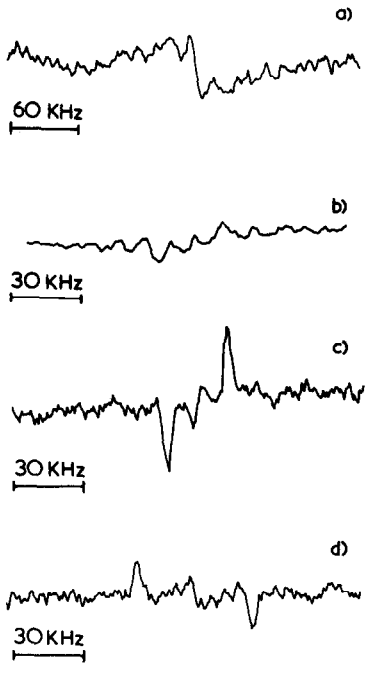

Fig. 1. DMR spectra of di(perdeuterio)myristoyl-Lα-lecithin–H_2O at (a) 10°, (b) 23.5°, (c) 30°, (d) 30°, 1:1 lecithin–cholesterol.

crystalline lecithin at 30°, that is, that the anisotropy of motion of the alkyl chains is quite high; this thus represents a considerable restriction on the freedom of motion of a large number of the acyl chain deuterons, and supports the suggestion made earlier [17] that cholesterol can inhibit the chain motion of some phospholipids that are in their liquid crystalline state. The central component is still present, however, and has a width of 4.5 ± 1 kHz. It would thus appear that the terminal end of the acyl chain is less restricted by the presence of cholesterol than the regions nearer the headgroup, which is in agreement with the ESR findings of Hubbell and McConnell [1].

Upon cooling to 10°, the spectrum changes little; the observed splitting increasing from 49.4 kHz to 53.2 ± 1.5 kHz, indicating a small increased restriction in mobility. However, the appearance of the spectrum is quite unlike that observed in the gel at 10°, and is in fact "liquid crystalline". The cholesterol is causing formation of a state of intermediate chain mobility, this view being in accord with our previous work [18].

From these results, it appears that DMR will be a valuable additional method with which to study membrane structure. One of the principal advantages of this technique is that the physical properties of a perdeuterated molecule are extremely close to those of a natural ^2H abundance molecule; in particular here, the gel → liquid crystal phase transition occurs at the same temperature (23°, in excess water) as normal dimyristoyl lecithin.

It is possible to further exploit these close similarities, and we have successfully isolated chain deuterated polar lipids from *Mycoplasma laidlawii* B, grown upon fatty acid depleted tryptose broth, supplemented with exogenous deuterated fatty acids. This and further studies will be presented elsewhere.

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