Determination of Torsion Angles in Proteins and Peptides Using Solid State NMR

Peter V. Bower, Nathan Oyler, Manish A. Mehta,[†] Joanna R. Long,[‡] Patrick S. Stayton,[‡] and Gary. P. Drobny*

Contribution from the Department of Chemistry, University of Washington, Seattle, Washington 98195-1700, and Department of Bioengineering, University of Washington, Seattle, Washington 98195-1700

Received April 26, 1999. Revised Manuscript Received June 25, 1999

Abstract: A combination of Solid State NMR (SSNMR) dipolar recoupling and double quantum CSA−CSA correlation experiments are used to determine the Ramachandran angles ϕ and ι in a crystalline tripeptide (AGG) and a 14 amino acid peptide designed to be helical. The advantage of the SSNMR approach described herein is the ability to measure both ϕ and ψ to high resolution using a single noncrystalline, doubly carbonyl labeled peptide. It is also shown that DRAWS and DQDRAWS data are insensitive to $^{14}N-^{13}C$ and $^{15}N-^{13}C$ dipolar couplings making corrections for these effects unnecessary. Extremes in secondary structure (e.g., α-helix vs β-sheet) can be discerned by simple inspection of DQDRAWS spectra. Subtleties in secondary structure (α-helix vs 3_{10}-helix) can be distinguished by simulation of the DQDRAWS spectrum.

The structure of a molecule is defined by bond lengths, bond angles, and the orientation of rigid groups of atoms about bonds (torsion angles). For peptides and proteins the bond lengths and angles are very nearly fixed, and variation of secondary structure is only achieved through rotation around the rotationally free bonds in the backbone. A sequence of N amino acids will contain $(3N - 1)$ chemical bonds in the backbone of which $(N - 1)$ are amide linkages, which are nearly planar and have no rotational freedom due to partial sp^2-hybridization of the CN peptide bond. This rotational angle is usually referred to as ω and is fixed close to 180°. Each of the remaining $2N$ bonds have rotational freedom, which is restricted by side-chain steric hindrance and electrostatic interactions. N angles ϕ describe the rotational state of the amide $N-C_\alpha$ bonds, and N angles ψ describe the rotation around C_α-carbonyl bonds as shown in Figure 1. The $2N$ angles are usually described as N pairs (ϕ,ψ) called the Ramachandran angles.[1,2] A complete set of these angles (N pairs) will fully describe the secondary structure of an N amino acid peptide/protein.

Solution structures of proteins are solved by nuclear magnetic resonance (NMR) utilizing the nuclear Overhauser effect.[3] X-ray diffraction has been successful in elucidating structures of crystalline peptides and proteins. However, these widespread and powerful techniques are not useful when a system of interest cannot yield diffractable crystals or does not exhibit favorable behavior in solution (i.e., rotational correlation time > ca. 10^{-9} s). Membrane proteins and surface-adsorbed molecules are examples of such systems.

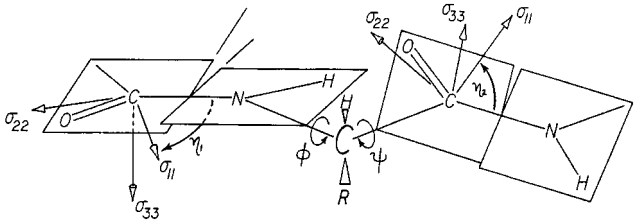

Figure 1. η_1 and η_2 describe the orientations of the CSA tensors relative to the C−N bonds. ϕ and ψ are the Ramachandran angles that describe rotational freedom of the backbone. The angle ω is depicted as the slight tilt between the $H-N-C_\alpha$ plane and the N−C−O plane.

Solid state NMR (SSNMR) can, in principle, yield high-resolution structural information on membrane proteins, surface adsorbed molecules, etc. However, anisotropic interactions such as the nuclear dipole−dipole coupling and the chemical shift anisotropy (CSA) present major difficulties in spectral analysis. The CSA for carbonyl ^{13}C spins results in line widths of approximately 200 ppm, which greatly exceeds the dipolar couplings of 150−400 Hz that are typical for distances between backbone carbonyls in peptides (2.7−3.7 Å). The introduction of a time dependency on the spatial part of the Hamiltonian by employing magic angle sample spinning[26,27] partially averages the SSNMR-Hamiltonian and yields far better spectral resolution through narrowing the resonance lines. However, the dipolar information will be averaged completely in the resulting spectrum and must be restored through the application of a second averaging process. A number of different rf dipolar recoupling techniques have been developed to accomplish this[4−13] including dipolar recoupling with a windowless sequence (DRAWS), which was developed in our lab.

[†] Present address: Department of Chemistry, Oberlin College, Oberlin, Ohio 44074.

[‡] Department of Bioengineering, University of Washington.

(1) Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. *J. Mol. Biol.* **1963**, *7*, 95.

(2) Ramachandran, G. N.; Sasisekharan, V. *Adv. Protein Chem.* **1968**, *23*, 281.

(3) *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Ernst, R. R., Bodenhausen, G., Wokaun, A., Eds.; Oxford: U.K., 1987.

(4) Nielsen, N. C.; Bildsøe, H.; Jakobsen, H. J.; Levitt, M. H. *J. Chem. Phys.* **1994**, *101*, 1805.

(5) Tycko, R.; Smith, S. O. *J. Chem. Phys.* **1993**, *98*, 932.

(6) Sun, B. Q.; Costa, P. R.; Kocisko, D.; Lansbury, P. T. J.; Griffin, R. G. *J. Chem. Phys.* **1995**, *102*, 702.

(7) Lee, Y. K.; Kurur, N. D.; Helme, M.; Johannessen, O. G.; Nielsen, N. C.; Levitt, M. H. *Chem. Phys. Lett.* **1995**, *242*, 304.

10.1021/ja991330q CCC: $18.00
Published on Web 08/25/1999

Thus far measurement of torsion angles in SSNMR has been approached by correlating heteronuclear couplings via homonuclear double-quantum experiments. This avenue has been explored in the case of the HCCH system[14] and the NCCN system.[15,30] Also heteronuclear HNCH double-quantum variations have been developed for measurement of ϕ.[16] Measurement of the Ramachandran angles have also previously been approached via spin−spin correlations produced through spin diffusion.[29] Although extremely useful, some of these techniques have limitations. For example, for the NCCN experiment, it is reported that an "ambiguity" occurs for $|\psi| \leq 120°$, degrees,[15,30] requiring that torsion angle estimation over most of the Ramachandran plane by N−C−C−N be supplemented by other information.

We have demonstrated earlier how orientational information can be obtained by correlating ^{13}C CSA tensors in double quantum MAS experiments, where the double quantum state was prepared using the DRAWS pulse sequence (DQDRAWS).[17] In the present paper we show how DRAWS distance measurements and the DQDRAWS experiment described in detail in ref 17 can be used to quantify the Ramachandran angles (ϕ,ψ) using carbonyl ^{13}C-labeled peptides. Two systems will be shown as examples: the tripeptide alanyl-glycyl-glycine (AGG) whose structure has been solved by X-ray diffraction,[18] and a 14 amino acid peptide Ac-LKKLLKL*L*KKLLKL (LK). LK was designed to exhibit α-helical secondary structure, a fact confirmed by circular dichroism.[19]

Ac-LKKLLKL*L*KKLLKL and two labeled varieties of A*G*G (Ala($^{13}C_1$)-Gly($^{13}C_1$)-Gly and Ala-($^{13}C_1$)-Gly-(^{15}N,$^{13}C_1$)-Gly-(^{15}N), where * indicates a ^{13}C label on the C_1-carbonyl) were synthesized using standard Fmoc chemistry.[20] In the case of labeling the C-terminal Gly the Fmoc-protected Gly was coupled to Wang resin by using standard protocols.[21] Coupling of amino acids was carried out on an Applied Biosystems 433A peptide synthesizer. Labeled AGG was diluted in a ratio of 1:10 with unlabeled AGG obtained from Bachem and recrystallized from aqueous solution. X-ray diffraction confirmed the correct unit cell dimensions and space group.[18]

NMR experiments were carried out on a 9.4 T magnet with a home-built spectrometer.[28] The probe used was a home-built HFXY probe[25] with the X-channel tuned to the ^{13}C Larmor frequency (100.6 MHz). Samples were spun at 4.000 or 4.082 kHz and ^{13}C rf irradiation levels were set synchronously to 34 or 34.694 kHz, respectively. During DRAWS recoupling and FID acquisition a 110 kHz ^{1}H decoupling level was used. For the DQDRAWS experiment 250 t_1 points and 1024 t_2 points were recorded with 128 transients (acquisition time of 36 h). Principal CSA values were determined using the Herzfeld−Berger analysis.[22] The orientation of the carbonyl CSA in the molecular frame is shown in Figure 1. σ_{11} and σ_{22} are assumed to be in the peptide plane with σ_{33} perpendicular to this plane. The rotation with respect to the C−N bond ($\eta_{1,2} \approx 45°$) is to a good approximation constant.[23]

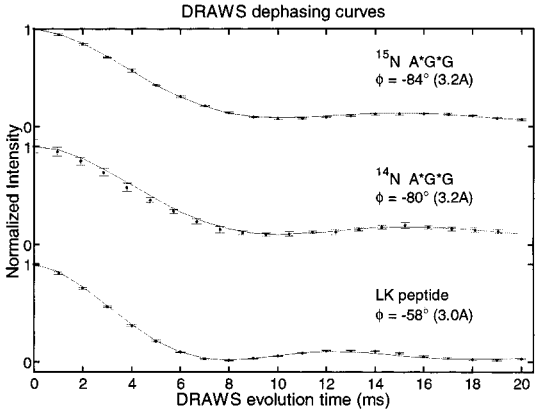

Figure 2. DRAWS dephasing curves with matching simulations (solid curves). Experimental parameters: trot = 250 μs.

The data-fitting program was based on home-built numerical density matrix simulation code written in C++. The calling program was written in Matlab and adapted to the various situations as needed. As described previously,[31] the equation of motion of the density matrix is integrated for each crystallite orientation by determining the quantum mechanical propagator for a short time increment, and multiplying these propagators together in a time-ordered fashion to obtain the numerically exact propagator for the DQDRAWS sequence.

Figure 1 clearly shows that the distance between two carbonyls depends only on the torsion angle ϕ. A measurement of the dipolar coupling between the two carbonyls can therefore yield direct information about the torsion angle ϕ. The direction of rotation cannot be determined since $\pm\phi$ results in the same carbonyl−carbonyl distances. However, peptides composed of L-form amino acids exhibit a preference for $-\phi$.[1,2] Using dipolar recoupling with a windowless sequence[13] (DRAWS) the dephasing curve obtained can be fit to a carbonyl−carbonyl distance. Figure 2 shows the experimental (points) and simulated (solid lines) dephasing curves for $^{13}C_1$-Ala-$^{13}C_1$-Gly-Gly, $^{13}C_1$-Ala-(^{15}N,$^{13}C_1$)-Gly-^{15}N-Gly, and Ac-LKKLLKL*L*KKLLKL. For AGG the carbonyl−carbonyl distance determined by DRAWS is in excellent agreement with the 3.19 Å reported in the X-ray diffraction study.[18]

(8) Hohwy, M.; Jakobsen, H. J.; Eden, M.; Levitt, M. H.; Nielsen, N. C. *J. Chem. Phys.* **1998**, *108*, 2686.
(9) Gullion, T.; Vega, S. *Chem. Phys. Lett.* **1992**, *194*, 423.
(10) Bennett, A. E.; Ok, J. H.; Griffin, R. G.; Vega, S. *J. Chem. Phys.* **1992**, *96*, 8624.
(11) Baldus, M.; Meier, B. H. *J. Magn. Reson.* **1997**, *128*, 172.
(12) Baldus, M.; Tomaselli, M.; Meier, B. H.; Ernst, R. R. *Chem. Phys. Lett.* **1994**, *230*, 329.
(13) Gregory, D. M.; Mitchell, D. J.; Stringer, J. A.; Kiihne, S.; Shiels, J. C.; Callahan, J.; Mehta, M. A.; Drobny, G. P. *Chem. Phys. Lett.* **1995**, *246*, 654.
(14) Feng, X.; Lee, Y. K.; Sandstrom, D.; Edén, M.; Maisel, H.; Sebald, A.; Levitt, M. H. *Chem. Phys. Lett.* **1996**, *257*, 314.
(15) Costa, P. R.; Gross, J. D.; Hong, M.; Griffin, R. G. *Chem. Phys. Lett.* **1997**, *280*, 95.
(16) Hong, M.; Gross, J. D.; Hu, W.; Griffin, R. G. *J. Magn. Reson.* **1998**, *135*, 169.
(17) Gregory, D. M.; Mehta, M. A.; Shiels, J. C.; Drobny, G. P. *J. Chem. Phys.* **1997**, *107*, 28.
(18) Lalitha, V.; Subramanian, E.; Bordner, J. *Ind. J. Pure Appl. Phys.* **1985**, *23*, 506.
(19) DeGrado, W. F.; Lear, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 7684.
(20) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
(21) Sieber, P. *Tetrahedron Lett. 28*, 6147.
(22) Herzfeld, J.; Berger, A. E. *J. Chem. Phys.* **1980**, *73*, 6021.
(23) Oas, T. G.; Hartzell, C. J.; McMahon, T. J.; Drobny, G. P.; Dahlquist, F. W. *J. Am. Chem. Soc.* **1987**, *109*, 5956.
(24) Gregory, D. M. Ph.D. Thesis, 1997, University of Washington, Department of Physics.
(25) Stringer, J. S. Ph.D. Thesis, 1998, University of Washington, Department of Chemistry.
(26) Andrew, E. R.; Bradbury, A.; Eades, R. G. *Nature* **1959**, *183*, 1802.
(27) Lowe, I. J. *Phys. Rev. Lett.* **1959**, *2*, 285.
(28) Gladden, J.; Drobny, G. P., unpublished results.
(29) Tycko, R.; Weliky, D. P.; Berger, A. E. *J. Chem. Phys.* **1996**, *105*, 7915.
(30) Feng, X.; Edén, M.; Brinkmann, A.; Luthman, H.; Erikson, L.; Gräslund, A.; Antzutkin, O. N.; Levitt, M. H. *J. Am. Chem. Soc.* **1997**, *119*, 12006.
(31) Mehta, M. A.; Gregory, D. M.; Kiihne, S. R.; Mitchell, D. J.; Hatcher, M. E.; Shiels, J. C.; Drobny, G. P. *Solid State Nucl. Magn. Reson.* **1996**, *7*, 211.

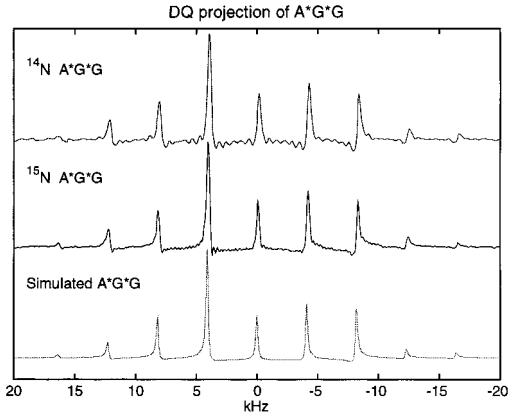

Figure 3. Experimental DQDRAWS projections for AGG, AGG (^{15}N), and simulated projection. Experimental parameters: dwell $t_1 = 20.42$ μs, $t_2 = 20$ μs, trot = 245 μs, 5 DRAWS periods applied for DQ excitation.

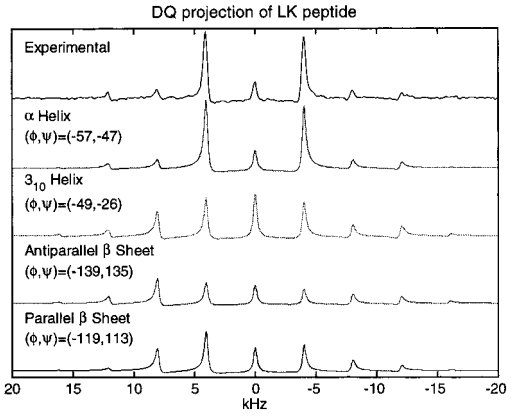

Figure 4. Experimental DQDRAWS projections for LK peptide and simulations for common structural motifs. Experimental parameters: dwell $t_1 = 20$ μs, $t_2 = 20$ μs trot = 250 μs, 4 DRAWS periods applied for DQ excitation.

The dipolar coupling and the tensor sum of the two carbonyl CSAs determine the evolution of double quantum coherence. The relative orientation of the highly anisotropic CSA tensors will therefore have a pronounced impact on the sideband intensity in the DQ spectrum. The dipolar coupling will likewise affect the DQ envelope. For peptides and proteins the backbone carbonyls of adjacent amino acids are prime candidates with their close proximity (2.7−3.7 Å) and their large CSAs (∼200 ppm). Figure 3 shows experimental DQDRAWS[17] data for crystalline AGG and a best-fit simulation ($\phi = -80$, $\psi = 164$) which is in very good agreement with the crystallographic torsion angles[18] ($\phi = -83$, $\psi = 169$). Experimentally, a DQ excitation efficiency of ∼30% was achieved. The previously mentioned NMR determined CSA orientations were used in the simulations.[23] It should be noted that the DRAWS dephasing curves and DQDRAWS spectra are insensitive to the ^{14}N−^{13}C and ^{15}N−^{13}C dipolar couplings, as shown in Figures 2 and 3.

Figure 4 shows experimental DQ data for the LK peptide juxtaposed with a best-fit simulation ($\phi = -59$, $\psi = -39$) and several simulations that are based on common secondary structural motifs. The DQDRAWS spectrum shows a marked sensitivity to small variations in ψ. For example, the double quantum spectrum for the α-helix ($\phi = -57$, $\psi = -47$) and 3_{10}-helix ($\phi = -49$, $\psi = -26$) are easily distinguishable, as shown in Figure 4. The same information could not be obtained from a carbonyl−carbonyl distance measurement alone.

Figure 4 also shows that extremes in canonical secondary structure, e.g., α-helix and β-sheet, can be identified virtually by inspection of the DQDRAWS spectrum. The trend shown in the DQDRAWS spectra in Figure 4 can be qualitatively explained in the following way. The DQDRAWS spectrum for two coupled ^{13}C carbonyl spins in an α-helical peptide result in a weak line at $\nu_1^{iso} + \nu_2^{iso}$ (the isotropic chemical shift sum), and intense first-order sidebands. This can be attributed to a small anisotropy of the CSA tensor sum.[24] Varying the secondary structure (i.e., the mutual orientation of the CSA tensors) can give a greater anisotropy for the CSA tensor sum, resulting in a more intense center peak at $\nu_1^{iso} + \nu_2^{iso}$ and more intense sidebands (i.e., 3_{10}-helix and β-sheet).

In conclusion we have demonstrated the use of DRAWS as a distance-measuring technique in peptides and shown that a simple correlation exists between the DRAWS dephasing curve for adjacent carbonyl ^{13}C spins and the torsion angle ϕ (accuracy of ±10°). The ψ angle can be determined from the mutual orientation of the two carbonyl CSA tensors, obtained by a DQDRAWS experiment. A possible complication for any CSA−CSA correlation experiment is variation of CSA orientation with respect to the molecular framework. Fortunately, the carbonyl ^{13}C CSA tensor is one of the best studied of the CSA tensors. The variation of carbonyl CSA tensors in numerous peptides and in particular in a homologous series of G−X dipeptides[23] has been reported. From this work, a typical variation of η for a chemically homologous series of peptides is less than 10°. Note that even with errors in ψ on the order of ±10° to account for the typical variations reported for carbonyl CSA tensor orientations in peptides, differences between extremes in secondary structure (e.g., α helix versus β sheet) can be easily distinguished via DQDRAWS, and even subtle differences in helical structures (i.e., a helix versus 3_{10} helix) can be distinguished (see Figure 4). Therefore, minor variations in carbonyl CSA tensor orientation notwithstanding, the DQDRAWS spectrum is a very useful probe of the torsion angles ψ. The combined use of DRAWS and DQDRAWS with peptides that are enriched with ^{13}C at adjacent carbonyl positions provides a method for determining secondary structure of membrane proteins and proteins adsorbed to biomaterials interfaces.

Acknowledgment. We gratefully acknowledge the support of the National Science Foundation (EEC-9529161 and DMR-9616212), and the University of Washington Center for Engineered Biomaterials (UWEB). P.V.B. acknowledges support from the Danish Research Academy.

JA991330Q