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Peptide Torsion Angle Measurements: Effects of Nondilute Spin Pairs on Carbon-Observed, Deuterium-Dephased PM5-REDOR

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Reintroducing dipolar coupling between spin-1/2 nuclei (e.g., ^{13}C, ^{15}N) and spin-1 ^{2}H, using phase-modulated deuterium dephasing pulses, provides a simple and efficient basis for obtaining peptide backbone torsion angles (ϕ, ψ) in specific stable-isotope enriched samples. Multiple homonuclear spin-1/2 interactions due to isotopic enrichment can arise between neighboring molecules or within a multiply labeled protein after folding. The consequences of ^{13}C homonuclear interactions present during ^{13}C-observed, ^{2}H-dephased REDOR measurements are explored and the theoretical basis of the experimentally observed effects is investigated. Two tripeptides are taken to represent both the general case of $^{2}H^{\alpha}$-alanine (in the tripeptide LAF) and the special case of $^{2}H_{2}^{\alpha}$-glycine (in the tripeptide LGF). The lyophilized tripeptides exhibit narrowed spectral linewidths over time due to reduced conformational dispersion. This is due to a hydration process whereby a small fraction of peptides is reorienting and the bulk peptide fraction undergoes a conformational change. The new molecular packing arrangement lacks homonuclear ^{13}C spin interactions, allowing determination of (ϕ, ψ) backbone torsion angles. © 2001 Academic Press

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INTRODUCTION

Solid-state NMR is a unique tool for obtaining molecular level structural details of a wide variety of compounds without inherent restrictions to size or molecular ordering. Specific stable-isotope labeling is an effective strategy for obtaining local structural information in biological systems (reviews: (*1–4*)). An advantage of magic-angle spinning (MAS) solid-state NMR among sophisticated high-resolution structural methods is the flexibility in preparation state of the sample: crystalline, lyophilized, precipitated, membrane-bound, flash-frozen, or frozen solutions are all viable options.

One of the most powerful and widely used MAS methods of modern solid-state NMR is rotational echo double resonance (REDOR) (*5*), which determines heteronuclear distances between spin-1/2 nuclei. The experiments are easy to perform and in most cases the REDOR decay signals can be analyzed with the help of a simple master curve. Internuclear distance measurements between spin-1/2 and spin-1 deuterium (^{2}H) nuclei can be accurately measured using variations of standard spin-1/2 REDOR (*6–12*). The method of PM5-REDOR (*12*) uses optimized phase-modulated pulses to obtain efficient ^{2}H dephasing.

PM5-REDOR was recently used with specific stable-isotope labeling to determine ϕ and ψ peptide torsion angles (*13*). Three peptide torsion angles define the backbone conformation of a protein, (ϕ, ψ, ω). A number of solid-state NMR approaches exist to measure peptide backbone torsion angles and provide definitive information of local protein structure (*14–22*). PM5-REDOR determines peptide backbone conformational angles (ϕ, ψ) by reintroducing ^{13}C–^{2}H or ^{15}N–^{2}H dipolar couplings during MAS using the labeling scheme shown in Fig. 1. Site-specific stable-isotope labeling can be obtained by starting with commercially available enriched amino acids for solid-phase peptide synthesis or for growth medium supplements in bacterial biosynthesis (*23*). The torsion angle, ϕ_i, can be measured by reintroducing the dipolar coupling between a nonexchangeable deuterium at the α-proton position ($^{2}H_i^{\alpha}$) and a ^{13}C-enriched $i-1$ carbonyl carbon. The torsion angle ψ_i is determined in a separate ^{15}N–$^{2}H_i^{\alpha}$ PM5-REDOR experiment between $^{2}H_i^{\alpha}$ and a ^{15}N-enriched $i+1$ amide nitrogen. As in REDOR, every resolved peak in the observed spectrum can be analyzed from one experimental run.

This scheme works well for both two-spin (e.g., $^{2}H^{\alpha}$-alanine) and three-spin ($^{2}H_2^{\alpha}$-glycine) cases. The two-spin ^{15}N–$^{2}H_i^{\alpha}$ PM5-REDOR experiment to obtain ψ_i is sensitive to β-sheet vs α-helical conformations even when signal-to-noise is relatively low. Comparing experimental data and theoretical calculations results in two possible values for L-amino acid torsion angles or four degenerate values of glycine torsion angles. Readily available constraints from Ramachandran plots of ^{13}C–^{15}N REDOR measurements on the same sample can result in unique torsion angles (*13*).

The labeling scheme shown in Fig. 1 is advantageous for extracting multiple structural constraints from a single sample. Four different nuclei can be observed, providing complementary spectroscopic information. The presence of multiple spin

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