## Title

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## DISCLAIMER

# MAGNETIC RESONANCE STUDIES ON MEMBRANE AND MODEL MEMBRANE SYSTEMS: I. PROTON MAGNETIC RELAXATION RATES IN SONICATED LECITHIN DISPERSIONS

by

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Evidence is accumulating that many biological membranes contain, to a greater or lesser extent, regions of lipid bilayers.[1-3] Since many conclusions drawn from models[4-8] of these systems may indeed be applicable to natural membranes, the structural and functional properties of lipid bilayers is an active area of research. Nuclear magnetic resonance (NMR) provides a powerful method for investigating some structural and dynamic properties of such systems. In particular, the longitudinal, $T_1$, and transverse, $T_2$, nuclear relaxation times are explicitly and implicitly related to molecular motions.[9] Recent advances in Fourier transform NMR spectroscopy provide the means for measuring these relaxation times in complex spectra.[10-12] Since sonication of aqueous dispersions of lecithin produces relatively reproducible and homogeneous vesicles which give rise to high resolution NMR spectra,[6-8,13] we have chosen this system for our initial measurements of the nuclear relaxation times in aqueous dispersions of phospholipid bilayers.

MATERIALS AND METHODS

Proton magnetic resonance measurements were performed on a Varian HR-220 spectrometer, extensively modified for Fourier transform operation. $^{31}P$ magnetic resonance measurements were performed at 24.3 MHz on an instrument of our own design. Both spectrometers are interfaced with a computer system designed in this laboratory. The $T_1$ measurements used the method of Vold et al.,[10] while the $T_2$ values were determined by a variation of the Carr-Purcell method.[14,15] A Carr-Purcell sequence is established and terminated after the 1,2,3...nth pulse in n experiments. In each experiment the echo following the final pulse is Fourier transformed to yield the partially (transversally) relaxed spectrum. The

decay of each individual line then establishes its value of $T_2$. The details of this method will be published elsewhere.[12] Non-spinning capillaries were used for the $T_2$ measurements. The resonance assignments followed those of Chapman and Morrison.[16]

Lecithin was prepared from hen egg yolks by the method of Singleton et al.,[17] and was further purified by chromatography on silicic acid. Unsonicated lecithin dispersions were prepared using 30-60 μmoles lipid/ml $D_2O$ by the method described by Demel et al.[18] The lipids were then sonciated for 15 min on ice using a Branson 185E sonicator and centrifuged at 17,300 x g for 30 min at 4°C. All samples were prepared and stored under argon in 0.15 M KCl and $10^{-4}$ M EDTA. The lipid concentration was determined by phosphate analysis,[19] and the purity was checked by thin-layer chromatography on silica gel developed with chloroform:methanol:water (65:25:4) and by the measurement of the oxidation index.[20]

In sonicated lecithin at 220 MHz, the choline N-methyl and the fatty acid methylene, allyl, vinyl, methyl, and α-carbonyl protons are among the resolved resonances.[9,16] Their $T_1$ values are given in Table I. Figure 1 shows the temperature dependencies of the values of the spin-lattice relaxation times for three of these resonances. In the temperature region investigated, this plot reveals two important points: (a) $T_1$ increases with temperature for each of these classes of protons, and (b) the $T_1$ of the terminal methyl protons is clearly longer than that of the choline or methylene protons, which in turn appear to differ from each other. The fatty acid methylene protons appear to be characterized by a single value of $T_1$, but a distribution of values

cannot be excluded. The data presented in Table I indicate that the $T_1$ values for the vinyl and α-carbonyl protons are similar to but different from those of the methylene protons. We may treat the data of Figure 1, as well as the data for the other resonances which have a similar dependence on temperature, as Arrhenius plots and derive the activation energies for the thermal relaxation processes.[21] The values so derived are given in Table I, and agree favorably with literature values for potential barriers to internal rotation in alkanes.[22] Since our observations extended over a small temperature range, we may be unable to detect a distribution of activation energies for the methylene protons if such were to exist.

In Table I are also listed preliminary values of the transverse relaxation times for some selected resonances in sonicated egg lecithin. The N-methyl, methyl, and phosphorus nuclei each appeared to relax according to a single exponential. For each of these groups the value of the transverse relaxation time that one estimates from the conventional linewidth, $T_2^*$, is less than or equal to our experimental $T_2$.[13] The methylene protons exhibited a heterogeneity of $T_2$ values. About 20% showed a single value of $T_2$ of about 0.056 sec; the remaining 80% were much shorter and non-exponential, implicating a distribution of of $T_2$ values. Aside from the methyl and N-methyl protons, the remaining proton resonances exhibited relatively short values of $T_2$. Similar results have been obtained with dimyristoyl L-α-lecithin (at temperatures above the transition point[23]).

Because of the implicit complexity of relaxation processes in general, and in these systems in particular, a quantitative discussion

lies outside the scope of this note.[11,21] Thus, the following comments on our relaxation results should be considered as suggestive. Let us assume, however, that the dominant relaxation results from modulation of the dipolar coupling to the nearest protons, e.g., the companion proton for the methylene group and the two companion protons on a methyl group.

For all proton resonances, $T_1$ increases with increasing temperature, indicating that at 220 MHz we are in the short correlation time regime. The data in Table I reveal the close agreement between the activation energies for thermal relaxation and for internal rotation; they provide strong evidence that the dominant source of thermal relaxation derives from modulation of the intramolecular dipolar interactions by the internal rotations. In the short correlation time regime, theories based on isotropic motion predict that $T_1=T_2$ which is at variance with our results that $T_1>T_2$. Thus, we are obliged to conclude that the motions are in fact anisotropic. Two classes of motion may be suggested which account for the observations: (1) relatively small displacements due to rotations of individual methylene carbon atoms which occur at high frequencies, and (2) relatively larger angular displacements of protons further down the chain which are a consequence of the high frequency rotations. The former motions, which are roughly constant along the fatty acid chains, would result in the longer roughly corstant values of $T_1$, in agreement with our observations. Manipulation of CPK space-filling models indicates that motions of the latter class may involve large segments of the fatty acid chains. That is, starting from the minimum energy, all trans conformation, rotation about a single

C-C bond would result, for example, in a gauche + conformation. That segment of the molecule between the origin of the rotation and the methyl end would execute a large displacement; a simultaneous gauche - rotation about the bond β (toward the methyl end) from the first bond, would virtually restore the original linear shape. Rotations about C-C bonds closer to the methyl end would lead to displacements requiring less volume, and would be less likely to lead to collisional encounters with neighboring fatty acid chains, and thus would be more probable and result in longer relaxation times. This is, of course, only one of many possible conformational transitions which could account for our observations. Such dynamics simultaneously maintain minimal displacements of large segments of the molecules, account for the observed activation energy for and nearly constant value of $T_1$ for the methlene protons, and finally provide a mechanism for the abrupt increase in transverse relaxation times for the methyl protons and those methylene protons which are probably near the methyl terminus.

Simplistic estimates of the correlation times corresponding to the measured values of $T_2$ lead to values in the range of $10^{-11} < \tau_c < 10^{-8}$ sec.[21] Since we have measurements at only 220 MHz, we do not assign a correlation time for the $T_1$ processes, but may safely state that it is certainly less than $10^{-9}$ sec. Since the minimum diameter for sonicated lecithin vesicles is about 250 Å, for which the Debye correlation time at 20°C is $\simeq 10^{-6}$ sec,[9] * we may confidently rule out the tumbling of the vesicles as a significant source of motion contributing to nuclear relaxation.

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*For comparison, at 20°C, for water $\tau_c \simeq 10^{-12}$ sec, and for hemoglobin $\tau_c \simeq 10^{-7}$ sec.

A recent paper[24] reports values of $T_1$ for the $^{13}C$ NMR of sonicated lecithin bilayers which show that the $^{13}C$ nuclei exhibit a distribution of thermal relaxation times. The shortest $T_1$ values apply to the carbons at the polar end of the molecules, while the values for carbon atoms 3-13 are longer and nearly equal. The three remaining carbons, 14-16, show increasingly longer times with the terminal methyl the longest. Although the relaxation processes or mechanisms for protons differ in detail from those for $^{13}C$, the relaxation times share a similar functional dependence on the correlation times. These $^{13}C$ data imply a relatively long correlation time at the polar end, a shorter and nearly constant value for carbon atoms 3-13, and still shorter values of correlation times as the terminal methyl is approached. Although not stated by the authors, a reasonable interpretation of these data is that a large segment of the molecule executes relatively uniform motion at a high frequency which is significantly slower than that executed at the terminal methyl end of the molecule. This interpretation is in accord with that offered above to account for the proton $T_1$ and $T_2$ data.

Proton relaxation rates in lecithin have been measured and discussed by other authors.[25-27] Their measurements, made by other methods, suggest that the protons of lecithin are characterized by a single value of $T_1$. Arguing in analogy with results from studies on solid n-alkanes,[28-30] a spin-diffusion mechanism has been proposed.[31] The spin-diffusion mechanism for these molecules proposes that spin-spin flip-flops between pairwise adjacent protons propagate along the aliphatic chain toward the terminal methyl. Because of its relative freedom to reorient, the methyl has a shorter $T_1$ than do the methylene protons, and thus serves as a heat

sink at these low temperatures. The entire molecule is then characterized by a single $T_1$. If a spin-diffusion mechanism were operative in lecithin, as has been suggested, then the value of $T_1$ would only provide information about the motion of the heat sink. In a recent paper, Chapman has tentatively proposed the choline headgroup as the heat sink.[27]

The results from Table I show that there is not a single spin-lattice relaxation time characterizing the protons of sonicated egg lecithin at 220 MHz. Further, the apparent existence of different relaxation times for protons along the methylene chain clearly excludes efficient coupling among all the methylene protons. If spin-diffusion contributes significantly to thermal relaxation in these molecules, it is likely restricted to short segments of the methylene chain. If so, one must inquire into the nature and location of the heat sinks.

The data in Table I suggests that the choline protons do not serve as a possible heat sink for the proposed spin-diffusion. Further evidence that the polar headgroups are magnetically isolated from the apolar regions derives from our observations on the effects of $Mn^{++}$ ions added to the external aqueous phase of the dispersions. $10^{-4}$ M $Mn^{++}$ ions produced a marked effect on the width of the N-methyl protons, notably reduced their value of $T_1$, but had little effect on the parameters of the methylene or methyl protons. We conclude then that spin-diffusion toward the polar headgroup is not responsible for thermal relaxation of the apolar region of lecithin in sonicated bilayers.

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TABLE I

Spin-lattice, $T_1$, and transverse, $T_2$, relaxation times and activation energies for some resonances of sonicated egg lecithin

| | $-\overset{+}{N}(CH_3)_3$ | $-CH_2-\overset{O}{\overset{\|}{C}}-O-$ | $-CH_2-$ | $-CH_2-\overset{H\ H}{\dot{C}=\dot{C}}-$ | -HC=CH- | $-CH_3$ | $^{31}P$ |
|---|---|---|---|---|---|---|---|
| $T_1$ (seconds) | 0.41 ± 0.02 | 0.34 ± 0.02 | 0.47 ± 0.03 | 0.41 ± 0.04 | 0.54 ± 0.03 | 0.76 ± 0.06 | 1.4 ± 0.1 (a)<br>8.5 ± 0.7 (b) |
| $T_2$ (seconds) | 0.075 | 0.008 | 0.056 (20%)<br><0.02 (80%) | 0.015 | 0.020 | 0.036 | 0.110 |
| $E_a$ (Kcal/mole) | 4.3 ± 0.3 | 2.8 ± 0.4 | 3.0 ± 0.2 | 2.7 ± 0.2 | 3.2 ± 0.3 | 4.2 ± 0.3 | --- |

FIGURE 1

Arrhenius plots of the spin-lattice relaxation time versus temperature for some selected resonances in sonicated egg yolk lecithin. The data presented were obtained from two different samples run on two different days.

TABLE 1

The $T_1$ values were determined at 40°C; for a given experiment the estimated error was within 10%, as indicated, however for experiments performed on different days with different samples the error sometimes exceeded this limit. The estimates of $T_2$ were made at 20°C; the text contains an explaination of the two relaxation times for the methylene protons. The phosphorus nuclear relaxation times were measured at 34°C and are included here for completeness and will be discussed in subsequent publications.[9] (a) refers to dimyristoyl L-α-lecithin and (b) refers to egg yolk lecithin.

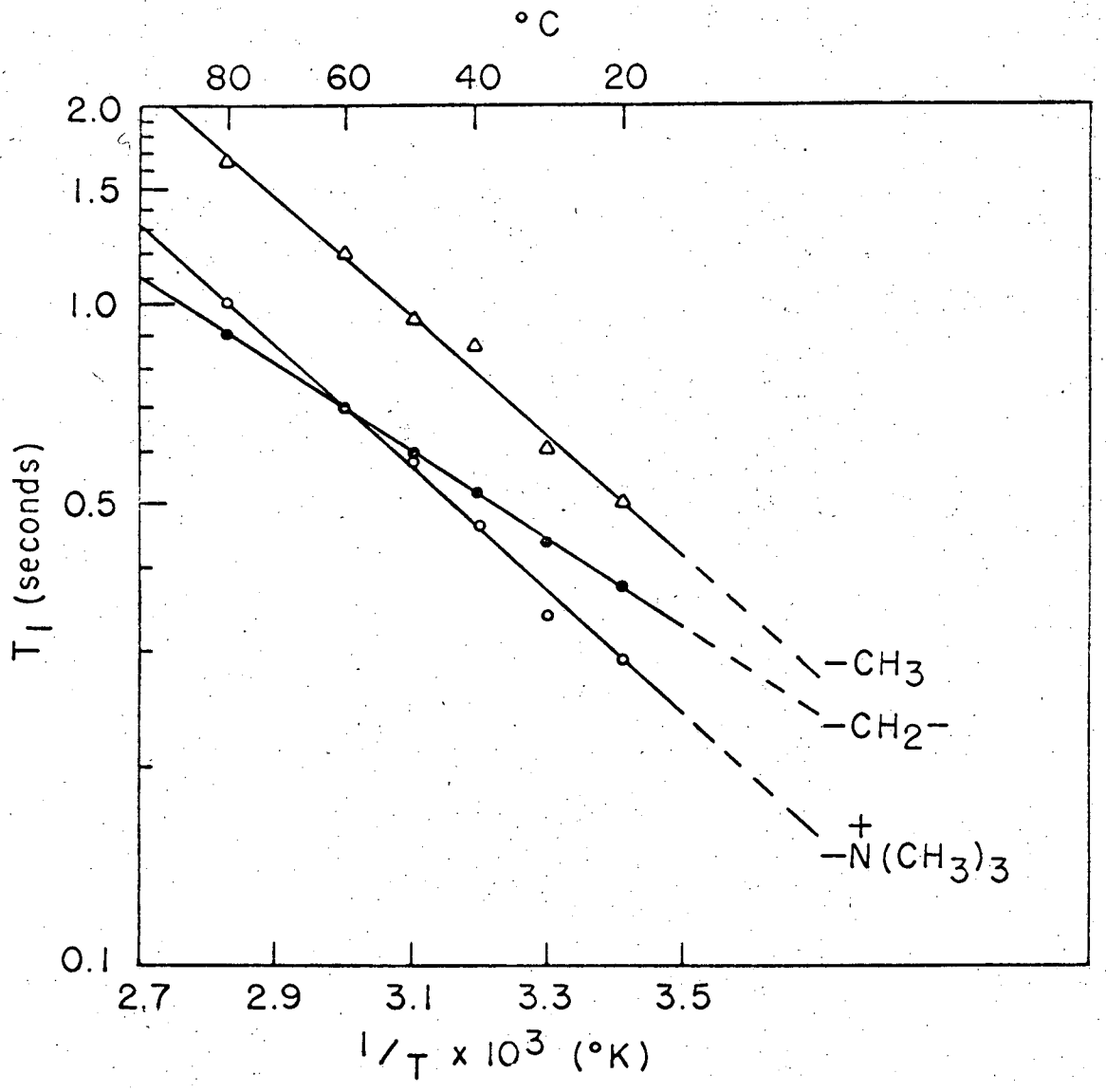


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Fig. 1

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