NMR OF GEL AND LIQUID CRYSTALLINE PHOSPHOLIPIDS SPINNING AT THE 'MAGIC ANGLE'

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1. Introduction

Recently Chapman et al. have been applying both natural abundance [1–4] and isotopic enrichment [5, 6] nuclear magnetic resonance spectroscopy (NMR) of ^1H, ^2H and ^{13}C nuclei to a study of molecular mobility in both model membrane [1–3, 5] and natural membrane [4, 6] systems. It has been established that the line broadening in such (unsonicated) systems is predominently dipolar [2, 7] in fields up to ~2T. Andrew et al. [8] and Lowe [9] have shown that dipolar broadening in solids can be greatly reduced by rapid sample rotation about an axis inclined at an angle $\beta = \sec^{-1}\sqrt{3}$ (the 'magic angle'), to the magnetic field H_0. Schneider et al. [10–14] and Cohn et al. [15] have extended these experiments to proton NMR in various polymers, e.g. solid polyethylene [12] and polybenzylglutamate in solution [14, 15] and in heterogeneous solid–liquid systems [10, 13]. Doskočilová and Schneider [10] have originally used Gutowsky and Pake's [16] linewidth formula for systems with anisotropic internal motion to characterise the motional requirements for line narrowing experiments to be effective, and Haeberlen and Waugh [17] and Andrew and Jasinski [18] have treated the problem theoretically in greater depth.

In the simple treatment [10] linewidth $\Delta\nu$ (arbitrarily defined with respect to line shape) can be expressed as

$$(\Delta\nu)^2 = A^2 \frac{2}{\pi} \operatorname{arctg}\left(\frac{\alpha\Delta\nu}{\nu_c}\right), \qquad (1)$$

where ν_c is the correlation frequency of molecular motion, A is the rigid lattice linewidth and α is a constant of order unity. Partial narrowing of a resonance line may occur by random motion, *isotropic* in space, such that $\nu_c > \Delta\nu$. In such cases, macroscopic rotation of the sample can further reduce the linewidth only if rotational frequencies $\nu_{rot} > \nu_c$ are reached. This is generally impracticable for protons. Partial narrowing of a resonance line may also occur by rapid internal motion which is *anisotropic* in space (e.g. oscillation of groups of nuclei about fixed axes, random reorientation of ionic groups fixed at crystal lattice points, etc.). In such cases, the linewidth can be described by the relation

$$(\Delta\nu)^2 = B^2 + C^2 \frac{2}{\pi} \operatorname{arctg}\left(\frac{\alpha\Delta\nu}{\nu_c}\right), \qquad (2)$$

where ν_c is the correlation frequency of the specialized motion, B is the linewidth in the extreme of very rapid reorientation and $B^2 + C^2 = A^2$. Residual dipolar interactions, summarized in B, can be effectively reduced by macroscopic rotation of the sample, irrespective of the value of ν_c.

Salsbury and Chapman [19] pointed out that restricted anisotropic rotation occurs in the liquid crys-

talline phase of phospholipid systems and Chan et al. [20] have recently used Woessner's [21] treatment to treat this anisotropic motion in some detail. Finer et al. [22], using the glass-slide oriented sample technique of de Vries and Berendsen [23], have shown that some reduction in linewidth of a broad unresolved lecithin spectrum could be obtained when a static oriented sample was aligned at the magic angle with respect of H_0, supporting the notion that the observed linewidths are of a dipolar origin. Accordingly, we have obtained the high-resolution 60 MHz proton NMR spectra of gel and liquid crystalline lecithins in excess D_2O when spinning at high frequency, at the magic angle.

2. Experimental

Spectra were obtained at 25°C on a JNM-3-60 spectrometer, using hexamethyldisiloxane as external reference. Spectra were calibrated using audio-frequency modulation sidebands, and the reported *tau* values were averaged from several consecutive runs. Samples were rotated at 0.3–3.5 KHz in a glass rotor cell in a gas driven turbine [11], about an axis inclined at 90° or at an angle $\sec^{-1}\sqrt{3}$ to the magnetic field H_0. Dipalmitoyl lecithin (DPL) was obtained from Koch-Light Ltd., Colnbrook, Bucks., England, and was pure by TLC ($CHCl_3$: MeOH: 7 M NH_4 OH, 230:90:15, Kieselgel G plates visualised with I_2), Grade 1 egg yolk lecithin (EYL) was from Lipid Products, South Nuttfield, Surrey, England, and was pure in the same solvent system. Samples were freeze dried twice from 99.7% D_2O (Prochem Ltd., Carolyn House, Croydon, England), and the solid lipids were then dispersed as 50 wt% (DPL) or 33 wt% (EYL) mixtures in D_2O.

3. Results and discussion

DPL at 25°C in excess water is in the α-crystalline gel state [24], and is well below its T_C transition temperature. Only a broad HOD band was detectable at 60 MHz on the sweep widths employed. On rotation at the 'magic angle' some narrowing of the absorption of the DPL gel was observed at 3.5 KHz, yielding a band of 250 Hz width at 6.8 τ assigned to the $\overset{+}{N}(CH_3)_3$ protons [25], in addition to the very sharp HOD line at 5.2 τ (fig. 1a).

For static EYL, a broad (~500 Hz) absorption was observed, similar to that reported previously [26]. On rotation of EYL at the 'magic angle', some structure was apparent at frequencies as low as 300 Hz. At 1 KHz the $\overset{+}{N}(CH_3)_3$ resonance at 6.8 τ reached a linewidth of 17 Hz, which was rotationally invariant at higher spinning speeds. The HOD signal was only 2.5 Hz. A prominent signal was also apparent at 9.1 τ, and is assigned to terminal methyl groups on the lipid acyl chains. The width of this resonance is difficult to estimate because of overlap with resonances from near-terminal methylene groups of the lipid chain. The resolution, however, continues to improve up to 3.5 KHz (fig. 1b). Any purely mechanical effect upon linewidth is excluded by comparison of spectra measured with rotation about an axis a) perpendicular and b) inclined at the 'magic angle' with respect of H_0, at equal spinning frequencies, in two consecutive runs spaced by a few seconds (fig. 2).

In the gel phase, only the HOD and $\overset{+}{N}(CH_3)_3$ protons are mobile enough to have static linewidths not exceeding attainable spinning speeds. The residual linewidth of the $\overset{+}{N}(CH_3)_3$ protons, which is a measure of the frequency and form of the rapid reorientations of this group [14, 18, 21], is of the order observed in amorphous solid polymers [10, 12].

In the liquid crystalline phase of egg lecithin, the $\overset{+}{N}(CH_3)_3$ groups have the narrowest linewidth (~ 50 Hz at 60 MHz), and this is further narrowed by 'magic angle' rotation at low frequencies. This indicates [14, 17] that the spectrum of microscopic motions in this liquid crystalline lipid contains modes with correlation times $> 10^{-4}$ sec. The limiting linewidth of the $\overset{+}{N}(CH_3)_3$ protons is only 17 Hz, more than an order of magnitude less than in the gel phase. This decrease of limiting linewidth may be caused both by increased frequency and by decreased anisotropy of $\overset{+}{N}(CH_3)_3$ group reorientations in the liquid crystalline phase [1b]. Of the other proton groups, only the CH_3 and perhaps 1 or 2 terminal CH_2 groups of the aliphatic chains are expected, and observed, to narrow at the rotational frequencies used (max. 3.5 KHz). Higher frequencies might result in better resolution of the aliphatic proton lines, permitting quantitative characterization of side-chain motions, as in polybenzylglutamate [14]. Since considerable line narrowing occurs at (or near) the 'magic angle' at these relatively low rotational frequencies, it

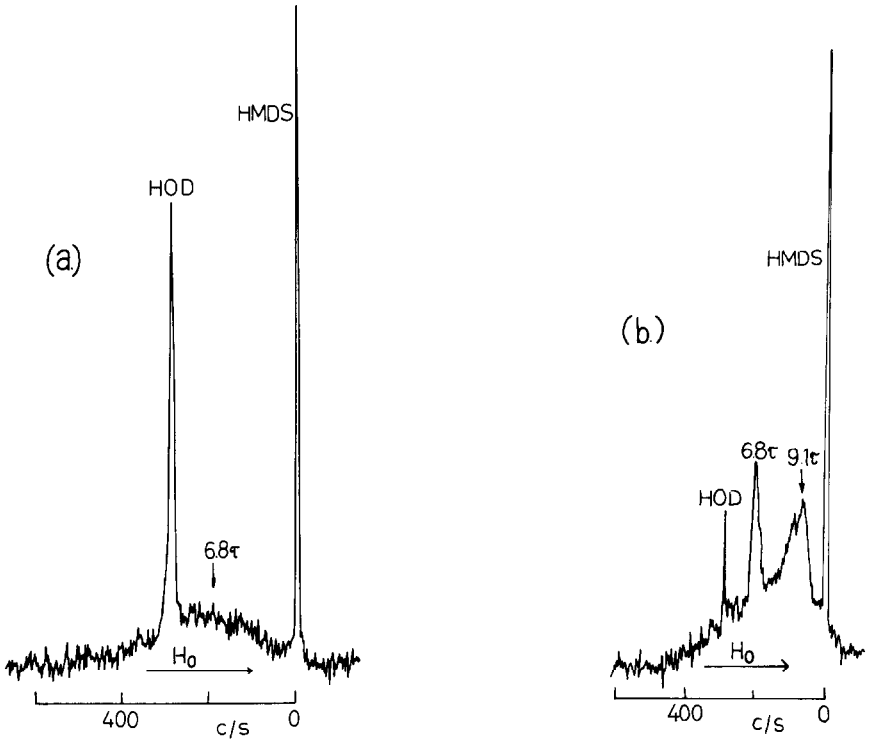

Fig. 1. 60 MHz ^{1}H-NMR spectra of a) dipalmitoyl lecithin (50 wt %)–D_2O, 25°C (gel state), ν_{rot} = 3.5 KHz, $\beta = \sec^{-1}\sqrt{3}$ b) egg yolk lecithin (33 wt %)–D_2O, 25°C (liquid crystalline state), ν_{rot} = 3.5 KHz, $\beta = \sec^{-1}\sqrt{3}$.

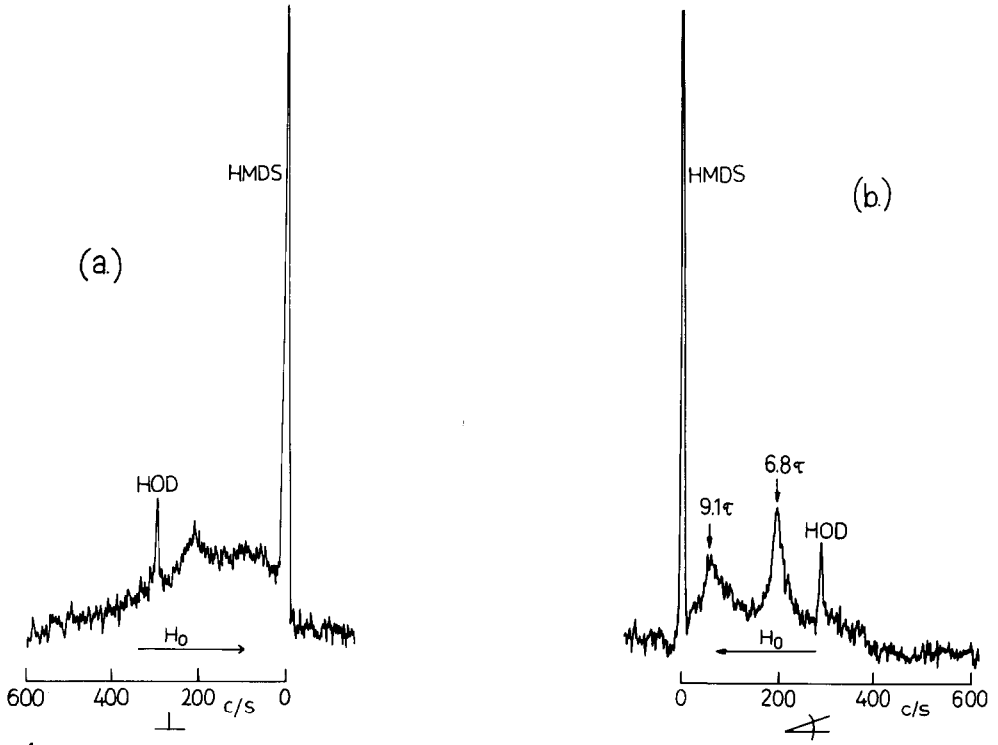

Fig. 2. 60 MHz ^{1}H-NMR spectra of egg yolk lecithin (33 wt %)–D_2O, 25°C (liquid crystalline state), ν_{rot} = 0.5 KHz, a) $\beta = 90°$; b) $\beta = \sec^{-1}\sqrt{3}$.

is likely that tumbling of *sonicated* lecithin vesicles be significant in the production of the well resolved spectra obtained with these systems as suggested by Finer et al. [22] and Penkett et al. [26].

These results indicate that internal motions of the lipid system can begin to be characterized and that further attempts at line narrowing experiments of these and other lipid membrane systems are well worthwhile. Similar studies on ^{13}C nuclei in higher fields, with lipids and membranes, where resolution is enhanced by an increased chemical shift range [3], may be particularly useful. Thus the resolution of the different carbon nuclei along the lipid chain in non-sonicated lipid systems and membrane systems should be further improved using the spinning experiment. In the case of cell membranes, useful information about the frequency and type of motion of particular groups in the intact membranes may become available. Other liquid crystalline systems, e.g. cholesteryl esters, soaps and detergents, should give interesting and informative results using this spinning method. We intend to carry out further experimental and theoretical studies on such systems.

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