

Solid-State NMR Determination of Peptide Torsion Angles: Applications of ^2H -Dephased REDOR

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Abstract: The backbone conformation of peptides and proteins is completely defined by the torsion angles (ϕ, ψ, ω) of each amino acid residue along the polypeptide chain. We demonstrate a solid-state NMR method based on heteronuclear distance measurements for determining (ϕ, ψ) angles. Simple and reliable deuterium phase modulated pulses (PM5) reintroduce dipolar couplings between ^2H and a spin- $1/2$ nucleus. Measuring the $^{13}\text{C}_{i-1}\{^2\text{H}_i^\alpha\}$ REDOR distance across a peptide bond results in the torsion angle ϕ_i as a consequence of the restricted geometry of the peptide backbone. The $^{15}\text{N}_{i+1}\{^2\text{H}_i^\alpha\}$ REDOR distance across a peptide bond defines the torsion angle ψ_i . This approach is demonstrated for both the 3-spin $\text{X}\{^2\text{H}_2\}$ REDOR case of glycine and the 2-spin $\text{X}\{^2\text{H}\}$ REDOR case, represented by L-alanine, using two different tripeptides. It is shown that the technique can handle multiple sample conformations. PM5-REDOR decay curves of the ψ angle show distinctly different behaviors between α -helix and β -sheet backbone conformations.

Introduction

The rapid rate at which high-resolution 3D protein structures are being solved is dramatic. The more than 13 000 structures of the Protein Data Bank (Research Collaboratory for Structural Bioinformatics, <http://www.rcsb.org/pdb>) attests to the success of protein structure determination in the crystalline state by X-ray crystallography and in solution by liquid-state nuclear magnetic resonance (NMR). Recent advances in both techniques suggest that the number of known structures will continue to escalate (see recent reviews, refs 1–4). Solid-state NMR provides a complementary high-resolution spectroscopic technique to determine local protein structure in disordered and anisotropic environments (reviews, refs 5–10). Routine high-resolution biomolecular structure determination of solids lacking long-range order may be feasible using solid-state NMR.^{11–15}

Solid-state NMR spectra contain information on anisotropic interactions (chemical shift, dipolar couplings, and quadrupolar couplings). Manipulation of the overabundance of information is necessary to obtain sufficient resolution to interpret the spectra. Of particular interest to protein structural studies have been methods measuring molecular orientations, internuclear distances, and the (ϕ, ψ) conformational angles.⁵ The 3D conformations of proteins are defined by the individual makeup of ϕ_i ($\text{C}_{i-1}\text{N}_i\text{C}_i^\alpha\text{C}_i$) and ψ_i ($\text{N}_i\text{C}_i^\alpha\text{C}_i\text{N}_{i+1}$) torsion angles of each amino acid residue along the polypeptide chain. Accurate knowledge of torsion angles is the basis of well-defined protein secondary structures. A number of techniques for determining peptide torsion angles using solid-state NMR were recently developed.^{12,16–26} The combination of multiple and highly

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(1) Beauchamp, J. C.; Isaacs, N. W. *Curr. Op. Chem. Biol.* **1999**, *3*, 525–529.

(2) Lindley, P. F. *Acta Crystallogr.* **1999**, D55, 1654–1662.

(3) Wider, G.; Wuthrich, K. *Curr. Op. Struct. Biol.* **1999**, *9*, 594–601.

(4) Siegal, G.; van Duynhoven, J.; Baldus, M. *Curr. Op. Chem. Biol.* **1999**, *3*, 530–536.

(5) Fu, R. Q.; Cross, T. A. *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 235–268.

(6) Bechinger, B.; Kinder, R.; Helmle, H.; Vogt, T. C. B.; Harzer, U.; Schinzel, S. *Biopolymers* **1999**, *51*, 174–190.

(7) Garbow, J. R.; Gullion, T. *Measurement of internuclear distances in biological solids by magic-angle spinning ^{13}C NMR*; Beckman, N., Ed.; Academic Press: San Deigo, 1995; pp 65–115.

(8) Griffin, R. G. *Nature Struct. Biol.* **1998**, *5*, 508–512.

(9) Wemmer, D. *Methods Enzymol.* **1999**, *309*, 536–559.

(10) Davis, J. H.; Auger, M. *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *35*, 1–84.

(11) Opella, S. J.; Marassi, F. M.; Gesell, J. J.; Valente, A. P.; Kim, Y.; Oblatt-Montal, M.; Montal, M. *Nature Struct. Biol.* **1999**, *6*, 374–379.

(12) Hong, M. *J. Magn. Reson.* **1999**, *139*, 389–401.

(13) Ketchum, R. R.; Roux, B.; Cross, T. A. *Structure* **1997**, *5*, 1655–1669.

(14) Marassi, F. M.; Ramamoorthy, A.; Opella, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8551–8556.

(15) Sun, B.-Q.; Rienstra, C. M.; Costa, P. R.; Williamson, J. R.; Griffin, R. G. *J. Am. Chem. Soc.* **1997**, *119*, 8540–8546.

(16) Bower, P. V.; Oyler, N.; Mehta, M. A.; Long, J. R.; Stayton, P. S.; Drobny, G. P. *J. Am. Chem. Soc.* **1999**, *121*, 8373–8375.

(17) Nomura, K.; Takegoshi, K.; Terao, T.; Uchida, K.; Kainosho, M. *J. Am. Chem. Soc.* **1999**, *121*, 4064–4065.

(18) Ishii, Y.; Hirao, K.; Terao, T.; Terauchi, T.; Oba, M.; Nishiyama, K.; Kainosho, M. *Solid State Nucl. Magn. Reson.* **1998**, *11*, 169–175.

(19) Bennett, A. E.; Weliky, D. P.; Tycko, R. *J. Am. Chem. Soc.* **1998**, *120*, 4897–4898.

(20) Costa, P. R.; Gross, J. D.; Hong, M.; Griffin, R. G. *Chem. Phys. Lett.* **1997**, *280*, 95–103.

(21) Hong, M.; Gross, J. D.; Griffin, R. G. *J. Phys. Chem. B* **1997**, *101*, 5869–5874.

(22) Feng, X.; Eden, M.; Brinkmann, A.; Luthman, H.; Eriksson, L.; Graslund, A.; Antzutkin, O. N.; Levitt, M. H. *J. Am. Chem. Soc.* **1997**, *119*, 12006–12007.

(23) Heller, J.; Laws, D. D.; Tomaselli, M.; King, D. S.; Wemmer, D. E.; Pines, A.; Havlin, R. H.; Oldfield, E. *J. Am. Chem. Soc.* **1997**, *119*, 7827–7831.

(24) Tycko, R.; Weliky, D. P.; Berger, A. E. *J. Chem. Phys.* **1996**, *105*, 7915–7930.

(25) Schmidt-Rohr, K. *Macromolecules* **1996**, *29*, 3975–3981.

(26) Creuzet, F.; McDermott, A.; Gebhard, R.; van der Hoef, K.; Spijker-Assink, M. B.; Herzfeld, J.; Lugtenburg, J.; Levitt, M. H.; Griffin, R. G. *Science* **1991**, *251*, 783–786.