Frequency dependent $^2$H N.M.R. relaxation rates of small unilamellar phospholipid vesicles

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Frequency dependent $^2$H N.M.R. relaxation rates of small unilamellar phospholipid vesicles

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Deuterium ($^2$H) N.M.R. spectroscopy has been used to investigate experimentally the molecular dynamics of phospholipid vesicles. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was deuterated specifically in both acyl chains, and aqueous suspensions of vesicles were studied above the main order-disorder transition temperature. $^2$H spin-lattice relaxation rates ($R_1$) were measured at nine different magnetic field strengths, corresponding to Larmor frequencies ($\nu_0 = \omega_0/2\pi$) spanning 2.5 MHz to 61.4 MHz. Of three models considered, a model in which $R_1 \propto \omega_0^{1/2}$ best described the $^2$H N.M.R. results, whereas fits to $R_1 \propto \omega_0^3$ or to a lorentzian plus a constant dispersion were less satisfactory. Thus a broad distribution of motions exists, which must be explained by any model for the N.M.R. relaxation of liquid-crystalline phospholipids.

Here we address the problem of interpreting the N.M.R. spin–lattice relaxation rates ($R_1$) of phospholipid vesicles. It is found experimentally that $^1$H, $^2$H, and $^{13}$C $R_1$ values of both unilamellar vesicles and multilamellar samples of liquid-crystalline phospholipids depend on the magnetic field strength, i.e. frequency [1–4]. The interpretation of the results in terms of molecular dynamics poses difficulties, however [4–10]. We have now acquired $^2$H $R_1$ data which enable distinction among various suggested relaxation models; a broad distribution of motions is evident.

Brown [5] suggests that collective order fluctuations in the MHz range may contribute to the dispersion. Such a dynamical process yields $R_1 \propto \omega_0^{1/2}$ in nematic liquid crystals, where $\omega_0$ is the Larmor frequency, and $^{13}$C N.M.R. data for phospholipid vesicles at relatively high frequencies can indeed be fit approximately to such a relaxation law [7]. Marqusee et al. [8], on the other hand contend that such a process should give rise to a $R_1 \propto \omega_0^{-1}$ dependence, owing to the bilayer dimensionality. In these continuum descriptions, a constant term is also included to account for the fast internal motions of phospholipids within the bilayer [9, 10]. A further possibility is a noncontinuum model, involving a lorentzian and a constant term [5, 6, 11, 12]. The latter model has been used extensively to explain frequency dependent data obtained for micellar systems [11–13], where it has been termed the

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'two-step' model. Finally, Rommel et al. [4] have interpreted the dispersion in the high frequency regime (>1 MHz) in terms of internal isomerizations and overall molecular rotational diffusion.

As far as results obtained at high frequencies (>15 MHz) go, each of the models can represent the data. However, previous $^2$H and $^{13}$C relaxation data are available over only a limited frequency range. To extend the measurements to lower frequencies, and thereby test the suggested relaxation laws, we have performed $^2$H N.M.R. studies of aqueous suspensions of sonicated vesicles of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) above the main order-disorder transition temperature. The $^2$H nucleus was chosen because its relaxation is intramolecular, and thus not complicated by problems of interpretation as found for the $^1$H nucleus [14]. The figure shows $^2$H $R_1$ values determined at 30 °C for vesicles labelled with deuterium at the third position of both acyl chains, viz. 1,2[3',3'-$^2$H$_2$] DMPC. The labelling of the acyl chains with $^2$H and synthesis of DMPC followed standard procedures [15, 16]. The spin-lattice relaxation rate, $R_1$, was measured by the

\[
J(\omega) = A + B/\omega
\]

\[
J(\omega) = (1-A)^2 B + A^2 C \left[ \frac{1}{1 + (\omega - c)^2} \right]
\]

$^2$H spin-lattice relaxation rates ($R_1$) as a function of the Larmor frequency ($\nu_0$/MHz) for vesicles of 1,2[3',3'-$^2$H$_2$] DMPC at 30 °C. Each reported value is the weighted average of at least two and typically three or more independent measurements of $R_1$ employing the same sample of DMPC vesicles. The weighting factors were obtained from standard deviations of three parameter fits of $R_1$ to the raw inversion recovery data; error bars correspond to standard deviations of the average $R_1$ values [23]. Where no error bars are indicated, the size of the symbol is larger than the error bar. Also given are the fits of the relaxation laws discussed in the text, using equation (1) and the expression for $J(\omega)$ as indicated in the figures as fitting functions. For clarity the figure has been divided into three panels; in each panel the performance of the indicated relaxation law is shown.
Frequency dependent N.M.R. relaxation rates of vesicles

inversion recovery method, using an electromagnet for frequencies between 2.5 and 13.8 MHz and superconducting magnets at higher frequencies. Data at nine magnetic field strengths are included, corresponding to Larmor frequencies between 2.5 MHz and 62.4 MHz. The results encompass 1.4 decades of frequency, and extend significantly previous 13C R1 studies spanning 0.9 decade [3, 7]. As can be seen, the values of R1 increase with decreasing frequency over the entire range. This behaviour is in contrast to that of surfactant micelles such as those formed by dodecyltrimethylammonium chloride, where a constant value of R1 is reached at sufficiently low frequencies [17, 18].

It should be noted that rotational tumbling of the vesicles and phospholipid lateral diffusion over the curved vesicle surface do not produce the dispersion in the figure. For these processes, the contribution to R1 can be estimated from the relations

$$R_1 = \frac{3\pi^2}{20} \chi^2 [J(\omega_0) + 4J(2\omega_0)].$$

Here \(\chi\) is the quadrupolar coupling constant, and set equal to 170 kHz, while

$$J(\omega) = S_{CD}^2 \tau_c/(1 + (\omega \tau_c)^2).$$

In (2) \(S_{CD}\) is the C-2-H bond order parameter [19] and the effective correlation time \(\tau_c\) is given in terms of the vesicle radius \(R_{ves}\), solvent viscosity \(\eta\), and phospholipid lateral self-diffusion coefficient \(D\) by

$$\frac{1}{\tau_c} = \frac{3k_B T}{(4\pi\eta R_{ves}^3) + 6D/R_{ves}^2}.$$ 

Taking \(S = -0.2\) [20], \(R_{ves} = 12.5\ nm\) [21], and \(D = 3 \times 10^{-12} m^2 s^{-1}\) at 30°C [22] yields \(R_1\) = 5 to 0.008 s^{-1} over the frequency range \(v_0 = 2.5\) to 61.4 MHz, which is small relative to the observed values of \(R_1\).

The three panels of the figure include the results of non linear regression fitting of the following relaxation laws: \(R_1 \propto \omega^{-1/2} + \text{const} , R_1 \propto \omega^{-1} + \text{const} ,\) and the 'two-step' model to the data. The error square sums ERRSUM and ERRSUMd [23] of the fits to the data are given in the table. As is evident from the figure and the table, the first of the models does indeed best match the experimental findings. Similar results are obtained for 1,2[7,7'-2H2] DMPC at 30°C, as well as for 1,2[3', 3'-2H2] DMPC and 1,2[7',7'-2H2] DMPC at 50°C. In this context it is worth mentioning the work of Pastor et al. [24], who use a model consisting of fast axial rotation and a slow noncollective wobble of lipids in the bilayer. These two motions are then superimposed on the fast internal motions [5, 6, 10, 24]. This motional model gives rise to a complicated relaxation law with several lorentzian terms. The

<table>
<thead>
<tr>
<th>Model</th>
<th>n†</th>
<th>ERRSUM</th>
<th>ERRSUMd</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R_1 = A + B\omega_0^{1/2})</td>
<td>2</td>
<td>11.11</td>
<td>1.587</td>
</tr>
<tr>
<td>(R_1 = A + B\omega_0^{-1})</td>
<td>2</td>
<td>198.9</td>
<td>28.41</td>
</tr>
<tr>
<td>Two-step noncollective</td>
<td>3</td>
<td>33.87</td>
<td>5.647</td>
</tr>
</tbody>
</table>

† The error square sums, ERRSUM, is defined as ERRSUM = \(\Sigma[(R_{obs}^i - R_{calc}^i)/\sigma_{R_i}]^2\), where \(R_{obs}^i\) and \(R_{calc}^i\) are the observed and calculated values of \(R_1\), respectively while \(\sigma_{R_i}\) is the standard deviation of \(R_1\), determined from replicate measurements of \(R_1\). In the fitting procedure, ERRSUM is minimized [29]. When comparing models with different numbers of adjustable parameters, the reduced error square sum (ERRSUMd), defined as ERRSUM/d, should be used [23]. Here \(d = N-n\) is the number of degree of freedoms, \(N\) the number of data points, and \(n\) the number of adjustable parameters.

† Number of adjustable parameters for the indicated model.
data in the figure can also be represented very well with two lorentzians and a constant, which is perhaps not surprising bearing in mind that the number of fitting parameters now totals five.

Thus a model yielding $R_1 \propto \omega^{-1/2} + \text{const}$ describes the $^2\text{H} R_1$ data over frequencies spanning 2.5 MHz to 60 MHz (actually from 2.5 MHz to 120 MHz, due to the dependence on the spectral density at twice the Larmor frequency, cf. equation 1). Moreover as shown elsewhere [25], the fit of the $^2\text{H} R_1$ data can be used to predict $^{13}\text{C} R_1$ rates at natural abundance for DMPC vesicles [26], which include frequencies up to 450 MHz. But given that a model approximated by $R_1 \propto \omega^{-1/2} + \text{const}$ accurately reproduces the $^2\text{H}$ and $^{13}\text{C}$ relaxation data over a broad frequency range, the hypothesis that collective order fluctuations govern the relaxation is by no means proven. In fact, other models based on a distribution of correlation times [27] also predict an $\omega^{-1/2}$ dependence. Recent $^2\text{H}$ N.M.R. studies of oriented multilamellar samples of DMPC are noteworthy in this regard [28], and are inconsistent with a formulation of order fluctuations solely in terms of motion of a local director axis (order-director fluctuations, ODF). What seems clear is that a broad distribution of motions exists, which is not described by a single lorentzian dispersion, and which depends approximately on $\omega^{-1/2}$ over the accessible range of frequencies. Evidently a comprehensive treatment of the relaxation should consider a hierarchy of motions, including local segmental [5, 9, 10] and molecular [4, 5] motions, in addition to concerted fluctuations of molecules within the bilayer.

Leaving aside the specific cause of the relaxation data presented in the figure, the observed dispersion suggests two things. First, there are motional components over a very wide time-scale in phospholipid vesicles. In the event that such motions have physiological implications in cell membranes, the actual dynamical process may be of lesser importance. Second, any model for the N.M.R. relaxation of vesicles must properly account for the dispersion reported in the figure.

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[29] The minimization was achieved by the general minimization routine STEPIT (Program No. 307, 'Quantum Chemistry Program Exchange', Department of Chemistry, Indiana University, Bloomington, Indiana 47401. Author: J. P. Chandler, Oklahoma State University, Stillwater, OK).