# Determination of Peptide Conformations by Two-Dimensional Magic Angle Spinning NMR Exchange Spectroscopy with Rotor Synchronization

David P. Weliky and Robert Tycko*

*Laboratory of Chemical Physics*
*National Institute of Diabetes and Digestive and*
*Kidney Diseases, National Institutes of Health*
*Bethesda, Maryland 20892-0520*

Solid state nuclear magnetic resonance (NMR) is an increasingly important tool in structural investigations of biopolymers. Structural studies by solid state NMR have been based primarily on two distinct approaches: (1) the determination of *interatomic distances* in unoriented samples from measurements of nuclear magnetic dipole−dipole couplings in magic angle spinning (MAS) experiments[1] and (2) the determination of *absolute orientations* of chemical groups in uniaxially oriented samples from measurements of dipole−dipole couplings, anisotropic chemical shifts, and nuclear quadrupole couplings in nonspinning experiments.[2] In this Communication, we describe a third approach in which information about the *relative orientations* of chemical groups in unoriented samples is obtained from two-dimensional (2D) NMR exchange spectroscopy synchronized with MAS at moderate speeds. This approach is related to earlier structural studies by nonspinning 2D exchange spectroscopy[3] in which 2D powder pattern shapes were analyzed in terms of molecular conformations or molecular packing. The use of MAS enhances the sensitivity by a factor greater than 10 and facilitates the quantitative analysis of the data, as described below. Rotor-synchronized 2D MAS NMR exchange spectroscopy has been developed previously for studies of slow molecular motions and chemical exchange.[4,5] The results in this paper demonstrate the utility of rotor-synchronized 2D exchange in structural studies.

Experiments were carried out at a $^{13}$C NMR frequency of 100.4 MHz on a 79 mg polycrystalline sample of the model tripeptide L-alanylglycylglycine (AGG), in which 5% of the AGG molecules were $^{13}$C-labeled at the carbonyl sites of both Ala-1 and Gly-2. 2D exchange spectra were obtained with the pulse sequence $(CP)_y$-$t_1$-$(\pi/2)_\phi$-$\tau$-$(\pi/2)_x$-$t_2$, in which $(CP)_y$ represents cross-polarization with a spin-locking pulse with phase $y$ applied to $^{13}$C nuclei, $t_1$ is the evolution period, $(\pi/2)_\phi$ is a $\pi/2$ pulse (5.0 $\mu$s long) with phase $\phi$, and $t_2$ is the period during which $^{13}$C NMR signals are detected. Intramolecular spin diffusion, driven by dipole−dipole couplings between $^{13}$C
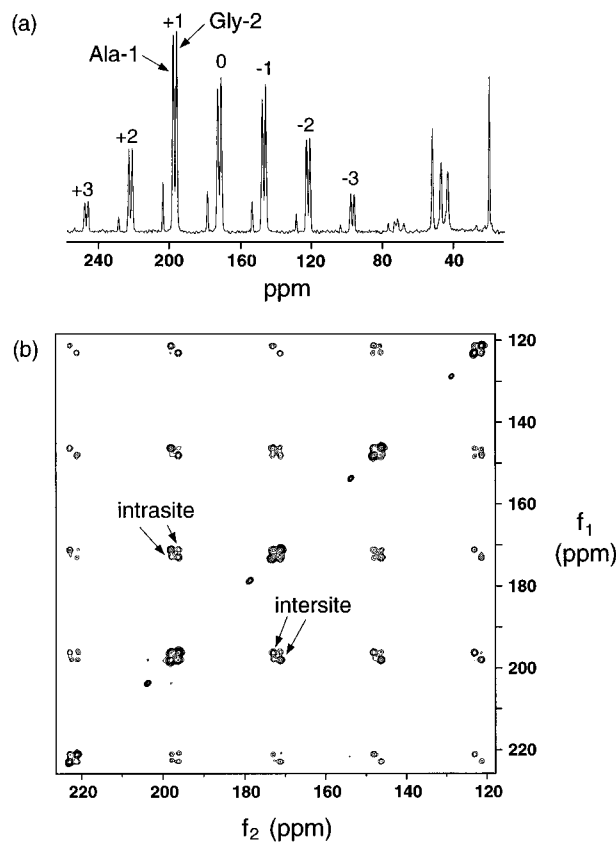


**Figure 1.** (a) 1D $^{13}$C NMR spectrum of polycrystalline 5% $^{13}$C$_2$-L-alanylglycylglycine, with magic angle spinning at 2.5 kHz. Spinning sidebands for the labeled Ala-1 and Gly-2 carbonyl carbons are numbered, with the centerband line defined to be 0. (b) Carbonyl region of the 2D $^{13}$C MAS NMR exchange spectrum, spinning at 2.5 kHz, at room temperature. Examples of intersite and intrasite crosspeaks are indicated. Exchange period $\tau = 500$ ms plus fractions of $\tau_R$ required for synchronization with sample spinning. Maximum $t_1$ value = 15.3 ms, 256 $t_1$ increments, 128 total scans per $t_1$ value.

labels, occurs during the exchange period $\tau$. Proton decoupling (80 kHz rf field) is applied during $t_1$ and $t_2$, but not during $\tau$. Spin diffusion during $\tau$ is therefore relatively rapid, independent of the MAS rotor period $\tau_R$, i.e., rotational resonance effects[6] do not play a role. Phase-sensitive 2D spectra are obtained by acquiring 2D data with $\phi = x$ and $\phi = y$, synchronizing the pulse sequence according to $\tau = n\tau_R$ and $\tau + t_1 = n\tau_R$, where $n$ is an integer, and processing the four data sets as originally described by Hagemeyer et al.[4,5]

The one-dimensional (1D) spectrum of the labeled peptide (Figure 1a) consists of single lines for each of the naturally abundant aliphatic carbons (16−52 ppm range) and series of spinning sideband lines for the naturally abundant carboxyl carbon and labeled carbonyl carbons. The assignment of the carbonyl resonances in Figure 1a was made tentatively from solution NMR measurements and was subsequently confirmed as described below. The 2D exchange spectrum with $\tau = 500$ ms (Figure 1b) exhibits three significant features. First, no crosspeaks connect the spinning sidebands of the carboxyl carbon with one another, demonstrating the absence of large-amplitude molecular motions on the time scale of $\tau$.[4,5] Second, crosspeaks that connect spinning sidebands of the Ala-1 carbonyl with spinning sidebands of the Gly-2 carbonyl (with volumes $V_{n,n'}^{i,j}$ for sideband $n$ of site $i$ in $f_1$ and sideband $n'$ of site $j$ in $f_2$) are apparent. These intersite crosspeaks arise from the exchange of nuclear magnetization via intramolecular spin

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(5) Luz, Z.; Spiess, H. W.; Titman, J. J. *Israel J. Chem.* **1992,** *32,* 145−160.

(6) Levitt, M. H.; Raleigh, D. P.; Creuzet, F.; Griffin, R. G. *J. Chem. Phys.* **1990,** *92,* 6347−6364.

diffusion during $\tau$. The experimentally observed dependence of all the intersite crosspeak volumes on $\tau$ is approximately $V_{n,n}^{i,j}(\tau) \propto 1 - \exp(-\tau/\tau_0)$ with $\tau_0 \approx 75$ ms, so that Figure 1b is a fully exchanged spectrum. Third, intrasite crosspeaks that connect the spinning sidebands of the Ala-1 carbonyl with one another and the spinning sidebands of the Gly-2 carbonyl with one another are also apparent and are explained below.

Structural information is contained in the volumes of the intersite crosspeaks in Figure 1b, because these volumes reflect the correlations between the anisotropic NMR frequencies of the two labeled carbonyl sites, which are in turn determined by the relative orientation of the two carbonyl groups within a molecule. From 1D MAS spectra,[7] the chemical shift anisotropy (CSA) tensor principal values $(\delta_{11}, \delta_{22}, \delta_{33})$ were determined to be (245,186,88) and (242,182,89) ppm for the Ala-1 and Gly-2 carbonyls, respectively. Studies of model peptides[8] indicate that the $\delta_{33}$ principal axes are perpendicular to the carbonyl $sp^2$ planes and that the angles between the $\delta_{11}$ axes and the C–N bonds are approximately 40°. Dipole–dipole couplings to amide $^{14}N$ nuclei also contribute to the carbonyl $^{13}C$ frequencies. For a C–N bond length of 1.32 Å, the $^{13}C$–$^{14}N$ coupling acts as an axially symmetric "frequency shift tensor" with an anisotropy of $(-2.83)m$ kHz, with $m = 1, 0,$ or $-1$ depending on the $^{14}N$ spin state. Assuming these tensors, planar peptide bonds with standard geometries, and complete intramolecular spin diffusion[9] and using the mathematical analysis in ref 5, the $V_{n,n}^{i,j}$ can be calculated numerically as functions of the dihedral angles $\phi$ and $\psi$ that define the peptide backbone conformation between the labeled carbonyl sites.[10] We then analyze the experimental 2D spectrum by computing the root-mean-squared deviation (RMSD) between experimental and calculated $V_{n,n}^{i,j}$ for a grid of possible $\phi,\psi$ values. Since only the relative values of the $V_{n,n}^{i,j}$ are measured experimentally, the calculated crosspeak volumes are scaled uniformly to minimize the RMSD for each $\phi,\psi$ pair. Crosspeak volumes $V_{n,n}^{i,j}$ with $-2 \leq n,n' \leq 2$, $n \neq n'$, $i,j = 1,2$, and $i,j = 2,1$ are included in the analysis. The results are plotted in Figure 2a. The global minimum RMSD occurs at $\phi,\psi = -78°,168°$, in good agreement with the values $\phi,\psi = -83°,170°$ determined from neutron diffraction measurements on crystalline AGG.[11] Figure 2b shows a plot of the RMSD between experimental and calculated quantities $V_{n,n}^{\text{sum}} = V_{n,n'}^{1,2} + V_{n,n'}^{2,1}$, mimicking a case in which the signals from Ala-1 and Gly-2 could not be resolved or assigned. The global minimum RMSD in Figure 2b occurs at $\phi,\psi = -72°,164°$. A calculation (plot not shown) in which the assignment of the Ala-1 and Gly-2 signals is deliberately reversed yields a global minimum at $\phi,\psi = -170°,-70°$ in clear disagreement with the diffraction measurements.

For reasons of symmetry, analysis of the 2D exchange spectrum cannot distinguish $\phi,\psi$ from $-\phi,-\psi$. However, for residues other than glycine, typically only one of these pairs of dihedral angles will be sterically allowed.[10] Additional ambiguities due to multiple local minima in the RMSD plot can be resolved by measurements of the carbonyl–carbonyl distance (which depends on $\phi$) or other independent structural measurements.[12]

The intrasite crosspeaks in Figure 1b are attenuated substantially in 2D exchange spectra obtained at $-120$ °C (not shown).

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(9) Simulations of 2D exchange data indicate that an 8° deviation from planarity in the Ala-1-Gly-2 peptide bond, as reported in ref 15, would introduce errors of 2° or less in the $\phi$ and $\psi$ values derived from our RMSD analysis. The assumption of complete intramolecular spin diffusion is experimentally validated, as described in the text.

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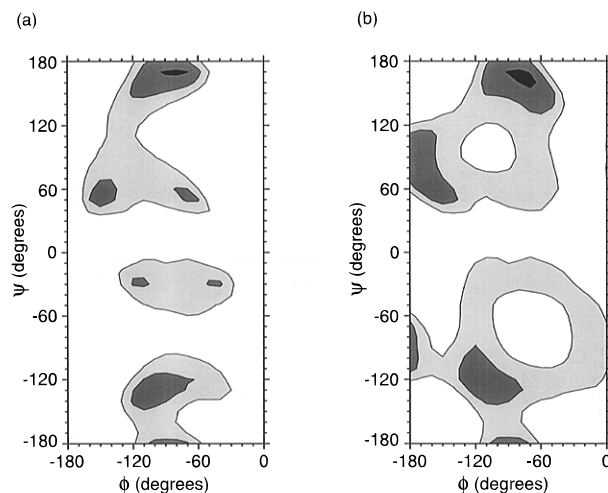


**Figure 2.** (a) Contour plot of RMSD between experimental and calculated intersite crosspeak volumes as a function of the $\phi$ and $\psi$ dihedral angles used in the calculation. Black, dark grey, light grey, and white regions correspond to RMSD values (per crosspeak) less than 6.5, less than 11.5, less than 16.5, and greater than 16.5, respectively, in units of the RMS noise volume in a 1 ppm × 1 ppm region of the spectrum in Figure 1b. At the global minimum $(\phi,\psi = -78°,168°)$, RMSD = 5.65. Neutron diffraction measurements[11] indicate $\phi,\psi = -83°,170°$. (b) Same as part a, but assuming unresolved or unassigned signals from the labeled carbonyl sites.

We attribute these crosspeaks to spin–lattice relaxation of amide $^{14}N$ nuclei during $\tau$. $^{14}N$ relaxation produces a change in the net frequency shift anisotropy tensor ($^{13}C$ CSA plus $^{13}C$–$^{14}N$ dipole–dipole coupling) of an individual carbonyl site, in turn producing intrasite crosspeaks in analogy to chemical exchange or slow molecular motions.[4,5] The $^{14}N$ spin–lattice relaxation times estimated from intrasite crosspeak intensities are roughly 10 ms at room temperature and roughly 400 ms at $-120$ °C.

The approach described above may have broad applications in biophysical chemistry, because it yields detailed structural information from a single doubly-labeled sample and a single 2D spectrum. High-speed MAS and very high-powered radiofrequency pulses are not needed. We have used pairs of carbonyl labels because the carbonyl $^{13}C$ CSA is large and well-characterized. Other schemes are possible, including labeling of the carbonyl and carboxyl carbons of glutamate or aspartate residues to investigate side-chain conformations and labeling of pairs of amide nitrogens with $^{15}N$ to investigate backbone conformations. The structural information in our approach is contained in the intensities of spinning sideband crosspeaks and is angular in nature, in contrast to earlier uses of exchange spectroscopy without rotor synchronization to obtain distance information.[13] Other NMR measurements that are sensitive to relative orientations of CSA tensors, such as magnetization exchange under rotational resonance or double-quantum spectroscopy with slow MAS, are difficult in systems with small isotropic shift differences, large CSA, and small homonuclear couplings, such as the doubly-labeled peptides discussed here.[12]

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