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Communications to the Editor

$^{13}C-^{17}O$ REAPDOR NMR as a Tool for Determining Secondary Structure in Polyamides

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Secondary structure of polyamides is determined largely by hydrogen bonding between the carbonyl oxygen and the amide proton. Nylon-6, with its α and γ crystalline forms, is the classic example of a synthetic polyamide where hydrogen bonding has a significant influence on structure, and the secondary structure of natural proteins and peptides is very much governed by hydrogen bonding.[1] X-ray crystallography is the primary tool for determining structures in crystalline macromolecules, but not all polyamides can be suitably prepared for X-ray diffraction experiments. For such samples solid-state NMR has been widely used for structural studies, including ^{13}C chemical shift analysis, $^{13}C-^{13}C$ spin-diffusion, and REDOR experiments.[2] Because the carbonyl oxygen serves as the proton acceptor and, consequently, plays such an important role in hydrogen bonding and formation of the secondary structure, we have begun to explore ^{17}O NMR as a structural tool in polyamides. This paper illustrates $^{13}C-^{17}O$ REAPDOR NMR as a structural tool for characterizing polyamides.

A common structural motif for polyamides is the sheet structure, which is stabilized by NH···O=C hydrogen bonds. Typically, the O=C bonds are lateral to the axis of the molecules, lie approximately in the plane of the sheet, and place the oxygen atom between two intrasheet molecules. From a solid-state NMR perspective this makes $^{13}C-^{17}O$ dipolar recoupling experiments desirable because the distance between an ^{17}O spin-label on one molecule and ^{13}C spin-labels on neighboring molecules can be designed by targeted isotopic labeling to be short within the sheet and significantly longer between sheets. Hence, it may be possible to produce a strong intrasheet $^{13}C-^{17}O$ dipolar coupling which would cause the spin system to evolve more or less as an isolated spin pair. Such behavior would lead to simpler data analysis when determining secondary structures. The use of $^{13}C-^{17}O$ REAPDOR NMR for measuring $^{13}C-^{17}O$ intermolecular distances in sheet-forming polyamides is demonstrated.[3] The experiment is illustrated on the tripeptide L-alanyl-alanyl-alanine (AAA). The use of AAA as a test sample is motivated by its simplicity and because it can be easily prepared either as a parallel β-sheet[4] (P-AAA) or as an antiparallel β-sheet[5] (AP-AAA). In addition, our interest in the structure of silk peptides makes AAA a good test system. Even though trialanine is not a silk per se, it is a good test system for demonstrating the application of NMR experiments to silk because some silk proteins, such as that produced by *Samia cynthia ricini* silkworm or spider silks, have poly(L-alanine)-rich regions in their primary sequences.[6]

Table 1. Internuclear Distances and Dipolar Couplings

parallel			antiparallel		
r (Å)	*D* (Hz)	type	*r* (Å)	*D* (Hz)	type
4.54	43.7	intrasheet	7.60	9.33	intrasheet
4.62	41.5	intrasheet	7.62	9.26	intrasheet
7.17	11.1	intersheet	6.35	16.0	intersheet
7.36	10.2	intersheet	6.43	15.4	intersheet
7.40	10.1	intersheet	6.44	15.3	intersheet
7.57	9.42	intersheet	6.57	14.4	intersheet

Samples were made with one part Ala-Ala-[1-^{13}C]Ala and four parts Ala-[^{17}O]Ala-Ala and prepared as P-AAA or AP-AAA by appropriate drying methods. Table 1 provides the two intrasheet and the four shortest intersheet $^{13}C-^{17}O$ distances determined by X-ray diffraction experiments[4,5] and their respective dipole couplings for the two samples. The labeling strategy used in this work was chosen to provide short intrasheet $^{13}C-^{17}O$ distances in the P-AAA sample and very long, >7.60 Å, intrasheet $^{13}C-^{17}O$ distances in the AP-AAA sample. This labeling scheme will illustrate the sensitivity of the $^{13}C-^{17}O$ REAPDOR experiment to sheet structure and placement of nuclear spin-labels. All six $^{13}C-^{17}O$ distances are long in the AP-AAA sample. The P-AAA sample has two short intrasheet distances of 4.54 and 4.62 Å, which are much shorter than the intersheet distances. The two intrasheet distances are slightly different from one another because the sample, as well as the AP-AAA sample, has two nonequivalent A and B molecules per unit cell.[4,5] The $^{13}C-^{17}O$ intrasheet dipolar couplings are much stronger than the intersheet dipolar couplings for the P-AAA sample. However, the $^{13}C-^{17}O$ intersheet dipolar couplings are larger than the intrasheet dipolar couplings in the AP-AAA sample. Figure 1 shows the two intrasheet carbon−oxygen contacts for the two samples.

$^{13}C-^{17}O$ REAPDOR dipolar dephasing experiments were performed with a home-built spectrometer having a proton operating frequency of 151 MHz. The intensity of ^{13}C dipolar dephased signals, S_r, is compared to the intensity of ^{13}C signals obtained in the absence of dipolar dephasing, S, through the ratio $\Delta S/S = 1 - S_r/S$ for various dipolar evolution times, τ. The REAPDOR dipolar dephasing is a measure of the strength of the $^{13}C-^{17}O$ dipolar coupling, D, and isolated spin pairs can be reasonably described up to $D\tau \approx 0.5$ by[2]

$$\Delta S/S^{R} = 0.63(1 - \exp[-(3.0D\tau)^2]) + 0.2(1 - \exp[-(0.7D\tau)^2]) \quad (1)$$

It has been shown elsewhere that fitting data with eq 1 can provide dipolar couplings that yield internuclear distances to

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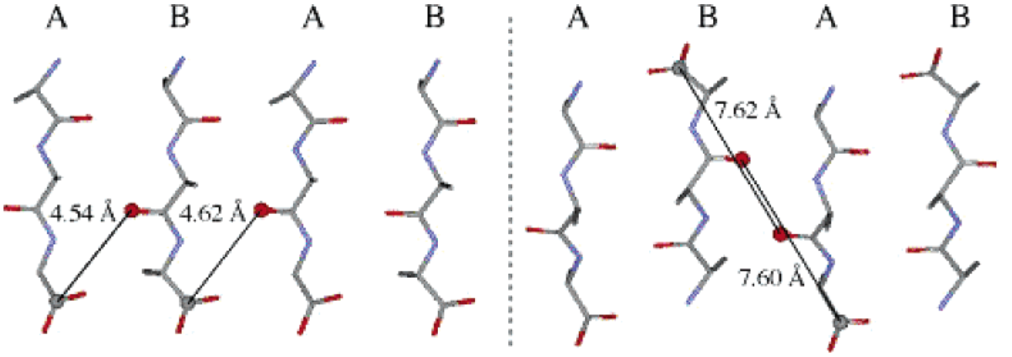

Figure 1. Schematic of intrasheet carbon−oxygen separations between ^{17}O- and ^{13}C-labeled sites for the P-AAA (left) and AP-AAA (right) samples.

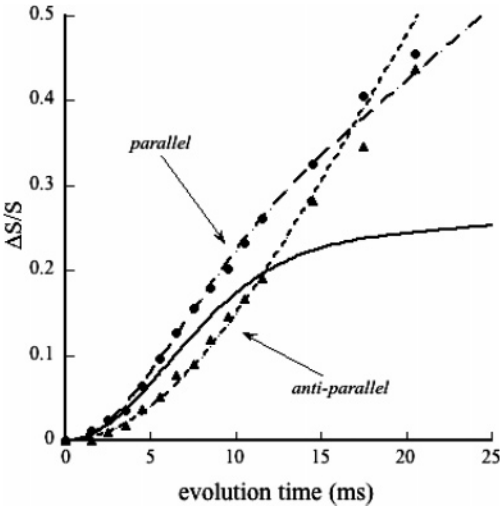

Figure 2. ^{13}C−^{17}O REAPDOR results. The circles and triangles are experimental data for the P-AAA and AP-AAA samples, respectively. Experiments were performed with a spinning rate of 4 kHz (stabilized to 0.2 Hz), 10 μs ^{13}C π pulses, proton decoupling rf field strength of 120 kHz, and an 83 μs, 60 kHz rf field strength ^{17}O adiabatic passage pulse. Solid line: $\Delta S/S = 0.36 \times \Delta S/S^{R}(38.7\ \text{Hz})$. Dotted line: $\Delta S/S = \Delta S/S^{G}(6.4\ \text{Hz})$. Dashed line: $\Delta S/S = 0.36 \times \Delta S/S^{R}(38.7\ \text{Hz}) + 0.64 \times \Delta S/S^{G}(4.56\ \text{Hz})$.

within 4% of their true value.[2] Measured $\Delta S/S$ ratios are shown for several dipolar evolution times in Figure 2 for the two samples. Inspection of Figure 2 shows the P-AAA ^{13}C signal undergoes the stronger dipolar dephasing, especially at the shorter evolution times. This occurs because of the significantly shorter ^{13}C−^{17}O distances in this sample, which results in significantly larger dipolar couplings. A given ^{13}C spin-label in the P-AAA sample has 0.36 probability of having either a 4.54 or 4.62 Å ^{13}C−^{17}O distance (a particular ^{13}C spin-label cannot be simultaneously coupled to two ^{17}O spins having the two shortest distances) and additional couplings to ^{17}O nuclei located over 7 Å away. Because the shorter distances correspond to dipolar couplings that are ~4 times stronger than the >7 Å distances, the ^{13}C dipolar dephasing behavior will be governed by the stronger dipolar couplings at the shorter dipolar evolution times. The solid line is a ^{13}C−^{17}O REAPDOR calculation generated from eq 1 with a 4.72 Å ($D = 38.7$ Hz) ^{13}C−^{17}O internuclear distance (within 4% of either of the two short distances) and weighted by 0.36. The solid-line simulation and data are in good agreement at short evolution times, showing that it is possible to measure the short intrasheet ^{13}C−^{17}O contacts. Data at longer evolution times begin to deviate from the solid line primarily because the solid-line simulation neglects the significant fraction (0.64) of ^{13}C spins that are coupled only to multiple ^{17}O spins located >7 Å away for this sample. The dipolar dephasing for ^{13}C nuclei coupled to many ^{17}O spins can be accounted using a moment analysis approach. Accordingly, an additional effective background dipolar coupling, D_e, of 4.56 Hz applied in the Gaussian form $\Delta S/S^{G} = 1 - \exp(-(2\pi D_e \tau)^2)$ with a weighting of 0.64 produces a reasonable fit (dashed line) for all the P-AAA data. These P-AAA results show that the REAPDOR experiment can detect and measure ^{13}C−^{17}O distances in labeled peptides.

The AP-AAA sample has no short ^{13}C−^{17}O contacts with the labeling scheme used in this work. Any particular ^{13}C spin-label is basically surrounded by many ^{17}O nuclei located over 6 Å away. The REAPDOR ^{13}C dipolar dephasing for this sample is weaker than that found for the P-AAA sample and can be simulated using $D_e = 6.4$ Hz, (dotted line). Of course, a different ^{13}C−^{17}O labeling scheme could have provided close ^{13}C−^{17}O contacts for this sample if desired.

This work shows the potential that ^{13}C−^{17}O REAPDOR has for providing structural information in polyamide sheet structures. Other heteronuclear solid-state NMR dipolar recoupling experiments using different spin-labels have been used to measure distances in polyamides, especially in peptides and proteins. For example, Schmidt-Rohr and Hong recently developed a REDOR experiment to specifically probe hydrogen bonding.[8] The experiment measures the ^{1}H−^{13}C dipolar interaction between the ^{13}C at the carbonyl position and amide proton on a neighboring molecule. Because of the large magnetogyric ratio of the proton, the corresponding ^{1}H−^{13}C dipolar coupling is large. Detection of the dipolar dephasing takes place by transferring the proton magnetization to the amide ^{15}N and acquiring the ^{15}N signal. For AAA, the shortest carbonyl carbon to amide proton intermolecular distance is ~3.40 Å. Since the carbonyl oxygen is oriented toward the amide group of neighboring molecules in AAA, the carbonyl oxygen to amide proton intermolecular distance is noticeably shorter and is ~2.25 Å. Hence, the ^{1}H−^{13}C dipolar coupling is 770 Hz and the ^{1}H−^{17}O dipolar coupling is 1430 Hz. Figure 3 compares simulations of the ^{1}H−^{17}O REAPDOR and ^{1}H−^{13}C REDOR experiments for the conditions expected for AAA. It is clear that the REAPDOR experiment undergoes stronger dipolar dephasing at much shorter evolution times, which is advantageous. Aside from the stronger ^{1}H−^{17}O dipolar coupling, there is another reason that the REAPDOR experiment dephases so much stronger than the corresponding REDOR experiment. The changes in quantum number, m, in REDOR is 1. In REAPDOR, the changes in m can be 1 and higher. Larger changes in m cause stronger dipolar dephasing.

This work shows that the ^{17}O isotope can be used in dipolar recoupling NMR experiments to obtain secondary structure in polyamides. Such experiments are useful because of the importance of oxygen in hydrogen bonding.

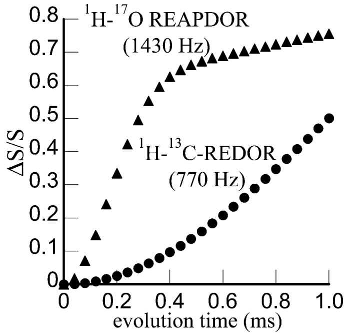

Figure 3. Comparison of ^{1}H−^{13}C REDOR (circles) and ^{1}H−^{17}O REAPDOR (triangles). The dipolar couplings used in the simulations do not take into account any scaling factor that arises from using multiple-pulse ^{1}H sequences such as MREV-8.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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