NOESY NMR Crosspeaks between Lipid Headgroups and Hydrocarbon Chains: Spin Diffusion or Molecular Disorder?

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The good resolution and long spin−lattice relaxation times of ^{1}H NMR lipid resonances in fluid membranes recorded with magic angle spinning (MAS) enable the study of cross-relaxation by two-dimensional NOESY experiments.[1] Intense crosspeaks between distant protons such as those of the headgroup choline and the terminal methyl group of lipid hydrocarbon chains have been observed.[1−9] This is due to close approach between these protons or the result of relayed magnetization transfer by spin diffusion along the proton network of lipids. There is disagreement regarding the contribution of spin diffusion to cross-relaxation in membranes. Although the magnitude of spin diffusion to crosspeak intensity in lipids was not determined, most authors consider spin diffusion to be important for magnetization transfer along lipid hydrocarbon chains.[2,4,5] In a recent paper, we presented the first quantitative analysis of cross-relaxation in membranes.[10] The cross-relaxation rates of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine at 30 °C in the biologically relevant liquid crystalline phase were mostly smaller than 1 s^{-1}, except for some rates between lipid hydrocarbon chain methylene groups and between the glycerol resonance signals. This implies that spin-diffusion can be safely neglected for most crosspeaks at mixing times up to 100 ms. Furthermore, we addressed the issue of the molecular origin of cross-relaxation and concluded that, except for magnetization exchange between lipid protons that are nearest neighbors by chemical bond, the major contribution to cross-relaxation is due to intermolecular lipid−lipid interactions. However, cross-relaxation rate analysis in lipid bilayers is complicated by resonance signal superposition. In particular, it cannot be determined how fast magnetization is transferred along the lipid hydrocarbon chains by spin diffusion. This paper specifically addresses the issue of intramolecular spin diffusion along lipid hydrocarbon chains and intermolecular spin diffusion as a cascade of magnetization transfer steps between lipid molecules.

The NOESY cross-relaxation rates between the methyl groups of the lipid choline and the terminal methyl groups of lipid hydrocarbon chains were determined for pure 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) at 50 °C. Cross- and diagonal peak volumes of all lipid resonances were measured as a function of mixing time in the range from 0 to 1 s. Cross-relaxation rates were determined by a matrix procedure that minimizes least-squares deviations between calculated and measured peak volumes.[11] Alternatively, the specific cross- and diagonal peak volumes of methyl group resonances were fit to the model of interacting spin pairs.[12] In the latter fit, cross-relaxation rates depend predominantly on crosspeak volumes at short mixing times where spin diffusion does not contribute to peak intensity. Both approaches resulted in identical headgroup-to-chain cross-relaxation rates. Reproducibility of the rates between independent experiments is better than ±15%. The rates of pure DPPC were compared with the values for binary mixtures composed of DPPC-d_{13} with a perdeuterated headgroup and DPPC-d_{62} with perdeuterated hydrocarbon chains. In a third experiment, spin diffusion along protonated lipid hydrocarbon chains was suppressed by mixing DPPC-d_{21}, with protonated palmitic acid chains that contain two deuterated methylene groups in the chain's center and a perdeuterated headgroup, and the chain-perdeuterated DPPC-d_{62}.

In the following, only the cross-relaxation rate between the most distant molecular groups of a lipid, the terminal chain methyl protons and the protons in the methyl groups of the PC headgroup, is discussed. Evidence of the purely intermolecular nature of that crosspeak comes from the comparison of cross-relaxation rates in pure DPPC and a 50/50 (mol/mol) mixture of headgroup perdeuterated DPPC-d_{13} and chain perdeuterated DPPC-d_{62}. In pure DPPC, each contact between headgroup and chain contributes to cross-relaxation. In an ideal mixture of headgroup and chain perdeuterated lipids, only one out of four contacts between headgroups and the ends of chains involves a proton at both sites. As expected, the absolute cross-peak intensity at short mixing times in the binary, partially deuterated lipid mixture is only 25% of the cross-peak intensity of DPPC, and the cross-relaxation rate between headgroups and the ends of the chains decreases by 50% (Figure 1).

Chen and Stark[4] reported that the latter experiment does not discriminate between a direct headgroup-to-hydrocarbon chain magnetization transfer and an indirect transfer from the headgroup to the upper chain methylene groups of neighbored lipid molecules in combination with a relay of magnetization down the lipid hydrocarbon chains by spin diffusion. Therefore, in the third experiment, spin diffusion along the protonated hydrocarbon chains was blocked by using DPPC-d_{21} with a perdeuterated PC headgroup and two deuterated methylene groups per hydrocarbon chain in positions 7 and 8. Within experimental error, the cross-relaxation rate for headgroup-to-chain interactions did not change, indicating that spin diffusion along lipid hydrocarbon chains is negligible (see Figure 1).

Intermolecular cross-relaxation may also relay magnetization from lipid headgroups to the hydrocarbon chains by an intermolecular, multiple-step magnetization transfer that involves more than two lipid molecules. An illustration of this process is given in Figure 2. If such a mechanism were important, then the head-to-chain cross-relaxation rates would depend in a nonlinear fashion on the DPPC-d_{21} mole fraction in the DPPC-d_{21} /DPPC-d_{62} binary mixture. For example, if magnetization is transmitted in a two-step process, then cross-relaxation rates would be proportional to the square of the mole fraction; a three-step transfer would result in proportionality to the third power, etc. (see Figure 2). The experimentally determined cross-relaxation rates (Figure 3) are a linear function of the mole fraction indicating that single-step, direct contacts between the choline methyl protons and the

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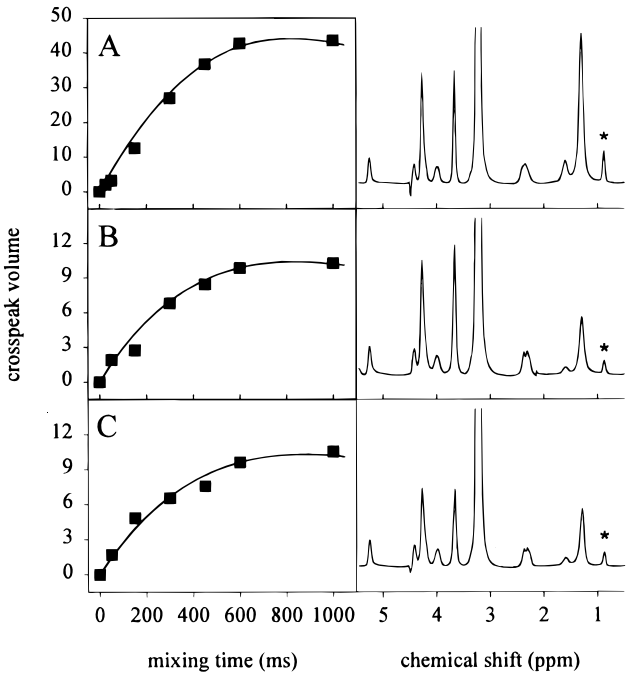

Figure 1. NOE build-up curves for the crosspeak between the lipid headgroup methyl protons and the palmitic acid chain terminal methyl protons of DPPC at 50 °C. Data points measure peak volumes of identical molar amounts of lipid per sample. The lines have been obtained by fitting peak volumes of all resolved lipid resonances by a matrix analysis with minimization of least-squares deviations between calculated and experimental values.[11] Indistinguishable rates have been obtained by fitting headgroup-to-hydrocarbon chain cross-peak volumes to the equation of interacting spin pairs.[12] Panel A shows pure DPPC yielding a cross-relaxation rate of $\sigma_{\text{head-chain}} = -0.011\ s^{-1}$, in panel B the 50/50 mixture of DPPC-d_{13}/DPPC-d_{62} is shown $\sigma_{\text{head-chain}} = -0.0059\ s^{-1}$, and panel C presents the 50/50 mixture of DPPC-d_{21}/DPPC-d_{62} ($\sigma_{\text{head-chain}} = -0.0055\ s^{-1}$). To the right, rows of the 2D NOESY spectra (3.23 ppm) acquired at a mixing time of 300 ms with the head-to-chain-cross-peak (highlighted with an asterisk) are shown. Intensities represent identical molar content of lipid per sample. Lipids were synthesized by Avanti Polar Lipids, Alabaster, AL using specifically labeled palmitic acid from Cambridge Isotopes, Cambridge, MA. Mixtures of lipids were prepared in chloroform, lyophilized, and hydrated to a D_2O content of 50 wt %. ^{1}H MAS NOESY spectra[14,15] were acquired on a Bruker DMX500 spectrometer at a resonance frequency of 500.13 MHz and a MAS spinning speed of 5 kHz in a phase sensitive mode using a spectral width of 3.3 kHz with 256 $t_1 \times 1024$ t_2 time increments, and 16 scans per t_1 increment. A squared sine-bell window function was used for processing in both dimensions. Peak volumes were determined with AURELIA software (Bruker Instruments, Inc., Rheinstetten, Germany).

chain methyl protons are the sole source of cross-relaxation. If there is any contribution from spin diffusion to these crosspeaks, it must be smaller than the experimental error.

The results demonstrate without doubt that crosspeaks between distant molecular groups in the lipid matrix, like headgroups and hydrocarbon chains, are caused by direct interactions between these protons. The intermolecular nature of headgroup-to-chain interactions was initially suggested by Xu and Cafiso based on experiments with sonicated small unilamellar vesicles (SUV)[3]. The small radius of curvature in SUV has some influence on lipid packing,[13] but it is unlikely that curvature seriously enhances the probability of head-to-chain contacts between lipids. Later experiments on multilamellar liposomes conducted with magic angle spinning did not observe intermolecular head-to-chain interaction.[2]

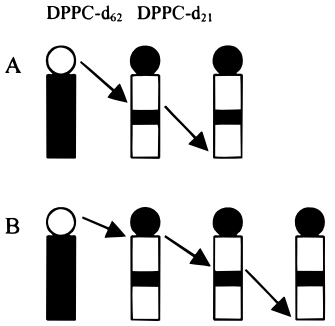

Figure 2. Hypothetical intermolecular two-step (A) and three-step (B) spin diffusion processes in DPPC-d_{21}/DPPC-d_{62} mixtures. Black areas on the lipid symbolize specific deuteration. The probability of the two-step transfer in an ideal binary lipid mixture is proportional to the square of the mole fraction of DPPC-d_{21} ($X_{\text{DPPC}-d_{21}}$), the probability of a three-step transfer to the third power of $X_{\text{DPPC}-d_{21}}$.

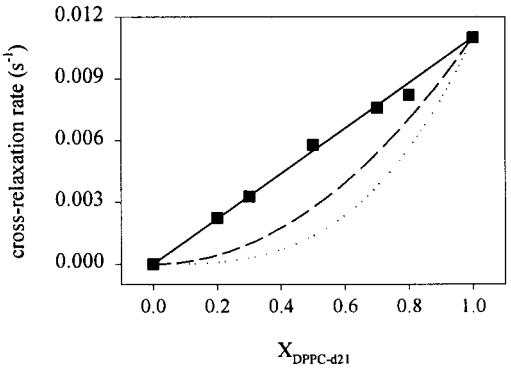

Figure 3. Intermolecular headgroup methyl-to-hydrocarbon chain methyl cross-relaxation rates as a function of mole fraction of DPPC-d_{21} in DPPC-d_{21}/DPPC-d_{62} mixtures. The linear dependence of cross-relaxation rates indicates direct contacts between headgroup and chain protons. Magnetization transfer by a cascade of intermolecular transfer steps would have resulted in a nonlinear dependence (dashed line, two-step transfer; dotted line, three-step transfer).

In more recent experiments,[4,7,10] the magnetization exchange between headgroups and the ends of chains was detected and the involvement of intermolecular magnetization transfer established. The quantitative analysis of cross-relaxation rates in this study proves that the crosspeaks between headgroups and hydrocarbon chains are not caused by spin diffusion but are the result of a direct approach (within 5 Å) of headgroup and chain protons.

The cross-relaxation rates in liquid crystalline bilayers reflect a tremendous amount of molecular disorder. Lipids change rapidly between a large number of conformations, and the position of molecular groups along the bilayer normal appears to be a broad distribution function. Contacts between molecular groups that are located closer to each other are distinct by having cross-relaxation rates more than an order of magnitude higher.[10] However, both conformational flexibility and variations in the location of lipids along the bilayer normal result in a finite probability of close approach even between the most distant molecular groups, like protons of the methyl groups of choline and those of the terminal methyl groups of lipid hydrocarbon chains. Current membrane models may need adjustment to better account for this very high degree of disorder.

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Supporting Information Available: ^{1}H MAS NMR spectrum of DPPC with signal assignments, contour plot of NOESY spectrum recorded at 300 ms mixing time, cross-relaxation rate matrix of DPPC at 50 °C (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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