Deuterium Relaxation and Molecular Dynamics in Lipid Bilayers

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The quadrupolar relaxation of deuterium-labeled lipid bilayers has been analyzed using standard Redfield theory and is discussed with regard to the problem of chain segmental motion and order in membranes. Considering the segmental reorientation as a stochastic process, the $T_1$ and $T_2$ relaxation rates are interpreted in terms of the rate of motion, characterized by one or more correlation times $\tau_{2M}$, and statistical amplitude, characterized by the segmental order parameter $S_{CD}$. For the case of phospholipid bilayers with $S_{CD} \approx 0.2$, the relaxation rates are predominantly determined by the rate of motion, rather than the ordering. Recently obtained $T_1$ relaxation data for selectively deuterated and perdeuterated multilamellar dispersions of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine are analyzed and compared to the results of previous carbon-13 $T_1$ relaxation studies. The available experimental results suggest that the fast segmental motions affecting $T_1$ in these systems can be treated to a reasonable degree of approximation in terms of a single effective correlation time.

INTRODUCTION

The proper assessment of contributions from molecular order and motion to the NMR relaxation rates of lipid bilayers has been a longstanding problem in membrane molecular biology (1-21). The lack of definitive experimental data has led to a number of models (22-30), which in many cases involve untested assumptions. With the advent of deuterium NMR as a tool for the investigation of lipid membranes (31,32), it has become possible to measure the order parameters of individual molecular segments (18,33-35), and, more recently, the spin–lattice relaxation time $T_1$ as a function of the fatty acyl chain position (36,37). The segmental order parameter is related to the statistical amplitude of molecular motions, and hence to the average molecular conformation. A quantitative analysis of the deuterium relaxation times can provide information regarding the rates of molecular motions, but is complicated in ordered media by the fact that the relevant spin interactions are not averaged over all space.

Lipid bilayers differ from nematic and other liquid crystalline mesophases in that the molecular constituents are highly flexible. Since the lipid molecules are presumably subject to an ordering potential similar to that experienced by nematogens (38), the motion of the fatty acyl chains can be analyzed in a relatively simple manner by applying concepts developed from studies of liquid crystals to the ordering and...
motion of each segment. In this manner, the complex many-body problem dealing with intra- and intermolecular interactions in the hydrocarbon interior of lipid bilayers is effectively reduced to an analysis of the behavior of individual chain segments. This approach represents a considerable simplification compared to models involving multiple internal rotations (22, 24, 27, 30), which require a number of assumptions that are of questionable applicability to ordered bilayer systems.

The relaxation of lipid bilayers will depend, in general, on both the rate and amplitude of the segmental motions. The appropriate molecular parameters which define the relaxation rates are (i) the orientation of the motional symmetry axis (known as the director) with respect to the applied magnetic field; (ii) the segmental ordering; and (iii) the frequency range of the molecular motions, characterized by one or more correlation times. The director orientation is usually known from the experimental geometry, while the order contributions can be estimated from the degree of motional averaging of various tensorial interactions, such as the deuterium quadrupole coupling. Therefore, in principle, it is possible to determine the motional correlation times from an analysis of deuterium relaxation rates. Knowledge of the various segmental order parameters and correlation times provides an experimental basis necessary for a physical understanding of lipid bilayers.

As a specific example of the sort of problem encountered in the analysis of NMR relaxation in ordered lipid bilayer systems, consider the data illustrated in Fig. 1. Here we have measured and compared the $T_1$ relaxation rates of selectively deuterated bilayers of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)\(^1\) to the corresponding deuterium order parameters as a function of chain position (37).

![Graph of $1/T_1$ vs. carbon number](image)

**Fig. 1.** Comparison of the deuterium spin–lattice relaxation rates (●) and segmental order parameters (○) of DPPC bilayers as a function of the labeled fatty acyl chain segment at 51°C (37).

\(^1\) Abbreviations used: DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine.
Both profiles are approximately parallel, particularly in that a "plateau" is observed up to about the middle of the chain, followed by a decrease in the central region of the bilayer. Since the $T_1$ relaxation times depend on both the segmental ordering and rate of motion, while the order parameter, obviously, only depends on order, do the two profiles manifest two independent quantities, viz., order and motion? Or is the similarity of the shapes of the two profiles governed by the profile of segmental order in the bilayer? It is the purpose of this article to discuss the interpretation of such relaxation data and to show how this information can be related to molecular motions in lipid membranes.

**THEORY**

*Irreducible Representation of the Quadrupolar Hamiltonian*

Nuclear interactions which are of bilinear form in the spin variable, e.g., the quadrupolar or dipolar interaction, can be most usefully represented in a spherical basis using irreducible tensor operators (39–41). The Hamiltonian for the coupling of a quadrupolar nucleus of spin $I$ with an electrostatic field gradient (41, 42) can be written as the scalar product of two second-rank irreducible tensors, as indicated below

$$H_Q = \left(\frac{3}{2}\right)^{1/2} \frac{eQ}{4I(2I-1)} \sum_{M=-2}^{2} (-1)^M V_M^{(2)} T_M^{(2)}.$$  

Here $V_M^{(2)}$ are the irreducible components of the electrostatic field gradient tensor and the $T_M^{(2)}$ are the irreducible tensor components of the spin angular momentum operators. The tensor $V_M^{(2)}$ is given by

$$V_0^{(2)} = V_{zz},$$
$$V_{\pm 1}^{(2)} = \mp \left(\frac{3}{2}\right)^{1/2} (V_{xx} \pm iV_{xy}),$$
$$V_{\pm 2}^{(2)} = \left(\frac{3}{2}\right)^{1/2} \left\{ \left(V_{xx} - V_{yy}\right) \pm iV_{xy} \right\},$$

where $V_{ij}$ are the Cartesian components of the electrostatic field gradient tensor at the nucleus. The irreducible components of the spin angular momentum operators are

$$T_0^{(2)} = \left(\frac{3}{2}\right)^{1/2} (3I_z^2 - I^2),$$
$$T_{\pm 1}^{(2)} = \mp (I_z I_\pm + I_\pm I_z),$$
$$T_{\pm 2}^{(2)} = I_\pm^2.$$

The field gradient tensor $V$ is diagonal in some coordinate system of the molecule or atom of interest, while the spin angular momentum $I$ is quantized in the laboratory coordinate system defined by the direction of the applied magnetic field $H_0$. By an appropriate rotation of either $V_M^{(2)}$ or $T_M^{(2)}$, it is possible to evaluate the various components of the quadrupolar Hamiltonian in a single coordinate system. This is most easily accomplished using Wigner rotation matrices (39). An irreducible tensor
of rank \( L \) transforms under rotations by

\[
T_{LM}^{(L)} = \sum_{K=-L}^{L} D_{KM}^{(L)}(\alpha, \beta, \gamma) T_{K}^{(L)},
\]

[4]

where the factored dependence of the rotation matrix on the three Euler angles \( \alpha, \beta \) and \( \gamma \) is

\[
D_{KM}^{(L)}(\alpha, \beta, \gamma) = e^{-iK\alpha} d_{KM}^{(L)}(\beta) e^{-iM\gamma}.
\]

[5]

The elements of the Wigner rotation matrices can be found elsewhere (39, 40, 43).

In liquid crystals, molecular motions are restricted in their angular fluctuations and are generally characterized with regard to an axis of rotational symmetry known as the director (44). For the case of lipid bilayers, a number of studies have shown that the average director is perpendicular to the bilayer surface (45–48). The field gradient tensor of carbon–deuterium bonds, the only case to be considered here, is approximately axially symmetric (asymmetry parameter \( \eta = 0; V_{zz} = eq \)). Therefore, for deuterium \((I = 1)\), the quadrupolar Hamiltonian can be expressed as

\[
H_Q(t) = \frac{3}{2} e^2 qQ \sum_{\alpha,\beta} D_{\alpha\beta}^{(2)}(\alpha(t), \beta(t)) D_{\alpha'\beta'}^{(2)}(\alpha', \beta', 0) T_{P}^{(2)},
\]

[6]

where we have applied Eq. [4] twice. In Eq. [6] the Euler angles \( \Omega' = (\alpha', \beta', 0) \) refer to rotation of the laboratory coordinate system \((x, y, z)\) to the director coordinate system \((x', y', z')\) and \( \Omega(t) = (\alpha(t), \beta(t), 0) \) to the time-dependent transformation from the director to the principal-axis system of the electrostatic field gradient \((x'', y'', z'')\) (cf. Ref. (39, p. 50)). The Euler angles are summarized below:

- \( \alpha' \) Rotation about \( H_0 (z) \) axis
- \( \beta' \) Angle between \( H_0 \) and director \((z')\) axis
- \( \alpha \) Rotation about director
- \( \beta \) Angle between field gradient \((z'')\) and director axes

Note that Eq. [6] can be written in the form

\[
H_Q(t) = \sum_{P} (-1)^P F_p^{(2)} T_p^{(2)},
\]

[7]

where \( F_p^{(2)} \) are functions of the lattice variables (42).

In general, the quadrupolar Hamiltonian for liquid crystals has a nonzero time average, in contrast to the situation for isotropic or anisotropic fluids. The time-averaged quadrupolar Hamiltonian \( \overline{H_Q} \) must, therefore, be subtracted from the total quadrupolar Hamiltonian to yield the fluctuating part \( H_Q'(t) \) which produces relaxation (42)

\[
H_Q'(t) = H_Q(t) - \overline{H_Q}.
\]

[8]

Since the time dependence of \( H_Q(t) \) is contained in the director \( \rightarrow \) field gradient coordinate transformation, we average over the Euler angles \( \alpha(t) \) and \( \beta(t) \) in Eq. [6] to obtain \( \overline{H_Q} \). The time-averaged Hamiltonian must be invariant to rotation about the director axis, so that terms containing the Euler angle \( \alpha \) must go to zero.
Therefore,

\[
\overline{H_Q} = \left( \frac{3}{2} \right)^{1/2} \frac{e^2 q Q}{4} \sum_F \delta_{00}(\beta(i)) \sum_{P} d^{(2)}_{P0}(\alpha', \beta') T^{(2)}_{P}.
\]  

[9]

In terms of the lattice functions the relaxation Hamiltonian (Eq. [8]) can be written as

\[
H'(t) = \sum_P (-1)^P [F^{(2)}_P - F^{(2)}_{-P}] T^{(2)}_{P}.
\]  

[10]

**Relaxation in Ordered Media**

Using the standard density matrix formalism introduced by Redfield (41, 49), one can readily derive equations of motion for the longitudinal \((M_z)\) and transverse \((M_x, M_y)\) magnetization components of a spin-1 system. This has been discussed by Vold and Vold (50, 51). Ignoring nonsecular contributions and denoting the two spectral magnetization vectors of a spin-1 nucleus by \(M^A\) and \(M^B\), one obtains

\[
\frac{d}{dt}(M^A_x + M^B_x) = -(R_{2323} + 2R_{1133})(M^A_x - M^A_0 + M^B_x - M^B_0),
\]  

[11a]

\[
\frac{d}{dt}(M^A_y + iM^B_y) = -(R_{2323} + i\omega_A)(M^A_y + iM^B_y) - R_{1223}(M^B_x + iM^B_y),
\]  

[11b]

and an analogous equation for \(d/dt(M^B_x + iM^B_x)\). In Eqs. [11], \(\omega_{A,B} = (\omega - \omega_0) \pm \frac{1}{2} \Delta \nu_Q\), where \(\omega_0\) denotes the Larmor frequency and \(\Delta \nu_Q\) is the quadrupole splitting. The quantities \(R_{aa',bb'}\) denote the elements of the Redfield relaxation matrix, the subscripts 1, 2, and 3 referring to the spin states \(-1, 0,\) and \(1\), respectively. The relaxation is exponential, with

\[
1/T_1 = R_{2233} + 2R_{1133},
\]  

[12a]

\[
1/T_2 = R_{2323} + R_{1223},
\]  

[12b]

where \(T_1\) is the longitudinal (spin–lattice) relaxation time and \(T_2\) is the transverse relaxation time. The Redfield coefficients \(R_{2233} (= R_{1122})\) and \(R_{1133}\) correspond to the \(W_{23}\) and \(W_{13}\) transition probabilities, while the coefficients \(R_{2323}\) and \(R_{1223}\) correspond to the transverse relaxation rate and the transverse cross-relaxation rate, respectively. For the case of equally spaced energy levels \((\omega_A = \omega_B)\), such as those found in liquids, both the Redfield coefficients \(R_{2323}\) and \(R_{1223}\) are secular and must be considered for evaluation of \(T_2\). If the energy spacings are nondegenerate, as is usually the case for liquid crystals, then the precessional frequencies of the two lines are different and the \(R_{1223}\) coefficient may be ignored.

The Redfield coefficients are given by

\[
R_{2323} = (1/\hbar^2)[3J_0(0) + 3J_1(\omega_0) + 2J_2(2\omega_0)],
\]  

[13a]

\[
R_{2233} = R_{1223} = (2/\hbar^2)J_1(\omega_0),
\]  

[13b]

\[
R_{1133} = (4/\hbar^2)J_2(2\omega_0),
\]  

[13c]
where we have neglected any small shifts of the magnetic energy levels due to nonzero quadrupole splittings. The spectral densities \( J_p(\omega_0) \) are Fourier transform partners of temporal autocorrelation functions of the fluctuating Hamiltonian (Eq. [10])

\[
J_p(\omega_0) = \int_0^\infty G_p(\tau) e^{-i\omega_0 \tau} \, d\tau,
\]

where

\[
G_p(\tau) = \left[ F_p^{(2)}(t) - F_p^{(2)}(t + \tau) \right]^*.
\]

It can be shown rigorously that cross-correlation terms between lattice functions with different values of \( p \) do not appear in Eqs. [13] (51).

**Rotational Dynamics in Lipid Bilayers**

The spectral densities (Eq. [14]) can only be determined from a study of the frequency dependence of the relaxation. This could be accomplished by measurements of \( T_1 \) and \( T_{1p} \) as a function of frequency or by selective pulse experiments (50). In the absence of such information, it is necessary to make assumptions regarding the nature of the molecular reorientation.

Since the viscosity of lipid bilayers appears to be significantly higher than that of simple (nonviscous) liquids (52, 53), we can neglect inertial effects and assume that the molecular or segmental reorientation takes place only by collisions with neighboring molecules. For either a strong collisional or diffusional model, the autocorrelation functions of the Wigner matrix elements relevant to the present problem are given by (54)

\[
[D^{(2)}_{M0}(\Omega(t)) - D^{(2)}_{M0}(\Omega(t + \tau))]^2 = \left[ \frac{[D^{(2)}_{M0}(\Omega(t))]^2 - D^{(2)}_{M0}(\Omega(t)) \delta_{M0}}{\delta_{M0}} \right] e^{-|\tau|/\tau_{2M}} \delta_{MM}.
\]

where the \( \Omega(t) \) are now the Euler angles for transformation from the director coordinate system to the principal-axis system for the motion, which is assumed to be axially symmetric, i.e., characterized by a single molecular order parameter \( S_{mol} \). In Eq. [16] it is assumed that the molecular motions are cylindrically symmetric about the bilayer normal (director) and subject to an orienting potential. Using Eq. [16] we obtain from Eqs. [6] to [10]

\[
G_p(\tau) = \frac{3}{32} (e^2 qQ)^2 \sum_M \left[ d^{(2)}_{M0}(\beta(t))^2 - d^{(2)}_{M0}(\beta(t)) \delta_{M0} \right] d^{(2)}_{PM}(\beta)^2 e^{-|\tau|/\tau_{2M}}.
\]

Equations [14] to [17] reflect the fact that the mean-square amplitude of the fluctuating quadrupolar interaction is reduced from the value \( \overline{H^2} \) in simple fluids to \( \overline{H^2} - \overline{H^2} \) in ordered media. For the strong collision model, the \( \tau_{2M} \) can be taken to be the correlation times for a symmetric rotor (39, 52, 54–56). If we assume a diffusional model, then the \( \tau_{2M} \) depend on both the degree of orientational order and
the degree of motional anisotropy $\eta = D_\|/D_\perp$ (54), where $D$ refers to the molecule- or segment-fixed rotational diffusion tensor.

Concise expressions for $T_1$ and $T_2$ can be derived from Eqs. [12] to [14] and [17] only with several simplifying assumptions. In general, since Eq. [17] involves squares of the second-order Wigner matrix elements, terms containing both $d_{00}^{(2)}(\beta(t))$ and $d_{00}^{(4)}(\beta(t))$ appear. Thus, measurements of the residual deuterium quadrupole splitting, indicated by

$$\Delta \nu_Q = \frac{3}{2} \left( \frac{e^2 qQ}{h} \right) \frac{d_{00}^{(2)}(\beta(t))}{d_{00}^{(2)}(\beta')},$$

are insufficient, in general, to account for the effect of restricted angular fluctuation on the $T_1$ and $T_2$ relaxation rates. Assuming that (i) the motional fluctuations with respect to the director can be described by a single correlation time $\tau_2$, and (ii) the relevant molecular motions are in the short correlation time regime ($\omega_0^2 \tau_2^2 < 1$), the following expression for $T_1$ is obtained:

$$\frac{1}{T_1} = \frac{3}{8} \left( \frac{e^2 qQ}{h} \right)^2 \left\{ 1 - d_{00}^{(2)}(\beta(t))d_{00}^{(2)}(\beta') - \frac{d_{00}^{(2)}(\beta(t))}{d_{00}^{(2)}(\beta(t))} \right\} \tau_2.$$

If we further assume that the magnetic energy levels are equally spaced (zero quadrupole splitting), terms containing $d_{00}^{(4)}(\beta(t))$ also drop out of the expression for $T_2$:

$$\frac{1}{T_2} = R_{2323} + R_{1223} = \frac{3}{8} \left( \frac{e^2 qQ}{h} \right)^2 \left\{ 1 + \frac{1}{2} d_{00}^{(2)}(\beta(t)) - \frac{1}{2} d_{00}^{(2)}(\beta') \right\} \tau_2.$$

In the above expressions ($e^2 qQ/h$ indicates the static quadrupolar coupling constant (170 kHz for carbon-deuterium bonds) (43). The Wigner matrix elements $d_{00}^{(2)}(\beta(t))$ and $d_{00}^{(2)}(\beta')$ are the second-order Legendre polynomials $P_2(\cos \beta(t))$ and $P_2(\cos \beta')$. Note that, for a perfectly ordered system with $\beta = 0$, $d_{00}^{(2)}(\beta(t)) = 1$ and hence there is no relaxation. In this case the carbon-deuterium bond vector (the electrostatic field gradient) is parallel to the director, with rotation about the director axis as the only possible degree of freedom. For the case of unrestricted reorientation $d_{00}^{(2)}(\beta(t)) = 0$ and we obtain the well-known results (42, 57) for quadrupolar relaxation due to rotational diffusion in isotropic fluids.

The model outlined here is also applicable to relaxation due to dipolar coupling. The Hamiltonian for the dipolar interaction between two identical spins ($I = \frac{1}{2}$) is given by Eq. [10] with the substitution $e^2 qQ/4 \rightarrow -\gamma^2 h^2/r^3$, where all symbols have their usual (42, 57) meanings. The corresponding relaxation rate expressions (Eqs. [12] to [14] and [17]) can be shown to reduce to the literature results for dipolar relaxation due to isotropic rotational diffusion (42) or anisotropic diffusion of ellipsoids of revolution (58, 59).

**RESULTS AND DISCUSSION**

The present treatment represents the simplest possible analysis of the quadrupolar relaxation of specifically deuterated lipid bilayers which takes into proper con-
sideration the statistical amplitude, symmetry, and rate of the molecular motions. Assuming that the segmental reorientation can be described as a stationary Markoff process, the degree of motional reorientation is characterized by statistical averages of the two Legendre polynomials $P_2(\cos \beta(i))$ and $P_4(\cos \beta(i))$. The second-order polynomial is simply related to the segmental order parameter $S_{CD}$, defined by

$$S_{CD} = \frac{1}{2}(3 \cos^2 \beta(i) - 1).$$

Thus, for cases in which the fourth-order terms can be neglected, the effects of ordering on the relaxation rates can be estimated from the deuterium quadrupole splittings without assumptions as to the form of the angular probability distribution. In particular, there is no need for recourse to "cone" models to describe the amplitude of the segmental reorientation (8, 23, 25, 26, 28). In the present treatment it is not necessary to make assumptions regarding correlated chain motions in lipid bilayers, as required by more complex models involving multiple internal rotations (24, 27, 30).

In order to interpret the spectral densities which enter into the relaxation time expressions, a model for the segmental reorientation must be assumed. Here we have only considered rather general models for the rotational motions of the various chain segments. The results of the present analysis are not expected to depend greatly on whether a strong collisional or diffusional model is chosen to characterize the segmental reorientation. In general, both models have proved satisfactory in previous spin label EPR studies of lipid bilayers (52, 56). The strong collisional model has the virtue of simplicity and can be solved analytically, while the diffusional model is advantageous in that it allows a simple physical interpretation of the relaxation time data in terms of the bilayer viscosity.

Regarding the experimental data depicted in Fig. 1, which were obtained using unsonicated lipid dispersions, we note first that the residual deuterium quadrupole splittings observed from multilamellar samples correspond to domains where the bilayer normal (director) is oriented perpendicularly to the external magnetic field. In this case, Eq. [19] can be written in the simple form

$$\frac{1}{T_1} = \frac{3}{8} \left( \frac{e^2 q Q}{\hbar} \right)^2 (1 + \frac{1}{2}S_{CD} - \frac{3}{2}S_{CD}^2) \tau_2. \tag{22}$$

For the phospholipid bilayer systems considered here, $|S_{CD}| \leq 0.2$ and the effect of order on the relaxation rates is predicted to be small or negligible. Thus, the present analysis suggests that the order profile has little to do with the shape of the $T_1$ profile shown in Fig. 1. Rather, the two sets of data represent the positional dependence of two independent parameters, viz., $\tau_2$ and $S_{CD}$, and consequently the similar shapes of the two profiles provide information regarding the forces stabilizing lipid bilayers, as discussed elsewhere (37).

These conclusions are supported by the relatively simple experiment shown in Fig. 2. Here we have measured the $T_1$ relaxation rates of multilamellar samples of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) selectively deuterated at the 9,10 double bond of the sn-2 chain. The two deuterons of the cis double bond are inclined, on time average, at different angles with respect to the bilayer normal (60) and give rise to different quadrupole splittings (12.3 and 2.1 kHz); yet little
difference in the $T_1$ relaxation times is apparent. In a further set of experiments, bilayers of DPPC deuterated at the C5 fatty acyl chain segment were oriented between stacks of glass plates and the $T_1$ relaxation times measured, but, again, a significant angular dependence could not be detected. In the latter case the quadrupole splittings vary from 0 to about 30 kHz. Vold and Vold (50) have performed elegant selective pulse experiments using CDCl$_3$ dissolved in the nematic liquid crystal MBBA that are also consistent with a weak order contribution to the deuterium relaxation rates. It must be emphasized, however, that for more strongly ordered systems, such as DPPC bilayers containing cholesterol ($S_{CD} = -0.4$) (61), order effects may become very important. In such cases one must inquire whether a single correlation time is adequate to characterize the motion, i.e., the assumption that $\eta = D_{\parallel}/D_{\perp} = 1$ may not be valid, and it may be necessary to estimate the fourth-order Legendre polynomial $P_4(\cos \beta(t))$, in addition to $P_2(\cos \beta(t))$.

As employed here, $\tau_2$ represents an effective or average correlation time for those motional fluctuations contributing to the $T_1$ relaxation. With regard to the strong collisional model, the use of a single correlation time amounts to neglect of motional anisotropy while, for the diffusional model, this approximation ignores both anisotropy and ordering effects on the motional correlation times. For the case of lipid bilayers with flexible chains consisting of

$$\begin{align*}
\text{CH}_2 \\
\text{C=C} \\
\text{H}
\end{align*}$$

and

$$\begin{align*}
\text{CH}_2 \\
\text{C=C} \\
\text{H}
\end{align*}$$

groups, the assumption that the segmental anisotropy ratio $\eta \approx 1$ is plausible; also,
the correlation times relevant to the present problem depend on order by only about 20–30\% (assuming that \( \eta = 1 \)) (54), so that analysis of the deuterium \( T_1 \) data in terms of a single correlation time treatment appears to be consistent with both motional models. Moreover, it appears from previous experimental results that any distribution in the correlation times for those motions affecting \( T_1 \) in lipid bilayers must be fairly narrow. For example, a full nuclear Overhauser enhancement (NOE) of 3.0±0.2 is observed in proton-decoupled carbon-13 NMR spectra of phospholipid vesicles (28); aside from establishing the source of the carbon-13 relaxation as dipolar in nature, this observation suggests that if any distribution of correlation times is present, it must lie predominantly within the extreme narrowing time regime at the resonance frequency employed (20 MHz). Given the magnitude of the motional correlation times, it is unlikely that the distribution at each segment position is very great. In a number of organic polymers, by contrast, a substantially reduced NOE is observed, even though the measured \( T_1 \) values suggest that the extreme narrowing condition should be fulfilled (62, 63).

In general, the use of a single correlation time has proved adequate in simulating the EPR spectra of nitroxide spin labels in lipid bilayers (52) and nematic liquid crystals (64). However, previous proton NMR studies have suggested that the relaxation of lipid bilayers (6, 9, 11, 19, 20, 25) and membranes (65, 66) may be frequency dependent, implying that effects due to anisotropic motion are important or, alternatively, that independent motional components are present which differ in terms of their amplitude and frequency range. With this in mind, I have compared the rotational correlation times derived from the deuterium \( T_1 \) data for selectively deuterated DPPC multilamellae (37) to those calculated for samples of (perdeuterated) DPPC-\( d_{62} \) (67) and from the carbon-13 \( T_1 \) values of sonicated vesicles of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) (27). The results are shown in Fig. 3. The various data are in broad agreement, but several points are noteworthy. First, the \( T_1 \) measurements were made at different resonance frequencies (54.4, 34.4, and 25.2 MHz, respectively). Therefore, if a broad distribution of independent motional components were present, not all of which were in the extreme narrowing time regime, or if the motional fluctuations near \( \omega_0 \) were significantly non-Lorentzian, different correlation times would be calculated from the various \( T_1 \) data. This does not appear to be the case. Second, in each instance the data are compared at approximately equivalent reduced temperatures (68). The data for DPPC and DPPC-\( d_{62} \) in Fig. 3A are compared at 51 and 45°C, since the phase transition temperatures (\( T_c \)) are 41.5 and 37°C, respectively (67, 69, 70). Both sets of data can be interpreted in terms of a single correlation time and agree well in the “plateau” region from C3 to C9. Beyond C10, the data diverge somewhat, which may be related to difficulties in evaluating peak amplitudes in the partially relaxed overlapping powder patterns of DPPC-\( d_{62} \). The correlation times derived from the deuterium \( T_1 \) values of the DPPC multilamellae at 80°C are in reasonable agreement with those calculated from the carbon-13 \( T_1 \) values of DMPC vesicles at 52°C (\( T_c = 23°C \)), except at the C3 position, where the discrepancy is not understood (Fig. 3B).

Thus the currently available experimental data appear to be consistent with the simple analysis described here to characterize the fast segmental motions affecting \( T_1 \).
Fig. 3. Comparison of rotational correlation times derived from deuterium and carbon-13 $T_1$ measurements at different resonance frequencies. In the upper (A) panel, the correlation times calculated from the $T_1$ relaxation times of selectively deuterated multilamellar dispersions of DPPC at 51°C and 54.4 MHz (●) (37) are compared to those calculated from $T_1$ data for DPPC-$d_{62}$-multilamellae at 45°C and 34.4 MHz (○) (67). The tentative peak assignments of Davis (67) were used to calculate the correlation time profile for DPPC-$d_{62}$; the bar (—) indicates the average correlation time corresponding to the C3 to C8 segment positions. The two data points for the C10–C15 segment positions of DPPC-$d_{62}$ refer to the nonequivalent resonances from the sn-1 and sn-2 chains. Identical relaxation rates were observed from the sn-1 and sn-2 chain resonances of the selectively deuterated DPPC multilamellar dispersions. In the lower (B) panel the correlation times calculated for selectively deuterated DPPC multilamellar dispersions at 80°C and 54.4 MHz (●) (37) are compared to those derived from the carbon-13 $T_1$ relaxation times of sonicated DMPC vesicles at 52°C and 25.2 MHz (○) (27). The carbon-13 correlation times were calculated by assuming that the relaxation is due to the $^{13}$C-$^1$H dipolar interaction between directly bonded nuclei (27, 28, 73). The symbol ω indicates the chain terminal methyl group.

in lipid bilayers, at least over the limited frequency range investigated (25–54 MHz). Nevertheless, it is conceivable that additional slow motions such as director fluctuations may exist in these systems near the gel to liquid crystalline phase transition temperature (29), and further experimental work is clearly desirable. If such slow motions are found to provide a significant contribution to the relaxation of phospholipid bilayers, then the present analysis will have to be extended to include this
possibility, although the primary conclusions are expected to remain unaltered. The present treatment may also be helpful in understanding other relaxation processes in membranes, such as fluorescence depolarization (71, 72).

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REFERENCES