The Value of Proton Relaxation Measurements in the Study of Aqueous Phospholipid Dispersions

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

(*Medical Research Council, Molecular Pharmacology Unit, Medical Scoool, Hills Road, Cambridge* CB2 2QD)

Summary Proton spin lattice relaxation times (T_1) are reported for the $-\overset{+}{N}Me_3$, $[CH_2]_n$, and terminal methyl protons in sonicated samples of aqueous dispersions of lecithins; these three resonances have distinct T_1 values at each temperature measured, so that spin-diffusion to the terminal methyl or the $-\overset{+}{N}Me_3$ cannot be the dominant relaxation mechanism; the effect of the crystalline to lamellar liquid crystalline phase transition is discussed.

THERE has recently been considerable interest in the properties of phospholipid dispersions as models for biological membranes, since phospholipids in aqueous dispersion are known to be in a bilayer configuration. E.s.r. studies of nitroxide labelled lecithins [phosphatidyl cholines; $RCO{\cdot}OCH_2{\cdot}CH(O{\cdot}CO{\cdot}R){\cdot}CH_2O{\cdot}P(:O)(O^-){\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}\overset{+}{N}Me_3$] incorporated into dispersions of natural phospholipids have been interpreted as showing that the hydrocarbon chains of phospholipid fatty acids become more 'fluid' towards the terminal methyl end.[1] Similarly, ^{19}F n.m.r. studies of monofluorostearic acids incorporated into lecithin vesicles formed by irradiation of aqueous lecithin dispersions with ultrasound, show an increase in motional freedom towards the centre of the bilayer.[2]

Relaxation measurements of ^{13}C nuclei in lecithin bilayers unperturbed by the presence of any probe molecules confirm the results of probe experiments.[3] Proton relaxation measurements on unmodified lecithin, however, appear to be at variance with these experiments. Barratt[4] observed a single spin-lattice relaxation time (T_1) of 350 ms for the $-\overset{+}{N}Me_3$ and $[CH_2]_n$ protons in sonicated dispersions of lecithin using a direct saturation method (neither the chemical composition of the lecithin nor the temperature were given). Chan *et al.*,[5] using a partially homogenised dispersion of egg lecithin in D_2O also found a single T_1 relaxation time of 220 ms at 30 °C for all the protons by free induction decay.

The observation of a single T_1 was in both cases attributed to a process of spin diffusion, in which a common spin temperature is maintained for a number of nuclei by spin diffusion to a small group of nuclei which are the most effectively coupled to the lattice. Chan *et al.*[5] were undecided as to whether spin diffusion was to the terminal methyl group or to 'some methylene protons further up the chain' whereas Salsbury *et al.*[4] interpreted Barratt's results to mean spin diffusion to the $\overset{+}{N}Me_3$ protons, *via* the non-magnetic –O–P–O– unit of the head group.

If the previous spin-label, ^{19}F, and ^{13}C n.m.r. experiments are correct, then we can only account for a single T_1 value for all the protons in the lecithin molecule by a process of spin diffusion, otherwise the proton relaxation times should increase from the glycerol bridge towards the terminal methyl of the fatty acid chains and also towards the $-\overset{+}{N}Me_3$ as indicated by ^{13}C n.m.r. T_1 relaxation, where spin diffusion cannot occur.

To resolve this problem, we have now measured the proton relaxation times T_1 in sonicated samples of aqueous dispersions of lecithins, using a Varian XL100/15 spectrometer and a Fourier transform technique. with a $(\pi\text{-}t\text{-}\pi/2)$ pulse sequence as described by Freeman and Hill,[6] where t is the time in seconds between the π and $\pi/2$ pulses. In principle this allows relaxation times to be obtained for all the resolvable signals in the n.m.r. spectrum. The table lists

TABLE

Proton relaxation times T_1 *for 20% sonicated dipalmitoyl lecithin in* D_2O *buffer* [NaCl (45 mmol), NaOAc (30 mmol), *sodium phosphates* (5 mmol) pD 7·8]

Temperature/°C	Protons $\overset{+}{N}Me_3$	Protons $[CH_2]_n$	Protons Me
65	0·73 ± 0·03	0·57 ± 0·04	0·83 ± 0·05
54	0·57 ± 0·02	0·53 ± 0·04	0·84 ± 0·08
45	0·50 ± 0·04	0·38 ± 0·02	0·57 ± 0·08
40	0·38 ± 0·03	0·32 ± 0·02	0·42 ± 0·03
35	0·30 ± 0·01	(0·35 ± 0·1)[a]	(0·35 ± 0·1)[a]
25	0·26 ± 0·02	—	—

[a] Value for the broad peak corresponding to both the residual $[CH_2]_n$ and Me protons observed in the spectrum.

the values obtained for the protons of the $\overset{+}{N}Me_3$ group, the methylene chains, and the terminal methyl groups of the fatty acid residues for sonicated aqueous dispersions of dipalmitoyl lecithin.

It is clear that in general the three resonances do not have the same T_1 value at each temperature and we conclude that spin diffusion to the terminal methyl or the $-\overset{+}{N}Me_3$ cannot be the dominant relaxation mechanism.

Since the T_1 values increase with temperature, it is clear that $\omega_0^2\tau_c^2 \ll 1$ so that $T_1 \propto 1/\tau_c$.[7] The differences in T_1 for the different groups reflect distinct rotational correlation times and are therefore able to provide information about molecular motion within the bilayer. We note particularly that the terminal methyl group has a longer T_1 than the $[CH_2]_n$ at each temperature consistent with the gradation of relaxation times for the ^{13}C nuclei of the methylene chain increasing towards the terminal methyl group.

Spin diffusion within the methylene chain itself cannot be ruled out completely on the basis of these results, since a single exponential decay characterised the intensity of the resonance due to the $[CH_2]_n$ protons as a function of pulse delay t. It is easy to show that this does *not* by itself imply that all the methylene protons have the same T_1 value; for example, a range of T_1 values from *ca.* 0·1 to 1·0 s for the methylene protons, the majority having the longer T_1's, would appear experimentally as a single T_1. If spin diffusion were the dominant relaxation mechanism in the chain, however, there is no obvious reason why a common spin temperature would not be established with the terminal methyl group.

At 43 °C, aqueous dispersions of dipalmitoyl lecithin undergo a crystalline to lamellar liquid crystalline phase transition. Below this temperature, sonicated aqueous dispersions show a considerable broadening of all the spectral lines, and below 35 °C only the absorptions due to the $\overset{+}{N}Me_3$ protons can be observed. Similarly, in the ^{13}C spectrum, only the carbons of the $\overset{+}{N}Me_3$ group are resolvable below the transition. Both n.m.r. and e.s.r. studies show that the transition change is completely reversible. The abrupt change in T_1 value for the protons of the $-\overset{+}{N}Me_3$ group over the transition suggests some rearrangement of the head group at this temperature, in addition to the considerable loss in motional freedom for the $[CH_2]_n$ and Me groups. The T_1 values for sonicated dispersions of egg lecithin (which has a transition temperature *ca.* −10 °C) show only a gradual change over this temperature range.

Distinct T_1 values are also observed for the $\overset{+}{N}Me_3$, $[CH_2]_n$, and Me protons in lecithins in methanol, in which they are weakly associated, and in chloroform, in which they are in the form of inverted micelles with the polar head-groups packed at the centre of the structure. Spin diffusion is therefore not a dominant relaxation mechanism in these systems either.

Proton T_1 values can, therefore, provide information about motion in lecithin bilayers, and the biological relevance of these measurements together with the ^{13}C studies will be discussed elsewhere.

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