Unified picture for spin-lattice relaxation of lipid bilayers and biomembranes

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The present study compares and interprets the $^1$H, $^2$H, and $^{13}$C spin-lattice ($T_1$) relaxation times of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), in the liquid crystalline phase, in terms of models for the molecular dynamics of lipid bilayers. The $^1$H $T_1$ times of the DPPC bilayer hydrocarbon region at two frequencies and $^{13}$C $T_1$ data at seven frequencies, for which the relaxation is dipolar in origin, as well as the $^2$H $T_1$ data at three frequencies, due to the quadrupolar interaction, can be unified and interpreted in terms of a collective model for order fluctuations. In normalizing the $^{13}$C $T_1$ data to the $^2$H and $^2$H $T_1$ values, a vibrationally corrected $^{13}$C-$^1$H distance parameter of $r_{CH} = 1.14$ Å has been assumed, rather than the equilibrium bond length of 1.09 Å. The analysis suggests that the behavior of the individual acyl chain segments of lipid bilayers, in the liquid crystalline phase, is similar to that of molecules in nematic fluids.

I. INTRODUCTION

Can the NMR relaxation data for different nuclei in lipid bilayers, i.e., $^1$H, $^2$H, and $^{13}$C, be interpreted quantitatively and self-consistently in terms of a single model for spin-lattice relaxation? In Ref. 1, and in the preceding paper, we have discussed various models which can be used to analyze the molecular dynamics of lipid bilayers in terms of the quadrupolar and dipolar relaxation mechanisms. If such a unified development is possible, then our confidence in the relaxation analysis for each of the nuclei is bolstered, and the underlying physical significance is enhanced. Previous work has tended to focus on the relaxation behavior of a single nucleus at one, or at most two, frequencies (magnetic field strengths); for earlier leading references, cf. Refs. 1 and 2. Here our objective is to show how, for the hydrocarbon region of bilayers of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), in the liquid crystalline state, the $T_1$ relaxation data for $^1$H at two frequencies, $^2$H at three frequencies, and $^{13}$C at seven frequencies can be interpreted in terms of a collective model for order fluctuations of the individual acyl chain segments. Since the magnetogyric ratios of each of these nuclei are different, the reported studies together encompass 12 different frequencies, spanning the range of 14–220 MHz. In addition, since the $^{13}$C relaxation depends on the sum and difference of the $^{13}$C and $^1$H frequencies, spectral density terms at up to $v_H + v_C = 500 + 126 = 626$ MHz are included in the analysis.

II. THEORY

It is first useful to summarize the results of the preceding paper for dipolar relaxation, as well as those of Ref. 1 for the case of quadrupolar relaxation. We shall focus our attention on the relaxation of $^1$H and $^{13}$C (dipolar relaxation mechanism) and $^2$H (quadrupolar relaxation mechanism) in lipid bilayers and biomembranes; the results can be generalized to other nuclei as well. We will also write the relaxation expressions in a somewhat more compact form for future reference, and will only consider the simplest case of the orientally averaged relaxation rates. As mentioned in Refs. 1 and 2, the dependence of the $T_1$ relaxation times on the angle $\beta$ between the bilayer normal and the main magnetic field direction (cf. Fig. 1 of Ref. 1) can be lost, i.e., averaged during the time course of the relaxation (ms→s), by rapid tumbling of small vesicles in aqueous suspensions, or in multilamellar dispersions by lateral diffusion of phospholipids about their curved surfaces or by liposome tumbling.

In Refs. 1 and 2, the observed spin-lattice relaxation rates of lipid bilayers are given in terms of independent contributions from fast or local segmental motions, in addition to slower motions of a more long-range nature; that is by:

$$1/T_1 = 1/T_{1f} + 1/T_{1s} \quad \quad (2.1)$$

In Eq. (2.1), $1/T_{1f}$ and $1/T_{1s}$ denote the spin-lattice contributions from the fast and slow motions, respectively, where cross correlations are ignored. The local motions are assumed to be diffusive in nature, and thus analogous to those of simple fluids, but restricted in their amplitude due to the presence of an ordering potential. Two models for the slow motions can be considered: (i) a simple one correlation time model in which long-range cooperative motions are not explicitly considered; we term such a model "noncollective" for this reason; and (ii) a collective model in which the slow motions are described by a continuous distribution of bilayer disturbances, leading to an $a^{-1/2}$ frequency dependence.

For each of the models considered, the short correlation time limit will be assumed for the local segmental fluctuations. Thus, the contribution to the dipolar or quadrupolar relaxation rates will be directly proportional to the effective correlation time $\tau^{(2)}_d$ of the fast motions. For a noncollective model, in which the correlation time $\tau^{(2)}_d$ for the lower fre-
quency fluctuations is assumed to fall into the slow motional limit (cf. Sec. IV A of Ref. 1, Secs. IV A.1 and IV B.1 of Ref. 2), the results for the orientationally averaged spin-lattice relaxation rates can be written as

\[
\langle 1/T_1 \rangle_{\alpha} = A \left( 1 - (S_{\text{CH}})_{\alpha} \right) r_{10}^2 + B' \left( S_{\text{CH}} \right)_{\alpha} (1 - S_{\beta}) r_{10}^{2(-1)} \omega_{\perp}^{-2}, \tag{2.2a}
\]

\[
\approx A r_{10}^2 + B S_{\text{CH}}^2 (1 - S_{\beta}) r_{10}^{2(-1)} \omega_{\perp}^{-2}. \tag{2.2b}
\]

Here \( A \) and \( B' \) are constants characteristic of the particular nuclei, e.g., \(^1\text{H}, ^2\text{H}, \text{or } ^{13}\text{C} \), as given in Table I, and \( \omega_{\perp} \) denotes the resonance frequency given a magnetic field strength \( (\omega_{\perp} = \gamma_i B_0) \). In the following it will be helpful to refer to Fig. 1 of Ref. 1. The brackets \( \langle \rangle_{\alpha} \) indicate that the relaxation rates are averaged over all bilayer orientations with respect to the main magnetic field \( B_0 \) characterized by the Euler angles \( \Omega \). Equations (2.2a) and (2.2b) correspond to Eqs. (3.20) and (4.10) of Ref. 1 and to Eqs. (3.2), (3.15), and (4.8) of Ref. 2. In Eqs. (2.2a)-(2.2b),

\[
\langle (S_{\text{CH}})_{\alpha} \rangle = \frac{\langle d_{ij} \beta \beta \cdot i \rangle_{ij} \tau_i^2}{3 \cos^2 \beta \beta \cdot i (1 - \tau_i^2)}. \tag{2.3a}
\]

\[
\approx A r_{10}^2 + B S_{\text{CH}}^2 (1 - S_{\beta}) r_{10}^{2(-1)} \omega_{\perp}^{-2}. \tag{2.3b}
\]

is an order parameter which describes slower fluctuations in the fast motions only, i.e., Euler angles \( \Omega \). In Eq. (2.2b), it is assumed that \( \langle S_{\text{CH}} \rangle \ll 1 \), where \( B = AB'C/S_{\beta}^2 \). For both the noncollective and collective models, the constants \( A \) and \( B' \) appropriate for the \(^1\text{H}, ^2\text{H}, \) and \(^{13}\text{C} \) \( T_1^{-1} \) relaxation rates of lipid bilayers are summarized in Table I.

Now, irrespective of whether a collective or noncollective model is assumed, the observed \( T_1^{-1} \) rates for the case of dipolar \(^1\text{H}, ^{13}\text{C} \) or quadrupolar \(^2\text{H} \) relaxation can be approximately written as

\[
1/T_1 = A \left[ r_{10}^2 + B' \langle 1/T_{1\text{norm}} \rangle \right]. \tag{2.4}
\]

\( (T_{1\text{norm}}) \) denotes the normalized contribution from the slow motions to the relaxation rates of the \(^1\text{H}, ^2\text{H}, \) and \(^{13}\text{C} \) nuclei, and is given by

\[
1/T_{1\text{norm}} = (1/AB') \left[ (1/T_1 - (1/T_{1\text{norm}})) \right], \tag{2.5a}
\]

\[
= (1/B') \left( 1/AT_1^{-1} - r_{10}^{-2} \right). \tag{2.5b}
\]

In the above equations, \((1/AB')\) is the normalization constant for the slow motional contribution; \( A \) depends on the nucleus employed and \( B' \) depends on both the nucleus and the nature of the model assumed for the slow motions; cf. Table I. Note that the above normalization is only possible if collection of the spectral density terms and factoring of the frequency contribution is possible in the relaxation expressions, e.g., an \( \omega_{\perp}^{-1/2} \) or \( \omega_{\perp}^{-2} \) dependence is assumed. Equations (2.5a) and (2.5b) have the same functional form for the various nuclei with respect to (i) frequency and (ii) ordering. In terms of the two models, we then have that:

**Noncollective:**

\[
1/T_{1\text{norm}} = (1/AB') \left( 1 - S_{\beta} r_{10}^{2(-1)} \omega_{\perp}^{-2} \right). \tag{2.6a}
\]

**Collective:**

\[
1/T_{1\text{norm}} = C (S_{\text{CH}})_{\beta \beta} \omega_{\perp}^{-1/2}. \tag{2.6b}
\]

III. COMPARISON TO EXPERIMENTAL DATA

From Eqs. (2.6a) and (2.6b), it is apparent that there are two ways to conveniently normalize the \( T_1 \) data obtained for

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>( A )</th>
<th>( B' ) (Noncollective)</th>
<th>( B' ) (Collective)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1\text{H}^c)</td>
<td>( 3/2 \omega_{\perp} \gamma_1^2 \beta \beta \cdot i \tau_i^2 )</td>
<td>2/5</td>
<td>3/10(1 + 2i2)</td>
</tr>
<tr>
<td>(^2\text{H}^c )</td>
<td>( 3\omega_{\perp} \gamma_1^2 \beta \beta \cdot i \tau_i^2 )</td>
<td>2/5</td>
<td>3/10(1 + 2i2)</td>
</tr>
<tr>
<td>(^{13}\text{C}^a)</td>
<td>( N_{\beta \beta} \gamma_1^2 \beta \beta \cdot i \tau_i^2 )</td>
<td>1/10(( \gamma_1^2 \gamma_c - 1 ))^{-1/2} + 3 + 6(( \gamma_1^2 \gamma_c + 1 ))^{-1/2}</td>
<td>3/20(( \gamma_1^2 \gamma_c - 1 ))^{-1/2} + 3 + 6(( \gamma_1^2 \gamma_c + 1 ))^{-1/2}</td>
</tr>
</tbody>
</table>

\( ^a \) Intrasegmental contribution assuming constant internuclear distances \( r_{10}^2 \) for \(^1\text{H} \) NMR and \( r_{1\text{CH}}^2 \) for \(^{13}\text{C} \) NMR. For the case of \(^{13}\text{C} \) NMR, \( N_{\beta \beta} \) denotes the number of directly bonded protons; all other symbols are defined in the text or have their standard meanings.
the acyl chain segments of the DPPC bilayer\footnote{4-7} employing different nuclei at different resonance frequencies, assuming either a noncollective model for the slow motions in the long correlation time regime [Eqs. (2.2)] or a collective model [Eqs. (2.3)]. One can plot (i) the functions \( \omega_{j}^{-2}(T_{1j}^{-1})_{\text{norm}} \) or \( \omega_{j}^{-1/2}(T_{1j}^{-1})_{\text{norm}} \) vs \((S_{CD})^{2}\), corresponding to the noncollective or collective models, respectively, where \( S_{CD} \) is the bond segmental order parameter from \(^2\)H NMR studies.\footnote{5,25,26} Alternatively (ii), one can plot the function \((S_{CD})^{-2}(T_{1j}^{-1})_{\text{norm}} \) vs \( \omega_{j}^{-2} \) (noncollective model) or \( \omega_{j}^{-1/2} \) (collective model). Such a procedure assumes that the amplitude of the fluctuations in the local ordering, given by \( S_{\mu\nu} \), is similar for the different acyl chain segments of the bilayer hydrocarbon region. We shall adopt the former approach.

Figure 1 shows plots of the function \( \omega_{j}^{-1/2}(T_{1j}^{-1})_{\text{norm}} \) vs \((S_{CD})^{2}\) for the DPPC bilayer assuming the collective model, where \((T_{1j}^{-1})_{\text{norm}} \) is given by Eqs. (2.5) and Table I. In normalizing the \(^1\)H \( T_{1j} \) data, a value of \( (\rho_{\text{CH}}^{2}Q/\hbar) = 170 \text{kHz} \) has been taken for the quadrupolar coupling constant;\footnote{26} an effective \(^{13}\)C-\(^1\)H bond distance of \( \rho_{\text{CH}} = 1.14 \text{ Å} \) has been used in normalizing the \(^{13}\)C \( T_{1j} \) data for reasons to be discussed shortly. In addition, an average value of \( \rho_{\text{CH}}^{2}Q/\hbar = 1 \times 10^{-11} \text{ s} \) over the range of temperatures employed has been assumed. While \( \rho_{\text{CH}}^{2} \) is expected to vary with both the (i) acyl chain segment position and (ii) temperature, the relatively close agreement of the intercepts of the plots of \( (N_{\text{CH}}T_{1j})^{-1} \) vs \( \omega_{j}^{-1/2} \) or \( (S_{CD})^{-2} \), for the case of the \(^{13}\)C data in Figs. 7 and 8 of Ref. 2, together with the relatively small values of the \((N_{\text{CH}}T_{1j})^{-1} \) contribution, imply that for the collective model significant error will not be introduced by taking an average over all segment positions and temperatures. The \(^{13}\)C \( T_{1j} \) data' for the acyl chain resonances of small unilamellar DPPC vesicles at seven frequencies (magnetic field strengths) are shown in the upper panel (a) of Fig. 1; the lower panel (b) shows the corresponding \(^2\)H \( T_{1j} \) results\footnote{5,6} for specifically deuterated DPPC multilamellar dispersions at three frequencies. (Only small differences are observed in the \( T_{1j} \) relaxation times of vesicle suspensions compared to unsonicated multilamellar dispersions; cf. Ref. 5). In each case, a linear relationship is apparent, in agreement with Eq. (2.6b) for the collective model. The observed linear dependence implies that the amplitude of the order fluctuations, characterized by the order parameter \( S_{\mu\nu} \), is similar for the various acyl chain segments of the DPPC bilayer. If the same data are plotted as \( \omega_{j}^{-2}(T_{1j}^{-1})_{\text{norm}} \) vs \((S_{CD})^{2}\) according to Eq. (2.6a), i.e., for the noncollective model in the long correlation time limit, then nonsense results. Thus, a noncollective model of the type suggested previously\footnote{27,28} can be clearly eliminated, as concluded in Refs. 1 and 2.

It should be noted that in panel (a) of Fig. 1 we have simply replotted the \(^{13}\)C \( T_{1j} \) data in Fig. 8 of Ref. 2 by assuming an \( \omega_{j}^{-1/2} \) frequency dependence; the normalized data for each of the seven frequencies are now superimposable within experimental error. Likewise, the \(^2\)H \( T_{1j} \) data and least squares fit of panel (b) of Fig. 1 are superimposable upon the \(^{13}\)C \( T_{1j} \) data of panel (a); the large number of data points is the only reason that the results are presented separately. Thus, we claim to have unified the \(^2\)H and \(^{13}\)C \( T_{1j} \) data for the DPPC bilayer, since the results for all the hydrocarbon chain segments (excluding the interfacial C-2 position), at all temperatures and frequencies for the two nuclei, can be shown to fall on a single straight line when a collective model is assumed and the data are normalized using Eqs. (2.5) and (2.6b). However, a noncollective model could also account for the data near a \( T_{1j} \) minimum for the slow motions (cf. Ref. 2), and thus relaxation time measurements are needed over a wider frequency range to unequivocally distinguish the two interpretations.

In Fig. 2 we show plots vs \( \omega_{j}^{-1/2} \) of the \(^1\)H, \(^2\)H, and \(^{13}\)C \( T_{1j} \) data obtained for the DPPC bilayer, in the liquid crystalline state at \( 50 \text{ °C} \) and 12 frequencies, normalized in accord with the collective model using Eq. (2.5). In addition to the \(^2\)H and \(^{13}\)C \( T_{1j} \) results discussed in conjunction with Fig. 1, we have now included the \(^1\)H \( T_{1j} \) data of Kroon et al.\footnote{4} for the acyl chain (CH\(_2\))\(_n\) resonance, corresponding to the C-3 to C-15 segments, of vesicles of (protonated) DPPC mixed with DPPC-\(d_{14}\) at two frequencies (100 and 220 MHz). In the latter studies the intermolecular contribution has been eli-
of a CH₂ group now appears to be supported by a recent direct determination of the \(^{13}\text{C}-^{1}\text{H}\) dipolar coupling constant employing solid-state NMR techniques.\(^{33}\) When the \(^{13}\text{C}\) data for the hydrocarbon region of the DPPC bilayer are normalized according to Eq. (2.5) using a value of \(r_{\text{CH}}^{0} = 1.14 \text{ Å}\) and plotted vs \(\omega_{r}/2\), the data for the three nuclei, viz \(^{1}\text{H},^{2}\text{H},\) and \(^{13}\text{C}\), are seen to fall on a single straight line [Fig. 2(a)]. Thus a collective model is capable of explaining the presently available \(T_{1}\) data for the hydrocarbon region of the DPPC bilayer, with the proviso that \(r_{\text{CH}}^{0} = 1.14 \text{ Å}\).

If we alternatively choose \(r_{\text{CH}}^{0} = 1.09 \text{ Å}\), i.e., the equilibrium bond distance is assumed, then the results shown in panel (b) of Fig. 2 are obtained. In this case, the normalized \(^{13}\text{C}\) \(T_{1}\) data do not fall on the same line as the normalized \(^{1}\text{H}\) and \(^{2}\text{H}\) \(T_{1}\) values, and we conclude that the choice of \(r_{\text{CH}}^{0} = 1.14 \text{ Å}\) better describes the results for the case of the collective model. For the DPPC bilayer at 65 and 80 °C, results similar to those in Fig. 2 are obtained (not shown). By contrast, if the noncollective model in the long correlation time regime is assumed, and the normalized \(T_{1}\) data plotted vs \(\omega_{r}/2\), , curved lines are obtained in all cases (not shown; cf. Ref. 7). Thus, we again conclude that such a model\(^{27,28}\) is not in accord with these most recent \(T_{1}\) results.

It should be mentioned that the disparity between the \(^{1}\text{H}\) and \(^{2}\text{H}\) results, on the one hand, and the \(^{13}\text{C}\) \(T_{1}\) data, on the other [Fig. 2(b)], could also arise from any one of several assumptions inherent in the analysis of the \(T_{1}\) data for the various nuclei. Thus, it is perhaps reassuring that a relatively simple analysis of the data appears to be possible. For the case of the \(^{1}\text{H}\) \(T_{1}\) values, we have only considered the intra-segmental contribution to the relaxation. Although as much as 80% of the observed residual second moment \(M_{2}\), of the multilamellar DPPC dispersions is believed to be intrasegmental in origin,\(^{30}\) neglect of \(^{1}\text{H}\) dipolar interactions between the various CH₂ groups of a given chain could introduce a significant error into the results. In addition, it is uncertain as to what extent the intra- and the intersegmental \(^{1}\text{H}\) dipolar interactions contribute to \(M_{2}\), averaged over the fast motions only, which would then be modulated by the slow motions to provide the additional relaxation contribution; cf. Sec. IV A of Ref. 2. For the case of \(^{2}\text{H}\) NMR, we have further neglected the possibility of \(^{2}\text{H}\) isotope effects in comparing the motion of the \(^{12}\text{C}-^{2}\text{H}\) groups to that of \(^{13}\text{C}-^{1}\text{H}\) groups\(^{34-36}\); these might lead to longer apparent correlation times for the \(^{2}\text{H}\) nucleus, corresponding to an increase in the normalized \(^{2}\text{H}\) \(T_{1}\) rates relative to those for \(^{13}\text{C}\) as observed [Fig. 2(b)]. In this respect it is encouraging that the normalized \(^{1}\text{H}\) and \(^{2}\text{H}\) \(T_{1}\) rates fall on the same line [Fig. 2(b)], in spite of the different assumptions inherent in the analysis for each nucleus. Also, for the case of the \(^{1}\text{H}\) and \(^{13}\text{C}\) \(T_{1}\) values, the relaxation is averaged over a fairly substantial number of chain segments corresponding to the unresolved (CH₂)ₙ resonances; the \(^{1}\text{H}\) data by contrast refer to DPPC with specifically labeled acyl chain deuteromethylene groups. Finally, since the \(^{1}\text{H}\) and \(^{13}\text{C}\) \(T_{1}\) data are for vesicles of DPPC, whereas the \(^{2}\text{H}\) \(T_{1}\) data are for DPPC multilamellar dispersions, it is always possible that small systematic differences in the \(T_{1}\) values of the two systems exist which

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{Plots of the normalized contribution from slow motions to the acyl chain segmental \(^{1}\text{H},^{2}\text{H},\) and \(^{13}\text{C}\) \(T_{1}\) relaxation rates, for DPPC in the liquid crystalline phase at 50 °C, as a function of \(\omega_{r}/2\) assuming a collective model; cf. text. (\(x\)) \(^{1}\text{H}\) \(T_{1}\) data (Ref. 4) for (CH₂)ₙ resonance of vesicles of 10% (protonated) DPPC/90% DPPC-d₂; (\(\bullet\)) \(^{2}\text{H}\) \(T_{1}\) data (Refs. 5 and 6) for C-4, C-8, and C-12 segments, respectively, of DPPC multilamellar dispersions; (\(\sigma\)) \(^{13}\text{C}\) \(T_{1}\) data (Ref. 7) for (CH₂)ₙ resonance of DPPC vesicles (corresponding to C-4 to C-13 segments). An \(^{1}\text{H}-^{2}\text{H}\) intranuclear distance of 1.78 Å and a quadrupolar coupling constant of \((e^{2}qQ/\hbar) = 170 \text{ kHz}\) have been used. In the top panel (a), an effective \(^{13}\text{C}-^{1}\text{H}\) distance of \(r_{\text{CH}}^{0} = 1.14 \text{ Å}\) has been assumed in normalizing the \(^{13}\text{C}\) \(T_{1}\) rates; in the bottom panel (b) \(r_{\text{CH}}^{0} = 1.09 \text{ Å}\), i.e., the equilibrium bond distance has been used.}
\end{figure}
may have been previously overlooked. With the above reservations, the fact that the $T_1$ data for each of the three nuclei can be explained in such a simple and internally consistent manner by a collective model is encouraging. It suggests that we have indeed been successful in accounting for many of the important motional features influencing the spin-lattice relaxation of the hydrocarbon chain segments of DPPC in the liquid crystalline state.

ACKNOWLEDGMENTS

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3Abbreviations used: NMR, nuclear magnetic resonance; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine.
15In the notation of S. I. Chan and colleagues, $S_{Cm} = S_p$, $S_{m} = S_{m}$, and $S_{m} = S_{m}$, where $S_{m} = S_{m}$.
32M. G. Munowitz and R. G. Griffin (personal communication).