8963

Interpretation of NOESY Cross-Relaxation Rates from Molecular Dynamics Simulation of a Lipid Bilayer

Scott E. Feller,*,† Daniel Huster,‡ and Klaus Gawrisch‡

Department of Chemistry, Wabash College
Crawfordsville, Indiana 47933
Laboratory of Membrane Biochemistry and Biophysics, NIAAA
National Institutes of Health, Rockville, Maryland 20852

Recent experiments have shown two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) to provide a powerful probe of the biologically relevant liquid crystalline phase of phospholipid bilayers.[1,2] Analysis of NOESY cross-relaxation rates quantifies the high degree of molecular disorder in biological membranes, showing a finite probability of close approach between even the most distant segments of neighboring lipid molecules (e.g., the methyl groups in the choline headgroup and the terminal methyl groups of the fatty acid chains). In addition to providing information on lipid structure, these rates are sensitive to the dynamics of membrane reorganization in the correlation time range from pico- to microseconds.

In the following, we describe our analysis of a 10-ns molecular dynamics (MD) simulation of a 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine (DPPC) bilayer, where the cross-relaxation rates between lipid resonances have been determined from the relevant autocorrelation functions for proton−proton dipolar interaction. The simulation results confirm the existence of direct magnetization exchange between distant lipid segments and allow interpretation of the underlying correlation function in terms of librational, rotational, and diffusive motions. The correlation function is extremely complex, with relaxation occurring on a variety of time scales, demonstrating that understanding NOESY cross-relaxation rates requires knowledge of both the probability of close approach for proton−proton interaction and the time scale with which the internuclear vector relaxes. The simulation suggests that lipid lateral diffusion is involved in the relaxation of the dipolar interaction correlation function. Although the present simulation is unable to sample motions on this time regime, combining simulation results with plausible estimates of the lateral diffusion rate results in excellent agreement between model and experiment. The results confirm the experimental observation that NOESY magnetization transfer in the lipid matrix occurs primarily via an intermolecular mechanism. Finally, the observation that cross-relaxation rates are sensitive to both the structure and dynamics of lipid bilayers demonstrates the value of these experiments in verifying the quality of MD simulations.

The NOESY experiment probes the transfer of magnetization, occurring over a time interval referred to as the mixing time, from a set of magnetically equivalent protons (producing resonance *i*) to a second set (producing resonance *j*). The calculation of these cross-relaxation rates from models was described by Brüschweiler and Wright[3] and is summarized here. The rates of magnetization transfer,

† Wabash College.
‡ NIAAA.
(1) Huster, D.; Arnold, K.; Gawrisch, K. *J. Phys. Chem. B* **1999,** *103,* 243−251.
(2) Huster, D.; Gawrisch, K. *J. Am. Chem. Soc.* **1999,** *121,* 1992−1993.
(3) Brüschweiler, R.; Wright, P. E. *Chem. Phys. Lett.* **1994,** *229,* 75−81.
(4) Feller, S. E.; Venable, R. M.; Pastor, R. W. *Langmuir* **1997,** *13,* 6555−6561.
(5) Pastor, R. W.; Feller, S. E. In *Biological Membranes: A Molecular Perspective from Computation and Experiment*; Merz, K. M., Roux, B., Eds.; Birkhauser: Boston, 1996; pp 3−30.
(6) Judd, C. M.; McClelland, G. H. *Data Analysis: A Model-Comparison Approach*; Harcourt Brace Jovanovich, Inc.: New York, 1989; pp 163−174.

$$\Gamma_{ij} = \zeta[3J_{ij}(2\omega_0) - {}^1\!/_2 J_{ij}(0)] \qquad (1)$$

are determined by the spectral density, $J_{ij}(\omega)$, where ω_0 is the proton Larmor frequency, and $\zeta = (2\pi/5)\gamma^4\hbar^2(\mu_0/4\pi)^2$, where γ is the gyromagnetic ratio. The spectral density is given by the Fourier transform of the sum of autocorrelation functions for the magnetic dipole−dipole interactions between all spins of resonances *i* and *j*:

$$J_{ij}(\omega) = \int_{-\infty}^{\infty} C_{ij}(t) \cos(\omega t)\, dt$$

$$C_{ij}(t) = \frac{4}{5}\sum_i\sum_j \left\langle \frac{Y_{20}(\vec{r}_{ij}(0))}{r_{ij}^3(0)} \frac{Y_{20}(\vec{r}_{ij}(t))}{r_{ij}^3(t)} \right\rangle \quad (2)$$

where $Y_{20} = [5/16\pi]^{1/2}(3\cos^2\theta - 1)$, and θ is the angle between the internuclear vector, $\vec{r}_{ij}$, and the *z* axis (normal to the membrane). The summations over *i* and *j* include all magnetically equivalent protons of each resonance.

The $C_{ij}(t)$ defined in eq 2 were calculated from the MD simulation for all pairs of protons defined in Figure 1. The simulation consisted of 72 DPPC molecules at full hydration with a fixed average surface area of 62.9 Å²/molecule, a constant normal pressure of 1 atm, and a constant temperature of 50 °C. The simulation protocol is described in more detail in ref 4. For each lipid in the simulation cell, the $C_{ij}(t)$ were calculated by summing over all possible proton pairs (including periodic images). Because the denominator of the correlation function depends on the sixth power of the separation, the contribution from distant pairs is negligible, and thus the simulated membrane patch (with lateral dimensions of approximately 50 Å square) is sufficient for calculating the $C_{ij}(t)$. Both the mean values of $C_{ij}(t)$ and their standard deviations were obtained by combining the results for the 72 lipids; a typical correlation function is presented in Figure 2. The spectral density functions can be obtained directly from the $C_{ij}(t)$ by fast Fourier transform (FFT) numerical methods. However, it is more instructive to fit the $C_{ij}(t)$ to a sum of exponential decay functions, allowing the $J_{ij}(\omega)$ to be calculated analytically in terms of the fit parameters.

$$C_{ij}(t) = \sum_n a_n\, e^{-t/\tau_n} \qquad J_{ij}(\omega) = \sum_n 2a_n\tau_n/(1 + \omega^2\tau_n^2) \quad (3)$$

This approach reports the contributions to Γ_{ij} from various motions within the bilayer as a function of their correlation times.

Figure 2 shows an example of a correlation function calculated from the MD trajectory (symbols). The figure also shows a fit of the data to a sum of four exponentials (solid line), with four adjustable intensities, three adjustable rate constants, and one fixed rate constant. Fit parameters for a variety of resonance pairs are given in Table 1. Based on previous analysis of the time scales of lipid dynamics,[5] these relaxation rates can be approximately attributed to various motions: <1 ps, bond vibrations; 50−100 ps, gauche/trans isomerization; 1−2 ns, molecular rotation and wobble; >100 ns, lateral diffusion. The intensities, a_n, report the relative weight of the various molecular relaxation processes to the decay of $C_{ij}(t)$. If we neglect differences in the distribution functions of proton−proton distances at close contact (<∼6 Å), then the sum of the intensities, a_n, is a representation of the statistics of proton−proton interactions in the bilayer.

The model correlation function with three adjustable decay times and one fixed decay time was obtained by starting with a single-exponential model, adding additional exponential functions, and subsequently using the *F*-test[6] to determine if the addition of parameters was statistically significant. We found that fitting the

Table 1. Exponential Fit Parameters for the $C_{ij}(t)$ Correlation Functions Calculated from the MD Trajectory[a]

peak	a_S	a_1/a_S	τ_1 (ps)	a_2/a_S	τ_2 (ps)	a_3/a_S	τ_3 (ps)	a_4/a_S	τ_4 (ns)	Γ (MD)	Γ^a
1:3	1.8×10^{-6}	0.478	13.2	0.305	149	0.179	1335	0.038	170	−0.008	−0.007
1:5	8.7×10^{-8}	0.293	5.0	0.210	55	0.343	932	0.154	170	−0.002	−0.003
1:10	7.9×10^{-7}	0.370	11.6	0.224	121	0.337	1187	0.069	170	−0.007	−0.006
3:5	3.3×10^{-6}	0.495	38.3	0.219	269	0.233	1755	0.052	170	−0.021	−0.012
3:10	1.4×10^{-5}	0.338	21.6	0.341	180	0.295	1987	0.025	170	−0.047	−0.044
5:10	2.2×10^{-5}	0.427	11.6	0.389	98	0.182	949	0.002	170	−0.006	−0.017

[a] The sums of the a_n are denoted a_S and are given in units Å^{-6}. The Γ_{ij} are calculated on a per proton basis and are reported in units of s^{-1}. NOESY NMR spectra of DPPC at 50 °C were acquired and analyzed as described previously.[1,2] Experiments were conducted at 10 kHz MAS spinning speed to obtain better agreement between peak intensity in the MAS center band and the number of protons per resonance. Furthermore, we employed an enhanced peak integration routine for integration of partially superimposed resonances. Small deviations from expected center band intensities were corrected by calculating effective number of protons per center band resonance.
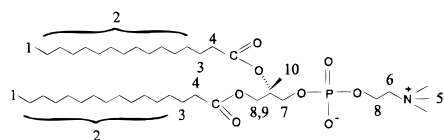
Figure 1. Structure of DPPC. The numbers refer to the peak assignments given in ref 1.
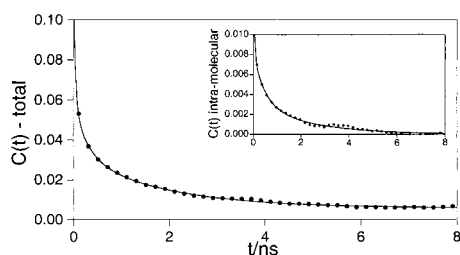
Figure 2. Correlation function, $C_{ij}(t)$, for resonances 3 and 5. Raw data points calculated from the MD simulation are represented as symbols, and the solid line gives the best fit of the data to a sum of exponential functions (inset shows the intramolecular contribution). In carrying out the fits, each data point was weighted by the inverse of its standard deviation. The small plateau value of the correlation function, arising from intramolecular vectors only ($\langle Y_{20}(\vec{r}(t))/r^3(t)\rangle^2$), was calculated from the MD simulation and subtracted from the correlation function.

simulation data to four exponentials can provide four intensities (a_n) but only three decay times in a statistically significant manner. This is not surprising since the slowest exponential represents a relaxation process that has been only partially sampled during the MD simulation; therefore, its decay constant cannot be determined unambiguously. Assuming that the slowest relaxation arises from lateral diffusion and that this motion is independent of the lipid segments involved, we fixed identical values of τ_4 when fitting each MD correlation function. Table 1 gives the best-fit parameters obtained assuming $\tau_4 = \nu^{-1} = 170$ ns, where ν is the frequency in a simple-jump model of lateral diffusion based on experimental measurements of the diffusion coefficient.[5] This simple assumption results in excellent agreement between the experimental and simulation cross-relaxation rates, Γ_{ij} (see Table 1). From these data, it is clear that the major contribution to the cross-relaxation rate is made by the slowest motions; i.e., the fast motions determine Γ_{ij} only by the extent to which they relax the correlation function. Thus, determining the magnitude of the slowest exponential, a_4, from the simulation $C(t)$ is critical in calculating a cross-relaxation rate. In this sense, our analysis of lipid NOESY experiments is similar to the "model-free" approach of Lipari and Szabo[7] for the interpretation of protein NMR, where the effect of all fast motions is contained within a single parameter, S^2, analogous to a_4 in the present description. The processes responsible for the slow relaxation, however, are different. While the slow relaxation in proteins arises from tumbling in solution (i.e., orientational averaging at finite internuclear distance), the

decorrelation in lipids is due to lateral diffusion (i.e., increasing intermolecular separations). Table 1 shows clearly that the absolute value of Γ_{ij} is influenced by the fast relaxation processes and cannot always be taken as proportional to the number of close contacts; i.e., the ratio of a_4/a_S is not uniform.

The MD simulation analysis allows the $C_{ij}(t)$, $J_{ij}(\omega)$, and Γ_{ij} to be separated into inter- and intramolecular contributions. Comparing the intramolecular $C_{ij}(t)$ in the inset of Figure 2 with the complete correlation function (also in Figure 2) leads to a qualitative explanation of the experimental finding that intermolecular contributions are dominant in determining the cross-relaxation rate. The magnitude of the intramolecular correlation function is observed to be approximately a factor of 10 less than the complete correlation function. Additionally, the intramolecular $C(t)$ has completely decayed on the time scale of 5 ns, while the intermolecular contribution has the slowly decaying residual tail that contributes most of the magnitude of Γ_{ij}. Calculating the cross-relaxation rate for this resonance pair (using eq 3) results in an intramolecular contribution of 0.56%. By this same method, the intramolecular contribution was calculated for the other resonance pairs and is always less than 10% (in most cases <1%), in accord with the experimental results in Table 4 of ref 1. These calculations again demonstrate the importance of both the magnitude and relaxation time of the correlation function in determining Γ_{ij} and highlight the complex nature of the analysis of NOESY experiments on fluid bilayers.

To summarize, the simulation results confirm that cross-relaxation is predominantly intermolecular and suggest that lateral diffusion of lipid molecules is a necessary component in relaxing the dipolar interaction correlation function to zero. Although, the diffusion process cannot be sufficiently sampled in a 10-ns MD simulation, the magnitude of the resulting decay can be estimated by examining how far the correlation function remains to be relaxed. Additionally, the present analysis demonstrates the potential for collaboration between laboratory and computational experiments. In the present work, we have focused on extracting information from simulation that is useful for the interpretation of experimental results. Conversely, since the NOESY experiment is probing intermolecular structure and dynamics, it can provide a new tool to verify the quality and integrity of MD simulation on lipid bilayers. In the past, simulations have relied primarily on ^{2}H NMR order parameters profiles and electron density distributions to assess bilayer structure; however, these data do not provide information on the lateral organization of the membrane. In addition to structural data, the NOESY experiment provides time-resolved dynamic information that complements C^{13} relaxation previously employed for validating MD simulations.[8] The NOESY experiment can also provide atomic level information on the interactions of membranes with a variety of solute molecules, including peptide species.

(7) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4546−4559.
(8) Venable, R. M.; Zhang, Y.; Hardy, B. J.; Pastor, R. W. *Science* **1993**, *262*, 223−226.