THE USE OF WIDE-LINE [^{14}N]NITROGEN NMR AS A PROBE IN MODEL MEMBRANES

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1. Introduction

A major aim of biological membrane research is to understand the relationship between membrane structure and function at the molecular level. The presently accepted fluid mosaic model [1] indicates that the membrane is basically an anisotropic fluid composed of a lipid bilayer in which proteins are embedded. At the molecular level it is necessary to describe both the average conformation of the molecules and their molecular dynamics. There is both translation and rotation of the lipid and protein molecules within the bilayer structure. For the lipids in particular, there are many internal degrees of freedom.

Of the many techniques [2] being used to probe the molecular environment within membranes, nuclear magnetic resonance (NMR) [3,4] has proven to be one of the most useful methods for investigating the dynamical state of the membrane. Work has concentrated on describing the dynamical behaviour of the lipid molecules and the interaction of these molecules with other membrane components such as various proteins [5,6] and cholesterol [7,8]. To do this, it is essential to probe various positions in the lipid molecules. Because of the overabundance of hydrogen atoms, proton NMR spectra have been difficult to interpret [9,10] and give little information about the motion of a single segment of the lipid molecule. ^{31}P NMR [11] has been successfully used to probe the head group region of phospholipids. Selective incorporation of ^{2}H [12], ^{13}C [13] and ^{19}F [14] into lipid molecules has been the most fruitful method of probing the molecular dynamics. However, considerable time and expertise are necessary for the synthesis of the labelled molecules required for a complete investigation of the lipid dynamics. The use of naturally abundant NMR probes is therefore advantageous whenever possible. One naturally abundant probe which has not been used to date is ^{14}N. ^{14}N, like ^{2}H, has nuclear spin $I = 1$ and in an anisotropic fluid is expected to exhibit a quadrupolar split spectrum. The quadrupolar coupling constant for ^{14}N is generally an order of magnitude larger [15] than for ^{2}H. The spectra are therefore expected to cover a wider frequency range. As is the case for ^{2}H the magnitude of the quadrupolar splitting is a direct measure of the order parameter for the symmetry axis of the electric field gradient tensor.

Nitrogen has the potential of being a very useful probe because it is found in natural abundance (99.4%) in a wide variety of phospholipids, particularly among the nitrogenous-base containing glycerophosphatides such as phosphatidylcholine and among the sphingosyl phosphatides such as sphingomyelin.

Here we report the first observation of quadrupolar split nitrogen spectra for a model membrane system consisting of an aqueous dispersion of dipalmitoyl phosphatidylcholine (DPPC). We have obtained spectra over 3–65°C. The observed quadrupolar splittings in both the liquid crystal and gel phases are of the order of 10 kHz, indicating a very small order parameter for the choline headgroup. The behaviour of the order parameter as a function of temperature agrees qualitatively with that observed [16] using deuterium NMR.

2. Materials and methods

DPPC was purchased from Sigma Chem. Co. (St Louis, MO) and was used without further purification. A multilayer dispersion of DPPC in excess water ($> 40\%$ H_2O) was prepared by mixing 0.25 g lipid with the required amount of water. This mixture was sealed in a 10 mm o.d. glass ampule under 0.5 atm. N_2, and left overnight to form a homogeneous mixture.

The ^{14}N spectra were obtained at a frequency of 19.438 MHz using a homebuilt spectrometer [17] and a Bruker superconducting solenoid (6.3 Tesla). Digitization and Fourier transformation of the nuclear signal were carried out using a Nicolet 1090 AR digital oscilloscope interfaced to a Digital PDP-8/A minicomputer.

In order not to lose the initial part of the free induction decay, and to minimize the effects of magnetic–acoustic ringing, a modified form of the quadrupole echo technique [18] was used. Changing the phase of the first 90° pulse by 180° on successive repetitions of the basic 90°_x–τ–90°_y quadrupole echo sequence, and then alternately adding and subtracting the nuclear signal when signal averaging, permitted the accumulation of the echo signal while averaging out any coherent ringing present. The 90° pulses were 15 μs in duration, and the recycle time was 0.17 s. The quadrupolar echo signal was digitized at a rate of once every 5 μs, corresponding to a spectral width of 200 kHz.

The sample was enclosed in a copper oven in the NMR probe. The oven temperature was electronically regulated to within ± 0.5°C and the temperature gradient across the sample was estimated to be <0.25°C.

3. Results and discussion

Several ^{14}N spectra taken at different temperatures are shown in fig.1. The width of the rf pulses was 15 μs and the maximum spectral width is ~32 kHz. As a result, there will be a small amount of spectral distortion which arises from a non-uniform distribution of rf power across the spectrum and because finite-width pulses were used in the formation [19] of the quadrupolar echo. All of the spectra show a single powder pattern [3] arising from the nuclear Zeeman interaction perturbed in first order by an axially symmetric nuclear quadrupole interaction. The frequency separation between the two peaks in the spectrum is given by [3]:

$$\Delta\upsilon = \frac{3}{4}\,\frac{e^2qQ}{h}\left(S_{zz} + \frac{1}{3}\eta\left[S_{xx} - S_{yy}\right]\right) \qquad (1)$$

where $e^2q\,Q/h$ is the nitrogen quadrupole coupling constant and η is the asymmetry parameter. The order parameters:

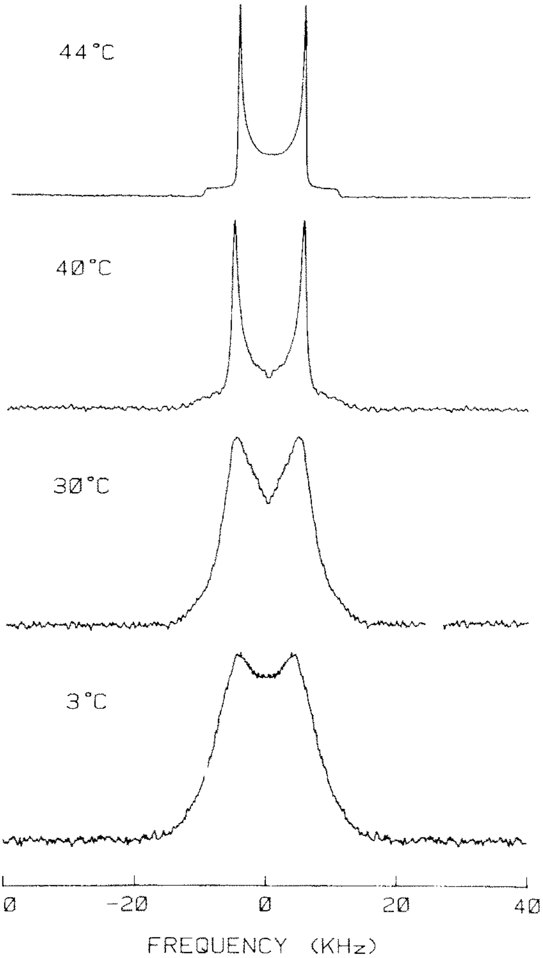

Fig.1. NMR spectra of ^{14}N in the choline headgroup of DPPC. The spectra were obtained using a modified quadrupole echo sequence with a repetition rate of 0.17 s. The spacing between the pulses, τ, was 280 μs for all of the spectra displayed except for that at 3°C where τ was 210 μs. To enhance the signal to noise ratio, 131 k scans were averaged except for the spectrum at 30°C, where 262 k scans were used. All the spectra have a 150 Hz line broadening due to exponential multiplication of the echo signal prior to Fourier transformation.

$$S_{ii} = \left(\frac{1}{2}\right)\overline{(3\mathrm{Cos}^2\theta_i - 1)} \qquad i = x, y, z$$

are a time average (indicated by the bar) of the angular fluctuations of the *i*th coordinate axis fixed in the molecular frame with respect to the bilayer normal. The electric field gradient at the nitrogen nucleus arises from the surrounding electronic and

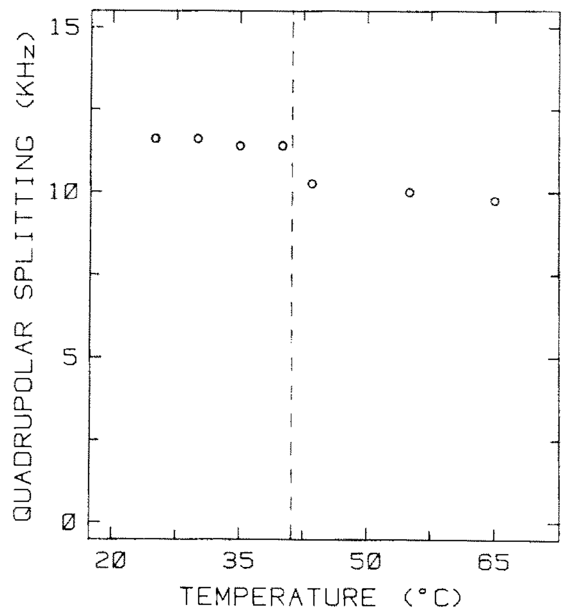

Fig.2. The ^{14}N quadrupolar splitting as a function of temperature for DPPC bilayers. Below 30°C, the splittings are known to ± 0.3 kHz and at 30°C or above they are known to ± 0.1 kHz. The dashed line marks the phase transition which occurs at 41°C for fully hydrated DPPC bilayers.

nuclear charge distribution. In the case of DPPC the major contribution will come from within the $C-N(CH_3)_3$ group. Since this group possesses a C_3 symmetry axis, the asymmetry parameter η is expected to be negligible. The quadrupolar splitting is therefore given by:

$$\Delta\upsilon = \frac{3}{4}\left(e^2qQ/h\right) S_{C_\beta-N} \qquad (2)$$

The quadrupolar splitting as a function of temperature is shown in fig.2. Results are for both the gel and liquid crystal phases. At the present time it is not possible to obtain accurate values of $S_{C_\beta-N}$ from the measured values of $\Delta\upsilon$ because e^2qQ/h is unknown for the nitrogen nucleus in a choline group. Gally et al. [16] have deduced a value for $S_{C_\beta-N}$ from their measurements of the deuteron quadrupolar splitting from a sample of DPPC containing $N-(CH_3)_2CD_3$ moieties. Using the bond angles proposed [20] they showd that the order parameter S_{C-D} obtained directly from the deuteron NMR results could be related to $S_{C_\beta-N}$ by the relation:

$$|S_{C_\beta-N}| = 10.8\,|S_{C-D}| \qquad (3)$$

provided the C–N–C and N–C–D bond angles are fixed. Rotation of the choline group about the C–N axis and methyl group reorientation is allowed. Because of eq. (3), the ^{14}N quadrupolar splitting and ^2H splittings should be proportional to each other. A comparison of the two sets of results shows that the temperature dependence is the same but the discontinuity at the phase transition is smaller for the ^{14}N results. From the analysis in [16] it was deduced that $S_{C_\beta-N}$ lies in the range 0.07–0.17 over 15–80°C.

Using the value for $S_{C_\beta-N}$ at 44°C and the corresponding nitrogen splitting at the same temperature, we would predict, using eq. (4), a value of 135 kHz for the quadrupolar coupling constant:

$$\frac{e^2qQ}{h} = \frac{4}{3}\,\frac{\Delta\upsilon_{N(CH_3)_3}}{S_{C_\beta-N}} = 135\ \text{kHz} \qquad (4)$$

This coupling constant is an order of magnitude smaller than that commonly observed for nitrogen in other compounds [15]. A value as small as 135 kHz could, however, arise [21] because of the nearly tetrahedral symmetry of the 4 nearest neighbouring carbon atoms surrounding the nitrogen nucleus.

The peaks in the spectra obtained below the phase transition are much broader and more poorly defined as compared to those in the liquid crystal phase. The broadening of the peaks increases as the temperature decreases. The spin–spin relaxation time T_2 was measured to be 510 μs in the gel phase (at 30°C) and 1560 μs in the liquid crystal phase (at 65°C). In the liquid crystal phase the observed spectrum could be reasonably well simulated using the expression for a quadrupolar powder pattern with Lorentzian broadening corresponding to the observed T_2. In the gel phase the observed broadening of the peak is far greater than what would be deduced from the measured T_2 value. A possible explanation is that slow anisotropic rotational diffusion [22] occurs in the gel phase giving rise to the observed lineshape.

4. Conclusions

We have demonstrated the feasibility of obtaining good quality wide-line ^{14}N spectra from unsonicated lipid bilayers. The ^{14}N spectra which have been obtained for the widely occurring phospholipid DPPC indicate that under favourable circumstances, where there is rapid large angle reorientation of the moiety containing the ^{14}N nucleus, the forbidingly large width of the ^{14}N resonance [15] in the solid state need not impede spectroscopy in the anisotropic fluid environment of lipid bilayers. We are presently extending ^{14}N spectroscopy to other nitrogenous lipids such as sphingomyelin and phosphatidylethanolamine. By serving as a complementary probe to ^{31}P and ^{2}H, ^{14}N NMR should make a useful contribution to the determination of headgroup conformation and dynamics in both model and biological membranes.

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