Chem. Phys. Lipids 7 (1971) 1–8

PROTON N.M.R. RELAXATION STUDY OF MOBILITY IN LIPID WATER SYSTEMS

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Received May 1971
Accepted June 1971

Both pulse and C.W. proton n.m.r. have been used for further study of the mobility of the hydrocarbon chains and headgroups in some smectic liquid crystalline phases. It is shown that dipolar broadening is responsible for the observed proton linewidths in these systems, in the frequency range 10–40 MHz. In lecithins in the liquid crystalline phase, the $-\overset{+}{N}Me_3$ group is relatively mobile.

I. Introduction

Early C.W. proton n.m.r. measurements indicated that the proton linewidths in smectic liquid crystalline phases were field dependent[1]). The linebroadening mechanism was not known. Kaufman et al.[2]), using spin-echo, suggested that magnetic field inhomogeneities were responsible, and Hansen and Lawson[3]), diffusion throught these gradients. De Vries and Berendsen[4]), however, showed that dipolar broadening appeared to be the major cause of linebroadening in a potassium oleate-D_2O system. Tiddy[5]) has recently shown that in another smectic phase, that the effective spin-spin relaxation time, T_2 eff. (defined as the time for the transverse magnetisation following a 90° pulse to fall to $1/e$ of its original value) is not field dependent, in the range 8–90 MHz.

We have studied the field dependence of T_2 eff. in erythrocyte ghost lipids, egg lecithin, dipalmitoyl lecithin and potassium laurate, in D_2O, together with the response of these nuclear spin systems to spin-echo ($90\text{-}\tau\text{-}180_{90}$) and solid echo ($90\text{-}\tau\text{-}90_{90}$) pulse sequences, and the spin lattice relaxation (T_1) behaviour of dipalmitoyl lecithin. Independently, Chan et al.[6]) have reported some similar preliminary results on egg yolk lecithin.

II. Experimental

Dipalmitoyl-Lα-lecithin was purchased from Fluka, Buchs, and was purified

on Mallinckrodt SilicAR CC-7 (100–200 mesh), eluting with chloroform-methanol (2:1).

Egg lecithin was extracted from *Gallus domesticus* eggs according to Singleton et al.[7]) and purified on Woelm alumina and SilicAR.

Palmitic acid was perdeuterated using the method of Stenhagen and Dinh-Nguyên[8]) and converted to the acid chloride with oxalyl chloride. Glyceryl phosphoryl choline was prepared according to Dawson[9]) and its cadmium chloride complex according to Tattrie and McArthur[10]); this was acylated with the perdeuterated palmitoyl chloride according to Baer and Buchnea[11]). The product was purified on SilicAR.

Potassium laurate was prepared from Fluka puriss. lauric acid, and crystallised from ethanol.

Erythrocyte ghosts were prepared according to Dodge et al.[12]) and the lipid and membrane samples prepared according to Kaufman et al.[2]).

Potassium laurate 70 wt.%–D_2O was made by centrifugal homogenisation at 90°. Other lipids were 25 wt.% dispersion in D_2O (99.95% Norsk-Hydro).

C.W. spectra were run on a Varian HR-220 spectrometer. Pulse measurements were made on Bruker B-KR 322s spectrometers operating between 10 and 60 MHz, using Data Laboratories DL-102S or DL-101S signal averages for processing of noisy signals. 90° pulse lengths were approximately 2μsec at frequencies above 30 MHz and 1 μsec at frequencies below 30 MHz.

III. Results

No field dependence in the range 10–40 MHz was observed for the effective spin-spin relaxation time, T_2 eff., in ghost lipids, dipalmitoyl lecithin (50 °C), egg lecithin and potassium laurate (90 °C) (fig. 1). The free induction decay of the ghost sample was obtained at 60 MHz only.

90-τ-180_{90},[13,14]) pulse sequences were applied to ghost lipids, dipalmitoyl lecithin, egg yolk lecithin, potassium laurate and erythrocyte ghosts, at 60 MHz, putting the 180° pulse just after the end of the observable FID. Small echoes, $T_2 \sim 6$ msec, were observed from the first three samples (fig. 2), none was observed from the potassium laurate sample, and a large signal, $T_2 = 50 \pm 20$ msec (50% total signal intensity) was observed from the ghost sample for which T_2 eff. $= 750 \pm 250$ μsec. The T_2 of water added to dipalmitoyl lecithin was measured using this sequence, T_2 varying from 1.0 ± 0.1 sec at 22 °C to 2.1 ± 0.1 s at 81 °C.

90-τ-90_{90},[15,16]) pulse sequences were applied to dipalmitoyl lecithin in the gel and liquid crystalline phases, with the resultant production of dipolar echoes (fig. 3).

The temperature dependence of the spin lattice relaxation time, T_1, of

dipalmitoyl lecithin, was measured in the range 36–48 °C, using the 90-τ-90 pulse sequence (fig. 4). Chain deuterated dipalmitoyl lecithin was dispersed in 99.7% D_2O above its gel → liquid crystalline transition temperature, 42 °C, and the 220 MHz proton n.m.r. spectrum was recorded at 35 and 60 °C, using TSP (sodium-3-trimethylsilyl propionate-2,2,3,3-d_4) as internal lock (fig. 5).

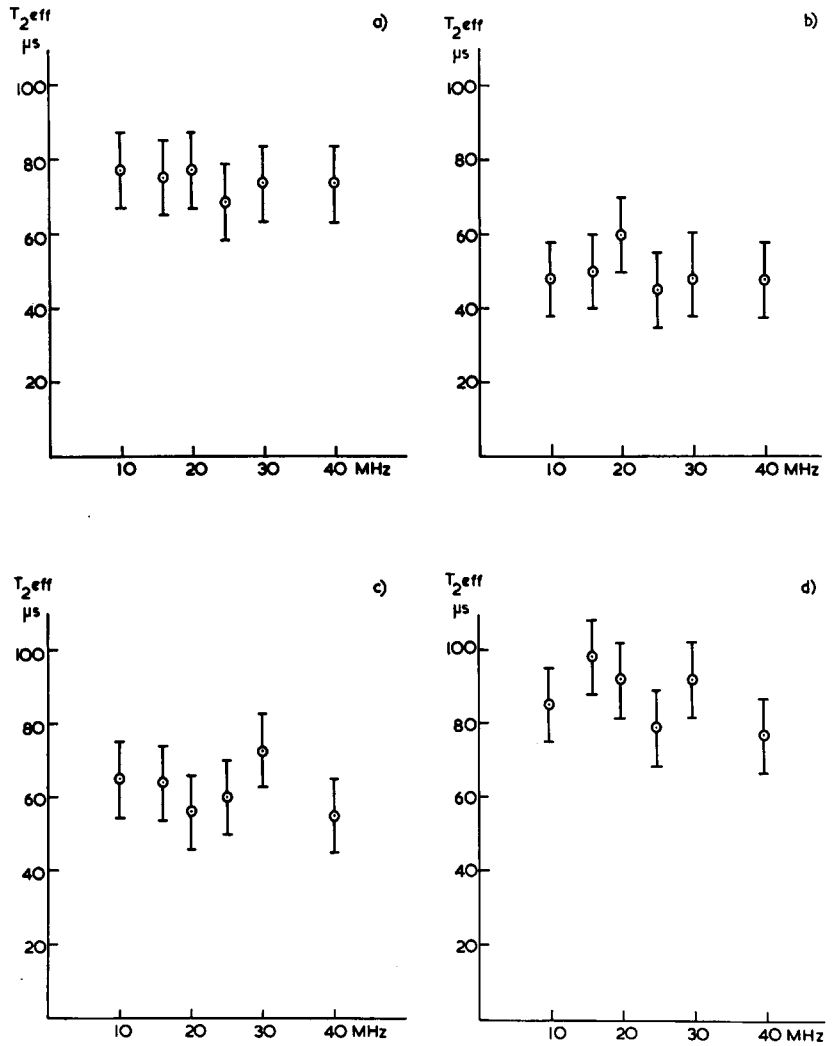

Fig. 1. Frequency dependence of T_2 eff. in (a) potassium laurate (70 wt.%) – D_2O (90 °C), (b) ghost lipids (25 wt.%) – D_2O, (c) dipalmitoyl lecithin (25 wt.%) – D_2O (50 °C), (d) egg yolk lecithin (25 wt.%) – D_2O.

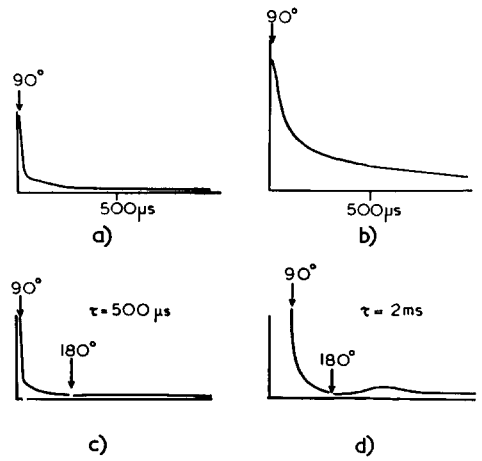

Fig. 2. Transient response of dipalmitoyl lecithin – D_2O spin system after (a) 90° pulse, 25°C, (b) 90° pulse, 50°C, (c) 90-τ-180_{90} sequence, 25°C, (d) 90-τ-180_{90} sequence, 50°C.

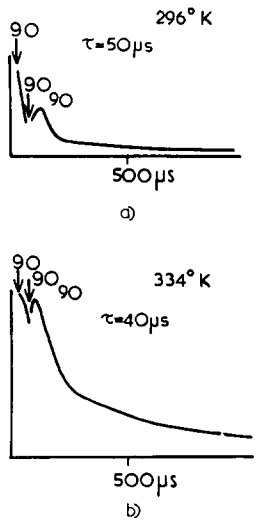

Fig. 3. Dipolar echoes obtained after 90-τ-90_{90} sequence in dipalmitoyl lecithin in (a) gel, 23°C and (b) liquid crystalline phase, 61°C.

IV. Discussion

Penkett et al.[1]) originally found that the half-height linewidths of egg lecithin in D_2O and potassium laurate 63% – D_2O were field dependent. It was proposed that this effect could be caused by a magnetic anisotropic effect, i.e. the observable linewidths were not totally due to incomplete averaging of the dipolar term.

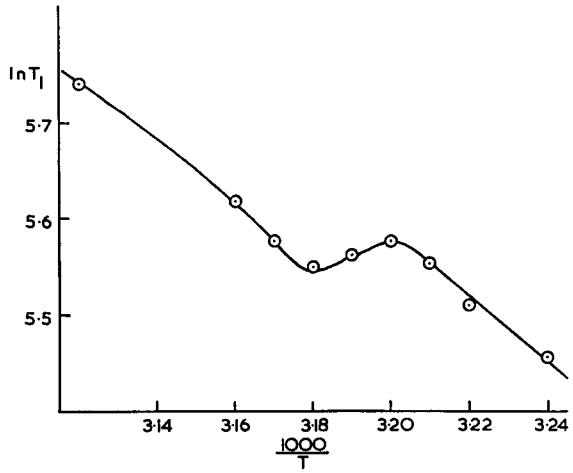

Fig. 4. T_1 run (90-τ-90 sequence) on dipalmitoyl lecithin from 36 → 48°C.

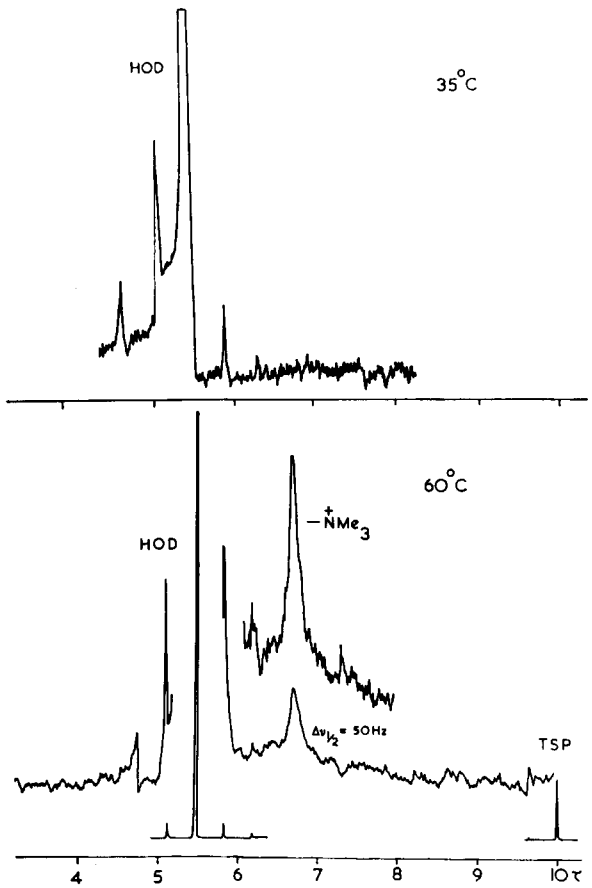

Fig. 5. 220 MHz proton n.m.r. spectrum of di(perdeutero)palmitoyl lecithin 5%) – D_2O at 35 and 60°C.

Apparent evidence for the existence of internal magnetic field gradients was then obtained by Kaufman et al.[2]), using spin-echo. Hansen and Lawson then proposed that self-diffusion through these internal magnetic field gradients might contribute to the spin-spin relaxation process. Tiddy[5]) has recently shown that in a sodium caprylate-decanol – D_2O smectic liquid crystalline system, T_2 eff. is not field dependent. However, in very high magnetic fields, it is possible that chemical shift anisotropy effects could contribute to the observed linewidths[17]).

In fig. 1 it is apparent that in the systems potassium laurate – D_2O, egg yolk lecithin – D_2O, dipalmitoyl lecithin – D_2O and ghost lipids – D_2O, that T_2 eff. is essentially frequency independent in the range 10–40 MHz.

After a $90\text{-}\tau\text{-}90_{90}$ sequence, dipolar echoes are obtained in both the gel and liquid crystalline phases of dipalmitoyl lecithin – D_2O (fig. 3). Using a $90\text{-}\tau\text{-}180_{90}$ sequence or $90\text{-}\tau\text{-}90_0$ no echoes are seen[15]). Similar dipolar echoes are obtained on the other lipids.

From a comparison of our T_2 eff. values for ghost lipids, egg yolk and dipalmitoyl lecithin, it is apparent that a very short T_2 component is present in the ghost lipids which is not observed in the other lipid systems. This probably represents the cholesterol in the lipid, the effect of cholesterol, in this case, being to decrease the mobility of the hydrocarbon chains of the unsaturated phospholipids[18]).

Using the Carr-Purcell spin-echo sequence[13]) as modified by Meiboom and Gill[14]), Kaufman et al.[2]) have obtained an echo train on ghost lipids, the major component having a $T_2 = 110$ msec, which would indicate that the proton absorption lines were being broadened by internal magnetic field inhomogeneities. We have not been able to reproduce this result.

A similar mobile component in *E. coli* membranes, a T_2 (FID) of 25 msec, accounting for a total of $14 \pm 2\%$ total signal intensity, has been observed by Steim[19]). Our spin-echo results with ghosts, prepared according to Kaufman et al.[2]), indicate a component ($50 \pm 10\%$ total signal intensity) with $T_2 \sim 50$ msec. Taken in consideration with the previous results and the wide line results of Clifford et al.[20]), this component is attributed HOD. Values of T_2 similar to this (55 msec and 52 msec) have recently been reported by Damadian[21]), for structured water in cell tissues.

Using spin-echo Hansen and Lawson[3]) have obtained very long T_2s when applying 180° pulses with a pulse spacing $\tau \simeq T_2$ eff. Interpretation of relaxation times obtained under these conditions can be misleading as they can produce a "line-narrowing" effect[22,23]). This effect may account for the result of Kaufman et al.[2]) on ghost lipid. Similar results are not to be expected from single shot or simple multiple pulse experiments when $\tau > T_2$ eff.

With the choline residue containing lecithins and ghost lipid, we have ob-

tained spin echoes with $T_2 \sim 6$ msec. This component accounts for $\sim 10\%$ total signal intensity, and corresponds to a half height linewidth, for a Lorentzian line, of ~ 50 Hz. With dipalmitoyl lecithin an echo is not observed until at 37 °C, just below the transition temperature (42 °C). This component is attributed to the choline $-\overset{+}{N}Me_3$ group (fig. 2) predominantly. By removing the wide obscuring line due to the acyl chain protons, by deuteration, it is possible to clearly define the $-\overset{+}{N}Me_3$ group, which has $\Delta\nu_{\frac{1}{2}} \simeq 50$ Hz. This group begins to be visible at 37°, and above the transition temperature is quite prominent (fig. 5), indicating relatively rapid motion. This conclusion is in agreement with our previous ^{13}C pulse measurements[24]).

Our spin-lattice relaxation results indicate a single T_1 in the dipalmitoyl lecithin – D_2O system between 25 and 60 °C (linear regression fits of >0.98 for all points) (fig. 4). If, in fact, the alkyl chain spin-spin relaxation is dominated by a dipolar mechanism then, since $T_2 \ll T_1$, it is possible that spin diffusion may occur[25]). A T_1 minimum is apparent in the region of the phase change from gel→l.c., this is expected and similar ill defined minima have been observed in the soaps[26]), a wide correlation time distribution along the alkyl chains is indicated from the shallowness of the T_1 minimum.

V. Conclusion

(a) The spin-spin relaxation in these systems is predominantly due to dipolar interactions.

(b) A spin-echo is seen with lecithin which is attributed to the choline $-\overset{+}{N}Me_3$ group. The T_2 agrees with that obtained from high-resolution spectra of a chain deuterated lecithin; this group is thus relatively mobile in the l.c. phase.

(c) The very long relaxation times previously observed with ghosts appear to be due to HOD.

Acknowledgements

We thank Professor W.J. Orville-Thomas and Professor E.R. Andrew for use of their Bruker spectrometers; Dr. W. Derbyshire and Dr. G.J.T. Tiddy for helpful discussions, and Dr. J. Carolan for valuable technical advice on taking pulse measurements. We are also grateful to M. Hutchins for obtaining some of the T_1 measurements, and to Miss M. Tyson for expert technical assistance.

E.O. thanks the S.R.C. for financial support and J.M. the S.R.C. and Messrs. Reckitt and Colman, Ltd.

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