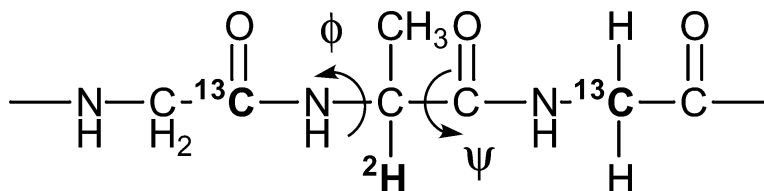


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Determining Dihedral Angles and Local Structure in Silk Peptide by ^{13}C - ^2H REDOR

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Silk fibroins from *Bombyx mori* silkworm consist mostly of the sequence (Gly-Ala-Gly-Ala-Gly-Ser)_n and comes in silk I and silk II structural forms.^{1,2} Silk II fibers are heterogeneous with 73% of the crystalline fraction in the antiparallel β -sheet motif.^{3,4} The silk I conformation is the silk structure that occurs just prior to the spinning process and remains poorly understood.⁵ Attempts to produce oriented silk I fibers of suitable quality for X-ray diffraction have not been successful. This work shows how ^{13}C - ^2H REDOR NMR can be used to provide structural details in peptides and will provide structural information about the elusive silk I conformation. Individual dihedral angles can be determined by suitable placement of ^{13}C and ^2H spin labels, and the method will be illustrated on labeled 30-residue silk I mimics (AlaGly)₁₅.^{6,7}

High-resolution MAS NMR dipolar recoupling methods have provided important structural information for biological solids.⁸ Most of these NMR experiments measure the dipolar coupling between ^{13}C - ^{13}C or ^{13}C - ^{15}N spin pairs that have been incorporated into the system. The distance between spin pairs can be extracted from the dipolar coupling. The use of the versatile ^2H spin label as a structural probe has not, unfortunately, been utilized much for quantitative distance measurements.⁹ The main difficulty associated with using ^2H is that efficient manipulation of the spin states by typical radio-frequency field strengths is not easy because the deuterium quadrupolar interaction spreads the ^2H resonances over a very large spectral range. This problem can be circumvented in REDOR by using a composite ^2H $\pi/2$ recoupling pulse of the form $17_x62_x99_x144_x$.¹⁰

The ^{13}C - ^2H REDOR experiment is characterized by a normalized difference signal, $\Delta S/S$, which is independent of all NMR parameters except the dipolar coupling. In terms of Bessel functions of the first kind, the ideal difference signal is given by eq 1 and shown as the solid lines in Figures 1–3.^{10,11} The dimensionless parameter λ is the product of the dipolar evolution time and the dipolar coupling, D , which has units of Hz.

$$\Delta S/S = 1 - \frac{1}{6} \left[1 + 4 \frac{\sqrt{2}\pi}{4} J_{1/4}(\sqrt{2}\lambda) J_{-1/4}(\sqrt{2}\lambda) + \frac{\sqrt{2}\pi}{4} J_{1/4}(2\sqrt{2}\lambda) J_{-1/4}(2\sqrt{2}\lambda) \right] \quad (1)$$

REDOR data are generated by two experiments, and the results are compared to eq 1 to determine the dipolar coupling. First, a ^{13}C control signal, S , is acquired without application of the deuterium recoupling pulse during the dipolar evolution period. Next, a dipolar dephased ^{13}C signal, S_d , is acquired by applying a ^2H $\pi/2$ dipolar dephasing pulse during the dipolar evolution period.

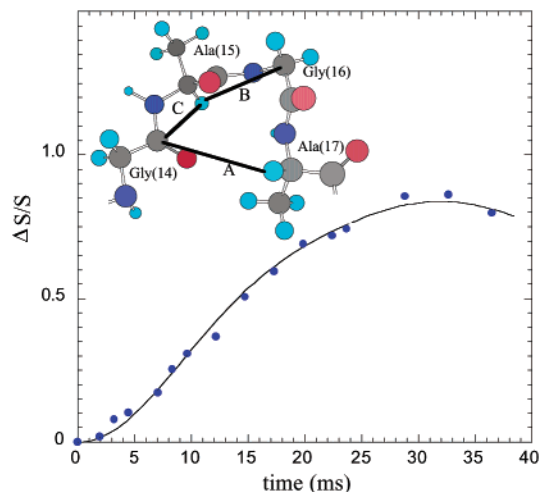


Figure 1. ^{13}C - ^2H REDOR results for **A** with $\rho = 0.856$ and $\alpha = 0.58$. The value of ρ is 0.856 for all samples and was calculated using the ^{13}C and ^2H enrichment factors and natural abundance contributions to the ^{13}C signal. The overall scaling factor $1/\rho\alpha$ was determined by requiring general agreement between data and theory at the $\Delta S/S$ maximum.

The two experiments produce the measured ratio $\Delta S/S|_m = 1 - S_d/S$. However, only a fraction ρ of the total signal contributing to S is specifically from the ^{13}C label. Furthermore, ^1H - ^{13}C cross-polarization enhancement of the ^{13}C signal may be scaled by a factor α for ^{13}C spins in the vicinity of a deuterium. Hence, a simple correction of the data is necessary for direct comparison with eq 1, and the corrected ratio is $\Delta S/S = (1/\rho\alpha)\Delta S/S|_m$.¹⁰

Three ^{13}C , ^2H -labeled (AlaGly)₁₅ samples were synthesized by solid-phase chemistry. The silk I conformation was prepared by dissolving the peptides in 9 M LiBr followed by dialysis against water and drying the precipitate.^{6,7} The ^{13}C chemical shifts are sensitive to conformation, and the chemical shifts of the alanine residues of the prepared peptides matched those of the silk I form of natural *B. mori* silk fibroin. Indicative of the formation of a unique structure is the 1.5 ppm ^{13}C line width of the resonance from the alanine C_β carbons. Sample **A** has the carbonyl carbon of Gly(14) enriched with ^{13}C and a deuterium on the C_α carbon of Ala(17). Sample **B** has the C_α carbon of Gly(16) enriched with ^{13}C and a deuterium attached to the C_α carbon of Ala(15). Sample **C** has the carbonyl carbon of Gly(14) enriched with ^{13}C and a deuterium on the C_α carbon of Ala(15). The distances probed by the three labeled spin pairs are illustrated by the molecular structure shown in Figure 1.

Figure 1 shows ^{13}C - ^2H REDOR data for 90 mg of **A**. The best fit of the data to eq 1 yields $D = 55 \pm 2$ Hz, corresponding to a carbon–deuterium distance of 4.36 ± 0.06 Å. The ^{13}C - ^2H distance would exceed 9 Å for a fully extended peptide. Hence, the relatively

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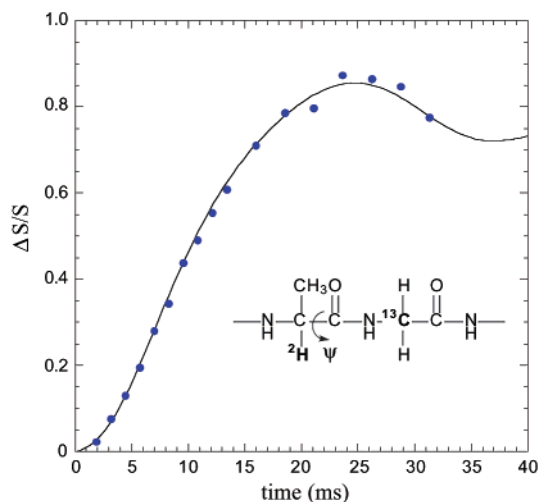


Figure 2. ^{13}C - ^2H REDOR results for **B** with $\rho = 0.856$ and $\alpha = 1$. REDOR experiments were performed on a home-built spectrometer with a ^1H Larmor frequency of 151 MHz. The ^{13}C 180° pulse length is $10\ \mu\text{s}$ and the ^2H 144° pulse length is $7.6\ \mu\text{s}$. The ^1H decoupling field strength, $\gamma B_1/2\pi$, is 110 kHz. The sample spinning period is $320\ \mu\text{s}$.

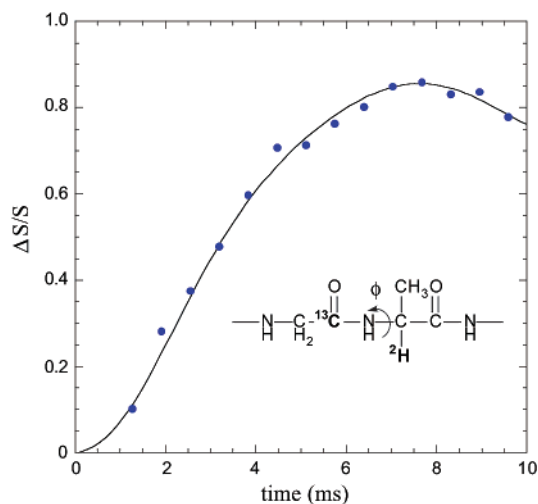


Figure 3. ^{13}C - ^2H REDOR results for **C** with $\rho = 0.856$ and $\alpha = 0.72$.

short distance found between the spin labels on Gly(14) and Ala(17) shows that the peptide has performed a major turn in the region of Gly(14)-Ala(17). This result led us to investigate the nature of the turn via REDOR experiments on **B** and **C** since these experiments provide the dihedral angles of Ala(15).

Figure 2 shows REDOR data for 28 mg of **B** and a structure showing the relative positions of the ^2H label on Ala(15) and the ^{13}C label on Gly(16). The distance between the two spin labels is determined solely by the dihedral angle ψ of Ala(15). The data fit eq 1 with $D = 72 \pm 2\ \text{Hz}$. The ^{13}C - ^2H distance is $3.99 \pm 0.03\ \text{\AA}$, and the possible ψ values consistent with the REDOR-determined distance are 92° and 140° .

Figure 3 shows ^{13}C - ^2H REDOR data for 28 mg of **C** and a structure showing the relative positions of the ^2H label on Ala(15) and the ^{13}C label on Gly(14). The distance between the two spin labels is determined only by the dihedral angle ϕ of Ala(15). The data fit eq 1 with $D = 230 \pm 2\ \text{Hz}$. The corresponding distance between the ^{13}C and ^2H labels is $2.71 \pm 0.01\ \text{\AA}$, and the two

possible ϕ values consistent with this distance are -63° and -172° . However, the $4.36\ \text{\AA}$ distance found for **A** eliminates the ψ value of -172° .

A repeated β -turn model for silk I has been proposed based on ^{13}C - ^{13}C spin-diffusion NMR, ^{13}C - ^{15}N REDOR NMR, and WAXS data.^{6,7,12} The Ala(15) and Gly(16) dihedral angles derived from analysis of those data are $(-60^\circ, 130^\circ)$ and $(70^\circ, 30^\circ)$, respectively. The ^{13}C - ^2H REDOR determined pair of $\phi = -63^\circ$ and $\psi = 140^\circ$ for Ala(15) found in this work supports the proposed model. The strong evidence for a turn provided by **A** and the specific values of the Ala(15) dihedral angles suggest that a type II turn is present in the region Gly(14)-Ala(17) for the silk I mimic. Our REDOR data are consistent with the proposed Gly(16) dihedral angles in the repeated β -turn model.

A significant advantage of using ^2H in the ^{13}C -observe REDOR experiment to determine dihedral angles is that the dipolar coupling of each of the ^{13}C - ^2H spin pairs in **B** and **C** is determined by only one dihedral angle. This is in contrast to ^{13}C - ^{15}N REDOR where there is no ^{13}C - ^{15}N labeling scheme such that the ^{13}C - ^{15}N dipolar coupling depends only on ϕ .¹³ As mentioned above, two values for each dihedral angle are possible for a REDOR-determined distance. For silk peptides, the ^{13}C chemical shifts of the C_α and C_β carbons can help eliminate the ambiguity in dihedral angles.⁶ This work shows the potential of ^{13}C - ^2H REDOR for determining dihedral angles and detecting turns in peptides. Because ^2H labeling of the prevalent proton sites in organic molecules has a rich history, the ^{13}C - ^2H REDOR experiment should be applicable to a wide variety of systems.

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