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PROTON MAGNETIC RESONANCE STUDIES OF LIPID BILAYER MEMBRANES

EXPERIMENTAL DETERMINATION OF INTER- AND INTRAMOLECULAR NUCLEAR RELAXATION RATES IN SONICATED PHOSPHATIDYLCHOLINE BILAYER VESICLES

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SUMMARY

We have determined the relative magnitudes of the intra- and intermolecular contributions to the nuclear magnetic relaxation rates of the methylene protons of the hydrocarbon chains in phosphatidylcholine bilayer vesicles over a range of temperatures and at two NMR frequencies (100 and 220 MHz). These measurements have been made by the isotopic dilution method using deuterated phosphatidylcholines containing fully deuterated hydrocarbon chains. The results showed that both the methylene linewidths and the spin-lattice relaxation rates are dominated by intramolecular dipolar interactions. Both the intra- and intermolecular contributions to the spin-lattice relaxation rate were found to decrease with increasing temperature and to exhibit a frequency dependence, the rates being higher at the lower NMR frequency in both cases. These observations indicate that both intra- and intermolecular dipolar interactions are modulated by anisotropic motions. In the case of the intermolecular dipolar fields, it is proposed that they are modulated both by the rapid rotational isomerization of the hydrocarbon chains as well as by lateral diffusion of the lipid molecules. That the hydrocarbon chain motion must be fairly effective in effecting efficient spin-lattice relaxation is evident from the negligible intramolecular interchain contribution to the relaxation found in the present work.

INTRODUCTION

Recent interest in the structural and dynamical properties of biological membranes has led to a number of nuclear magnetic relaxation studies of phosphatidyl-

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choline multilayers and sonicated bilayer dispersions [1–5]. In principle, nuclear relaxation measurements offer a powerful approach to elucidate the motional state of these systems. However, owing to complications from motional restriction [1, 5] and motional anisotropy [1, 2, 4, 5], the interpretation of these data in terms of motional characteristics is far from straightforward. In addition, there is the question of the relative magnitudes of the intramolecular and intermolecular dipolar fields on the nuclear magnetic relaxation rates, particularly in the case of proton magnetic resonance measurements, and the role which the rapid lateral diffusion of the phospholipid molecules play in modulating these dipolar fields. Several groups of workers have assumed or argued that the relaxation rates are dominated by intramolecular effects and have interpreted their relaxation results accordingly [1, 2, 5]. While this assumption is certainly reasonable for the transverse relaxation rate, T_2, in phosphatidylcholine multilayers, where the intramolecular dipolar interactions are not completely averaged out spatially [5], the validity of this assumption is less obvious for the longitudinal relaxation rate and for sonicated bilayer vesicles. Lee et al. [3] have stressed the importance of the intermolecular contribution to both the proton magnetic resonance linewidths and T_1 values. In fact, these workers have gone so far as to argue that the proton magnetic resonance linewidths in these systems may provide a lower limit to the surface diffusion coefficients of the lipid molecules, and on the basis of this assumption, have obtained estimates of the lateral diffusion coefficients of the lipid molecules in bilayer vesicles as well as for several intact biological membranes.

These conflicting views can only be resolved via experimental determination of the relative magnitudes of the inter- and intramolecular contributions to the nuclear magnetic relaxation rates. Towards this goal, we have synthesized a number of deuterated phosphatidylcholines [6]. The various relaxation contributions to the methylene linewidth and T_1 can be effectively sorted out by isotopic "dilution" experiments with deuterated hydrocarbon chains. For example, if we take a phosphatidylcholine molecule and surround it by phosphatidylcholine molecules with deuterated hydrocarbon chains, we reduce the intermolecular contribution to the methylene linewidth and the longitudinal relaxation rate by a factor of approx. 16. In the process of this study, we have also prepared phosphatidylcholine molecules where one of two hydrocarbon chains has been replaced by a fully deuterated chain, so that the interchain contribution to the intramolecular relaxation rates can also be determined.

It should be stated that a similar attempt to separate the intermolecular and intramolecular contributions to the proton magnetic relaxation rates by isotopic dilution has previously been carried out by Lee et al. [3]. Unfortunately, these data were limited to one temperature, and were subject to such large experimental uncertainties that definite conclusions would have been unwarranted. Our present studies differ from the earlier work of Lee et al. in at least one important respect. Not only are the data we are presenting here more precise and extensive, but we have also taken precautions to ensure the reproducibility of the lipid dispersions used. These latter controls were apparently not taken in the earlier work.

EXPERIMENTAL

Materials

Normal 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (dipalmitoyl phosphatidylcholine) was obtained from General Biochemicals. Deuterated phospholipids were synthesized using known procedures [7]. 1,2-Diperdeuteropalmitoyl-*sn*-glycero-3-phosphorylcholine (dipalmitoyl phosphatidylcholine-d_{62}) was prepared by acylating *sn*-glycero-3-phosphorylcholine with perdeuteropalmitic anhydride. 1-Palmitoyl-2-perdeuteropalmitoyl-*sn*-glycero-3-phosphorylcholine (dipalmitoyl phosphatidylcholine-d_{31}) was synthesized from 1-palmitoyl-*sn*-glycero-3-phosphorylcholine (lysophosphatidylcholine) and perdeuteropalmitic anhydride. The *sn*-glycero-3-phosphorylcholine was obtained from egg-yolk lecithin [8] which was extracted from commercial heneggs. Lysophosphatidylcholine was prepared enzymatically from dipalmitoyl phosphatidylcholine [9]. The fatty acid anhydrides were made using dicyclohexyl carbodiimide [10]. Perdeuteration (98.8 %) was achieved catalytically [11]. The high deuterium content was a result of two successive deuterations. The extent of deuteration was estimated from a comparison of the methyl and chain proton NMR resonances of the methyl ester of perdeuteropalmitic acid. All lipids were extensively purified by silicic acid chromatography.

Sample preparation

Samples containing deuterated dipalmitoyl phosphatidylcholine and protonated dipalmitoyl phosphatidylcholine were prepared by dissolving weighed amounts of the lipids in chloroform in a 3 ml centrifuge tube. Most of the chloroform was removed by placing the centrifuge tube in a sand bath at approx. 50 °C and directing a stream of dry nitrogen into the tube. The remaining viscous solution was frozen in liquid nitrogen and dried overnight under high vacuum. ^2H_2O (1 ml) was added to the sample, which was then sonicated for 20 min using an MSE 150 W ultrasonic disintegrator at high power. During sonication the sample was partially immersed in a glycerol cooling bath to avoid overheating. Thin-layer chromatography revealed no degradation of the dipalmitoyl phosphatidylcholine during sonication under these conditions. Samples containing a single lipid component were prepared in the same way, except that the chloroform step was omitted. All samples contained 5 % (weight/volume) of lipid.

Instrumentation

220 MHz NMR measurements were made using a Varian HR-220 spectrometer equipped with a Fourier transform accessory and interfaced with a Varian 620i 16 K computer. Measurements at 100 MHz were made using a Varian XL-100 system equipped with Fourier transform accessories. The probe temperature in either case was regulated (± 1 °C) by a Varian 4540 variable temperature unit. The probe temperature was monitored by measuring the ethyleneglycol splitting. Temperature calibration charts were prepared using a copper-constantan thermocouple.

T_1 measurements were made using the (π-τ-$\pi/2$) inversion recovery sequence. Typically 200 transients were collected for samples containing normal dipalmitoyl phosphatidylcholine dispersed in deuterated dipalmitoyl phosphatidylcholine. T_1 values were calculated from the amplitude of each line in the partially relaxed spec-

trum by plotting ln $(M_z(\infty)-M_z(\tau))$ versus τ. The slope of this plot is $-1/T_1$. The T_1 values are accurate and reproducible to within 5 %.

The T_1 values reported here are for samples which had not previously been deoxygenated. Comparison of these results with those obtained for deoxygenated samples showed that the effects of dissolved oxygen on the T_1 values are within the experimental errors inherent in these measurements. The presence of traces of oxygen in the sample is not expected to affect the proton NMR linewidths.

300 MHz PMR spectra were obtained on a Varian SC-300 spectrometer, courtesy of Varian Associates, Palo Alto, California. 360 MHz PMR spectra were recorded on a Bruker HX-360 spectrometer at the Stanford Magnetic Resonance Facility.

RESULTS

A meaningful comparison of the methylene linewidths and T_1 values* of dipalmitoyl phosphatidylcholine dispersed in a protonated vs. a deuterated lipid matrix is valid only under the conditions that (i) the presence of the deuterated dipalmitoyl phosphatidylcholine does not change the nature of the bilayer, and (ii) random mixing of the deuterated and protonated phospholipids occurs. The first question is of concern since the bilayer owes part of its stability to the van der Waals interaction between the hydrocarbon chains, which may be different for deuterated chains. Actually, the phase transition temperature of dipalmitoyl phosphatidylcholine-d_{62} is about 4 °C lower than that of normal dipalmitoyl phosphatidylcholine [6]. Thus, the van der Waals interaction between deuterated chains is slightly weaker than that between protonated chains. However, this difference is probably too small to cause pronounced changes in the bilayer structure except near the phase transition. The second question is important since the intermolecular effects in question can be ascertained only if the deuterated and protonated phospholipid molecules mix randomly. Recent differential thermal analysis experiments have shown that phospholipid molecules containing deuterated and protonated chains do in fact form ideal solutions, as expected [6].

Experimentally, the proton T_1 values of the choline methyl groups can be used to monitor the physical state of the bilayer vesicles, particularly the size distribution of the vesicle dispersion. Previous work from this laboratory has shown that these T_1 values are sensitive to the size distribution of the bilayer vesicles, an observation which has been interpreted in terms of the effect of surface curvature on the spatial packing of the headgroups. In Fig. 1, we compare these choline methyl T_1 values at 220 MHz between two vesicle dispersions, one of normal dipalmitoyl phosphatidylcholine and the other of 10 % normal dipalmitoyl phosphatidylcholine dispersed in dipalmitoyl phosphatidylcholine-d_{62}, prepared otherwise under identical conditions. The results here show that these T_1 values are the same between the two systems within experimental error, suggesting that the two bilayer dispersions have the same

* It should be pointed out that the various CH_2 groups of the fatty acid chains give rise to an envelope of overlapping resonances and the observed changes in the proton NMR linewidths and T_1 values caused by ^2H dilution represent an average change for all the CH_2 groups which contribute to the bulk methylene signal.

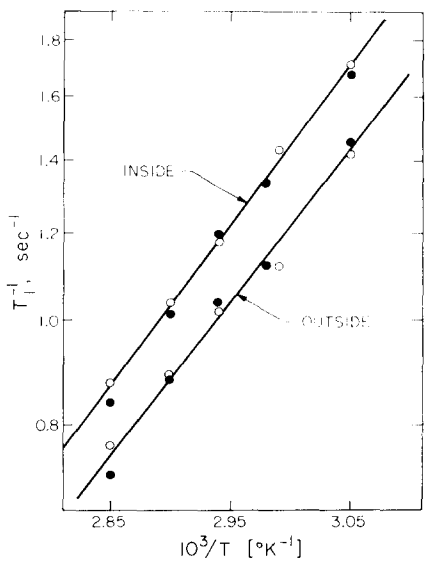

Fig. 1. Spin-lattice relaxation rates ($1/T_1$) at 220 MHz as a function of reciprocal temperature of the choline methyl protons for the inner and outer monolayers of small sonicated phosphatidylcholine bilayer vesicles. ●, normal dipalmitoyl phosphatidylcholine; ○, 10 % dipalmitoyl phosphatidylcholine dispersed in 90 % dipalmitoyl phosphatidylcholine-d_{62}.

physical state. Note the difference in T_1 values between the choline headgroups on the two halves of the bilayer membrane. This T_1 difference is probably a manifestation of the different packing of the headgroup moieties on the inner and outer monolayers. In a small bilayer vesicle, the inner and outer bilayer surfaces have quite different surface curvatures [12], both in magnitude and sign, and the outside cholines are expected to be somewhat more loosely packed than the inside choline groups. Accordingly, the outside cholines should exhibit greater mobility and a longer T_1 than the inside cholines, as observed.

Intermolecular and interchain contributions to the linewidth

In order to obtain an estimate of the intermolecular contributions to the methylene linewidth we have compared the spectrum of dipalmitoyl phosphatidylcholine in a protonated dipalmitoyl phosphatidylcholine matrix with that of dipalmitoyl phosphatidylcholine dispersed in a deuterated dipalmitoyl phosphatidylcholine matrix. Fig. 2 shows the results at 100 and 220 MHz and over a temperature range of 50–90 °C.

We note that the 100 MHz linewidths are smaller in dipalmitoyl phosphatidylcholine-d_{62} than in a normal dipalmitoyl phosphatidylcholine matrix, especially at low temperatures. The 220 MHz data are somewhat surprising in that the linewidths are the same for both systems. It must be remembered, however, that the methylene signal is somewhat magnetic-field dependent, owing to dispersion of chemical shifts for these protons, and the larger chemical shift broadening at 220 MHz tends to mask the small intermolecular contribution to the linewidth. This phenomenon is clearly evident at 300 and 360 MHz, where the greater dispersion of chemical shifts at these

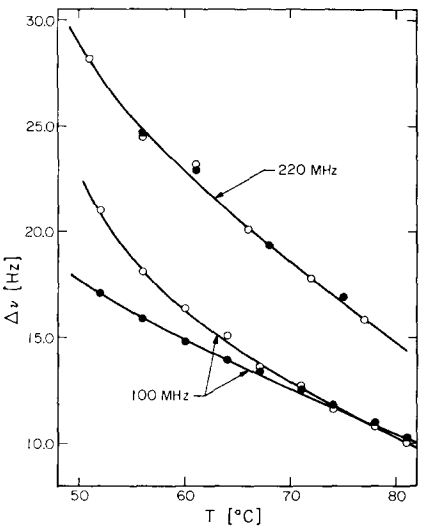

Fig. 2. Observed linewidths of the hydrocarbon chain methylene proton signal for small sonicated phosphatidylcholine bilayer vesicles as a function of temperature and at NMR frequencies of 100 MHz and 220 MHz. ○, normal dipalmitoyl phosphatidylcholine; ●, 10 % dipalmitoyl phosphatidylcholine dispersed in 90 % dipalmitoyl phosphatidylcholine-d_{62}.

higher frequencies of observation lead to asymmetric lineshapes for the methylene signal (Fig. 3). At these higher magnetic fields, the intermolecular contributions to the linewidth were also found to be small, no more than 3–4 Hz at approx. 50 °C, and we consider these data to be barely outside of our error of measurements. In Fig. 4, we have plotted the observed apparent linewidth of the methylene signal for small dipalmitoyl phosphatidylcholine bilayer vesicles as a function of NMR frequency and at two temperatures, 50 and 80 °C. Extrapolation of these data to zero NMR frequency (magnetic field) yields values of approx. 18 and approx. 8 Hz for the homogeneous part of the methylene linewidth at the two temperatures respectively.

Interchain contributions, i.e. contributions to the linewidth from the hydrocarbon chain belonging to the same phosphatidylcholine molecule, were examined by comparing the spectrum of dipalmitoyl phosphatidylcholine with that of dipalmitoyl

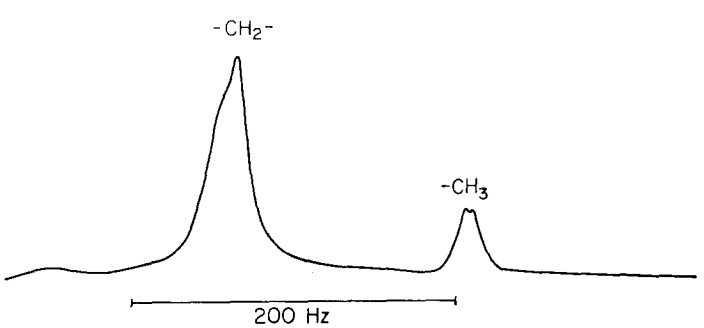

Fig. 3. 360 MHz spectrum of small sonicated dipalmitoyl phosphatidylcholine bilayer vesicles at 80 °C.

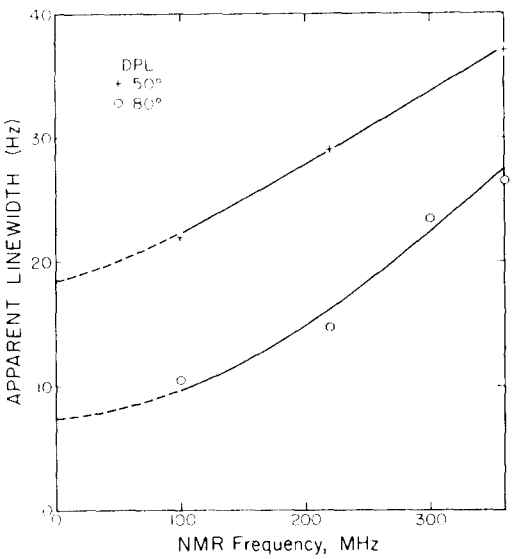

Fig. 4. Observed linewidths of the hydrocarbon chain methylene proton signal for small sonicated dipalmitoyl phosphatidylcholine bilayer vesicles as a function of NMR frequency at two temperatures.

phosphatidylcholine-d_{31}, both dispersed in a dipalmitoyl phosphatidylcholine-d_{62} matrix. The percentage of dipalmitoyl phosphatidylcholine-d_{31} used (20 %) was twice that of dipalmitoyl phosphatidylcholine (10 %) to keep the ratio of protonated

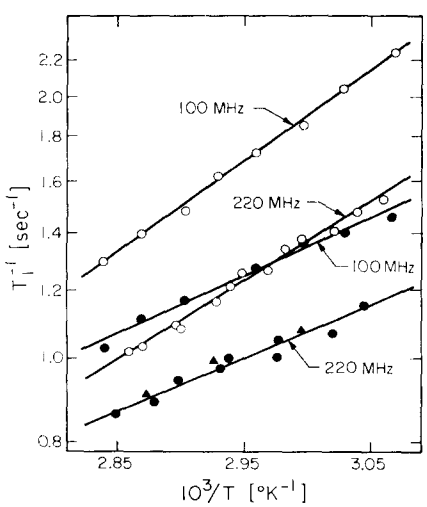

Fig. 5. Spin-lattice relaxation rates ($1/T_1$) of the hydrocarbon chain methylene protons for small sonicated phosphatidylcholine bilayer vesicles as a function of reciprocal temperature and at NMR frequencies of 100 and 220 MHz. ○, normal dipalmitoyl phosphatidylcholine; ●, 10 % dipalmitoyl phosphatidylcholine dispersed in 90 % dipalmitoyl phosphatidylcholine-d_{62}; ▲, 20 % dipalmitoyl phosphatidylcholine-d_{31} dispersed in 80 % dipalmitoyl phosphatidylcholine-d_{62}.

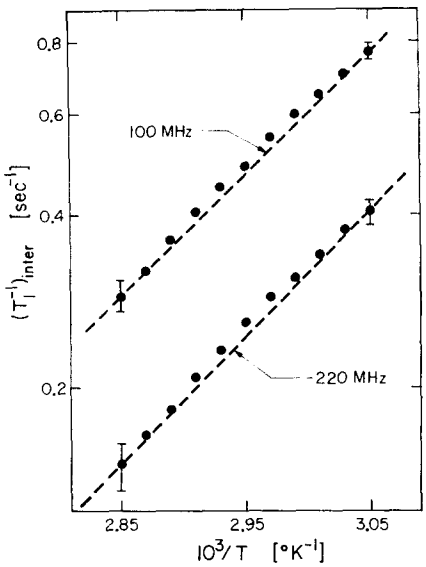

Fig. 6. Intermolecular contribution to the spin-lattice relaxation rates of the hydrocarbon chain methylene protons of small sonicated dipalmitoyl phosphatidylcholine vesicles as a function of reciprocal temperature and at two NMR frequencies.

to deuterated chains constant. Within experimental error no interchain contribution was detected.

Intermolecular and interchain contributions to T_1

A comparison of the hydrocarbon methylene T_1 values for dipalmitoyl phosphatidylcholine in a protonated dipalmitoyl phosphatidylcholine matrix with those of dipalmitoyl phosphatidylcholine-d_{62} matrix is shown in Fig. 5. Several points are noteworthy in these data. First, the intermolecular contribution is small, at most 30 %. Secondly, both the inter- and the intramolecular relaxation rates are frequency dependent. Thirdly, both the inter- and the intramolecular contributions to the relaxation rates decrease with increasing temperature (see also Fig. 6).

Interchain contributions to T_1, obtained by comparing the T_1 values of dipalmitoyl phosphatidylcholine-d_{31} with those of normal dipalmitoyl phosphatidylcholine, both dispersed in a dipalmitoyl phosphatidylcholine-d_{62} matrix, are small (Fig. 5). In fact, no effect is observed within experimental error.

Corrections to the intermolecular and interchain contributions to the relaxation rates for finite concentrations of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine-d_{31}

In order to obtain the true intermolecular contributions to the linewidths and T_1 values, the data reported in the previous sections must be corrected for the finite concentration of protonated molecules used in the measurements. This correction can be made as follows.

The phospholipid molecules in a bilayer are believed to be arranged in such a way that their fatty acid chains form a hexagonal lattice. Accordingly, a particular

fatty acid chain is surrounded by six other chains, one of which belongs to the same lipid molecule. In the case of measurements on dipalmitoyl phosphatidylcholine, this will be a protonated chain, whereas in the case of measurements on dipalmitoyl phosphatidylcholine-d_{31}, it will be a deuterated chain. Of the remaining five lattice sites, each can be treated as a random variable, i.e. it can be occupied by a protonated or deuterated chain. The expectation value for each of these sites to be occupied by a protonated chain is given by f, where f is the fraction of protonated hydrocarbon chains in the mixture. Since the expectation value of a sum of random variables is equal to the sum of the respective expectation values for each lattice site*, the total expectation value for the five lattice sites to be occupied by protonated chains is $5f$. The observed relaxation rate $(1/T_1)_{obs}$ can then be expressed in terms of the intermolecular relaxation contribution per chain, $(1/T_1)_{inter/chain}$, as follows

$$\left(\frac{1}{T_1}\right)_{obs} = \left(\frac{1}{T_1}\right)_{intra} + 5f\left(\frac{1}{T_1}\right)_{inter/chain} \tag{1}$$

for a given f. The experimentally measured difference in the relaxation rates $\Delta(1/T_1)_{obs}$ between protonated and deuterated matrices is therefore

$$\Delta\left(\frac{1}{T_1}\right)_{obs} = 5(1-f)\left(\frac{1}{T_1}\right)_{inter/chain} \tag{2}$$

from which it follows that the intermolecular contribution to the relaxation rate for normal dipalmitoyllecithin is

$$\left(\frac{1}{T_1}\right)_{inter} = \frac{1}{(1-f)}\Delta\left(\frac{1}{T_1}\right)_{obs} \tag{3}$$

In our experiments on dipalmitoyl phosphatidylcholine dispersed in dipalmitoyl phosphatidylcholine-d_{62}, $f = 0.1$, so that the correction factor to the measured $\Delta(1/T_1)_{obs}$ is 10/9. These corrected intermolecular T_1 contributions are plotted in Fig. 6 as a function of temperature at two NMR frequencies. We have not corrected the relaxation rates for dipole-dipole interactions with deuterated hydrocarbon chains. This correction is of the order of

$$\frac{\gamma_{^2H}^2 I_{^2H}(I_{^2H}+1)}{\gamma_H^2 I_H(I_H+1)} \approx 6\,\% \tag{4}$$

All of the above arguments obviously hold for T_2 or the linewidth as well.

In our experiments on dipalmitoyl phosphatidylcholine-d_{31} dispersed in dipalmitoyl phosphatidylcholine-d_{62}, the fraction of protonated chains was chosen to be 0.1, so that the observed difference in the relaxation rates between 20 % dipalmitoyl phosphatidylcholine-d_{31} in dipalmitoyl phosphatidylcholine-d_{62} vs. 10 % dipalmitoyl phosphatidylcholine in dipalmitoyl phosphatidylcholine-d_{62} gives the interchain contribution to the intramolecular relaxation rates.

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* We thank Professor Gary A. Lorden of Caltech for pointing out this result to us.

DISCUSSION

We have determined experimentally in this work the intermolecular contributions to the linewidths and T_1 values for dipalmitoyl phosphatidylcholine vesicles. These relaxation parameters have been obtained as a function of temperature and at two NMR frequencies.

The effects of intermolecular dipolar fields on the methylene linewidths have been found to be small. The intermolecular contribution decreases with increasing temperature and becomes essentially negligible at temperatures above approx. 60 °C. Thus the homogeneous part of the linewidths of sonicated phosphatidylcholine vesicles are dominated by intramolecular dipolar fields. The proton NMR linewidths, therefore, cannot provide a realistic lower-limit estimate of the self-diffusion coefficient of the lipid molecules in bilayer systems, contrary to the earlier suggestion of Lee et al. [3].

The spin-lattice relaxation rates for the hydrocarbon chain protons in these systems were also found to be dominated by intramolecular effects. Here the intermolecular contribution is not negligible, typically 20 %, but even under the least favorable circumstance (at 50 °C and at 100 MHz), it amounts to no more than one third of the overall relaxation rate. Both the intra- and intermolecular contributions to $(1/T_1)$ were found to decrease with increasing temperature. However, only the intramolecular component exhibits true Arrhenius behavior, with an apparent activation energy of approx. 3.1 kcal/mol for the T_1 data at both 100 MHz and 220 MHz. Similar presentations of the intermolecular relaxation rate vs. $1/T$ reveal some curvature in these plots (Fig. 6) although these data can probably also be fitted to a straight line if the large experimental error inherent in these measurements is included. If this is done, one obtains an apparent activation energy of approx. 10 kcal/mol for the intermolecular mechanism. Both components were also found to be frequency dependent. The rates were observed to be higher at the lower NMR frequency in both cases, but the observed frequency dependence was much more pronounced for the intermolecular contribution.

Proton magnetic relaxation results for sonicated bilayer vesicles have previously been interpreted by Horwitz and Klein [2] in terms of anisotropic chain motion, assuming that the relaxation is dominated by intramolecular dipolar effects. The intermolecular dipolar contribution has been shown to be small in this work, so the Horwitz-Klein analysis is substantially correct. However, since a frequency dependence is observed for $(1/T_1)_{\text{intra}}$, the longer of the two correlation times $\tau_\perp$ must be longer than $1/\omega_0$ or 10^{-9} s. In order to account for the observed temperature behavior of $(1/T_1)$, the shorter correlation time, $\tau_\parallel$, must be in the extreme narrowing limit, i.e. $\omega_0\tau_\parallel \ll 1$, and in fact be of the order of 10^{-10}–10^{-11} s. if the T_1 data are to be fitted. These results are in qualitative agreement with the earlier conclusions of Seiter and Chan [5]. The standard theories of NMR relaxation in the presence of anisotropic motion suggest that $(1/T_1)_{\text{intra}}$ should have the form [5, 13] $A\tau_\parallel + B\tau_\perp{}^{-1}\omega_0{}^{-2}$ in the limit of $\omega_0\tau_\parallel \ll 1$ and $\omega_0\tau_\perp > 1$. This expression predicts that $(1/T_1)\omega_0{}^2$ should vary linearly with $\omega_0{}^2$, which can be shown to be the case for phosphatidylcholine multilayers, if the T_1 frequency dependence data previously reported by Feigenson and Chan [1] are treated in this manner. In addition, this theory predicts that the frequency dependence of $(1/T_1)$ may become vanishingly

small at sufficiently high NMR frequencies. Preliminary T_1 measurements at 360 MHz on a sample of 10 % dipalmitoyl phosphatidylcholine dispersed in a dipalmitoyl phosphatidylcholine-d_{62} matrix showed that the spin-lattice relaxation rates at this frequency are almost identical within experimental error, to those reported here for 220 MHz, lending further evidence to the motional picture described here, and suggesting $\tau_\perp$ to be in the range of 10^{-9}–10^{-8} s.

The interpretation of $(1/T_1)_{inter}$ is unfortunately not straightforward and must await further developments in NMR relaxation theory. The analysis of these data is complicated by the fact that the intermolecular dipolar fields can be modulated both by the rapid rotational isomerization of the hydrocarbon chain as well as by lateral diffusion of the lipid molecules. We expect *trans-gauche* rotations about C-C bonds to provide important contributions to the spectral density at the Larmor frequency. Rather than single *trans-gauche* rotations, however, we envisage coupled rotations in which pairs of *gauche* configurations of opposite polarities occur on sites separated by one (β-coupled) or more carbon atoms. These conformations which are usually referred to as kinks and jogs, result in a roughly straight hydrocarbon chain and avoid the encounters with neighboring chains that result from single *gauche* conformations [2, 5, 14]. That these hydrocarbon chain motions must be fairly effective in effecting spin-lattice relaxation is evident from the negligible intramolecular interchain contribution to the relaxation rate demonstrated in this work. The temperature and large frequency dependences observed for $(1/T_1)_{inter}$ clearly indicate that the motion modulating the dipolar fields must be anisotropic. If these motions can be described by two correlation times, then our $(1/T_1)_{inter}$ data suggests that one correlation time is much shorter than 10^{-9} s and the other longer than this time scale. The obvious procedure here is to assign the faster motion to rotational isomerization of the hydrocarbon chain and the slower process to lateral diffusion of the lipid molecules. If this correspondence is taken, then the T_1 data suggest a lateral diffusion coefficient $D \ll 10^{-6}$ cm^2/s. On the other hand, if the shorter correlation time corresponds with the time scale of lateral diffusion of the lipid molecules, then the relaxation results imply a $D \gg 10^{-5}$ cm^2/s, a result which we consider highly unlikely. These conclusions are qualitative. Nevertheless, they permit us to set a definite reasonable upper limit to the lateral diffusion coefficient in sonicated bilayer systems. These considerations also underscore the futility of interpreting T_1 data for anisotropic systems without knowledge on the frequency dependence of T_1. In the particular case of lipid bilayer systems, it is clear that $(1/T_1)_{inter}$ does not afford a straightforward determination of the lateral diffusion coefficients of the lipid molecules.

Brûlet and McConnell [15] have recently worked out a theory to treat the effect of translational diffusion on the nuclear relaxation rates in bilayer membranes. This Brûlet-McConnell theory is a two-dimensional theory to mimic the two-dimensional lateral diffusion of the lipid molecules in the plane of the bilayers. We contend that this two-dimensional theory is inadequate here as it fails to take into consideration the contribution of intramolecular rotatory-type motions to the spectral density at the Larmor frequency. In the Brûlet-McConnell theory, the spins are confined to parallel planes. In the case of the methylene protons under consideration here, processes such as kink creation, destruction and propagation cause the spins to change their depth in the membrane, and hence modulate the internuclear vectors between spins located on different molecules as well as on the same molecule. Be-

cause of the rod-like shape of the lipid molecules, small amplitude intramolecular chain motions perpendicular to the bilayer surface are significantly more effective in averaging the intermolecular dipolar interactions, particularly those between close by protons on neighboring chains which dominate the intermolecular dipolar fields anyway, than lateral movements of comparable distances in the plane of the bilayer membrane. The situation, hopefully, can be rectified by treating the problem in three dimensions and considering anisotropic diffusion. We plan to present an analysis of the data which we have reported here in terms of such a theory in due course.

Finally, we point out that Lee et al. [3] had previously interpreted the intermolecular contributions to the linewidth in terms of the translational diffusion theory of Torrey [16] and Kruger [17]. Whether the Torrey-Kruger theory, which assumes simple isotropic translational diffusion or small-jump vacancy diffusion in three dimensions is sufficiently satisfactory to account for the linewidths of the bilayer systems under consideration when compared with the more correct anisotropic diffusion model remains to be ascertained. For what it may be worth, our linewidth data when interpreted in terms of the Torrey-Kruger theory yield $D \cong 2.5\text{–}5.5 \cdot 10^{-8}$ cm^2/s. This value agrees well with ESR spin label and fluorescence measurements, which give values ranging from 10^{-8} to 10^{-7} cm^2/s [18].

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