THE USE OF PROTON-ENHANCED, NATURAL ABUNDANCE ^{13}C NMR TO STUDY THE MOLECULAR DYNAMICS OF MODEL AND BIOLOGICAL MEMBRANES

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1. Introduction

The effect of intrinsic membrane protein on the mobility of lipid bilayers has been the subject of many nuclear magnetic resonance (NMR) and electron spin resonance (ESR) investigations [1–6]. Early ESR experiments employing nitroxide probes were interpreted to show that some of the lipid within the membrane is immobilized by the presence of the protein [3]. This result has been subsequently confirmed in a number of reconstituted and model lipid–protein systems [3–6]. Recent deuterium NMR studies of a series of lipid–protein dispersions have shown, however, that the quadrupolar splittings, derived from lipids with deuterated methylene groups, are frequently reduced compared to those obtained from the same labels in pure lipid bilayers [2,7]. These observations have been interpreted as indicating an increase in the amplitude of the low frequency ($\simeq 10^5$ Hz) motion undergone by the lipid acyl chains in the presence of the protein [2]. It is argued that the frequency of this additional lipid motion is insufficiently rapid to cause a reduction in the hyperfine field splittings seen in the ESR spectrum.

Here we use proton-enhanced NMR [8] of the natural abundance ^{13}C nuclei to study the lipid mobility in dispersions containing cholesterol, the polypeptide gramicidin A, and in membrane preparations derived from spinach chloroplasts and bovine brain myelin.

2. Materials and methods

Dimyristoyl phosphatidylcholine (DMPC) was purchased from Calbiochem, La Jolla, CA and dispersed 50% (w/w) with H_2O. The purity of the DMPC was checked as in [9]. Cholesterol was purchased from Calbiochem and gramicidin A from Koch Light, Colnbrook. Dispersions of the cholesterol or gramicidin A with DMPC were prepared by rehydrating freeze-dried powders as in [10]. The mixed powders were hydrated to 50% of total solids to H_2O.

Myelin was prepared from bovine brain white matter by the method in [11] as modified [12].

Chloroplasts were prepared from spinach-beet leaves as in [13]. An additional final wash of the chloroplast fragments in 0.001 M tricine buffer (pH 7.6) containing 10 mM $MgSO_4$ was given to ensure that the thylakoid membranes remained stacked.

The proton enhanced ^{13}C spectra were obtained using a commercial Bruker CXP-100 spectrometer operating at 90 MHz for H^+ and 22.63 MHz for ^{13}C. The Hartmann-Hahn conditions were satisfied using a τ_{90° pulse duration of 9.5 μs. The detailed spectral conditions are shown in the figure captions.

3. Theory

The primary advantage of the proton-enhanced NMR technique is that it provides an increased sensitivity for the detection of the resonance spectra of rare spins with a low magnetic moment. By coupling to the polarization produced in an adjacent, abundant spin population possessing a relatively large magnetic moment, the polarization and therefore the sensitivity of the rare spins may be increased. Here, where the rare spins are the natural abundance ^{13}C nuclei and the abundant spins are the sample H^+, the theoretical

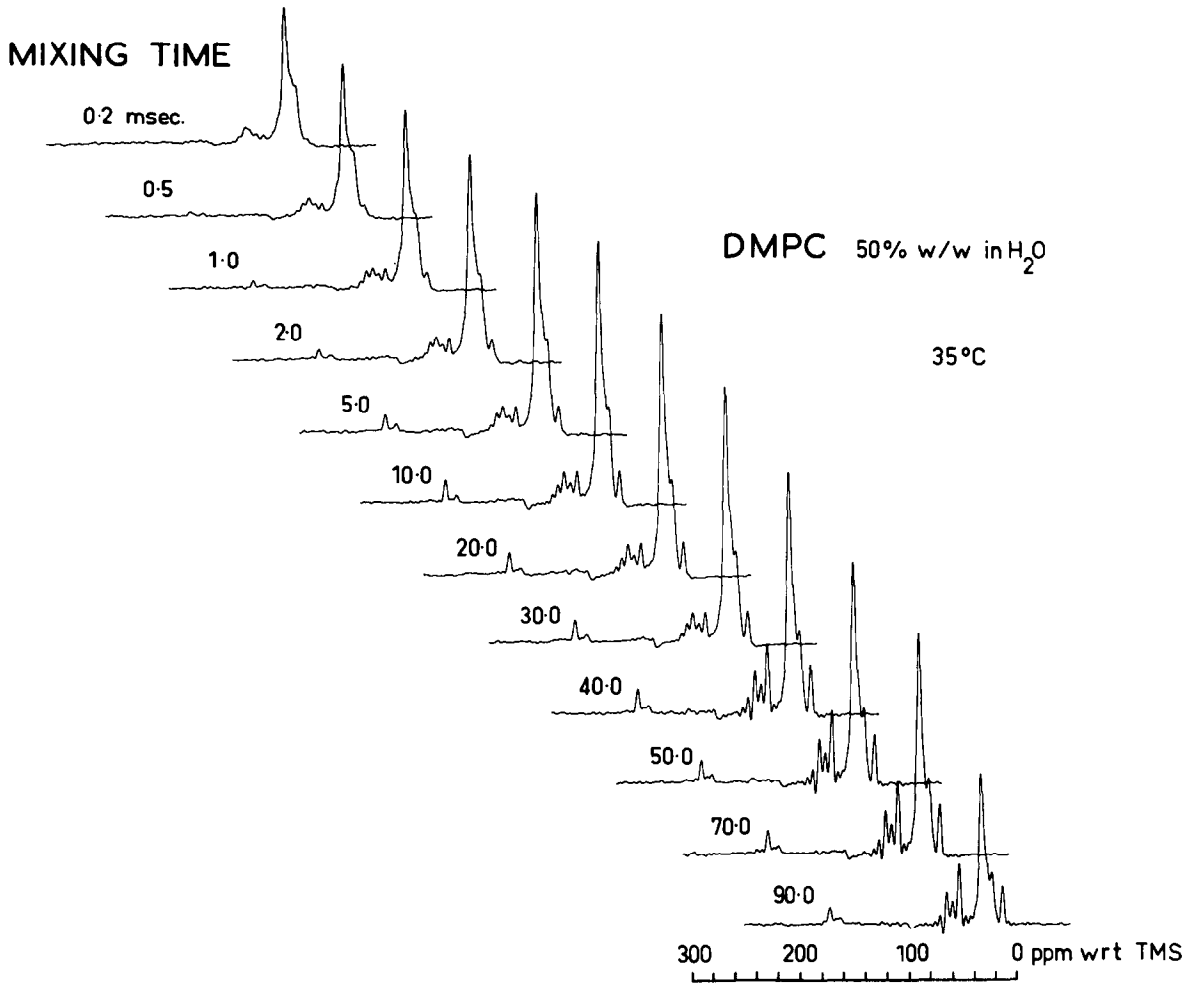

Fig.1. Proton-enhanced natural abundance ^{13}C NMR spectra of DMPC dispersed 50% (w/w) in H_2O. The spectra were obtained under the following conditions: 20 kHz sweep width; 20 ms acquisition time; 1 s sequence repetition time; 20 Hz line broadening; 10 000 scans/spectrum.

enhancement of the ^{13}C sensitivity is given by [8] as:

$$\gamma \text{ protons}/\gamma \text{ carbon} \simeq 4$$

The time required to achieve a particular signal quality is further improved over conventional NMR since the sequence repetition time is now limited by the proton spin lattice relaxation rate which can be an order of magnitude faster than that of the carbons.

The intensity of a proton-enhanced spectrum is therefore dependent on both the time required to exchange the spin polarization from the proton to the carbon nuclei, and on the rate at which the spin polarization of the proton population is lost due to spin lattice relaxation effects. Thus by varying the contact time prior to observing the spectrum, the proton–carbon cross polarization time, T_{C-H}, and the rotating frame proton spin lattice relaxation, $T_{1\rho}$, may be determined for the protons coupled to each resolved carbon resonance. In the case of both the cross polarization and the spin lattice relaxation processes being exponential the overall signal size is given by:

$$\exp(-t_m/T_{1\rho})\,[1-(\exp(-t_m)/T_{C-H})] \qquad (1)$$

where t_m is the contact time.

$T_{1\rho}^{-1}$ may be shown to be approximately proportional to the spectral density function near the

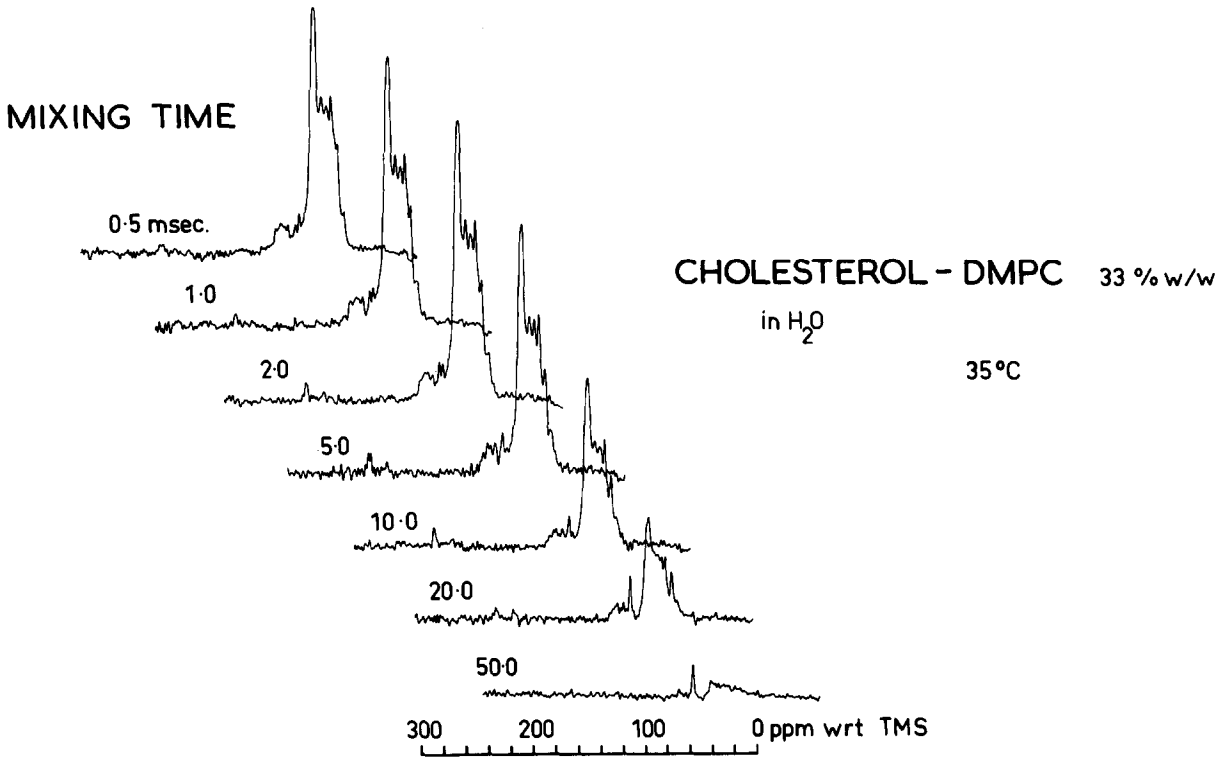

Fig.2. Proton-enhanced natural abundance ^{13}C NMR spectra of cholesterol–DMPC 30% (w/w) dispersed in H_2O. The H_2O : total solids ratio was 50% (w/w). The same spectral conditions apply as in fig.1. Both the spectra seen here and those in fig.1 are in good agreement with proton-enhanced spectra of phospholipid and phospholipid cholesterol dispersions obtained [13,14].

rotating frame precession rate, $\omega_1 = \gamma H_1$ and provides a direct measure of the low frequency motion undergone by the resonant group. $T_{C-H}{}^{-1}$, however, is related to the carbon–proton dipolar line width contribution to an undecoupled conventional ^{13}C NMR spectrum. In general the greater the reorientation freedom permitted a particular resonant group the smaller will be the residual dipolar interaction and the longer T_{C-H}.

4. Results and discussion

Figures 1–5 show proton-enhanced natural abundance ^{13}C spectra obtained from a variety of model and biological membrane systems. The data were tested against expressions of the form in eq. (1). In general it was not possible to fit the cross polarization region of the data at short t_m to a single exponential increase. At longer times in the region dominated by the spin lattice relaxation processes the signal decay was found to obey the $\exp(-t_m/T_{1\rho})$ part of expression (1). Table 1 summarizes the $T_{1\rho}$ and T_{C-H} values obtained in the 5 cases studied here. In order to obtain a single measure of the non-exponential cross polarization behaviour, the value quoted in table 1 is the time required to allow the signal intensity to reach 2/3rds of its maximum value. This time is denoted $T_{C-H}{}^*$.

The clear result of this investigation is that the addition of cholesterol or gramicidin A to a DMPC dispersion dramatically shortens the $T_{C-H}{}^*$ and $T_{1\rho}$ relaxation times of the DMPC methylene peak. Comparison with the two natural membrane systems studied here indicates that they behave in a manner very similar to the cholesterol or gramicidin A dispersions. An immediate conclusion from this result is that in all the mixed systems the motion of the hydrocarbon chains is very restricted relative to that of the pure DMPC dispersion. The shortened values of $T_{C-H}{}^*$ indicate that the amplitude of the motion undergone by the methylene groups is reduced

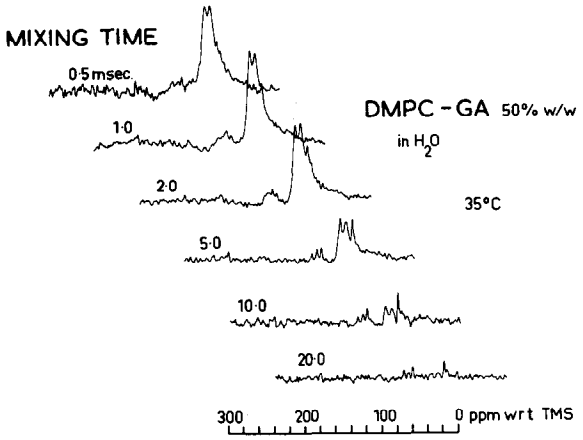

Fig.3. Proton-enhanced natural abundance ^{13}C NMR spectra of gramicidin A–DMPC 50% (w/w) dispersed in H_2O. The same spectral conditions apply as in fig.1.

resulting in a larger residual carbon–proton dipole interaction. In addition since $T_{1\rho}$ is also shorter in the mixed systems, the relative intensity of the low frequency motion undergone by the acyl chains has increased. This supports the observations in [2] and shows that on the NMR timescale employed here ($\sim 10^{-5}$ s) the effect of lipid–protein or lipid–cholesterol interactions is the production of an increase in the low frequency dynamic disorder. Examination of the order resonant peaks shows that apart from minor relative changes in the T_{C-H} and $T_{1\rho}$ of the headgroup and terminal methyl peaks, the reduction is a general property of the mixed systems.

Table 1

The rotating frame spin-lattice relaxation time, $T_{1\rho}$, and effective cross polarization time $T_{C-H}{}^*$ (see text) of the major methylene peak for hydrated dispersions of pure DMPC and DMPC with 30% (w/w) cholesterol or 50% (w/w) gramicidin A

Sample	$T_{C-H}{}^*$ (ms)	$T_{1\rho}$ (ms)
DMPC in H_2O	4 ± 0.5	150 ± 5
Cholesterol–DMPC 30% (w/w) in H_2O	0.5 ± 0.2	17 ± 2
Gramicidin A–DMPC 50% (w/w) in H_2O	0.25 ± 0.1	5 ± 2
Myelin	0.25 ± 0.1	11 ± 2
Chloroplasts	0.25 ± 0.1	6 ± 2

A comparison is made with the same parameters measured for myelin and chloroplast membranes. The spectra from which these data were obtained are shown in fig.1–5. The assignment of the methylene peak was made using spectra acquired at the optimum mixing time for much longer data acquisition periods than used to obtain the spectra in fig.1–5

This suggests that the disorder is an overall wobble of the molecular long axis rather than localized segmental motion. Further evidence of the restriction to the amplitude of all types of motion can be seen in the broadened methylene peak envelopes.

These data show that lipid–cholesterol and lipid–polypeptide interactions restrict the amplitude of the motion undergone by the lipid molecules in mixed bilayer dispersions, and increase the relative intensity of their low frequency disorder. Similar effects are seen in natural membrane dispersions.

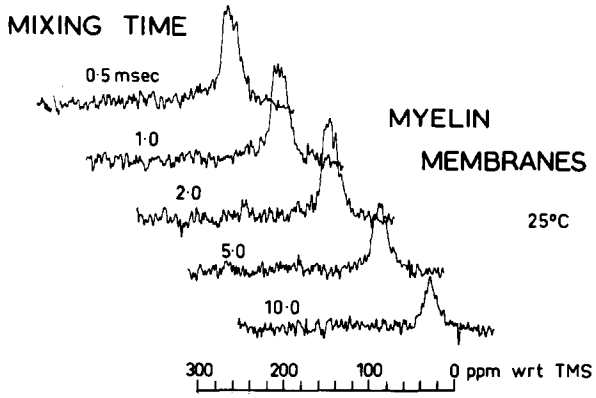

Fig.4. Proton-enhanced natural abundance ^{13}C NMR spectra of bovine brain myelin. The spectral conditions of fig.1 apply apart from an increased line broadening factor of 50 Hz.

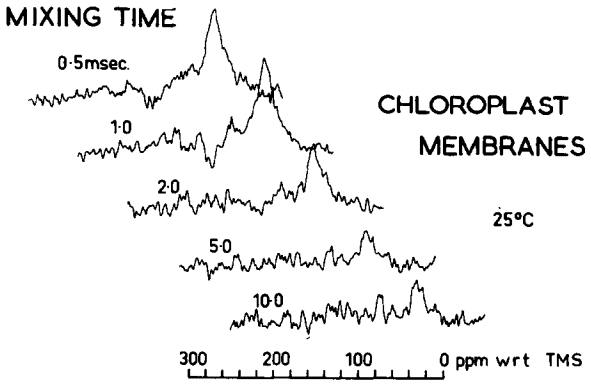

Fig.5. Proton-enhanced natural abundance ^{13}C NMR spectra of chloroplast membranes. The same spectral conditions apply as in fig.4.

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