# Water Translational Motion at the Bilayer Interface: An NMR Relaxation Dispersion Measurement

Melinda Whaley Hodges, David S. Cafiso, Carl F. Polnaszek, Cathy C. Lester, and Robert G. Bryant
Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901 USA

ABSTRACT   Nuclear magnetic relaxation rates for water protons in aqueous palmitoyloleoylphosphatidylcholine vesicle suspensions containing different nitroxide free radical spin labels are reported as a function of magnetic field strength corresponding to proton Larmor frequencies from 10 kHz to 30 MHz. Under these conditions the water proton relaxation rate is determined by the magnetic coupling between the water protons and the paramagnetic nitroxide fixed on the phospholipid. This coupling is made time-dependent by the relative translational motion of the water proton spins past the nitroxide radical. Using theories developed by Freed and others, we interpret the NMR relaxation data in terms of localized water translational motion and find that the translational diffusion constant for water within approximately 10 Å of the phospholipid surface is $6 \times 10^{-10}$ m$^2$ s$^{-1}$ at 298 K. Similar results are obtained for three different nitroxide labels positioned at different points on the lipid. The diffusion is a thermally activated process with an activation energy only slightly higher than that for bulk water.

## INTRODUCTION

The motion of water near a surface is fundamental to a number of processes in chemistry and biology. Nuclear magnetic relaxation dispersion, the measurement of nuclear magnetic relaxation rates as a function of the magnetic field strength, provides a method for measuring localized diffusion coefficients for the solvent. The technique depends on electron-nuclear dipole-dipole spin relaxation. If the paramagnetic center has a long electron-spin relaxation time, the electron-nuclear dipole-dipole interaction, which dominates the solvent nuclear spin-lattice relaxation rate, is made time-dependent by the relative translational motion of the interacting electron and nuclear spins. If the radical is co-valently bound on a surface, then the relative translational motion sensed by the solvent spin relaxation interaction is that of the solvent only. Since the strength of the magnetic dipole-dipole coupling decreases very rapidly with distance, even after appropriate averages over volume are considered, the method provides a measure of the translational motion of the observed nuclear spins within approximately 1 nm of the radical at the surface. Previous work from this laboratory has exploited this approach to characterize water translational motion very near a protein surface (Polnaszek and Bryant, 1984a,b) and silica surface (Polnaszek et al., 1987). We report here applications of the method to a measurement of the water molecule mobility in close proximity to a palmitoyloleoylphosphatidylcholine (POPC) vesicle surface.

## MATERIALS AND METHODS

Nuclear magnetic relaxation measurements were made on a field cycling magnetic resonance spectrometer of the Redfield design constructed in this laboratory with the collaboration of Dr. Seymour Koenig and Dr. Rodney Brown, III at the IBM Watson Research Laboratories, Yorktown Heights, NY. This spectrometer operates by switching the magnetic field in real time from zero field to a field of 0.7 T for a time sufficient for the spins to achieve Boltzmann equilibrium. Then, the field is switched to a value of interest in which the spins are permitted to relax for a specified time after which the field is switched to a resonance value of 0.17 T, where the free induction decay following a 90° pulse or a spin echo following a 90–180° pulse pair is sampled. Each relaxation rate was calculated from a fit of 30 data points to an exponential by a least squares procedure. The errors in the relaxation rates are 4% or less. Samples were contained in 10-mm Pyrex test tubes and the temperature controlled in the spectrometer by a flow of liquid trichlorotrifluoroethane or perchloroethylene that was thermally regulated in an external Neslab RTE 8 temperature controller.

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids (Birmingham, AL). 3-Doxyl-5α-cholestane spin label (CSL) was purchased from Aldrich Chemical Company, and 4-octadecanoylamino-2,2,6,6,tetramethylpiperidine-1-oxyl (TSSLP), and 5-doxyl and 12-doxyl stearate spin labels were purchased from Molecular Probes, Junction City, OR. Lipid and spin label at 5 mol % were mixed in chloroform solution, evaporated under a stream of nitrogen, and in most cases placed under vacuum to remove the final traces of chloroform. The lipid was then dispersed in a 100 mM MOPS buffer and frozen and thawed five times using a dry ice/acetone bath to ensure homogeneity of the lipid suspension. Unilamellar vesicles were prepared by extruding the aqueous lipid suspension through 0.05-μm pore Nucleopore filters a minimum of 20 times to yield a mildly opalescent solution. Spin label concentrations were measured by comparison of the electron paramagnetic resonance spectrum of the lipid with a standardized spin label sample.

## RESULTS AND DISCUSSION

Representative relaxation data are shown in Fig. 1 for POPC vesicle suspensions doped with 12.3 mM 5-doxyl stearate spin label at pH 7.0, 6.0 mM 12-doxyl stearate label at pH 8.0, or 6.2 mM TEMPO labeling the headgroup at pH 7.0. These relaxation rates arise from the sum of the paramagnetic contribution and a nearly negligible diamagnetic contribution, which was measured independently and sub-
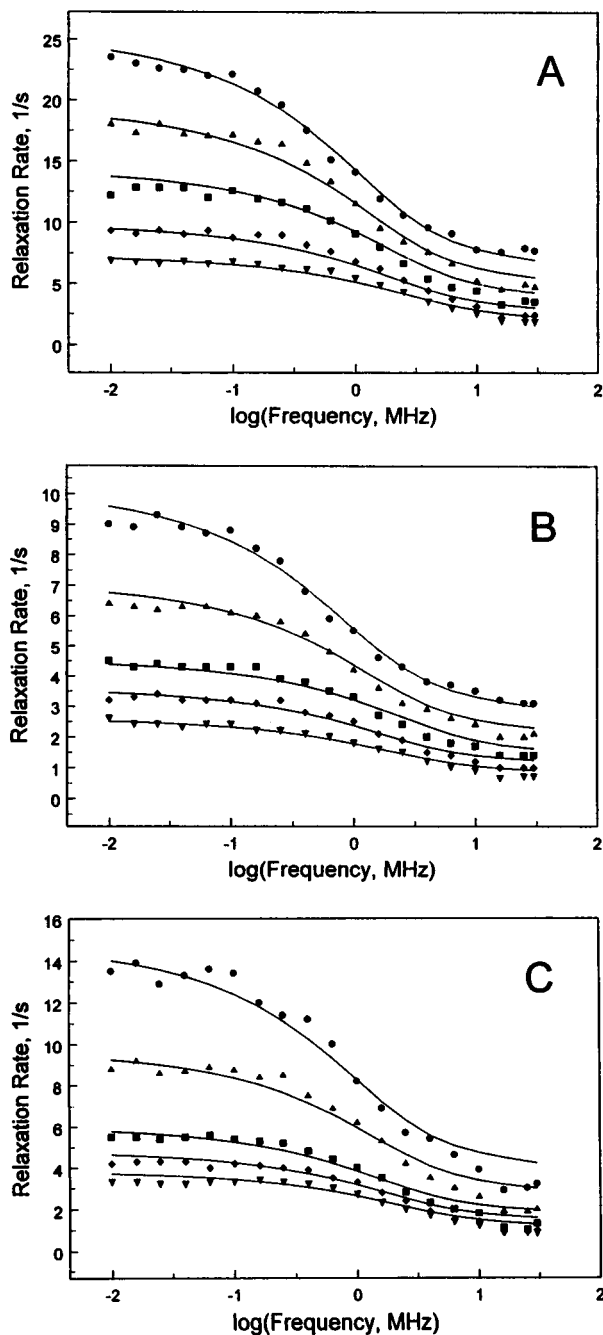
FIGURE 1   The water proton nuclear spin-lattice relaxation rate as a function of the magnetic field strength reported as the proton Larmor frequency for POPC small unilamellar vesicles containing nitroxide spin labels at different lipid positions. (A) 12.3 mM 5-doxyl stearic acid spin label at pH 8.0 in 100 mM MOPS buffer for different temperatures: 278 K (●), 288 K (▲), 298 K (■), 308 K (♦), and 318 K (▼). The spin label is on the hydrocarbon side of the lipid-water interface just below the headgroup. (B) 6.0 mM 12-doxyl stearate label at pH 8.0 in 100 mM MOPS buffer for different temperatures: 278 K (●), 288 K (▲), 298 K (■), 308 K (♦), and 318 K (▼). The spin label is on carbon-12 well below the headgroup. (C) 6.2 mM TEMPO labeling the headgroup at pH 7.0 in 100 mM MOPS buffer for different temperatures: 278 K (●), 288 K (▲), 298 K (■), 308 K (♦), and 318 K (▼).

tracted. The paramagnetic contribution is dominated by the intermolecular electron-nuclear dipole-dipole interaction, the time dependence of which is provided by the relative translational diffusion of the water molecules and the radicals that are incorporated into the vesicle. The water spin-lattice relaxation dispersion profile is dominated by the translational contributions that have been addressed theoretically by several authors (Hubbard, 1965; Hwang and Freed, 1975; Freed, 1978; Nientiedt et al. 1981; Noack, 1986). We utilize the Freed formulation for the case where diffusion dominates the correlation function that gives the spin-lattice relaxation rate as

$$\frac{1}{T_1} = \frac{32\pi}{405} \gamma_I^2 \gamma_S^2 \hbar^2 S(S+1) \frac{N_A}{1000} \frac{[S]}{bD}$$

$$\cdot [j_2(\omega_S - \omega_I) + 3j_1(\omega_I) + 6j_2(\omega_S + \omega_I)] \quad (1)$$

where

$$j_1(\omega) = j_2(\omega) = \frac{1 + \frac{5z}{8} + \frac{z^2}{8}}{1 + z + \frac{z^2}{2} + \frac{z^3}{6} + \frac{4z^4}{81} + \frac{z^5}{81} + \frac{z^6}{648}}, \quad (2)$$

$$z = \left[\frac{2\omega b^2}{D}\right]^{1/2}, \quad (3)$$

and

$$\tau_{\text{translation}} = \frac{b^2}{D}. \quad (4)$$

Here, $\gamma_I$ and $\gamma_S$ are the proton and electron magnetogyric ratios, respectively; $\omega_I$ and $\omega_S$ are the nuclear and the electron Larmor frequencies; $\hbar$ is Planck's constant divided by twice $\pi$; $S$ is the electron spin (1/2 for the nitroxide); $N_a$ is Avogadro's number, the square brackets indicate molar concentration; $b$ is the distance of closest approach between the centers of the nitroxide-labeled molecule and the water protons; and $D$ is the relative translational diffusion constant, i.e., the sum of the water and nitroxide diffusion constants. These equations assume that the relaxation observed is dominated by the electron-nuclear dipole-dipole coupling that is modulated by the relative translational motion of the spins that suffer no strong attractive or repulsive forces. This approach assumes that the motion may be described by a relative translational diffusion constant and neglects any rotational or scalar contributions to the correlation function, which would be necessary if there were evidence for long-lived water molecule/nitroxide complexes formed in water. A formalism derived to take into account electron spin relaxation rates that may be comparable to the translational correlation times is not needed for the present discussion because the electron relaxation times are longer than the translational correlation times deduced from the relaxation dispersion data and the simpler model accounts for the data. The solid lines drawn through the points are

**TABLE 1  Relaxation dispersion**

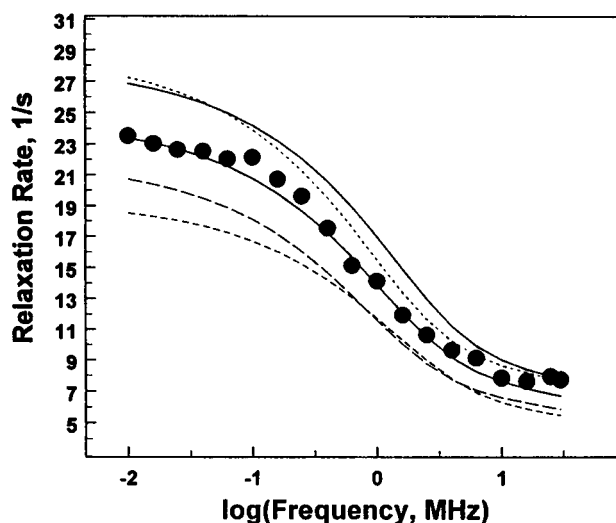| Label | $T$ (K) | $b$ (Å) | $10^6 \times D$ (cm$^2$ s$^{-1}$) |
|---|---|---|---|
| TEMPO (pH 7.0) | 278 | 3.9 | 3.1 |
| | 288 | 4.1 | 4.6 |
| | 298 | 4.7 | 6.6 |
| | 308 | 4.8 | 8.1 |
| | 318 | 4.8 | 10.1 |
| 12-Doxyl (pH 8.0) | 278 | 4.6 | 3.8 |
| | 288 | 4.6 | 5.6 |
| | 298 | 4.4 | 9.4 |
| | 308 | 5.4 | 9.7 |
| | 318 | 5.6 | 13 |
| 5-Doxyl (pH 8.0) | 278 | 4.0 | 3.5 |
| | 288 | 4.1 | 4.5 |
| | 298 | 4.1 | 6.2 |
| | 308 | 4.5 | 8.2 |
| | 318 | 4.5 | 11 |



FIGURE 2  The water proton nuclear spin-lattice relaxation rate as a function of the magnetic field strength reported as the proton Larmor frequency for POPC small unilamellar vesicles containing 12.3 mM 5-doxyl stearic acid spin label at 278 K for several choices of the parameters in Eqs. 1–4. From top curve to the bottom curve the values for $b$ and $D$ are respectively: $4.0 \times 10^{-8}$ and $0.30 \times 10^{-5}$ cm$^2$ s$^{-1}$; $3.5 \times 10^{-8}$ and $0.35 \times 10^{-5}$ cm$^2$ s$^{-1}$; $4.0 \times 10^{-8}$ and $0.35 \times 10^{-5}$ cm$^2$ s$^{-1}$; $4.5 \times 10^{-8}$ and $0.35 \times 10^{-5}$ cm$^2$ s$^{-1}$; $4.0 \times 10^{-8}$ and $0.45 \times 10^{-5}$ cm$^2$ s$^{-1}$.

computed from the parameters summarized in Table 1. Spin labels were incorporated at the headgroup, carbon-5, and carbon-12; data for all are summarized in Table 1.

The data in Fig. 1 demonstrate that the water proton-spin relaxation rates are higher at lower temperatures which is consistent with the usual models for the temperature dependence of the translational mobility. Similar results obtained for the doxyl spin labels at carbon-5 and carbon-12 using POPC vesicles and the results for the diffusion constants are summarized in Table 1. The same experiments were conducted on dilaurylphosphatidylcholine using 3-doxyl-5a-cholestane spin label and 4-octadecanoylamino-2,2,6,6-tetramethylpiperidine-1-oxyl with very similar results; however, the relaxation data do not fit the theory over the temperature range studied particularly well, perhaps because of changes in the lipid system with temperature and spin labeling conditions. Therefore, we focus on the POPC vesicle system. The solid lines in Fig. 1 were computed using the values summarized in Table 1. The sensitivity of the computed lines to the choices of the parameters $b$ and $D$ is demonstrated in Fig. 2. As discussed by Polnaszek and Bryant, the diffusion constant derived from this procedure senses the diffusional motions of the water molecule spins very near the paramagnetic centers located on the lipid molecules. For diffusing protons, approximately 90% of the relaxation is caused by motions within the first 10 Å of the nitroxide center; however, there is no provision in the models we have used for a dependence of the diffusion constant on distance from the surface. The values in Table 1, therefore, represent the effects of diffusive motion integrated over the whole sample volume, but the distances closest to the surface are inherently weighted very heavily by the distance dependence of the dipole-dipole coupling. The entries in Table 1, then, represent a good approximation to the dynamic characteristics of the water molecules in the first few monolayers of the lipid surface. Because the statistical precision of the derived parameters is good, better than implied by the limitations of the model, we suggest that the entries in Table 1 be interpreted cautiously.

The translational diffusion constants shown in Table 1 are relative diffusion constants, which are the sum of the dif-

fusion constants for the radical and the water. However, because the radical is constrained to the lipid vesicle, the vesicle diffusion constant, even when enhanced by the lateral diffusion of the lipid within the membrane, is much smaller than that for the water. Thus, the relative diffusion constant becomes essentially a report of the water molecule diffusion constant. The measurement actually depends on the translational motion of the protons in the system and proton jump mechanisms may enhance the apparent diffusion constant; however, the mean residence times of protons on a water molecule at neutral pH values and in the absence of catalysts is on the order of a millisecond (Graf et al., 1980). The translational correlation times that affect these measurements are approximately 7 to 8 orders of magnitude shorter than this residence time. Therefore, even if the phosphate headgroups catalyze the proton exchange by several orders of magnitude, it is still the diffusion of the whole water molecule that will be sensed by these measurements.

For the vesicles used here, the placement of the label is symmetrical with respect to the internal and external vesicle surfaces. Therefore, the measurements reported represent an average of the dynamics for the inside surface and the outside surface. However, as discussed above, the distances of merit that dominate the measurement are on the order of 10 Å, which is small compared with the vesicle diameter and sufficiently small that the distinctions between inside and outside the vesicle should be minor. These extruded vesicles are on the order of 400–500 Å, so that lipid packing differences, which are seen in small sonicated vesicles, should not affect the interpretation.

The magnitude of the diffusion constant sensed by the nitroxides is nearly independent of the spin label placement and approximately 1/3 that of the bulk water value. The decrease in translational mobility is consistent with expectations that the charged bilayer interface will inhibit the mobility of a good hydrogen bonding solvent. However, the reduction in the translational mobility is modest, only a factor of 3.

The entries in Table 1 for $b$, the distance of closest approach between the water molecule protons and the nitroxide radical considered as a point magnetic dipole, are remarkably similar even though very different label positions were employed. None violates van der Waals contact. Even though the 12-doxyl label is expected to be well within the lipid membrane because of the C-12 position on the chain, the parameter for the distance of closest approach only increases to 5.6 Å. The similarity of this parameter to those for the labels located at the interface, including the headgroup, suggests that the water and nitroxide come into close proximity, regardless of the nitroxide chain position, on the time scale of the measurement, which is the order of the proton $T_1$ in the samples or between 0.1 and 1 s. From neutron diffraction, water is known to reside in the upper portions of the acyl chains in bilayers (White and Wiener, 1995). A close approach of water and a chain-labeled nitroxide might result from further water penetration into the hydrocarbon domain or acyl chain librations that bring the nitroxides in close proximity to the interface on the time scale of this measurement.

The temperature dependence of $b$ is expected to be minor because it should be limited by van der Waals contacts unless nitroxide accessibility changes significantly with temperature. If the membrane system opened and became more labile with increasing temperature, then $b$ would decrease with increasing temperature. While $b$ is not absolutely constant, the values change relatively little with temperature, although the higher temperatures yield a larger value of $b$, opposite to what would be expected if penetration or accessibility of the solvent were the main contribution to the diffusion barrier. This observation may result from an increase in surface roughness with increasing temperature; however, we do not wish to use these measurements as significant evidence to support such a conclusion. Nevertheless, the diffusion constants for the water that are derived from these data are nearly the same regardless of where the label is located on the labeled lipid molecule.

The temperature dependence of the translational diffusion constant permits estimation of the activation parameters for diffusion in close proximity to the phospholipid surface. Representative data are shown in Fig. 3. The activation energy derived from the Arrhenius plots are 5.4 kcal/mol for the headgroup TEMPO label, 5.2 kcal/mol for the 5-doxyl stearate label, and 5.0 kcal/mol for the 12-doxyl stearate label. The values reported for pure water are $4.6 \pm 0.1$ kcal/mol (Wang et al., 1953), so that the activation barriers for molecular diffusion are only slightly larger at the membrane surface, even though the local vicinity is significantly
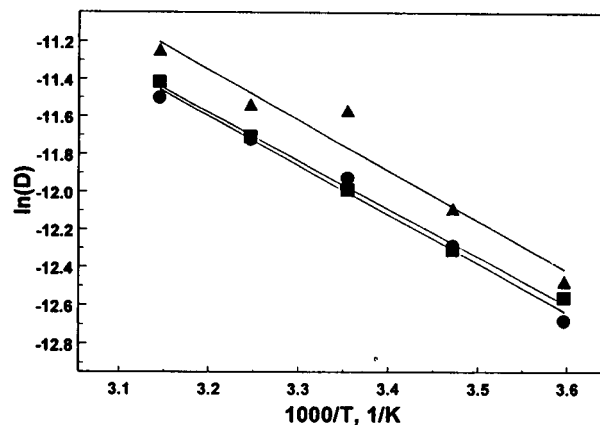


FIGURE 3 The temperature dependence of the derived translational diffusion constants for the headgroup TEMPO label, (●) the 5-doxyl stearate label (■), and the 12-doxyl stearate label (▲) in POPC vesicles; pH values of 7.0, 8.0, and 8.0, respectively.

charged and may provide relatively fixed molecular barriers to transport perpendicular to the surface director.

Comparison of these results with other measurements of localized diffusion constants of water is difficult because schemes for isolating just the surface motions are not common. Dye molecules such as acridine orange have been studied by fluorescence methods and 35-fold reductions in the diffusion constants are reported for silica surfaces treated with C-18 alkylating agents (Zulli et al., 1994), which should cause entanglements with diffusing probe molecules. Photobleaching methods have also yielded intramembrane diffusion constants, which may be remarkably large (Smith and McConnell, 1978; Hansen and Harris, 1995, 1996; Huang et al., 1994); however, these measurements do not provide information directly about the solvent motions.

In summary, the dynamic changes in the translational diffusion of water at the phosphatidyl choline vesicle membrane surface are small; typically within a factor of 3 of that in the bulk water.

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