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Solid-State NMR Spectroscopy Method for Determination of the Backbone Torsion Angle ψ in Peptides with Isolated Uniformly Labeled Residues

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Solid-state nuclear magnetic resonance (SSNMR) has proven to be indispensable for the structural elucidation of noncrystalline biological solids.[1] Several SSNMR techniques, based on the principle of spin interaction tensor correlation,[2−6] have been suggested for the determination of peptide backbone torsion angles ϕ and ψ under magic-angle spinning (MAS). Most techniques for ψ determination require isotopic labeling of two consecutive residues.[7−14] However, in several recent structural studies by SSNMR,[15,16] it has proven useful to label multiple isolated residues or short peptide segments (rather than the entire peptide chain) uniformly with ^{13}C and ^{15}N to facilitate spectral resolution and resonance assignment. Thus, techniques that require labeling of two consecutive residues are not generally applicable for ψ determination at all sites. Ishii et. al. have demonstrated the determination of ψ by correlation of the carbonyl (C′) ^{13}C chemical shift anisotropy (CSA) and the $^{13}C_\alpha-^1H_\alpha$ dipolar tensors within a *single* labeled residue in a two-dimensional (2D) powder pattern spectrum.[17] Although this is an attractive approach, the specific relayed anisotropy correlation (RACO) technique demonstrated by Ishii et al. works well only in the regime of slow MAS (<5 kHz) and is therefore not optimal for systems with multiple uniformly ^{13}C-labeled residues. A major problem has been the difficulty of recoupling the carbonyl CSA under fast MAS (>10 kHz) while retaining a static CSA powder pattern line shape (to maximize sensitivity to ψ) and simultaneously avoiding significant recoupling of $^{13}C-^{13}C$ dipolar interactions. This difficulty has recently been alleviated by the ROCSA (recoupling of chemical shift anisotropy) technique developed in our laboratory.[18]

Here we show that it is possible to combine ROCSA and $^{13}C_\alpha-^1H_\alpha$ dipolar dephasing by Lee−Goldburg (LG) irradiation[19,20] to correlate the ^{13}C′ CSA and the $^{13}C_\alpha-^1H_\alpha$ dipolar tensors. The ψ angle in any single uniformly labeled residue can then be determined under fast MAS, to within ambiguities dictated by symmetry. The implementation of the ROCSA-LG technique is shown in Figure 1. We measure $^{13}C'\rightarrow^{13}C_\alpha$ cross-peak intensities in a series of 2D $^{13}C-^{13}C$ correlation spectra with variable ROCSA period and fixed LG period. Intensities arising from the real and imaginary ^{13}C′ transverse magnetization components after N ROCSA cycles are denoted ReN and ImN. Numerical simulations indicate that essentially all information about the ψ angle is contained in Re0 (or Re1), Im1, Re2, Im2, and Re3. Thus, it is sufficient to record only five 2D spectra with the pulse sequence in Figure 1. Additionally, ^{13}C NMR signals are detected under high-resolution MAS conditions, resulting in significant improvements in sensitivity compared with results using the original RACO technique.[17]

Experiments were performed on two synthetic peptides with uniformly labeled residues. The 17-residue peptide MB(i+4)EK was synthesized with uniform labeling of Ala9 and examined in lyophilized form, where MB(i+4)EK has been shown to be highly α-helical.[12,21] The 15-residue amyloid-forming peptide Aβ_{11-25} was

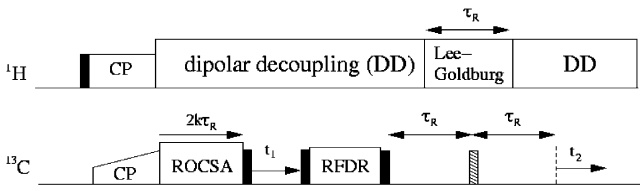

Figure 1. ROCSA-LG pulse sequence. Black and shaded rectangles represent $\pi/2$ and π pulses. ^{13}C′ polarization is first modulated by CSA interactions using the ROCSA sequence[18] for an even number of MAS rotor periods τ_R. After evolution at the isotropic C′ chemical shift for t_1 and polarization transfer to $^{13}C_\alpha$ under the radio frequency-driven recoupling (RFDR) sequence,[23] the polarization dephases due to $^{13}C_\alpha-^1H_\alpha$ couplings under LG irradiation. $^{13}C_\alpha$ signals are detected in t_2.

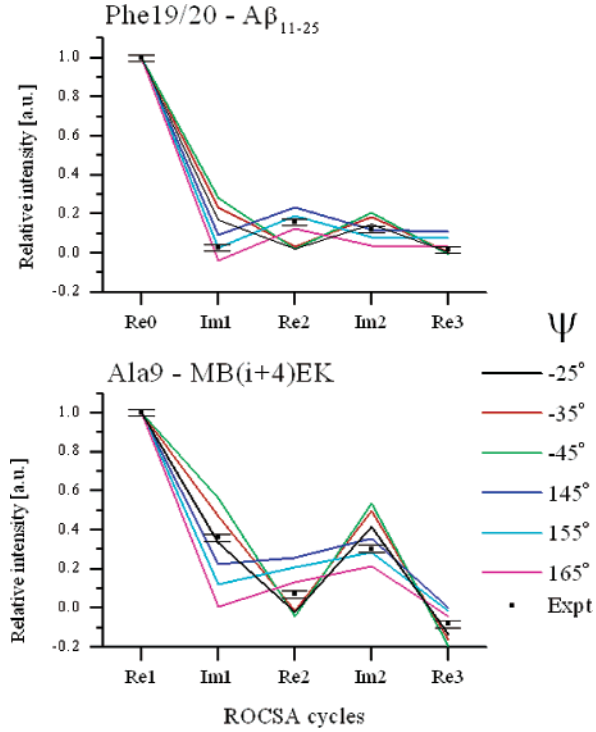

Figure 2. Experimental $^{13}C'\rightarrow^{13}C_\alpha$ cross-peak intensities in ROCSA-LG spectra for β-sheet (Phe19 and Phe20 of Aβ_{11-25}) and α-helical (Ala9 of MB(i+4)EK) peptides, and simulations for indicated values of the backbone torsion angle ψ. Experiments were carried out at 100.8 MHz ^{13}C NMR frequency and 14.0 kHz MAS frequency.

prepared with uniform labeling of Val18, Phe19, Phe20, and Ala21, fibrillized at pH 7.4, and lyophilized. In amyloid fibril form, residues 18−21 of Aβ_{11-25} reside in a β-strand.[18,22]

Figure 2 compares experimental ROCSA-LG data with numerical simulations. Simulations include the ^{13}C′, $^{13}C_\alpha$, and $^1H_\alpha$ spins in the ROCSA, RFDR polarization transfer,[23] and LG periods. Proton decoupling at 120 kHz, matching experimental conditions, is

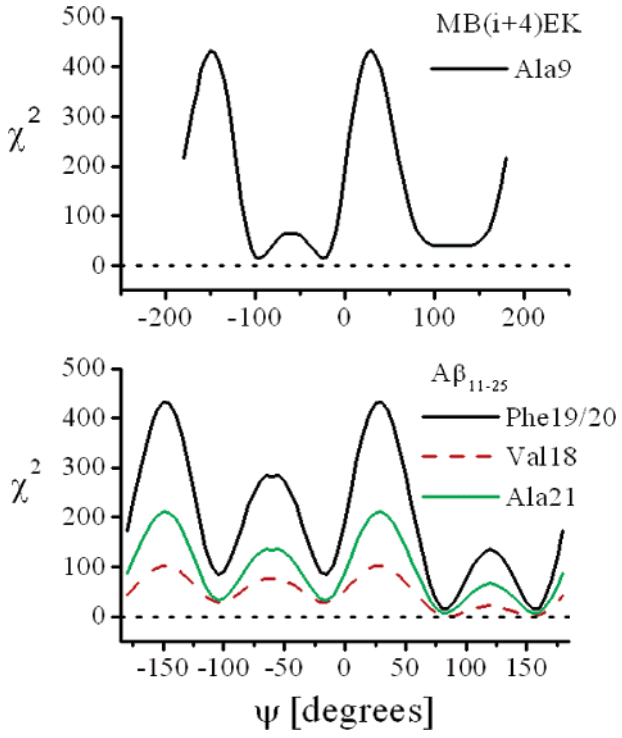

Figure 3. Total squared deviations between experimental and simulated data normalized by mean squared noise (χ^2) as a function of the backbone torsion angle ψ for lyophilized MB(i+4)EK (Ala9) and fibrillized Aβ_{11-25} (Val18, Phe19/Phe20, and Ala21).

included. Carbonyl CSA principal values were determined experimentally by ROCSA,[18] and principal axis directions were taken from model compound studies.[24] The $^{13}C_\alpha-^1H_\alpha$ bond length was set to 1.11 Å, as determined for MB(i+4)EK from LG dephasing curves. Figure 2 demonstrates both the sensitivity of the ROCSA-LG technique to the ψ value and the dependence of experimental ROCSA-LG data on the peptide conformation.

Figure 3 shows the χ^2 deviation between experiments and simulations as a function of the ψ value assumed in the simulations. Reflection symmetries about $-60°$ and $120°$ are due to the symmetry properties of the CSA and dipolar interactions[17] and are therefore unavoidable. Minimum χ^2 values for Ala9 in MB(i+4)EK occur at $-25°$ and $-95°$, consistent with the expected $\psi = -40° \pm 15°$ for an α-helical conformation. Minimum χ^2 values for the labeled residues in Aβ_{11-25} fibrils occur at $150-160°$ and $80-90°$, consistent with the expected $\psi = 140° \pm 20°$ for a β-strand conformation. Thus, the ROCSA-LG technique can distinguish the two principal polypeptide secondary structures from one another and from other possible conformations at a site-specific level. Analysis of simulations (see Supporting Information) indicates that an experimental signal-to-noise of 10 in Re0 permits a unique determination of ψ to within roughly $\pm 20°$ over the full range of ψ values, apart from the symmetry-related pairs.

We anticipate applications of the ROCSA-LG technique, together with previously reported techniques for ϕ angle determination,[25,26] in many structural problems where limited spectral resolution requires that uniformly labeled residues be introduced at isolated positions in a peptide sequence. One such application is structural studies of amyloid fibrils formed by relatively long peptides,[15] where achievable ^{13}C MAS NMR line widths are limited by the residual disorder inherent in a noncrystalline solid and where spectral resolution is additionally restricted by the predominance of β-strand conformations. Other possible applications include structural studies of peptides involved in biomineralization[27] and structural studies of membrane-associated peptides and proteins, where the predominance of α-helical conformations frequently restricts spectral resolution[28] and therefore may make uniform labeling of isolated residues an especially productive approach.

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Supporting Information Available: Details of experiments and simulations; simulated contour plot demonstrating the sensitivity of ROCSA-LG data over the full range of ψ values (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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