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Determination of the Complete Structure of a Uniformly Labeled Molecule by Rotational Resonance Solid-State NMR in the Tilted Rotating Frame

Kaoru Nomura,[†] K. Takegoshi,[†] Takehiko Terao,[*,†]
Kenichi Uchida,[‡] and Masatsune Kainosho[§]

Department of Chemistry, Graduate School of Science
Kyoto University, Kyoto 606-8502, Japan
Department of Biosciences
School of Science and Engineering, Teikyo University
Utsunomiya 320-8551, Japan
Department of Chemistry, Graduate School of Science
Tokyo Metropolitan University,
Hachioji, Tokyo 192-0397, Japan

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Solid-state NMR is becoming an important means for obtaining structural information, particularly on biological systems, requiring neither long-range order nor solubility. In structural studies by solid-state NMR, usually one specific internuclear distance[1−7] or dihedral angle[8−15] is determined using a selectively isotope-labeled sample. If we try to determine the complete structure by this approach, many selectively pair-labeled samples are required.[16] Recently, we proposed the R2TR (rotational resonance in the tilted rotating frame) method[17,18] which allows us to selectively recouple a particular homonuclear dipolar interaction under fast magic-angle spinning (MAS). It has a potential for obtaining structural information using a uniformly isotope-labeled powder sample, which significantly reduces experimental labor for preparing isotope-labeled samples. In this paper, we demonstrate the first solid-state NMR experiment for the determination of the complete three-dimensional (3D) molecular structure in a powder sample; using a uniformly ^{13}C,^{15}N-labeled glycylisoleucine (Gly-Ile; see inset in Figure 1), all of the six dihedral angles necessary to construct the 3D structure have been obtained by five R2TR experiments.

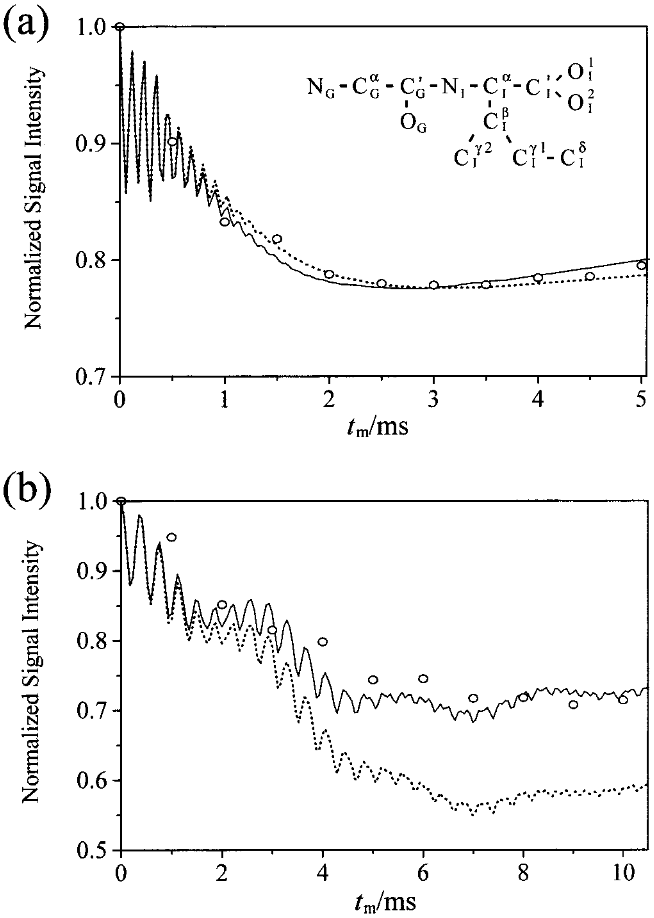

Figure 1. Recoupling-time (t_m) dependence of the normalized magnetization of C'_I in 10% fully ^{13}C,^{15}N-labeled glycylisoleucine under a R2TR condition $\omega_{eI} + \omega_{eS} = \omega_R$. Experiments were performed at a ^{13}C resonance frequency of 100.7 MHz. Experimental points are given by circles. The solid line is the curve calculated for dihedral angles (ω, ϕ_I, ψ_I) = (180°, −76°, 144°), whereas the dotted line for (ω, ϕ_I, ψ_I) = (0°, −86°, 169°). (a) The condition was satisfied for C'_I and C'_G by applying the rf field with the intensity of 8500 Hz on resonance to C'_I under spinning with ω_R = 17054 Hz. (b) The condition was satisfied for C'_I and C^{α}_{G} by applying the rf field with the intensity of 2800 Hz on resonance to C'_I under spinning with ω_R = 17030 Hz.

In R2TR, a rf field being applied, rotational resonance (R^2)[5,19−22] occurs in tilted rotating frame under a condition $\omega_{eI} + \omega_{eS}$, $\omega_{eI} - \omega_{eS}$, ω_{eI}, or $\omega_{eS} = n\omega_R$, where ω_{eX} is the effective field intensity for the X spin and ω_R is a spinning frequency.[17] R2TR can be applied to a pair of spins with a small chemical shift difference even under very fast spinning, contrary to R^2. Furthermore, while R^2 takes place frequency difference-selectively, R2TR occurs frequency selectively, providing much more efficient selectivity in dipolar recoupling than R^2. We frequency-

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[*] To whom correspondence should be addressed.
[†] Kyoto University.
[‡] Teikyo University.
[§] Tokyo Metropolitan University.
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selectively recoupled several spin pairs one by one under a R2TR condition $\omega_{eI} + \omega_{eS} = \omega_R$ to determine the dihedral angles.

Figure 1a shows the observed recoupling-time dependence of the spin-locked magnetization of C'_I under a R2TR condition satisfied between the resonances of C'_G and C'_I, which has been corrected regarding the relaxation using the $T_{1\rho}$ measured under an off-R2TR condition with shifting the rf-field frequency. If only the dipolar interaction between C'_G and C'_I contributes to the result, only the absolute value of the dihedral angle $\phi_I C'_G{-}N_I{-}C^{\alpha}_I{-}C'_I)$ can be determined from this experiment. However, owing to the presence of the chemical shift anisotropy (CSA) tensors of C'_G and C'_I, the dihedral angles ω $(C^{\alpha}_G{-}C'_G{-}N_I{-}C^{\alpha}_I)$ and ψ_I also affect the result, where ψ_I is defined as the dihedral angle between the $N_I{-}C^{\alpha}_I$ bond and the $O^1_I{-}C'_I{-}O^2_I$ plane. Therefore, we simulated the experimental curve by taking the three dihedral angles ω, ϕ_I, and ψ_I as adjustable parameters with the signs, using typical bond distances and bond angles. The anisotropy δ and asymmetry parameter η of the CSA tensor were assumed to be $(\delta, \eta) = (-79.3$ ppm, 0.82) and (-71.0 ppm, 0.84) for C'_G and C'_I, respectively. The former is the mean values of those for the carbonyl carbons on the peptide bonds in AcGlyGlyNH$_2$, AcGlyAlaNH$_2$, AcGly-TyrNH$_2$, and GlyGly·HCl[23] and the latter is those for L-alanine.[24] The σ_{33} principal axis of an sp^2 carbon is known to be perpendicular to the sp^2 plane.[25] The σ_{11} principal axis was assumed to be along the $C^{\alpha}_I{-}C'_I$ bond for C'_I [25] and make the angle of 34.5° with the $C'_G{-}N_I$ bond toward the $C'_G{-}O_G$ direction for C'_G.[23] For the dipolar interaction, we included not only the relevant spins of C'_G and C'_I but also C^{α}_G, C^{α}_I, and C^{β}_I for compensating incomplete selectivity in R2TR recoupling. Here, we simplify the fitting procedure assuming the planar configuration for the face (O_G, C'_G, N_I, C^{α}_I), namely we restricted ω to 0° or 180°. Although ω can be 0° only in limited cases, we examined the possibility of determination whether ω=0° or 180°. The global minimum of the root-mean-square deviation (rmsd) between the experimental data and simulated curves obtained for various ϕ_I and ψ_I occurs at $(\phi_I, \psi_I) = (-76°, 144°)$ for $\omega = 180°$, and $(-86°, 169°)$ for $\omega = 0°$. As shown in Figure 1a, simulations for the two sets of dihedral angles are not largely different from each other, so that we cannot choose the correct set out of the two from this result. To conclude which set of dihedral angles is correct, we made a similar recoupling experiment and analysis for the dipolar interaction between C^{α}_G and C'_I. The recoupling-time dependence is given by ω, ϕ_I, and ψ_I. Figure 1b shows the recoupling-time dependence of the spin-locked magnetization of C'_I together with the curves calculated for the two sets of parameters $(\omega, \phi_I, \psi_I) = (180°, -76°, 144°)$ and $(0°, -86°, 169°)$. Clearly, the former set reproduces the observation, being concluded to be the set of correct dihedral angles. These results show that two recoupling experiments per amino acid residue are enough to determine the dihedral angles (ψ, ω, ϕ) defining the 3D backbone structure of a peptide. The other dihedral angle of the main chain, ψ_G $(N_G{-}C^{\alpha}_G{-}C'_G{-}N_I)$, was determined to be 180° by another recoupling experiment on N_I and N_G.

We also determined the side chain conformations. The dihedral angle χ_{I1} $(C'_I{-}C^{\alpha}_I{-}C^{\beta}_I{-}C^{\gamma1}_I)$ was similarly obtained to be 179° from the recoupling experiment for C'_I and $C^{\gamma1}_I$. To determine the dihedral angle χ_{I2} $(C^{\alpha}_I{-}C^{\beta}_I{-}C^{\gamma1}_I{-}C^{\delta}_I)$, we observed the $C^{\gamma1}_I{-}C^{\delta}_I/C^{\alpha}_I{-}C^{\beta}_I$ dipolar correlation 2D (two-dimensional) spectrum, because it is difficult to determine χ_{I2} by a similar recoupling experiment for C^{α}_I and C^{δ}_I owing to small chemical-shift differences between C^{α}_I and C^{β}_I between $C^{\gamma1}_I$ and C^{δ}_I. In the t_1 period of this experiment the $C^{\gamma1}_I{-}C^{\delta}_I$ dipolar interaction is recoupled, in the mixing period the C^{δ}_I magnetization is transferred to the C^{α}_I spin, in the t_2 period the C^{α}_I magnetization is evolved under the recoupled $C^{\alpha}_I - C^{\beta}_I$ dipolar interaction, and then the dipolar-correlated 2D powder pattern is obtained by observing the high-resolution ^{13}C MAS spectrum in the t_3 period. From the rmsd between the experimental and simulated 2D spectra, the best-fit χ_{I2} was found to be ±160°. The sign of χ_{I2} cannot be determined from this experiment. The dihedral angle $(C^{\gamma2}_I{-}C^{\beta}_I{-}C^{\gamma1}_I - C^{\delta}_I)$ is calculated to be 39° for $\chi_{I2} = -160°$ and 79° for $\chi_{I2} = 160°$, suggesting that steric hindrance is smaller for the latter case; therefore, here we tentatively determine the sign to be positive. It would be worthy to note that the CSA tensors of these aliphatic carbons, which are not well established, were found not to affect the simulations.

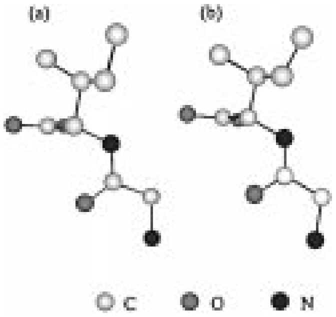

Figure 2. Three-dimensional structure of glycylisoleucine. (a) Present NMR study. (b) X-ray crystallography.

For comparison, we have also made an X-ray structural analysis. Figure 2 shows the 3D structures determined by the present NMR study and by the X-ray crystallography. The dihedral angles determined by NMR and X-ray are (ψ_G, ω, ϕ_I, ψ_I, χ_{I1}, χ_{I2}) (180°, 180°, −76°, 144°, 179°, 160°) and (165°, 170°, −70°, 153°, 178°, 170°), respectively. We could not prepare a good sample for the X-ray crystallography, so that the final conventional *R* factor is considerably large (11.8%). Nevertheless, the agreement of both structures is pretty good, indicating that the present approach is useful for structural determinations.

The present approach can be applied to analyze the structure of a restricted region important to biological function, using a sample in which the region is uniformly labeled. Also, in principle, we can apply this approach to larger systems, as long as necessary recoupling can be selectively realized. So far, quantitative structural information obtained by solid state NMR has been almost limited to a single internuclear distance or dihedral angle. The present approach substantially increases the obtainable quantity of structural information in a single powder sample.

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