Magnetic Resonance Spectra of Membranes

(spin label/two-dimensional diffusion/lipid bilayers/relaxation theory)

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ABSTRACT A number of electron and nuclear magnetic resonance studies of model membranes, and biological membranes, involve time-dependent magnetic interactions between membrane components that undergo lateral diffusion relative to one another. The two-dimensional character of this motion can have a special, large effect on magnetic resonance line shapes, and relaxation rates, because of the long-time tail of the correlation function for magnetic interactions modulated by this motion. Equations are given for the specific case in which nuclear relaxation rates are enhanced due to dipolar interactions with membrane-bound spin labels. An experimental study of spin-label-enhanced 13C nuclear relaxation in unsonicated dispersions of phosphatidylcholine is accounted for with this theory, together with the previously reported lipid diffusion constant of \( D \approx 2 \times 10^{-4} \text{ cm}^2/\text{sec} \). Our analysis of previously reported \(^1\text{H}\) and \(^13\text{C}\) nuclear relaxation rates in small phospholipid vesicles produced by sonication suggests that the rate of lateral diffusion in these small vesicles may be significantly larger than \( 10^{-4} \text{ cm}^2/\text{sec} \).

Electron and nuclear magnetic resonance spectroscopy have been used extensively for studies of kinetic and structural properties of model membranes (lipid bilayers) and biological membranes. Some of these studies have taken advantage of magnetic interactions between spin magnetic moments on different membrane-bound molecules in order to derive diffusion constants for their relative lateral motion in the plane of the membrane. However, none of these latter studies have specifically included the effects of two-dimensional diffusion in the theoretical analysis of magnetic resonance line shapes, and relaxation rates. It is known from experimental and theoretical studies of other systems that there can be major differences in the relative effects of two versus three-dimensional motion on magnetic resonance line shapes, and relaxation rates (1-4).

The purpose of the present communication is to treat one special problem that arises in the study of bilayers and membranes, namely, spin-label induced nuclear relaxation. In this case a low concentration of electron spins is present in the membrane, specifically attached to one membrane component (a lipid or protein), and there is an associated enhancement of nuclear relaxation in other membrane molecules (e.g., phospholipids). This type of experiment was first employed by Kornberg and McConnell (5), to demonstrate that lateral diffusion of phospholipid molecules in membranes is rapid. These authors were not able to estimate a diffusion constant since the theory developed in the present paper was not then available. The methods developed here are also applicable to certain other types of magnetic resonance studies of membranes, such as those involving magnetic interactions between pairs of nuclear spins on different molecules (6).

THEORETICAL RELAXATION RATES

We consider a biological membrane, or a model membrane (phospholipid bilayer), in which one or more membrane components undergoes two-dimensional lateral diffusion. A membrane component is specifically labeled with a spin label, for example, one containing the nitroxy group,

![Diagram of nitroxy group]

The large paramagnetic moment of the unpaired electron produces an enhanced relaxation of nuclei in other membrane molecules. Some of these molecules may diffuse relative to the spin-labeled molecule. It is assumed that the two-dimensional lateral diffusion of the spin label is confined to one plane, and that the nucleus of interest undergoes two-dimensional lateral diffusion in a second plane. The two planes are parallel, but need not coincide. The present calculation is applicable, for example, to experimental studies of spin-labeled lipids, or proteins, that enhance the nuclear relaxation in other lipids (5-9). It will be assumed that lipids whose nuclei undergo enhanced relaxation are in the "fluid" state, for which earlier studies of the rates of lateral diffusion have yielded diffusion constants \( D \) of the order of \( 2 \times 10^{-4} \text{ cm}^2/\text{sec} \) (10, 11). The magnetic field at a given nucleus fluctuates in time for (at least) two reasons. First, the spin label (on one molecule) and the nucleus (on a second molecule) diffuse relative to one another in the plane of the membrane and thereby modulate the strength of the spin–spin dipolar interaction. Second, the electron spin undergoes paramagnetic relaxation, which also modulates the local field at the nucleus. These two modulations of the local field at the nucleus can safely be assumed to be uncorrelated. Of course any relative motion of the two spins modulates the dipolar energy and thus affects nuclear resonance line broadening and relaxation. We neglect intramolecular motions on the ground that their amplitudes are much smaller than the amplitudes associated with the diffusion process. The discussion below follows, insofar as possible, the mathematical formalism that has been used previously in spin-label literature (12), and in the text on nuclear magnetism byAbragam (13).

Abbreviation: HGSL, the "head-group spin label" in which an N-methyl group of phosphatidylcholine is replaced by the paramagnetic nitroxy group I.

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The electron spin denoted $S$ is restricted to lateral diffusion in one plane that is parallel to a second plane in which the diffusion of the nuclear spin $I$ is restricted. The parallel planes are separated by a normal distance $z_M$. The coordinate system $x_M$, $y_M$, $z_M$ is fixed in the membrane, and the coordinate system $x_L$, $y_L$, $z_L$ is fixed in the laboratory. The $z_L$ coordinate is parallel to the applied magnetic field direction, and $x_M$ and $z_M$ are parallel and perpendicular to the plane of the paper. The smallest allowed value of $y_M$ is $d$, corresponding to the distance of closest approach of the two molecules, one containing the unpaired electron, and the other containing the nucleus of interest. The cylindrical coordinates $z$, $\rho$, $\phi$ (not shown here) are based on the membrane frame, where $z = z_M$, and $x_M = \rho \cos \phi$, $y_M = \rho \sin \phi$.

The magnetic dipole–dipole interaction between an electron and a nucleus can be represented by a spin Hamiltonian $\mathcal{H}$ (12),

$$\mathcal{H} = \hbar \mathbf{S} \cdot \mathbf{I}.$$  \[1\]

The hyperfine coupling dyadic can be expanded in irreducible (tensor) dyadics $T_m^M$ (12),

$$\mathbf{T} = \sum_m A_m^M T_m^M.$$  \[2\]

Here the superscript $M$ refers to a coordinate system fixed in the plane of the membrane; $\mathbf{i}^M, \mathbf{j}^M, \mathbf{k}^M$ are the unit vectors and $\mathbf{k}^M$ is normal to the plane of the membrane. The quantities $A_m$ are conveniently expressed in terms of cylindrical coordinates, $z$, $\rho$, $\phi$. This coordinate system is given in Fig. 1, and values of $A_m$ can be obtained from the values of $B_m$ given in Table 1, where

$$A_m = \gamma S I h^2 h/2 \left(\rho^2 + z^2\right)^{\gamma/2} \exp(-i m \phi) B_m.$$  \[3\]

The irreducible dyadics can then be transformed to a second set $T_{\phi}^E$ fixed in the laboratory, where $\mathbf{k}^L$ is parallel to the applied field direction, using the Wigner rotation matrices $D_{m\phi}(\alpha \beta \gamma)$. Thus,

$$\mathcal{H} = h \sum_{m\phi} A_m D_{m\phi}(\alpha \beta \gamma) \mathbf{S} \cdot \mathbf{T}_{\phi}^E \mathbf{I}.$$  \[4\]

![Fig. 1. Coordinate system. The electron spin denoted S is restricted to lateral diffusion in one plane that is parallel to a second plane in which the diffusion of the nuclear spin I is restricted. The parallel planes are separated by a normal distance z_M. The coordinate system x_M, y_M, z_M is fixed in the membrane, and the coordinate system x_L, y_L, z_L is fixed in the laboratory. The z_L coordinate is parallel to the applied magnetic field direction, and x_M and z_M are parallel and perpendicular to the plane of the paper. The smallest allowed value of y_M is d, corresponding to the distance of closest approach of the two molecules, one containing the unpaired electron, and the other containing the nucleus of interest. The cylindrical coordinates z, \rho, \phi (not shown here) are based on the membrane frame, where z = z_M, and x_M = \rho \cos \phi, y_M = \rho \sin \phi.](image)

This dipolar Hamiltonian is now in a form to which Redfield relaxation theory can be directly applied. In the notation of ref. 13, Eq. (67), p. 289, this Hamiltonian is written

$$\hbar \mathcal{C}_I = \sum_q \mathcal{E}_q A_q^O \mathcal{E}_q^*$$  \[5\]

where the $A_q^O$ are spin operators, and the $\mathcal{E}_q$ are space operators. Each term $A_q^O$ differs from $\mathbf{S} \cdot \mathbf{T}_L \cdot \mathbf{I}$ by a numerical coefficient; thus the $\mathcal{E}_q$ values are readily expressed in terms of the $A_m$ and $D_m$. The correlation functions $G^I(t)$ and spectral density functions $J^I(\omega)$ are then

$$J^I(\omega) = \int_{-\infty}^{+\infty} G^I(t) e^{-i\omega t} dt.$$  \[6\]

In the formulas for the nuclear relaxation rates given in ref. 13, Eqs. (88) and (89), pp. 295 and 296, we neglect all terms involving the electron resonance frequency itself ($\nu_e$) since the relatively slow diffusion motion ($\lesssim 10^{-7}$ sec$^{-1}$) has virtually no Fourier component at the electron resonance frequency ($\nu_e \approx 10^{11}$–$10^{12}$ rad sec$^{-1}$). The simplified expressions for the relaxation rates are

$$\frac{1}{T_1} = \frac{9}{8} \gamma S I h^2 \hbar I^{(1)}(\omega)$$  \[7\]

$$\frac{1}{T_2} = \frac{1}{2T_1} + \frac{1}{8} \gamma S I h^2 J^{(0)}(\nu_e).$$  \[8\]

The relaxation rates given in Eqs. 8 and 9 are then due to the component of the electron spin magnetization in the external field direction. However, this electron spin magnetization has a relaxation rate itself that is reasonably fast [$T_{1e} = 6.6$ usec (14), at an applied field of 3900 G], and this contribution to the fluctuating field at the nucleus must be taken into account. This can be done simply using the following modified equation for the spectral densities $J^I(\omega)$.

$$J^I(\omega) = \int_{-\infty}^{+\infty} G^I(t) \exp\left[-(i\omega + 1/T_1)t\right] dt.$$  \[9\]

The calculation of the correlation function $G^I(t)$ for the diffusional motion follows the work of Torrey (15; see also ref. 16), as described in ref. 13, pp. 301 and 302, except that here the motion is two-dimensional. We use the symbol 2D to represent the relative diffusion constant of the two spins, so that the diffusion equation is

$$\frac{\partial \Psi}{\partial t}(r,t) = 2 D \nabla^2 \Psi(r,t)$$  \[10\]

where $r = i \rho \cos \phi + j \rho \sin \phi$ (membrane axis system). The correlation functions $G^I(t)$ are

$$G^I(t) = \left(N/8 \pi D_0 \right) \mathcal{F}_I \left[F^I(t) \right] \times \exp\left[-(r - r_0)^2/8D_0 \sigma_0^2\right]$$  \[11\]

where $r = \rho \cos \phi$, $\rho = \rho \sin \phi$, and $N$ is the number of electron.
For example, calculated nuclear relaxation rates $T_1^{-1}$ and $T_2^{-1}$ of $^{13}C$ nuclei in small phosphatidylcholine vesicles. These calculated nuclear relaxation rates use the following parameters, $d = 6 \AA$, $z = 0$, $T_{1e} = 6.6 \mu$s and for planar nonrotating membranes, $\beta = \pi/2$ (applied field parallel to the surface of the membrane). For the small 300 $\AA$ diameter vesicles the assumed rotational correlation time is 1 $\mu$s. The phospholipid head group spin label concentration is 1.4 mole %. Enhanced $T_1^{-1}$ relaxation for (a) $^{13}C$ at 67.8 MHz for planar membrane, $\beta = \pi/2$, (b) $^{13}C$ at 25 MHz for vesicles, (c) $^1H$ at 100 MHz for vesicles.

spins per unit area. The following expression for the exponential,

$$\exp \left(-\frac{r^2}{8Dt}\right) = \frac{4D}{2\pi} \int \exp(-2Dk^2t) \exp i\mathbf{r} \cdot \mathbf{r}_0 dk d\phi_k$$

[13] can immediately be integrated over $\varphi$, $\varphi_0$, and $\varphi_k$ using the Jacobi-Anger expansion,

$$\exp i\mathbf{r} \cdot \mathbf{r}_0 = \sum_{m=-\infty}^{\infty} r^m J_m(k \rho) \exp [im(\varphi_k - \varphi)].$$

[14] The remaining algebra using Eqs. 8-13 is straightforward, leading to the following expressions for $T_1^{-1}$ and $T_2^{-1}$.

$$\frac{1}{T_1} = 2\pi N \gamma_1^2 \gamma_s^2 \hbar^2 \left(\int_0^{\infty} \frac{(2Dk^2 + 1/T_{1e})kdk}{(2Dk^2 + 1/T_{1e})^2 + \omega_s^2} K_1(k, d, \beta, z) \right)$$

[15]

$$K_a(k, d, \beta, z) = \sum_{m=-2}^{m=2} P^a(k, d, m, z) D_m(\alpha \beta)$$

[16]

$$P(k, d, m, z) = \int_{p=d}^{\infty} B_m(\rho, \varphi) J_m(k \rho) d\rho$$

[17]

$$\frac{1}{T_2} = \frac{1}{2T_1} + \frac{4\pi}{3} N \gamma_1^2 \gamma_s^2 \hbar^2 \sum_{m=-2}^{m=2} kdk K_2(k, d, \beta, z).$$

[18]

In Eq. 17, $d$ is the smallest allowed value of $\rho$. The resulting distance of closest approach of the two spins is $(\rho^2 + z^2)^{1/3}$, where $z$ is the distance between the two parallel planes, one containing the electron spins, and the other containing the nuclear spins of interest. An important feature of these calculated relaxation rates is that they are highly anisotropic. For example, when $z = 0$ the enhanced longitudinal relaxation rate $T_1^{-1}$ approaches zero as $\beta \to 0$, that is, when the applied field is perpendicular to the surface of the membrane. isotropically oriented membranes should, therefore, show an enhanced nonexponential decay of the longitudinal nuclear magnetization, even with cylindrical sample spinning. For purposes of semiquantitative comparisons with experimental data, we give in Figs. 2 and 3 calculated values of $T_1^{-1}$ and $T_2^{-1}$ for $^{13}C$ and $^1H$ nuclei for typical applied magnetic fields and resonance frequencies. These calculations use a lateral distance of closest approach, $d = 6 \AA$, $z = 0$, a mole-fraction spin label concentration equal to 1.4%, and an area per lipid molecule of 75 $\AA^2$, and $\beta = \pi/2$.

Although the above relaxation Eqs. 15-18 were derived for planar membranes, they are also applicable to small ($\sim 300 \AA$ diameter) phosphatidylcholine vesicles. In this case the rotational motion of the vesicle affects the spin-label enhancement of the relaxation rates. That is, $1/T_{1e}$ in Eqs. 15 and 18 must be replaced by $1/T_{1e} + 1/\tau_e$, where $\tau_e$ is the isotropic rotational correlation time of the vesicle. The terms $|D_m(\alpha \beta)|^2$ are then replaced by their time-average value, $1/\tau_e$. This application of the relaxation equations to small vesicles, of course, requires that $\tau_e^{-1} \gg T_1^{-1}$, $T_2^{-1}$. (Note, however, that the inner and outer layers of these bilayers may not rotate at the same rate.)

Figs. 2 and 3 also give calculated enhanced nuclear relaxation rates for vesicles, assuming the correlation time $\tau_e = 1$ $\mu$s, and the same parameters ($d = 6 \AA$, $z = 0$) used for the above calculations for planar, nonrotating membranes. Fig. 4 gives the calculated dependence of the enhanced relaxation rate $T_1^{-1}$ for $^{13}C$ at 25 MHz as a function of $z$.

The usual condition invoked for the validity of Redfield relaxation theory is that the relaxation times $T_1$, $T_2$ be much longer than the correlation time describing the interaction.
Fig. 4. Dependence of enhanced $^{13}$C nuclear relaxation rate $T_1^{-1}$ on distance $z$ between the plane in which the paramagnetic spin label undergoes lateral diffusion, and the plane in which the $^{13}$C nucleus undergoes lateral diffusion. Parameters used for the calculation are for phosphatidylycholine vesicles: $\tau_c = 1 \mu$sec, $D = 3 \times 10^{-8} \text{ cm}^2/\text{sec}$, 25 MHz, $d = 6 \AA$, 75 $\text{Å}^2$ per phospholipid and 1.4 mole $\%$ HGSL.

giving rise to the relaxation (13). In the present work there is no single correlation time, but the correlation function decays with a time constant at least as short as $T_{1e}$, so the validity of Redfield theory is assured.

**DISCUSSION**

The present work provides a theoretical foundation for the analysis of spin-label-enhanced nuclear relaxation in membranes where lateral diffusion is rapid. The theory is relevant to the experimental determination of lateral diffusion constants and the detection of specific interactions between membrane components. There are, however, a number of important quantitative limitations to the applicability of this theory for the analysis of experimental data. The theory contains parameters ($d, a$) that are not known accurately, and also makes a number of physical approximations, an important one being the neglect of internal molecular motion. At the present time there are also significant experimental uncertainties. For example, the angular anisotropies of enhanced nuclear relaxation rates [$T_1(\theta)$]$^{-1}$, [$T_2(\theta)$]$^{-1}$ have not been measured for oriented multilamellar arrays of phospholipid bilayers. Also, the electron paramagnetic relaxation time $T_{1e} = 6.6 \mu$sec was determined at an applied field of 3300 G (14) and may have other values at the higher fields used in the nuclear resonance experiments. Further, accurate values of enhanced transverse relaxation rates $T_2^{-1}$ are often difficult to measure. In spite of all of these difficulties, the brief comparison of observed and calculated enhanced $^1H$ and $^{13}$C nuclear relaxation rates $T_1^{-1}$ given below provides encouragement for pursuit of these studies.

In experiments reported here for the first time, we observed enhanced $^{13}$C $T_1^{-1}$ relaxation rates for choline methyl groups enriched 90% in $^{13}$C, at 67.8 MHz, 48°C, 1.4 mole $\%$ HGSL, in unsonicated dipalmitylophytosphatidylcholine. (HGSL is “head-group spin label,” with nitroxide I replacing an N-methyl of phosphatidylcholine.) An enhanced longitudinal relaxation rate of 0.5 sec$^{-1}$ was observed using $\pi, \tau, \pi/2$ pulse sequences. It will be seen from Fig. 2, curve (a), that this result is consistent with a diffusion constant of $\sim 4 \times 10^{-4}$ cm$^2$/sec, using the parameters given in the legend. [This diffusion constant is close to that obtained elsewhere using entirely different methods (10, 11).] Unfortunately, there are at present no other enhanced $^{13}$C or $^1H$ nuclear relaxation data at other frequencies that can be used to test the significance of this agreement. Because of the biphase character of the calculated $T_1^{-1}$ versus $D$ curve, an enhanced nuclear relaxation rate of 0.5 sec$^{-1}$ is consistent with two diffusion constants, $D \approx 4 \times 10^{-8} \text{ cm}^2/\text{sec}$ and $D \approx 7 \times 10^{-7} \text{ cm}^2/\text{sec}$. A very rough estimate of the $T_1^{-1}$ enhancement from the observed line width enhancement (\sim 65 sec$^{-1}$) agrees best with the lower diffusion rate (Fig. 2a).

All other studies of spin-label-enhanced $^{13}$C and $^1H$ nuclear relaxation rates have utilized sonicated dispersions of phosphatidylcholine. Our discussion of these data entails uncertainties concerning the homogeneity of these vesicle preparations together with the question of the range of lateral diffusion of lipids in vesicles having a small radius of curvature.

Levine et al. (7) have observed a HGSL-enhanced N-methyl $^{13}$C relaxation rate of $T_1^{-1} = 0.95 \text{ sec}^{-1}$ at 25 MHz in sonicated dipalmitylophytosphatidylcholine vesicles at 52°C containing 1.33 mole $\%$ HGSL. Because of the biphase nature of the curve in Fig. 2b, we can only say from these data that the enhanced $^{13}$C relaxation rate is consistent with a lateral diffusion constant of $3 \times 10^{-8} \text{ cm}^2/\text{sec}$, or $\sim 3 \times 10^{-7} \text{ cm}^2/\text{sec}$, using the parameters given in the legend to this figure.

Lee et al. (8) have observed HGSL-enhanced $^1H$ relaxation rates $T_1^{-1}$ in sonicated phosphatidylcholine vesicles at 100 MHz and at several temperatures between 62° and 44°C, where the enhanced $^1H$ relaxation rates range below $T_1^{-1} = 3.6 \text{ sec}^{-1}$ and 6.7 sec$^{-1}$. From Fig. 2, curve (c) we see that these enhanced relaxation rates are consistent with lateral diffusion constants in the range $10^{-7} - 10^{-6} \text{ cm}^2/\text{sec}$ [or even higher values if points to the right of the peak of curve (c) are selected]. There is one apparent discrepancy between theory and these experimental results. The larger nuclear relaxation rates are observed at the lower temperatures. This apparent discrepancy could arise in a number of ways: Due to intramolecular motions the effective distance of closest approach $d$ may be temperature-dependent, so that $d$ decreases with decreasing temperature. It is conceivable, but unlikely, that $D$ increases with decreasing temperature above the chain melting phase transition. Finally, it is possible that the rate of lateral diffusion in small phosphatidylcholine vesicles is so high that they are above $3 \times 10^{-7} \text{ cm}^2/\text{sec}$. Under these conditions an increasing temperature with increasing diffusion constant would result in a reduction in the enhanced relaxation rate, as observed. The reported HGSL-enhanced line width (7) corresponds to an enhanced $T_1^{-1}$ of the order of 100 sec$^{-1}$. From Fig. 2, curve (c), we see that this enhanced transverse relaxation rate agrees best with a large diffusion constant, $\sim 10^{-6} - 10^{-7} \text{ cm}^2/\text{sec}$.

Kornberg and McConnell (5) have reported an enhanced $^1H$ relaxation rate $T_1^{-1}$ of $\sim 13 \text{ sec}^{-1}$ in phosphatidylcholine vesicles at 220 MHz in the presence of 1% HGSL. Calculations using Eqs. 15–18 yield enhanced relaxation rates of 1.8 and 3.2 sec$^{-1}$ for lateral diffusion constants of $3 \times 10^{-8}$ and $3 \times 10^{-7} \text{ cm}^2/\text{sec}$. Again, reported line width enhancements agree best with the higher diffusion constant.

Godici and Landsberger (9) have carried out a very interesting study of $^{13}$C nuclear relaxation enhanced by fatty acid
nitrooxide spin labels in phospholipid bilayers. Their observations are relevant to our calculations illustrated in Fig. 4, in that they have varied the position of the nitrooxide group on the fatty acid chain, and have also determined enhanced $^{13}$C nuclear relaxation rates at various positions in host phosphatidylcholine molecules. Again, observed and calculated enhanced nuclear relaxation rates are in order-of-magnitude agreement. Because of the obvious importance of internal motions of the fatty acid chains of both the phospholipids and spin labels, no attempt will be made here to make a detailed comparison between experiment and theory. Our approach does provide an appropriate starting point for this comparison.

In summary, it is clear that there is reasonable agreement between observed and calculated spin-label-enhanced nuclear relaxation rates using plausible values for the parameters that appear in the theory. In particular the experimental data for planar phosphatidylcholine bilayers are in good order-of-magnitude agreement with the phospholipid diffusion constant $D \approx 2 \times 10^{-4}$ cm$^2$/sec obtained from earlier work using the paramagnetic resonance spectra of spin-labeled phospholipids (10, 11). The present work does suggest that lateral diffusion constants for lipids in small phosphatidylcholine vesicles produced by sonication may be significantly larger.

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