

Figure 7. Plot of $(D_m - D_{app})^{1/2}$ vs. $[I_t^+]^{-1}$. The straight line is drawn by the least-squares method.

Substituting the solution $\delta_1(t)$ from eq 1 and 2 into the following expression for the current i

$$i = nFA[D_m \cdot CMC / \delta_1(t)] \quad (3)$$

where n is the number of electrons per molecule oxidized, F the faraday, and A the area of an electrode, we get

$$i = nFA[I_t^+]D_{app}^{1/2} / 2t^{1/2} \quad (4)$$

where

$$D_{app} = (CMC/[I_t^+])(2 - CMC/[I_t^+])D_m + (1 - CMC/[I_t^+])^2 D_M \quad (5)$$

Equation 5 is rewritten as

$$(D_m - D_{app})^{1/2} = -CMC(D_m - D_M)^{1/2}[I_t^+]^{-1} + (D_m - D_M)^{1/2} \quad (6)$$

Figure 7 shows the plot of $(D_m - D_{app})^{1/2}$ vs. $[I_t^+]^{-1}$. The CMC and D_M values of 0.20 mM and $0.84 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, respectively, were determined from the slope and the intercept of the plot by using the foregoing D_m value of $5.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The solid line in Figure 2 was drawn by using these values of D_M and CMC and the experimental value of D_m . A good linearity of the plot of Figure 7 and a satisfactory fitness of the calculated curve with experimental points in Figure 2 suggest that the electrode reaction of I^+ is reasonably explained by the model in Figure 6. There is a small difference in CMC between the experimental value (0.5 mM) and the calculated value (0.2 mM). It may come from the simplicity of the model used. Further details of this model are now under discussion.

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Dependence of Deuterium Spin-Lattice Relaxation Rates of Multilamellar Phospholipid Dispersions on Orientational Order¹

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Abstract: ^2H NMR studies of a homologous series of 1,2-diacyl-*sn*-glycero-3-phosphocholines with perdeuterated saturated chains, ranging in length from C12:0 to C16:0, have been performed with use of quadrupolar echo techniques at a resonance frequency of 55.4 MHz. Randomly oriented, multilamellar dispersions containing 50 wt % H_2O in the liquid crystalline (L_α) phase have been employed. The ^2H spin-lattice relaxation times (T_1) and $\text{C}-^2\text{H}$ bond segmental order parameters (S_{CD}) of each of the resolved quadrupolar splittings have been obtained from the powder-type spectra, corresponding to a random distribution of orientations, as well as from the 0°C oriented subspectra obtained by numerical deconvolution (de-Pakeing). Evidence that the spin-lattice relaxation rate profiles as a function of chain position $T_1^{-1}(i)$ are related to the corresponding order profiles $S_{CD}(i)$ by a square-law functional dependence has been obtained, indicative of a contribution from relatively slow fluctuations in the local bilayer ordering to the relaxation. The results suggest that two broad classes of motions influence the ^2H spin-lattice relaxation rates of lipid bilayers: rapid local motions, most likely due to bond rotational isomerizations and long-axis rotational diffusion of the lipid chains, as well as slower director fluctuations as found in other liquid crystalline mesophases.

I. Introduction

Deuterium (^2H) NMR has been widely utilized to investigate the molecular properties of lipid bilayers and biological membranes.²⁻⁵ The ^2H nucleus has a spin $I = 1$, and the quadrupolar coupling with the electrostatic field gradient arising from its bonding environment is large relative to the magnetic dipolar interactions among ^2H and ^1H nuclei; thus a single interaction is dominant in most cases. While the relatively large size of the electric quadrupolar interaction introduces difficulties in obtaining ^2H NMR spectra, the interpretation of the results is correspondingly simplified.² Owing to the different time scales, ^2H NMR studies are complementary to ^{13}C NMR and similar in-

formation can be obtained in both cases. In addition to providing new structural knowledge, ^2H NMR studies of lipid bilayers may also prove useful in interpreting the more complex proton (^1H) NMR spectra and relaxation properties of the membrane constituents of cells and tissues. Since the quadrupolar Hamil-

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tonian for the ^2H nucleus with $I = 1$ is formally equivalent to that for the direct dipolar interaction between two identical $I = 1/2$ ^1H nuclei, ^2H NMR is tantamount to studying the pairwise ^1H dipolar interactions which are frequently obfuscated in ^1H NMR. Finally, the relatively low natural abundance of the ^2H nucleus (0.00156%) makes it possible to obtain information on the behavior of individual molecular segments by means of ^2H isotopic labeling. The above make ^2H NMR an attractive and valuable technique for studying the orientational ordering and dynamics of lipid bilayers and biomembranes at the molecular level.

One aspect of the ordering of lipid bilayers is reflected in the C- ^2H bond segmental order parameter, S_{CD} , which varies in absolute value from zero for the case of isotropic motion to a maximum of one for complete alignment along a given axis.^{2,4} Profiles of the bond segmental order parameter as a function of chain position, denoted by $S_{\text{CD}}(i)$ where i indicates the ^2H -labeled segment, have been obtained⁶⁻¹¹ and lend themselves to a simple rotational isomeric interpretation for the case of saturated bilayers in the liquid crystalline (L_α) phase;^{2,8,11,12} further quantitative analysis, however, has proved controversial.^{2,7,13-15} NMR relaxation studies^{16,17} can provide a better understanding of the types of motions occurring in these and related anisotropic systems and may allow one to begin to disentangle the various contributions to the disordering observed with NMR and other techniques. We and others have previously suggested various motional models which can be used to analyze the ^2H spin-lattice (T_1) relaxation times of lipid bilayers at the segmental level.¹⁴⁻²⁵ Recent evidence²⁶⁻³¹ suggests a cooperative picture,²²⁻²⁴ in which the local ordering set up by restricted segmental motions^{2,8,12} is modulated by slower, larger amplitude disturbances as in other liquid crystalline media.^{22-24,26,31-33} The latter may involve collective

tilting of the lipid acyl chains.^{13,30} Here we describe newly obtained ^2H NMR results which favor a substantial contribution from relatively slow order fluctuations^{22-24,34} to the spin-lattice relaxation of lipid bilayers in the L_α phase.

II. Theoretical Background

The ^2H spin-lattice relaxation is due to fluctuations in the nuclear quadrupolar interaction with the electrostatic field gradient of the C- ^2H bond. Molecular or segmental reorientation with respect to the main applied magnetic field direction provides a time-dependent perturbation of the nuclear Zeeman levels and thus an efficient means of exchanging energy with the surroundings. In what follows, it is assumed that the spin-lattice relaxation rates of the individual ^2H -labeled chain segments, denoted by $T_1^{-1}(i)$, are governed by rapid intramolecular motions, such as isomerizations, torsional oscillations, and long-axis rotational diffusion of the lipid molecules,^{17,22,31} in addition to any slower reorientations of the chains with respect to the macroscopic bilayer normal, an axis of cylindrical symmetry known as the director. Such a model²²⁻²⁴ represents a first approximation to what is doubtlessly a complex dynamic situation. The local motions yield a relaxation Hamiltonian of the form $(\mathcal{H}_Q) - (\mathcal{H}_Q)_{\Omega'}$ where $\Omega' = (\alpha', \beta', \gamma')$ are Euler angles³⁵ describing the relatively fast orientational fluctuations and the brackets indicate a time or ensemble average.²² The residual quadrupolar Hamiltonian left over by the faster motions $(\mathcal{H}_Q)_{\Omega'}$ can then be modulated by any slower disturbances. This leads to an additional relaxation contribution characterized by $(\mathcal{H}_Q)_{\Omega'} - (\mathcal{H}_Q)_{\Omega''}$ where $\Omega'' = (\alpha'', \beta'', \gamma'')$ describe the slower fluctuations.²² $(\mathcal{H}_Q)_{\Omega''}$ represents the residual quadrupolar Hamiltonian further averaged by any additional slow motions. Assuming that the rapid local motions involve threefold or higher symmetry about a preferred axis,³⁶ both $(\mathcal{H}_Q)_{\Omega'}$ and $(\mathcal{H}_Q)_{\Omega''}$ are proportional to or scale as $(P_2(\cos \beta'; t))$, where P_2 indicates the second Legendre polynomial and β' is the time-dependent angle between the principal symmetry axis for the local motion and the instantaneous bilayer normal.²² Thus, the transition probabilities³⁷ which determine the slow motional contribution will depend on $(P_2(\cos \beta'; t))^2$, which is in turn related to the square^{22,38} of the observed ^2H bond segmental order parameter $S_{\text{CD}}(i)$. The above is a simple consequence of the assumed axial symmetry of the motion²² together with the "golden rule" of perturbation theory.³⁹

Neglecting cross-correlations between the relatively fast (f) and slow (s) motions, one obtains that $T_1^{-1}(i) = T_{1f}^{-1}(i) + T_{1s}^{-1}(i)$, leading to²²⁻²⁴

$$1/T_1(i) = A\tau_f(i) + B(i)f(\omega_0)|S_{\text{CD}}(i)|^2 \quad (1)$$

In the above, A and $B(i)$ are collections of constants; $\tau_f(i)$ is an effective correlation time for the fast or local motions of the i th segment; and $f(\omega_0)$ describes the dependence of the relaxation on the resonance frequency ω_0 .^{3,14-17,22-24,26,27,29,30,32,40} The square-law functional dependence of $T_1^{-1}(i)$ on $S_{\text{CD}}(i)$ as given by the second term of eq 1 can be regarded as a characteristic signature of a contribution from relatively slow, axially symmetric motions to the relaxation.²² The values of $S_{\text{CD}}(i)$ are in turn directly proportional to the observed residual ^2H NMR quadrupolar splittings, $\Delta\nu_Q(i)$. Thus, for a multilamellar dispersion of a phospholipid with perdeuterated acyl chains, $T_1^{-1}(i)$ is expected to vary across the ^2H NMR spectrum as $|\Delta\nu_Q(i)|^2$, given that (i) the experimental

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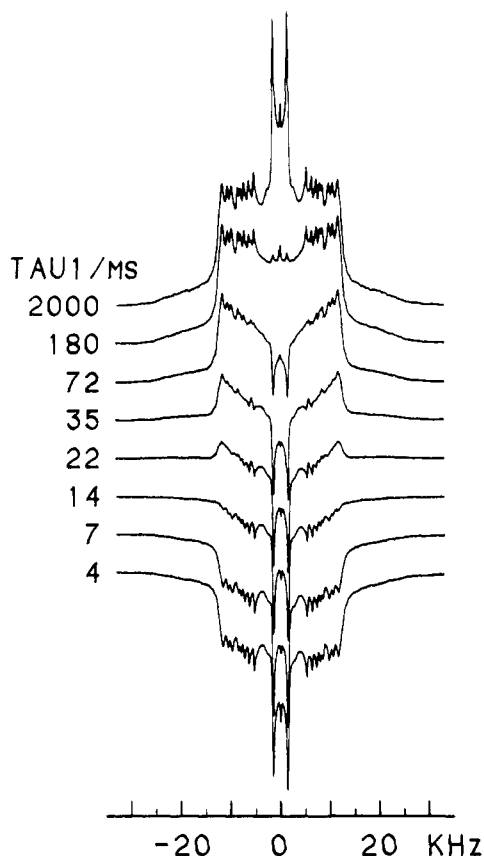


Figure 1. Representative partially relaxed ^2H NMR spectra of randomly oriented multilamellar dispersion of DLPC- d_{46} , containing 50 wt % H_2O in the L_α phase at 30 $^\circ\text{C}$, obtained by using the quadrupolar echo method. Values of the delay τ_1 between the inverting and sampling pulses are indicated.

values of $T_1^{-1}(i)$ are not greatly influenced by overlap of the spectral components,^{4,26,28} (ii) the orientational anisotropy of the relaxation is small,^{17,29} (iii) the amplitude of the order fluctuations is similar along the chains so that to a first approximation $B(i) \sim B$ for all segment positions,²² and (iv) that the values of $\tau_f(i)$ are small or do not vary strongly with chain position.²³ The above conclusion is independent of any assumptions as to the assignments of the overlapping $I = 1$ powder patterns.

III. Results and Discussion

Randomly Oriented Multilamellar Dispersions. To test the above, ^2H spin-lattice relaxation studies of a homologous series of saturated 1,2-diacyl-*sn*-glycero-3-phosphocholines⁴¹ with perdeuterated alkyl chains ranging in length from C12:0 to C16:0 were performed at a single resonance frequency of 55.43 MHz. Figure 1 shows a representative set of partially relaxed ^2H NMR spectra obtained for a randomly oriented, multilamellar dispersion of DLPC- d_{46} in the L_α phase at 30 $^\circ\text{C}$. Up to nine individual quadrupolar splittings $\Delta\nu_Q(i)$ are resolved, corresponding to 90 $^\circ$ orientations of the bilayer normal (director) with respect to the main magnetic field.^{2,4,28} As can be seen, the relaxation rates of the resolved components $T_1^{-1}(i)$ increase with $\Delta\nu_Q(i)$; note particularly those spectra with intermediate values of the delay τ_1 where the outer components with larger quadrupolar splittings are clearly relaxing most rapidly. Within experimental error, differences in the spin-lattice relaxation rates of the sharp edges due to the 90 $^\circ$ orientation and the shoulders corresponding to the 0 $^\circ$ orientation of the director are not distinguishable.²⁹

The above observations are qualitatively consistent with the type of model described by eq 1. For the case of rapid, local fluctuations, the interaction being modulated is the static quadrupolar

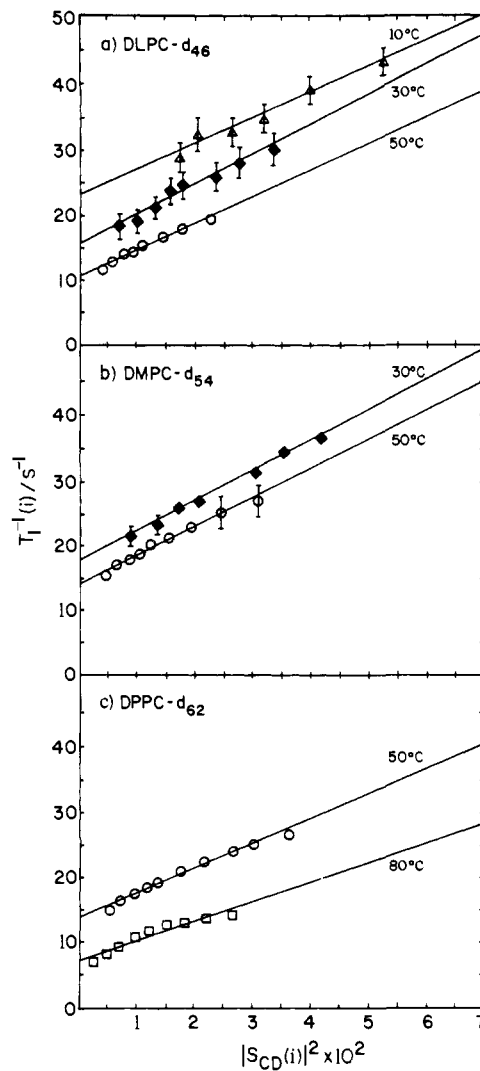


Figure 2. Representative plots of ^2H spin-lattice relaxation rates $T_1^{-1}(i)$ vs. $|S_{\text{CD}}(i)|^2$ for homologous series of chain-perdeuterated 1,2-diacyl-*sn*-glycero-3-phosphocholines in the L_α phase (cf. eq 1 of text). Points corresponding to different quadrupolar splittings, i.e., acyl chain index i , are not distinguished and data for terminal methyl groups have been omitted.²² The symbols refer to data obtained at temperatures of 10 $^\circ\text{C}$ (Δ), 30 $^\circ\text{C}$ (\blacklozenge), 50 $^\circ\text{C}$ (\circ), and 80 $^\circ\text{C}$ (\square). (a) DLPC- d_{46} (C12:0 acyl chains); (b) DMPC- d_{54} (C14:0 acyl chains); (c) DPPC- d_{62} (C16:0 acyl chains).

coupling, and if these are the only type of motions which are important, then $\Delta\nu_Q(i)$ or alternatively $|S_{\text{CD}}(i)|$ describes the residual interaction which does not contribute to the relaxation of the i th segment or group of segments.^{17,27} This results in a correction to the relaxation rate^{17,27,29} given by $(1 - |S_{\text{CD}}(i)|^2)$, which can be neglected for typical values of $|S_{\text{CD}}(i)|$. Thus, $T_1^{-1}(i)$ would depend primarily on the rate of the motions,^{17,27} characterized in eq 1 by $\tau_f(i)$. For the relaxation contribution due to any relatively slow motions,²² however, the interaction being modulated is not the static quadrupolar interaction, which is the same for all C- ^2H segments,² but rather the residual interaction scaled or left over by the more rapid orientational fluctuations, which can vary as a function of chain segment position.²² Assuming that the amplitude of the relatively slow motions is similar at the various chain positions, the magnitude or strength of the fluctuating interaction would be greatest for the largest $\Delta\nu_Q(i)$ values, i.e., $|S_{\text{CD}}(i)|$ (vide supra). It follows that the spin-lattice relaxation rates $T_1^{-1}(i)$ would be expected to increase with increasing $\Delta\nu_Q(i)$ as found experimentally. Thus Figure 1 suggests, but does not prove, that the relaxation is predominantly influenced by relatively slow fluctuations which average the local ordering set up at each segment position by faster motions.

(41) Abbreviations used: DLPC, 1,2-dilauryl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

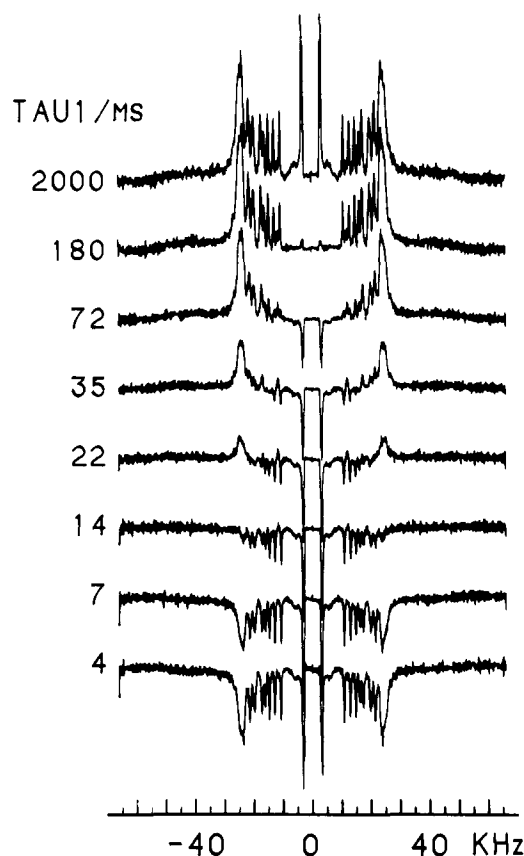


Figure 3. Representative partially relaxed ^2H NMR spectra of DLPC- d_{46} in the L_α phase at 30 °C, obtained by deconvolution of the spectra in Figure 1 (de-Pakeing).⁴³

The values of $T_1^{-1}(i)$ measured for each of the resolved splittings of the overlapping powder patterns of the chain perdeuterated 1,2-diacyl-*sn*-glycero-3-phosphocholines are plotted vs. the corresponding values of $|S_{\text{CD}}(i)|^2$ in Figure 2 for several different temperatures in the L_α phase; i.e., it is assumed that the two observables are functionally linked or dependent as described by eq 1. In general, both of the quantities $T_1^{-1}(i)$ and $|S_{\text{CD}}(i)|$, in the liquid crystalline state, are observed to decrease with increasing temperature^{22,27} (cf. Figure 2). As can be seen, $T_1^{-1}(i)$ is found to vary approximately linearly with $|S_{\text{CD}}(i)|^2$ along the saturated hydrocarbon chains in most cases, consistent with eq 1 provided that the other parameters do not depend strongly on the chain segment position i . These recent spin-lattice relaxation data agree with earlier, less extensive investigations²² of DPPC with specifically deuterated²⁷ and perdeuterated²⁸ acyl chains. Similar results have been obtained for *p*-(octyloxy)-*p'*-pentylphenyl thiobenzoate with perdeuterated alkyl chains in the nematic phase.⁴²

Oriented Subspectra Obtained by de-Pakeing. To ascertain to what extent the apparent $T_1^{-1}(i)$ values estimated for the 90° orientations (edges) of the overlapping powder patterns of the chain perdeuterated phospholipids are influenced by spectral overlap, the partially relaxed ^2H NMR spectra were numerically deconvoluted ("de-Paked")⁴³ to yield the 0° oriented subspectra. The deconvolution procedure⁴³ assumes that the ^2H NMR powder-type spectra are axially symmetric and scale as $P_2(\cos \beta''')$, where β''' denotes the angle^{22,34} between the macroscopic bilayer normal (director) and the main magnetic field direction. In principle, an orientational anisotropy of the spin-lattice relaxation rates of the different superposed components across the powder-type spectra is to be expected.^{17,27} This would lead to line shape changes

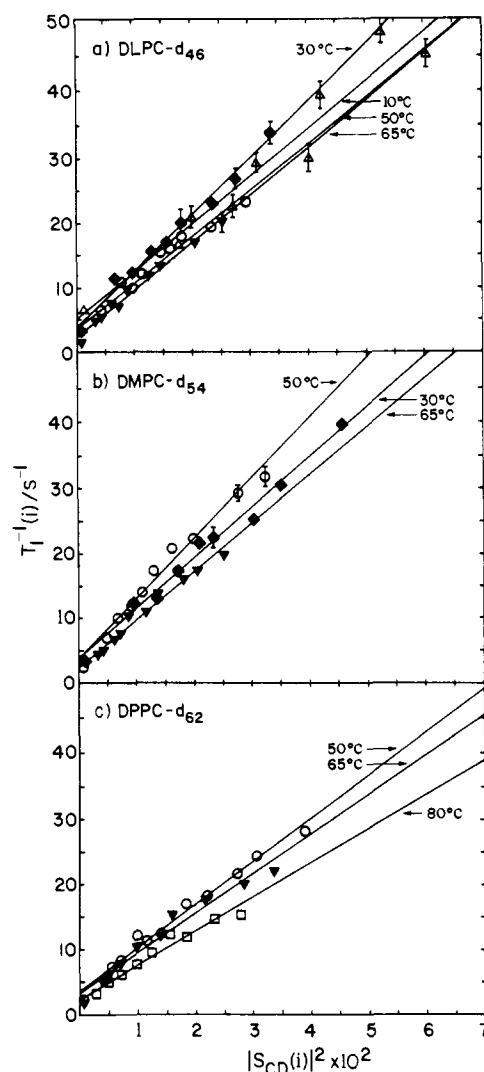


Figure 4. Plots of ^2H spin-lattice relaxation rates $T_1^{-1}(i)$ vs. $|S_{\text{CD}}(i)|^2$ obtained by de-Pakeing. Different acyl chain segments are not distinguished. The symbols refer to data obtained for the homologous series of chain-perdeuterated phospholipids in the L_α phase at temperatures of 10 °C (Δ), 30 °C (\blacklozenge), 50 °C (\circ), 65 °C (\blacktriangledown), and 80 °C (\square). (a) DLPC- d_{46} ; (b) DMPC- d_{54} ; (c) DPPC- d_{62} .

as a function of the delay between the inverting and sampling pulses, thereby affecting the assumption made in the de-Pakeing algorithm.⁴³ However, as shown by Brown and Davis,²⁹ rapid orientational averaging occurs on the time scale of the ^2H spin-lattice relaxation (milliseconds) or less in 50 wt % aqueous multilamellar dispersions of phospholipids such as DPPC. As a consequence, the axial symmetry and shape of a given superposed powder pattern are expected to remain invariant as a function of the delay time; deconvolution of the spectra is then warranted. The above may not always be the case, and when in doubt it is advisable to perform hole-burning or magnetization-transfer experiments as described by Brown and Davis.²⁹ For the case of DPPC bilayers containing cholesterol, a distinct orientational anisotropy of the spin-lattice relaxation rates across the powder pattern is observed.⁴⁴

Upon de-Pakeing the powder-type ^2H NMR spectra, a further increase in resolution is evident.⁴³ Figure 3 shows typical results obtained from the partially relaxed ^2H NMR spectra of DLPC- d_{46} at 30 °C included in Figure 1. The frequency axis of the 0° oriented subspectra is increased twofold relative to the sharp edges of the powder-type spectra; a loss of signal/noise is also seen as expected.⁴³ The $T_1^{-1}(i)$ rates evaluated from the de-Paked ^2H

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NMR spectra are plotted against the corresponding values of $|S_{CD}(i)|^2$ for the homologous series of 1,2-diacyl-*sn*-glycero-3-phosphocholines at different temperatures in Figure 4. In all cases a linear relationship is observed to within experimental error, consistent with Figure 2. The results of Figure 4 are in agreement with the model described by eq 1 and suggest that any dependence of the other parameter values on the chain segment index i can be neglected to a first approximation (*vide supra*). It should be noted that although the de-Pakeing procedure yields the 0° oriented subspectra, the measured $T_1(i)$ values reflect an average over all orientations.²⁹ Comparison of the results of Figure 4 to those in Figure 2 suggests that both the slopes and intercepts of the plots in the latter case are influenced by systematic errors. Thus, caution should probably be exercised in determining $T_1(i)$ from the overlapping powder patterns of multilamellar lipid dispersions with perdeuterated acyl chains.^{26,28,44,45} The $T_1^{-1}(i)$ values obtained from the de-Paked ^2H NMR spectra are somewhat longer than those evaluated from the unoriented powder patterns for the components with smaller quadrupolar splittings near the center of the spectra. These differ in their relaxation rates²⁷ and overlap substantially. The $T_1^{-1}(i)$ rates and values of $\Delta\nu_Q(i)$ for the components with largest quadrupolar splittings, by contrast, are similar²⁷ and are not as affected by spectral overlap.

The data summarized in Figure 4, together with earlier studies of DPPC with specifically deuterated acyl chains,^{22,27} suggest that the $T_{1f}^{-1}(i)$ contribution from local motions in eq 1 is relatively small for most of the segments.³⁰ Thus, while a profile or variation of $T_{1f}^{-1}(i)$ along the chains is to be expected,^{17,27,46} the dependence of the observed relaxation rate $T_1^{-1}(i)$ on chain segment position, acyl chain length, and temperature would appear to arise predominantly from the second term of eq 1 describing slower collective motions.^{22-24,30} It should be noted that the results obtained from the powder-type spectra of the chain perdeuterated 1,2-diacyl-*sn*-glycero-3-phosphocholines suggest a larger contribution from local motions to the $T_{1f}^{-1}(i)$ values, and due to the larger ordinate intercepts the plots in Figure 2 do not overlay at the different temperatures. By contrast, the results in Figure 4 obtained from the de-Paked partially relaxed spectra are largely superimposable at the different temperatures studied. Thus, the temperature dependence of $T_1^{-1}(i)$ appears to be correlated with that of $|S_{CD}(i)|^2$, suggesting that the coefficients $B(i)$ in eq 1 do not depend strongly on chain position or temperature to a first approximation.²² This explanation differs from the conventional interpretation of the temperature dependence of the spin-lattice relaxation rate in terms of an activation energy.²² The a - c plots in Figure 4 are also seen to be approximately superimposable to within experimental error, suggesting that any variations in $T_1^{-1}(i)$ among the homologous series are to a large extent correlated with differences in $|S_{CD}(i)|^2$, i.e., the coefficients $B(i) \sim B$ in eq 1 appear not to depend strongly on chain length from 12–16 carbons. Further work is desirable, however.

Finally, in Figure 5 the results obtained by de-Pakeing for acyl chain perdeuterated DPPC- d_{62} are compared to previous results acquired for DPPC with specifically deuterated chains^{22,27} at approximately the same magnetic field strength (frequency). Good agreement is evident between the results of the two studies. The congruence is less satisfactory, however, when the results obtained from the randomly oriented powder-type ^2H NMR spectra of DPPC- d_{62} (cf. Figure 2c) are compared to those for DPPC with specifically deuterated acyl chains (cf. Figure 5c–d). Thus it appears that the de-Pakeing procedure substantially reduces systematic errors due to spectral overlap for the case of phospholipids with perdeuterated acyl chains. In general, the currently available ^2H spin-lattice relaxation data appear described by eq 1 and support application of such a model²²⁻²⁴ to phospholipid bilayers with saturated acyl chains.⁴⁷

IV. Summary

In this work we have obtained further evidence that the spin-lattice relaxation rates $T_1^{-1}(i)$ and quadrupolar splittings $\Delta\nu_Q(i)$ obtained from ^2H NMR studies are correlated via a square-law dependence²² for the acyl chain segments of a homologous series of saturated 1,2-diacyl-*sn*-glycero-3-phosphocholines in the L_α phase. These new findings employing phospholipids with perdeuterated acyl chains lend additional support to the conclusions of earlier studies of DPPC with specifically deuterated acyl chains.^{22,27} The currently available ^2H NMR results suggest that the dominant contribution to the ^2H spin-lattice relaxation rates arises from collective fluctuations in the local ordering set up by faster motions, such as bond rotational isomerizations and long-axis diffusion of the chains. In addition, we have shown how systematic errors in evaluating $T_1^{-1}(i)$ from the overlapping powder patterns of multilamellar dispersions of chain perdeuterated phospholipids containing 50 wt % H_2O can be largely removed by deconvolution to obtain the 0° oriented subspectra.⁴³

V. Experimental Section

Fatty acids were obtained from Sigma ($\geq 99\%$ pure) and were perdeuterated by catalytic exchange of ^2H for ^1H over a 10% Pd-charcoal

(47) A referee has commented that the local $T_{1f}^{-1}(i)$ contribution estimated from the ordinate intercepts of Figures 4 and 5, neglecting any positional dependence, should be compared to the ^2H $T_1^{-1}(i)$ rates of the methylene groups of DPPC in chloroform/methanol (ref 27), and to the intramolecular contribution to the methylene ^1H T_1^{-1} rates of DPPC vesicles measured at 220 MHz (Kroon, P. A.; Kainosho, M.; Chan, S. I. *Biochim. Biophys. Acta* **1976**, *433*, 282–293). Given the model of eq 1, a value of $T_{1f}^{-1}(i)$ in the range of ~ 2 – 4 s^{-1} is obtained from the ordinate intercepts of Figures 4 and 5, from which it can be estimated that the correlation times of the local segmental motions fall in the range of ~ 5 – 10 ps (ref 22). The estimated values are substantially less than those obtained from the above ^2H and ^1H spin-lattice relaxation data for the methylene groups of DPPC in chloroform/methanol or DPPC vesicles if an analysis in terms of a single correlation time is assumed. How could the "fast" motions of the individual methylene groups in multilamellar dispersions of DPPC be faster than in solvent-dispersed lipids or in sonicated DPPC vesicles? It turns out that the latter measurements may not be appropriate for comparison to the $T_{1f}^{-1}(i)$ values estimated for DPPC in the liquid crystalline phase by extrapolation of the data in Figures 4 and 5. The reason lies in the fact that a single motional process with a cutoff frequency in the short correlation time regime may not suffice to account for the spin-lattice relaxation data in the latter two instances. For the case of DPPC dissolved in chloroform/methanol, it is probable that small inverted micelles exist, and thus the observed segmental relaxation rates $T_1^{-1}(i)$ can be influenced by multiple internal rotations of the polymethylene chains, as well as slower overall tumbling of micellar aggregates and other processes (ref 27). At present there is insufficient data to warrant a detailed comparison to the results of ^2H NMR investigations of DPPC in the L_α phase (ref 27). With regard to the ^1H NMR studies of DPPC vesicles, a clear frequency dependence of the intramolecular contribution to the methylene spin-lattice relaxation rates between 100 and 220 MHz is evident, and thus it is doubtful whether the results at 220 MHz can be compared to the $T_{1f}^{-1}(i)$ contribution obtained by extrapolation in Figures 4 and 5. (Only small differences in the spin-lattice relaxation rates of unilamellar vesicles and multilamellar dispersions exist; ref 27 and 30.) While the assumption of a single effective correlation time for the motions of each of the individual chain segments may represent a reasonable first approximation for the interpretation of data acquired at relatively high frequencies (ref 17 and 27), a detailed comparison of the results obtained for different nuclei must consider the relaxation frequency dependence (ref 22 and 24). We have suggested elsewhere that a more appropriate system to which the $T_{1f}^{-1}(i)$ contribution for DPPC in the L_α phase may be compared would be a neat paraffinic liquid such as *n*-hexadecane (ref 23 and 30). The results obtained by extrapolation of the methylene ^{13}C spin-lattice relaxation rates of vesicles of DPPC to infinite frequency (ref 23 and 30) or extrapolation of the methylene ^2H $T_1^{-1}(i)$ values of DPPC multilamellar dispersions to zero ordering (Figures 4 and 5) are in good agreement with the corresponding frequency independent ^{13}C and ^2H relaxation rates of liquid *n*-paraffins (ref 22 and 23). Thus, the magnitudes of the local contribution to the overall spin-lattice relaxation rates obtained for the acyl chain methylene groups of DPPC in the liquid crystalline state are indeed physically plausible, and further suggest that the bilayer microviscosity is on the order of that of an *n*-paraffin of similar chain length in the liquid state (ref 23 and 30). In passing, it should also be noted that the correlation time of ~ 5 – 10 ps estimated for the local contributions from Figures 4 and 5 is consistent with recent theoretical molecular dynamics studies of bilayers comprised of saturated paraffinic chains (Van der Ploeg, P.; Berendsen, H. J. C. *Mol. Phys.* **1983**, *49*, 233–248). Finally, we have shown elsewhere how the available ^1H , ^2H , and ^{13}C spin-lattice relaxation data for vesicles and multilamellar dispersions of DPPC in the liquid crystalline phase can be explained in a simple, unified fashion by an analysis of the type discussed here to a good degree of approximation (ref 24). For a more detailed discussion of these and related points, readers are referred to ref 22–24.

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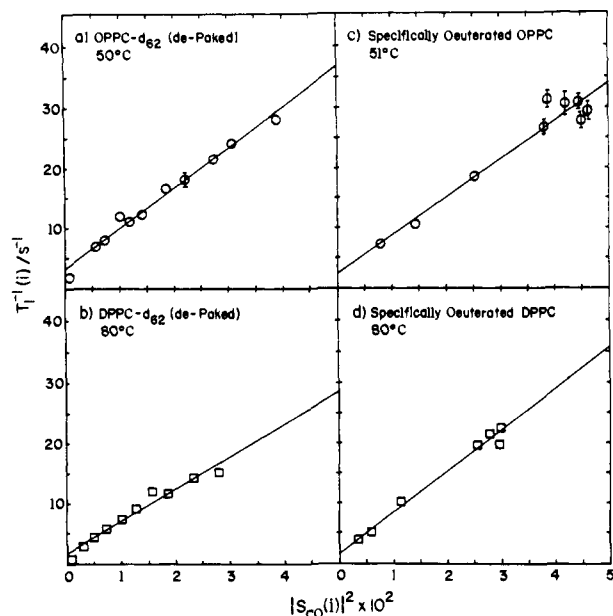


Figure 5. Comparison of plots of ²H $T_1^{-1}(i)$ rates vs. $|S_{CD}(i)|^2$ obtained for DPPC- d_{62} at 55.4 MHz by de-Pakeing to results obtained for DPPC with specifically deuterated acyl chains at 54.4 MHz.^{22,27} Data for different temperatures in the L_α phase are shown. (a) DPPC- d_{62} , 50 °C; (b) DPPC- d_{62} , 80 °C; (c) specifically deuterated DPPC, 51 °C; (d) specifically deuterated DPPC, 80 °C. Different acyl chain segments are not distinguished.

catalyst (Aldrich) at 200 °C.⁴⁸ ²H₂ gas was generated electrolytically from ²H₂O (99.8 atom %; Aldrich) with an Aadco Model 1150 hydrogen generator. The product fatty acids were characterized by mass spectroscopy and ¹H NMR to determine the percent incorporation of ²H and their methyl esters by gas-liquid chromatography on 10% SP-2330 (Supelco) in an all-glass system with a Varian Model 3700 gas chromatograph. The perdeuterated fatty acids were typically 92–96% labeled with ²H and were >99% pure by gas-liquid chromatography. Phospholipids were synthesized from the cadmium chloride adduct of *sn*-glycero-3-phosphocholine and the corresponding fatty acyl anhydrides following a modification of the procedure of Mason et al.⁴⁹ In some cases, acylation was performed by using fatty acyl-imidazoles to conserve starting materials.⁵⁰ Typical yields based on *sn*-glycero-3-phosphocholine were 75–90%. After purification by column chromatography on silicic acid (Bio-Sil A, 100-200 mesh; Bio-Rad), all phospholipids gave single spots upon thin-layer chromatography in CHCl₃:MeOH:H₂O (65/35/5) followed by charring with 40% H₂SO₄ in EtOH. Samples were prepared for ²H NMR studies by vortexing approximately 100–150 mg of phospholipid together with an equal weight of ²H-depleted ¹H₂O (Aldrich) above the bilayer phase transition temperature. The 50 wt % multilamellar dispersions were centrifuged and sealed in cutoff ca. 6 or 10 mm diameter Kimax or Pyrex type glass culture tubes with Teflon plugs. The samples were stored at –85 °C when not in use.

²H NMR spectra were acquired at 55.43 MHz with a phase-cycled, composite $\pi-\tau_1-(\pi/2)_x-\tau_2-(\pi/2)_y-\tau_2$ -echo sequence.^{51,52} Recycle times were generally greater than twice the longest T_1 value and typically

600–2400 echoes were collected and Fourier transformed. A Nicolet (General Electric) NT-360 spectrometer was employed together with a Henry Radio Tempo 2006 radio frequency boost and homebuilt, thermostated horizontal solenoidal probe with high-voltage Polyflon Corp. capacitors. Typical $\pi/2$ pulse lengths with the narrow-bore magnet were <5 μ s for the 10 mm diameter radio frequency coil and <3–4 μ s for the 6 mm diameter coil. Data were acquired with an external digitizer consisting of a Nicolet Explorer 2090 digital oscilloscope plus interface using both quadrature data channels. No first-order phase correction was applied to the spectra.^{4,53} The radio frequency coil was enclosed in a glass dewar and the temperature was controlled with a flow of compressed air by the Nicolet variable temperature unit interfaced to a heater/sensor located in the probe. The sample temperature was monitored before and after each experiment with a thermistor inserted directly above the radio frequency coil and was usually found to vary <0.5 °C during a given T_1 run. The temperatures are estimated accurate to within ± 1 °C of the reported values. The thermistor also served as an antenna to observe the radio frequency pulses. Thin-layer chromatography of the samples after the experiments were completed indicated the presence of some minor hydrolysis products which are not believed to significantly influence the results.⁵⁴

Powder-type ²H NMR spectra were analyzed by measuring the residual quadrupolar splittings, $\Delta\nu_Q(i)$, of the resolved sharp edges corresponding to the $\beta''' = 90^\circ$ orientation of the director with respect to the main magnetic field direction. Values of $S_{CD}(i)$ were calculated from the relation²

$$\Delta\nu_Q(i) = 3/2(e^2qQ/h)P_2(\cos \beta''')|S_{CD}(i)| \quad (2)$$

with $P_2(\cos \beta''') = -1/2$. A value of $(e^2qQ/h) = 170$ kHz was taken for the static quadrupolar coupling constant.² The spin-lattice relaxation times of each of the resolved splittings of the powder-type ²H NMR spectra, $T_1(i)$, were obtained by nonlinear regression fitting of the amplitudes measured from a flat base line to a three-parameter exponential function.⁵⁵ At least 13 different values of the delay τ_1 were employed in each case. Control studies of ²H₂O showed that T_1 was constant as a function of the spectral offset from the carrier frequency. The ²H NMR spectra were then transferred via a 9600 baud line from the Nicolet 1280 computer to a CDC Cyber 180/855 main-frame computer for further manipulations. The de-Pakeing algorithm was carried out in Fortran V with a modification of the program of Sternin et al.^{43,56} A single iteration using about 1000 spectral points was generally required to obtain accurate amplitudes. Control studies of 30 wt % potassium palmitate in the L_α phase⁴³ showed that the resolved peak areas of the de-Paked ²H NMR spectra were proportional to the number of ²H nuclei within experimental error. The order parameters $|S_{CD}(i)|$ of the resolved splittings of the 0° oriented subspectra were obtained with use of eq 2 with $P_2(\cos \beta''') = 1$. The corresponding relaxation times $T_1(i)$ were analyzed as described above for the powder-type ²H NMR spectra.

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