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TEMPERATURE AND FREQUENCY DEPENDENCE OF LONGITUDINAL PROTON RELAXATION TIMES IN SONICATED LECITHIN DISPERSIONS

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SUMMARY

The temperature dependence of the longitudinal relaxation time, T_1, for various protons in sonicated dipalmitoyllecithin vesicles at 60 and 220 MHz are compared. In order to qualitatively explain the frequency dependence of T_1 values the anisotropy of motion of individual protons in the phospholipid molecule must be explicitly taken into account. The difficulties in interpreting NMR relaxation times in terms of specific motions in the phospholipid molecule are discussed.

INTRODUCTION

There is now considerable evidence for the occurrence of lipid bilayer regions within the mosaic structure of biological membranes[1,2]. Consequently, it is reasonable to consider the intra- and intermolecular motions of lipid molecules in bilayer model membranes as being a good approximation to those motions present in bilayer regions of natural membranes. X-ray diffraction studies[3] have shown that above the thermal transition (>42 °C) bilayers formed from either dipalmitoyllecithin or natural lipids exhibit similar fatty acid chain packing and transverse electron density profiles. The dipalmitoyllecithin bilayer can therefore be taken as a reasonable model for bilayer membranes composed of natural lipids. In addition, many physical-chemical studies have indicated that the small-diameter single-bilayer vesicles, obtained by mild sonication of aqueous lipid dispersions, are representative of more extended forms of bilayer membranes[4–7]. Since single-bilayer preparations give more highly resolved nuclear magnetic resonance spectra than multilayer aggregates[6], they appear to be an ideal model system to assess the potential of nuclear magnetic resonance for elucidating the details of inter- and intramolecular motion of lipid molecules in a biological membrane.

We report here a comparison of the temperature dependence of the longitudinal relaxation time, T_1, at 60 and 220 MHz, for various dipalmitoyllecithin protons in preparations of small-diameter single-bilayer vesicles. Although studies of NMR relaxation times have been reported on this and related phospholipid bilayer systems[8–11] our results suggest that it may be more difficult than previously imagined

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to determine the nature of the motions which determine these relaxation times even in these simple systems.

This work has been briefly reported elsewhere[12].

MATERIALS AND METHODS

Synthetic β,γ-dipalmitoyl-L-(α)-lecithin was purchased from Calbiochem. Deuterium oxide (99.8%) was obtained from Thomson-Packard. Palmitic acid was purchased from Applied Sciences Laboratories; all other reagents were reagent grade and were used without further purification. Suspensions of dipalmitoyllecithin bilayer vesicles were obtained by sonication of coarse dispersions of dipalmitoyllecithin (36.6 mM) in a ^2H_2O solution (30 mM sodium acetate, 45 mM NaCl, 5 mM sodium phosphate, p^{2}H (20 °C) = 7.6). Free palmitic acid was added to the lecithin dispersion (molar ratio palmitic acid/dipalmitoyllecithin, 0.05) in order to give the lecithin bilayer a defined surface charge. A 5-min low-power sonication at 20 kHz was applied with a sonifier cell disruptor (Branson model W185) to degassed lecithin dispersions. During the sonication procedure the sample was maintained under nitrogen, and the temperature was kept above the transition temperature (>42 °C). In order to avoid thermal gradients between the sample and the bath, 30-s intervals of sonication were alternated with 30-s waiting intervals.

After transfer into a 5-mm diameter NMR tube the sample was again degassed with nitrogen and kept above the transition temperature during the period required for the NMR experiments (about 60 h). Between two successive experiments the sample was kept at least 30 min in a bath maintained at 60 °C to minimize temperature hysteresis effects. The T_1 measurements were carried out using 60-MHz and 220-MHz spectrometers, both preadjusted to the same probe temperature. The sample was thermally equilibrated in the probe for more than 1 h. The reversibility of the linewidth and T_1 measurements was determined by making the measurements alternately at low and high temperatures, instead of at monotonically increasing (or decreasing) temperatures.

NMR spectra and pulsed NMR measurements of the longitudinal relaxation times of individual protons in the phospholipid NMR spectra were performed at 60 MHz using an extensively modified Varian HA-60 spectrometer, and at 220 MHz using a Varian HR-220 spectrometer equipped with a frequency sweep unit. The HA-60 spectrometer was used in the field-locked mode while the HR-220 spectrometer (which employs a superconducting magnet) did not require field stabilization. Both the HA-60 and the HR-220 spectrometers were equipped with Varian Model V-4340 variable temperature accessories. The same Varian 620-i computer was used to control the widths and timing of the transmitter radio-frequency pulses for both instruments, and to collect the free induction decay from the receiver after an appropriate cycle of pulses. The radio-frequency pulse sequence employed was: ($\pi/2$, homospoil, τ, $\pi/2$, free induction decay, homospoil)[13], although one experiment was done with the ($\pi/2$, free induction decay, π, τ, $\pi/2$, free induction decay) sequence[13,14]. The pulse sequence and the collection of data were repeated as many times as necessary for a given delay time, τ, in order to achieve the desired signal-to-noise ratio. Typically, 100 transients were collected. The 620-i computer calculated the Fourier transform of the free induction decay to give the appropriate partially relaxed spectrum

for each value of τ. The T_1 for each respective line was calculated by a computer (PDP-10) analysis of the amplitude of each line in the partially relaxed spectrum as a function of the delay time, τ (ref. 13).

It was desirable to check that any differences in relaxation times measured with the 60-MHz instrument and the 220-MHz instrument were due to a frequency dependence of the relaxation times, and not a systematic error between the two instruments. For this purpose the T_1 values of a manganese-doped water sample, measured on the 60-MHz and the 220-MHz instruments, were compared. The manganese-doped sample was chosen instead of pure water as its longitudinal relaxation time could be adjusted to approx. 500 ms, which is the region of interest for most of the observed phospholipid proton relaxation times, and is also the region at which most accurate T_1 measurements may be made. From the data of Reuben and Cohn[15] it can be calculated that the T_1 of a manganese-doped water sample should be 3% smaller at 60 MHz than at 220 MHz. The measured values of T_1 for this sample were found to be identical (within 3%) showing that, within the statistical error of the measurement, there is no apparent systematic error in the measurements of T_1 at the two frequencies.

RESULTS

Examples of high resolution NMR spectra obtained from sonicated aqueous suspensions of dipalmitoyllecithin above the thermal transition at 220 and 60 MHz are shown in Fig. 1. The proportion of the hydrocarbon-chain protons contributing

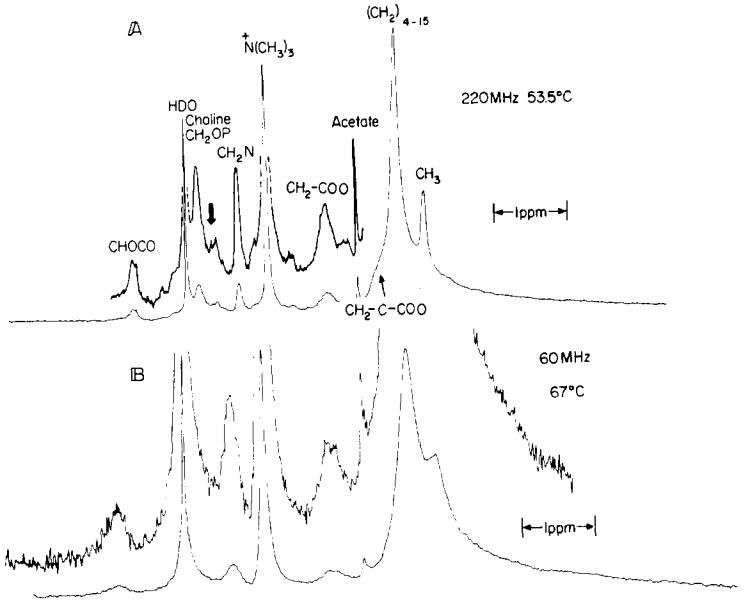

Fig. 1. NMR spectra of sonicated aqueous dispersions of dipalmitoyllecithin (36.6 mM) above the thermal transition: (A) at 220 and (B) at 60 MHz. Peak assignments were made according to ref. 5. The origin of the two scales is chosen in order to bring the acetate peaks (internal reference) into coincidence; their respective ppm units are indicated. The broad arrow indicates the position of the peak which is tentatively assigned to the glycerol CH_2OP group (see text).

to the high-resolution spectrum is estimated to be about 90% of the corresponding proportion of the $N^+(CH_3)_3$ protons throughout the considered temperature range. The peak assignments follow those of Finer *et al.*[5] for egg yolk lecithin. The spectra at 220 MHz clearly show the signal due to the proton on the β-carbon of the glycerol backbone, CHOCO*, which had been previously obscured by a water sideband[5]. A rather broad peak is also detected in the region between the choline CH_2OP and CH_2N peaks (indicated in Fig. 1 by an arrow) and is tentatively attributed to one of the glyceride methylene groups. This resonance is also unobserved in previous work because of interfering water sidebands. The present spectra do not allow resolution of the two methylene peaks on the α- and γ-carbon positions of the glycerol moiety. However, a comparison of the chemical shift with that exhibited by a solution of dipalmitoyllecithin in [^{2}H]chloroform (see Table I) suggests that this signal should be assigned to the glycerol CH_2OP group; the glycerol CH_2OCO resonance is probably obscured by the choline CH_2OP signal. Chemical shifts (measured with respect to the terminal methyl peak) for [^{2}H]chloroform solutions of dipalmitoyllecithin and sonicated dispersions of dipalmitoyllecithin in ^2H_2O are shown in Table I. Previously reported results for egg yolk lecithin[5], comparing the chemical shifts for a solution in [^{2}H]chloroform and a sonicated dispersion in ^2H_2O, showed considerable chemical shift changes at the headgroup but not in the hydrocarbon core. The present data for dipalmitoyllecithin is consistent with these results. Moreover, under the same conditions, slight downfield shifts are observed for the dipalmitoyllecithin

* Nomenclature is taken from Finer *et al.*[5].

TABLE I

PROTON CHEMICAL SHIFTS (WITH RESPECT TO THE TERMINAL METHYL GROUP) OF DIPALMITOYLLECITHIN IN [^{2}H]CHLOROFORM SOLUTION AND IN SONICATED AQUEOUS DISPERSIONS

Positive chemical shifts correspond to lower field resonances with respect to the reference. The use of CH_3 as internal reference in the two systems is justified by the fact, demonstrated by Finer *et al.*[5], that the position of this peak appears unaltered in the transfer of egg lecithin from [^{2}H]chloroform to ^2H_2O (bilayer).

	*Chemical shift (ppm)**	
	[^{2}H]Chloroform (solution)	*^2H_2O (bilayer)*
$N^+(CH_3)_3$**	2.48	2.36
CH_2N	2.92	2.79
CH_2OP (choline)	3.41	3.39
CHOCO (glycerol)	4.29	4.36
CH_2OP (glycerol)	3.03	3.10
CH_2OCO (glycerol)	3.20	obscured by choline CH_2OP
CH_2–COO	1.40	1.46
$(CH_2)_n$	0.36	0.39
CH_3	0.00	0.00

* Error in shift measurements is ± 0.02 ppm.

** Peak assignments follow those by Finer *et al.*[5].

TABLE II

PROTON SPIN-LATTICE RELAXATION TIMES T_1(s) OF DIPALMITOYLLECITHIN IN AQUEOUS SONICATED SOLUTION

	^{13}C (25.1 MHz) 52 °C*	^1H (220 MHz)				^1H (60 MHz)			
		53.5 °C	66.5 °C	78 °C	92 °C	54 °C	69 °C	78 °C	92 °C
$^+N(CH_3)_3$	0.57	0.58	0.83	1.15	1.41	0.51	0.67	0.84	1.05
CH_2		0.48	0.74	0.83	1.27	0.36	0.45	0.47	0.85
CH_2		0.38	—**	0.62	1.14	—***	—	—	—
O									
O–P–$\overline{O}$									
O									
H_2C–CH–CH_2		0.48	0.54	0.47	0.54	—***	0.16	0.29	0.50
O O									
C=O C=O									
CH_2 CH_2	0.10	0.39	0.54	0.63	0.88	—***	0.37	0.22	0.50
CH_2 CH_2	0.52	—***	—	—	—	—***	—	—	—
$(CH_2)_{4-13}$ $(CH_2)_{4-13}$	0.55								
CH_2 CH_2	1.14	0.63	0.78	0.91	1.10	0.35	0.43	0.58	0.69
CH_2 CH_2	1.76								
CH_3 CH_3	3.34	0.92	1.45	1.75	3.03	—***	1.01	1.28	1.63

* J. C. Metcalfe *et al.*[17].

** Obscured by the water peak.

*** Resonance observed, but T_1 not determined.

resonances assigned to the CHOCO proton (0.07 ppm) and for the glycerol CH_2OP protons (0.07 ppm) (see Table I).

T_1 relaxation times were measured for the choline trimethylammonium group, $N^+(CH_3)_3$; the choline methylene group adjacent to the quaternary nitrogen, CH_2N; the choline methylene group adjacent to the phosphate group, CH_2OP; the proton on the β-carbon of the glycerol backbone, CHOCO; the first methylene group in the fatty acid chain, CH_2–COO; the bulk of the methylene groups in the fatty acid chain, $(CH_2)_n$; and the terminal methyl group of the fatty acid chain, CH_3. The T_1 values for these protons as a function of temperature at 60 and 220 MHz are shown in Table II.

The partial overlapping of the fatty acid terminal methyl peak with the bulk methylene band makes an accurate T_1 analysis for the methyl group difficult, especially at 60 MHz and lower temperatures. The errors in T_1 measurement vary with the signal-to-noise ratio of each peak, ranging from less than 5% for the major resonances (*i.e.* the choline trimethylammonium group) to roughly 20% for the minor resonances (*i.e.* the glycerol proton). Table II shows that the relative order of T_1 values for protons in different regions of the phospholipid molecule generally remains constant for both frequencies and all temperatures. The proton in the glycerol backbone has the shortest T_1 while T_1 increases on proceeding from the glycerol backbone along the polar head-group towards the choline trimethyl ammonium group or on proceeding down the fatty acid chain towards the terminal methyl group. The T_1 for the second methylene group in the fatty acid chain, CH_2–C–COO, was not determined throughout the complete temperature range as the ($\pi/2$, τ, $\pi/2$) radio frequency pulse sequence made it difficult to separate this group from the "bulk" methylene band, $(CH_2)_{4-15}$ (see Fig. 2A). However, the results of a single experiment at 71 °C (Fig. 2B) using the (π, τ, $\pi/2$) radio frequency pulse sequence show that the null point of the shoulder containing the second metyhlene group occurs at a shorter delay time, τ, than the null point for the central portion of the bulk methylene band. This implies that the T_1 for the second methylene group in the fatty acid is shorter than the T_1 for the bulk methylene groups. In principle, this analysis could be quantitatively extended to the whole

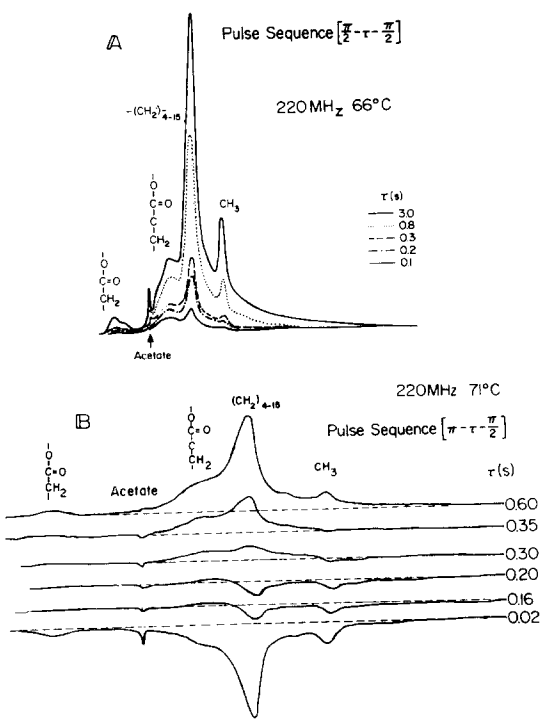

Fig. 2. Recovery of longitudinal magnetization of the various groups along the alkyl chain, $-(CH_2)_{2-15}$ and $-CH_3$, of dipalmitoyllecithin in sonicated aqueous dispersions studied at 220 MHz by means of: (A) radio frequency pulse sequence ($\pi/2$, τ, $\pi/2$); (B) radio frequency pulse sequence (π, τ, $\pi/2$).

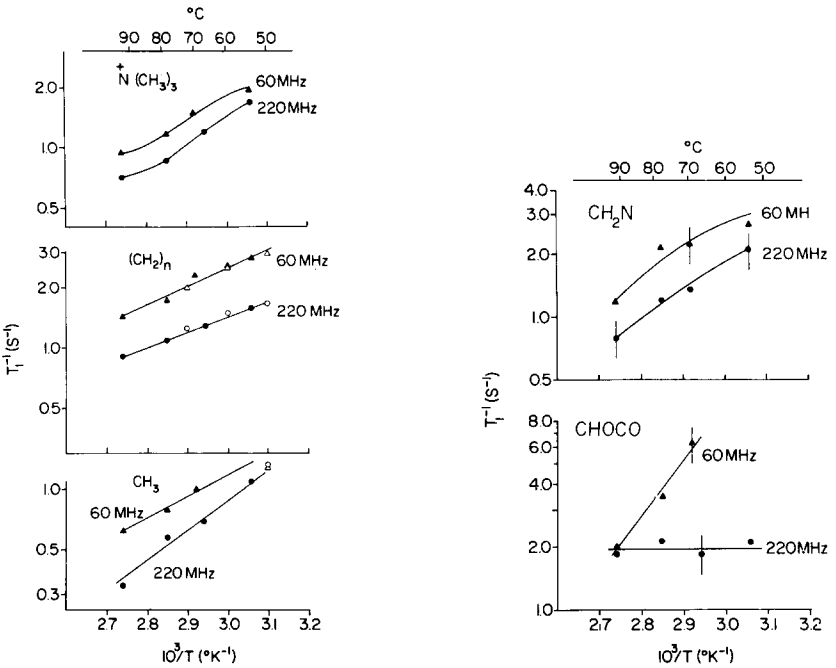

Fig. 3. Proton spin-lattice relaxation rates of the choline trimethylammonium group, $N^+(CH_3)_3$; the bulk of the methylene group of the fatty acid chain, $(CH_2)_n$; and the terminal methyl group, CH_3, for sonicated aqueous dipalmitoyllecithin at 220 and 60 MHz. Experimental errors are less than 5% for the choline trimethylammonium group and the methylene band, and about 20% for the terminal methyl signal. Empty and solid symbols refer to different experiments carried out on two different samples.

Fig. 4. Proton spin-lattice relaxation rates for the proton on the β-carbon of the glycerol backbone CHOCO, and the choline methylene group, CH_2N, for sonicated aqueous dispersions of dipalmitoyllecithin at 220 and 60 MHz.

methylene band. It would then be possible to determine if the central portion of the chain exhibits a distribution of T_1 values or a single T_1 value. For comparison, the T_1 relaxation times for carbon atoms at various positions in the phospholipid molecule, as determined by Metcalfe and coworkers[16,17], are shown in the first column of Table II.

The values of $1/T_1$ for the choline trimethylammonium group, the bulk methylene band, and the terminal methyl group of the fatty acid chain, taken from Table II, are plotted *versus* the reciprocal temperature for both frequencies in Fig. 3. It is seen that while the $1/T_1$ values have qualitatively the same temperature dependence at both frequencies, the absolute values of $1/T_1$ are quantitatively different throughout the entire temperature range. The difference between the relaxation rates measured at the two frequencies is greatest for the methylene protons (approx. 100%) and least for the choline trimethylammonium protons (approx. 20%). Fig. 4 shows a plot of $1/T_1$ for the proton on the β-carbon of the glycerol backbone at the two frequencies as a function of reciprocal temperature. The $1/T_1$ values at 220 MHz show no observable temperature dependence within the given error, while the $1/T_1$ values at 60 MHz increase substantially as the temperature is lowered.

TABLE III

NMR LINEWIDTHS OF DIPALMITOYLLECITHIN IN SONICATED AQUEOUS DISPERSIONS

	ν_0 (*MHz*)	*Temperature range* (°C)	$\Delta\nu_{1/2}$*
$N^+(CH_3)_3$**	220	54–92	10– 5
	60		6– 5
CH_2N	220	54–92	20–14
	60		18–14
CHOCO	220	67–92	26–20
	60		17–15
CH_2COO	220	67–92	30–28
	60		- -
$-(CH_2)_{4-15}-$	220	54–92	33–18
	60		30–14
CH_3	220	67–92	16–15
	60		11– 8

* Accuracy of linewidth measurements is approx. 2 Hz.
** Assignments follow those of Finer *et al.*[5].

The range of observed linewidths, $\Delta\nu_{1/2}$, for the given ranges of temperature for various dipalmitoyllecithin resonances in aqueous sonicated dispersions are shown in Table III. T_2 values cannot be simply determined from measured linewidth values as all of the peaks, except perhaps the $N^+(CH_3)_3$ peak, are expected to have extensive unresolved multiplet structure. In fact, the triplet structure of the terminal methyl group, (CH_3), can be resolved at higher temperatures (78 °C). However, T_2 cannot be determined for the $N^+(CH_3)_3$ peak from the measured linewidth as this resonance is known to consist of two peaks[18].

DISCUSSION

The chemical shifts reported in Table I provide an indication of the solvent effects on proton groups in different regions of the lecithin molecule. In particular they offer some information on the molecular arrangement of the bilayer. The upfield shifts exhibited by the $N^+(CH_3)_3$ and CH_2N peaks in the comparison of a dipalmitoyllecithin solution in [^{2}H]chloroform to a dipalmitoyllecithin dispersion in ^2H_2O can be attributed to the effect of two different reaction fields[19] created by the polar headgroups, which are located on the surface of the bilayers in aqueous dispersions, but in the interior of the inverted micelles[20] formed in [^{2}H]chloroform. Similarly the downfield shifts exhibited by the glycerol CHOCO and CH_2OP peaks and the first methylene group in the fatty acid chain suggest that water probably penetrates into the bilayer up to the level of the glycerol backbone. In agreement with Finer's results[5] on egg lecithin, the fatty acid chains appear exposed to a nonpolar environment in both solvent systems. The results, which are also in agreement with analogous hydration studies on micellar systems formed by other amphipatic molecules such as detergents[21], show that the degree of water penetration into the hydrophobic core of these aggregates is very little, if any.

The usefulness of T_1 and T_2 measurements on individual protons in the phospholipid molecule arises from the sensitivity of magnetic relaxation times to the detailed motion of neighboring magnetic dipoles (such as protons on the same or adjacent carbon atoms). The time dependence of different modes of motion of neighboring protons may be generally characterised by distinct correlation times, τ_{ci}. However, if it is naively assumed that the motion of near-by protons can be described by a single isotropic correlation time, τ_I, the contribution to the relaxation rate, $1/T_1$, from the motion of equivalent near-by protons can be described by the equation[22,23]:

$$1/T_1 = \frac{nA}{r^6} f_1(\tau_I) \tag{1}$$

where:

$$f_1(\tau_I) = \frac{\tau_I}{1+\omega^2\tau_I^2} + \frac{4\tau_I}{1+4\omega^2\tau_I^2} \simeq \frac{5\tau_I}{1+2.5\omega^2\tau_I^2},$$

r is the distance between the observed proton and the proton whose motion affects the relaxation rate, A is a known numerical constant, n is the number of equivalent neighboring protons, and ω is the radio frequency of the spectrometer.

The only source of temperature dependence in Eqn 1 arises from the temperature dependence of the correlation time, τ_I[23]. As the value of τ_I is increased the value of $1/T_1$ increases, reaches a maximum value, and then decrases; the maximum value of $1/T_1$ occurs at the value $\tau_I = 1/1.6\omega$. The region $\tau_I \ll 1/\omega$ (the "short-correlation-time" limit) is easily experimentally distinguished by the observed positive temperature dependence of $1/T_1$. Eqn 1 shows that in this limit T_1 is inversely proportional to τ_1.

Since the observed positive temperature dependence of T_1 (see Fig. 3) suggests the "short-correlation-time" limit (*i.e.* the direct proportionality of T_1 to $1/\tau_I$) it is tempting to interpret the relative differences among T_1 values (see Table II) as indicating relative differences in the correlation times in different regions of the phospholipid molecule. Using this criterion, the data in Table II imply that in the bilayer structure the glycerol is the most rigid part of the phospholipid molecule, with the mobility increasing along the polar headgroup up to the choline trimethylammonium group, and along the fatty acid chain down to the terminal methyl group. The relative order of T_1 values (and thus, by inference, the relative mobility) for protons in different regions of the phospholipid molecule is the same as the relative order of T_1 values for ^{13}C nuclei in different regions of the phospholipid molecule, as found by Metcalfe *et al.*[17]. The broader linewidths for the glycerol proton and the first methylene group in the fatty acid chain, as compared to the choline protons, could also be qualitatively interpreted in the same model[24] as implying that the mobility is lowest for the protons in the glycerol backbone region. It is interesting to note that the linewidth of the bulk methylene band of the fatty acid chain is the same at 60 and 220 MHz, in contrast to the apparent frequency dependence of the linewidth of this band in unsonicated dipalmitoyllecithin dispersions[6].

A less equivocal method for determining relative and absolute values of correlation times may be the frequency dependence of T_1 (ref. 25). The experimental results indicate that T_1 is different for the two frequencies (see Fig. 3). The temperature dependence at both frequencies, however, indicates the "short-correlation-time"

region, for which the simple model assuming only one isotropic correlation time predicts that T_1 should be independent of frequency (see Eqn 1). It is necessary to decide, then, what assumptions have to be added to the simple model in order to qualitatively interpret the observed frequency dependence.

The most obvious limitation of the simple model discussed above is the assumption that the motion determining T_1 is isotropic. The effect of anisotropic motion on T_1 relaxation times of small molecules in the solid phase has been discussed by Stejskyl *et al.*[26] and Woessner[27]. If the correlation times, τ_{ci}, for each distinct anisotropic motion are sufficiently different $f_1(\tau_c)$ in Eqn 1 may be approximately replaced by the sum, $\Sigma\alpha_i f_1(\tau_{ci})$, where the amplitude factors, α_i, will generally be of the same order of magnitude but will be sensitive to the degree of anisotropy of each respective motion. Their data for the temperature dependence of T_1 for *tert*-butyl chloride demonstrated two clear minima, as would be expected if two correlation times were involved in the anisotropic motion. They ascribed these two anisotropic motions to the rotation of individual methyl groups about each respective carbon–carbon axis, and the rotation of the tertiary butyl group around the carbon–chlorine axis[27].

The application of this theory to complicated systems such as phospholipids requires considerable caution. If the T_1 of a specific proton in the dipalmitoyllecithin molecule were dominated by an interaction with one particular proton it would then be feasible to quantitatively apply the above theory to determine the rates of each distinct anisotropic motion available to this proton. Also, the degree of anisotropy of each motion could be determined. An example of two different anisotropic motions which could contribute to T_1 relaxation times would be the restricted rotation of the methylene group about the hydrocarbon chain axis, as contrasted to the reorientation of that portion of the hydrocarbon chain axis. However, theoretical calculations on model hydrocarbon systems indicate that, in general, one interaction does not substantially predominate. Andrew and Eades[28] have calculated from the crystal structure of *n*-hexane that less than 50% of the dipolar interactions of a methylene proton with all of its neighboring protons arises from the interactions with the geminal protons. The remaining 50% arises from intermolecular interactions between protons on adjacent hydrocarbon chains and intramolecular interactions between protons on adjacent carbon atoms in the same hydrocarbon chain. Without a detailed model to explicitly take into account all of these different interactions in the phospholipid system it is impossible to rigorously treat the effects of anisotropic motion on T_1. It is worthwhile, however, to incorporate anisotropy into a drastically simplified model to ascertain if the assumption can qualitatively explain the observed experimental data.

The simplest assumption which incorporates anistropy is that the motion of any individual proton in the phospholipid molecule can be characterized by two correlation times. To simplify even further, it is assumed that the T_1 relaxation time of any individual proton is dominated by the interaction with its nearest neighbor (in most cases this will be a geminal proton). The above theory then predicts that the observed relationship between $1/T_1$ and $1/T$ should be reasonably approximated by the sum of two of the usual $f_1(\tau_c)$ curves (see Eqn 1). The relative position of the two maxima will depend on the relative values of the two correlation times; the relative amplitude at the two maxima (*i.e.* the relative values of $1/T_1$ at the two

maxima) will depend on the degree of anisotropy inherent in each motion. Since frequency dependence will be observed on the "long-correlation-time" side (the "low-temperature" side) of each of the two maxima, the region between the two maxima will, in general, be frequency dependent. Some theoretical curves, drawn for different ratios of the two correlation times, with roughly equal amplitudes, are shown in Fig. 5. For simplicity, the same activation energy has been assumed for the two motions. It is clear that the qualitative features of the data shown in Fig. 4 can easily be reproduced by appropriate choices for the values of the two correlation times and the two amplitude factors.

Since with only two correlation times there are at least four adjustable parameters it is difficult to extract unique values for the correlation times from the limited range of data given in Fig. 4. However, the T_1 of the glycerol proton appears to have no temperature dependence at 220 MHz (see Fig. 5). This behavior is characteristic of being at one of the maxima of the $f_1(\tau_1,\tau_2)$ curve. The "short" correlation time can then be calculated as the reciprocal of the observation frequency, giving $\tau_1 \simeq 7 \cdot 10^{-10}$ s. The temperature dependence of the relaxation time for the other protons suggests that the "short" correlation time for each of the other protons is less than

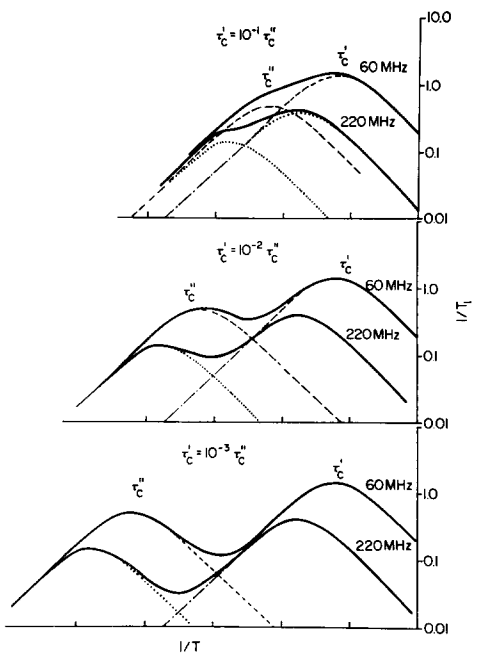

Fig. 5. Schematic theoretical curves representing the expected temperature dependence of the spin-lattice relaxation rates at 60 and 220 MHz for anisotropic motion of a proton pair (———). The anisotropic motion is characterized by two correlation times: τ_c' and τ_c''. The dashed lines represent the separate contributions from the fast (τ_c') and slow (τ_c'') anisotropic motions, respectively, at 60 MHz. The dotted lines represent the separate contributions from the fast (τ_c') and slow (τ_c'') anisotropic motions, respectively, at 220 MHz. The solid lines represent the sum of the two contributions from the fast and slow motions for each respective frequency. For simplicity, equal activation energies and roughly equal relative amplitudes are assumed for the contributions from the two motions.

10^{-9} s. All that can be said about the "longer" correlation time for each proton is that it appears to be substantially (at least one order of magnitude) greater than 10^{-9} s. Because of the extreme simplicity of the model it is difficult to put meaningful physical interpretations on these two correlation times. The pair of correlation times could be two distinct anisotropic correlation times; for instance, different characteristic times for restricted methylene rotation and segmental motion of the hydrocarbon chain, respectively. It could also be a mixture of anisotropic and isotropic correlation times; for instance, different characteristic times for restricted methylene rotation and vesicle tumbling, respectively. One possible interpretation of the "short" correlation time for the glycerol proton ($7 \cdot 10^{-10}$ s) could be the rotation time of the whole phospholipid molecule about the long axis.

From the observed frequency dependence of the phospholipid T_1 values it can be concluded that in order to adequately describe the anisotropic motion of any specific proton at least two correlation times must be considered. The longest of these correlation times (providing that it is shorter than the reciprocal of the solid-state linewidth) may dominate the T_2 relaxation time, while the correlation times in the region $\tau \simeq 1/\omega$ will tend to dominate the T_1 relaxation time[23,29]. The conclusions of Horwitz *et al.*[11] that T_1 and T_2 are determined by different correlation times are therefore consistent with the above model. With the data presently available it is, however, impossible to assign these correlation times to specific modes of motion in the phospholipid molecule. Since activation energies have been used to determine the specific motion governing T_1 it is important to note that if T_1 is a function of two correlation times the observed activation energy may not correspond to the real activation energy for either of the two processes, even in an apparently linear region of the Arrhenius plot (see Fig. 5). Also, with limited data it is difficult to unequivocally interpret changes in T_1 in terms of specific changes in molecular motions. Because T_1 appears to be determined by multiple correlation times a change in T_1 will be a complicated function of changes in all the relevant correlation times, as well as changes in the geometrical factors concerning the anisotropy of motion.

Perhaps the most interesting question is whether the apparent anisotropy is an inherent property of the phospholipid molecule, or is due to the anisotropic alignment of the phospholipid molecules in the membrane. These considerations are currently being investigated by a comparison of the frequency dependence of T_1 for aqueous solutions of fragments of phospholipid molecules (such as choline or glycerol-phosphorylcholine) to the frequency dependence of T_1 for these components in intact bilayer membranes.

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