Communication

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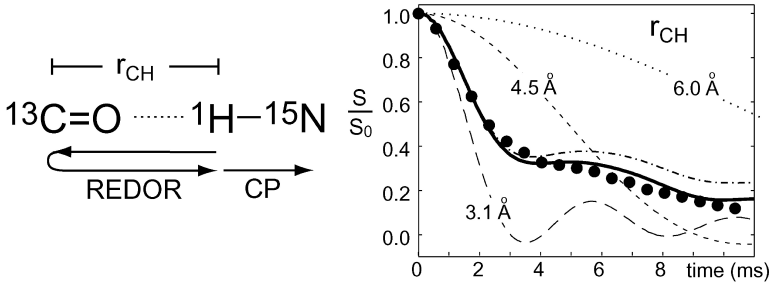

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Measurements of Carbon to Amide-Proton Distances by C−H Dipolar Recoupling with ^{15}N NMR Detection

Klaus Schmidt-Rohr and Mei Hong*

Department of Chemistry, Iowa State University, Ames, Iowa 50011

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Internuclear distance measurements are an important aspect of solid-state nuclear magnetic resonance (NMR) structure determination of biomolecules. The most frequently used method for determining heteronuclear distances is rotational-echo double resonance (REDOR), where rotor-synchronized 180° pulses recouple the heteronuclear dipolar interaction under magic-angle spinning (MAS).[1−5] In proteins, ^{13}C−^{15}N internuclear distances are usually measured using specifically ^{13}C,^{15}N-labeled samples.[6,7] However, the relatively small magnetic dipole moment of ^{15}N limits the maximum distance detectable.

In this Communication, we demonstrate how additional important internuclear distances, between the ^{13}C and the amide proton (H^N) bonded to the amide ^{15}N, can be determined up to at least 6 Å. The ^{13}C−H^N distance is particularly important for defining the hydrogen bonding geometry between ^{13}C═O and H^N−^{15}N groups. Because of the 10-fold difference between the ^{1}H and ^{15}N magnetic dipole moments and the multiple-pulse scaling factor of about 0.5, the effective ^{13}C−^{1}H dipolar interaction in our technique is 5-fold stronger than the ^{13}C−^{15}N dipolar coupling for the same internuclear distance. In addition, in hydrogen bonded ^{13}C═O···H−^{15}N systems, the relevant internuclear ^{13}C−$^1H^N$ distance is typically reduced by ∼1 Å as compared to the ^{13}C−^{15}N distance.[8,9] Thus, the effective ^{13}C−$^1H^N$ coupling will be about 10 times stronger than the corresponding ^{13}C−^{15}N interaction. This large coupling strength permits distances up to at least 6 Å to be determined reliably.

Figure 1 shows the pulse sequence for this ^{15}N-detected C−H REDOR experiment. As in the related medium- and long-distance (MELODI) heteronuclear correlation technique,[10,11] the magnetization of each proton initially evolves in the dipolar field of the ^{13}C spin. The ^{1}H homonuclear couplings are suppressed by a multiple-pulse decoupling sequence. As indicated in Figure 1a, we used MREV-8[12] without special tune-up and found it to be highly effective for amide protons bonded to ^{15}N nuclei, yielding ^{1}H T_2 relaxation times of up to 5 ms. Because undisturbed MAS averages out the C−H dipolar interaction, two ^{13}C 180° pulses per rotation period are applied to recouple the C−H dipolar interaction, as is common in REDOR experiments. The ^{1}H isotropic chemical shift is refocused by a ^{1}H 180° pulse in the center of this C−H dephasing period. The magnetization of each proton is modulated independently by its coupling to the ^{13}C nucleus, resulting in simple spin-pair REDOR curves that depend only on the C−H internuclear distance. To selectively detect the modulation of the H^N magnetization, a short Lee−Goldburg cross polarization[13−15] from ^{1}H to ^{15}N is applied before ^{15}N detection. The dephased signal S is recorded as a function of the C−H dephasing time t_{CH}. The reference signal S_0, without the C−H dipolar dephasing but otherwise with identical relaxation behavior, is obtained by switching off the ^{13}C 180° recoupling pulses. The normalized dephasing S/S_0, plotted as a function of t_{CH}, depends exclusively on the C−H^N distance.

When the ^{13}C−$^1H^N$ distance of interest is large, dephasing of the H^N proton by nearby natural-abundance ^{13}C (1.1%) produces

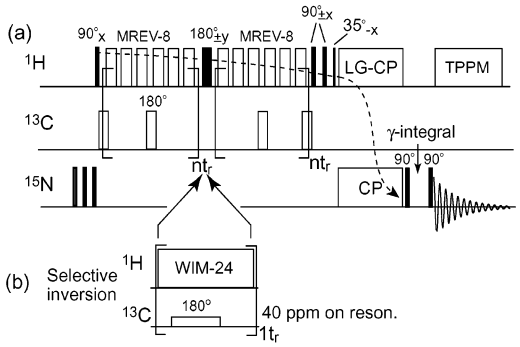

Figure 1. Pulse sequence for ^{15}N-detected ^{13}C−^{1}H REDOR NMR. (a) Basic sequence. ^{1}H magnetization evolves under the influence the recoupled ^{13}C−^{1}H dipolar coupling, while homonuclear couplings are removed by multiple-pulse irradiation. We used six cycles of MREV-8 per rotor period, 3.4 μs 90° ^{1}H pulses, and a cycle time of 48 μs. Each ^{13}C 180° pulse is simultaneous with a long window of MREV-8 and the following ^{1}H pulse. A short (50−100 μs) Lee−Goldburg CP from H^N to ^{15}N and a z-filter incremented in two steps of $\tau_r/2$ (γ-integral[16]) were applied before ^{15}N detection. (b) Selective inversion scheme to reduce dephasing by natural-abundance ^{13}C sites. ^{1}H evolution during the soft ^{13}C pulse is minimized by the WIM-24 time-suspension sequence. In ^{13}Cα,^{15}N-labeled *N*-*t*-BOC-glycine, the inversion pulse reduced aliphatic-carbon-induced dephasing of H^N protons by a factor of 5.

detectable dephasing. For instance, a reduction of $\Delta S/S_0$ by 15% is expected and observed (not shown) after 6 ms of MREV-8 decoupling in a typical ^{15}N-labeled peptide. For measurements of ^{13}CO−H^N distances, this effect can be minimized by adding a selective-pulse scheme,[7] as indicated in Figure 1b. The natural-abundance aliphatic-carbon coherence can be inverted by a soft on-resonance 180° pulse and thus does not dephase the protons, while the ^{13}C═O signal remains essentially unaffected. We applied a 73 μs 180° pulse, corresponding to a field strength of 6.8 kHz, at the 40 ppm ^{13}C frequency. This inverts the aliphatic carbons over a total range of ca. ±30 ppm. The ^{13}CO coherence nutates around an effective field that makes an angle of 27° with the *z*-axis. Its isotropic-shift component undergoes a 390° rotation and thus returns mostly to the *z*-axis, without having had more than a minor transverse component that could be affected by CSA evolution. To prevent ^{1}H evolution during the ^{13}C pulse, a time-suspension sequence with vanishing average Hamiltonian, for example, WIM-24,[17] is applied to the ^{1}H spins. The rotor synchronization of REDOR requires this time to be one rotation period, even if the ^{13}C pulse is shorter.

Figure 2 shows experimental ^{15}N-detected REDOR dephasing of the H^N magnetization by the ^{13}Cα spin in ^{13}Cα,^{15}N-labeled *N*-*t*-BOC-glycine. The data were collected without the selective inversion pulse (Figure 1a) because the coupling of interest is strong. The two-bond Cα−H^N distance in this compound is 2.18 Å. The curve simulated using this distance and the ideal MREV-8 scaling factor of 0.47 agrees well with the experimental data (●). The

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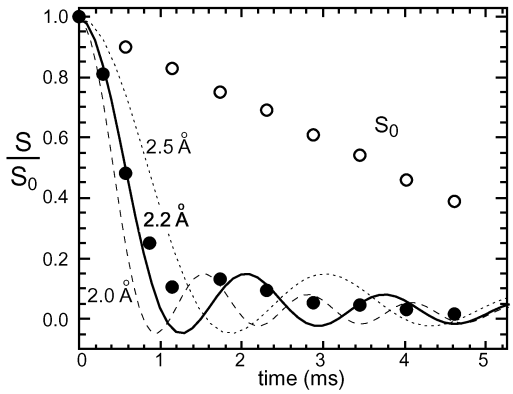

Figure 2. $^{13}C-H^N$ distance measurements in $^{13}C\alpha,^{15}N$-labeled *N-t*-BOC-glycine by ^{15}N-detected $^{13}C-^1H$ REDOR (with 50 μs $^1H-^{15}N$ CP). The normalized C−H dephasing (●) is recorded as a function of the C−H REDOR time using the sequence of Figure 1a. Also shown are calculated REDOR curves for three C−H distances, including the best-fit distance of 2.2 Å. The ○ symbols show the T_2 decay of the reference intensity S_0, which should be as slow as possible to maximize the sensitivity of the experiment. The experiments were performed using a Bruker DSX-400 spectrometer and a triple-resonance probe at a spinning speed of 3.47 kHz for the 4 mm rotor. The ^1H multiple-pulse decoupling works best in this slow-spinning regime. For selectively labeled samples, faster spinning does not provide any significant enhancement in resolution or sensitivity.

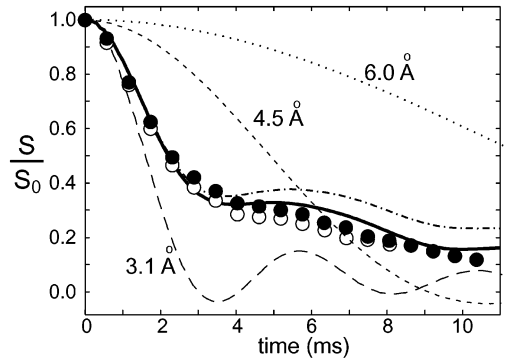

Figure 3. Intermolecular $^{13}CO-H^N$ distances in a 50:50 mixture of ^{13}-COO-labeled and ^{15}N-labeled *N-t*-BOC-glycine measured by ^{15}N-detected $^{13}C-^1H$ REDOR (with 100 μs $^1H-^{15}N$ CP). ○ (to 8 ms): S/S_0 obtained using the pulse sequence of Figure 1a. ●: S/S_0 obtained including the selective inversion of Figure 1b. The undesirable dephasing by natural-abundance aliphatic ^{13}C sites has been reduced. Thick line: parameter-free fit based on the crystal structure of *N-t*-BOC-glycine and statistical labeling. For simplicity, the minor effects of two-carbon dephasing of H^N were treated using a spin-pair approximation. Other curves indicate the REDOR curves for single C−H distances of 3.1 Å (− − −), 4.5 Å (- - -), and 6.0 Å (• • •). The dash−dotted curve indicates how a simulation omitting a 4.5 Å intermolecular distance that occurs with only 12.5% probability would lead to a clear discrepancy with the experimental data.

reference intensity S_0 (○) decreases with a relatively long T_2 time of 5 ms, indicating efficient homonuclear decoupling of the amide proton.

The ability of this method to reliably detect much longer, intermolecular $^{13}CO-H^N$ distances is demonstrated in Figure 3. In a 50:50 mixture of ^{13}COO-labeled and ^{15}N-labeled *N-t*-BOC-glycine, the contacts between the ^{13}COO and $H^N-^{15}N$ moieties are all intermolecular. Figure 3 shows the ^{15}N-detected C−H REDOR data acquired without (○) and with (●) the desirable selective inversion of natural-abundance aliphatic ^{13}C. The thick line is the calculated dephasing curve based on the crystal structure and the 50% ^{13}COO labeling. This simulation fits the experimental data quite well without any adjustable parameters. The crystal structure shows the closest ^{13}COO and H^N contacts of 2.85 and 3.25 Å for the two molecules in the asymmetric unit cell.[18] These are indicative of hydrogen bonding. The fast initial decay results from these nearest-neighbor distances and exhibits the characteristic oscillation found in a simulation for a 3.1 Å distance (− − −). Because of the statistical labeling, 50% of all ^{15}N have the nearest ^{13}COO neighbors at longer distances, causing slower decay at longer times. If a 4.5 Å intermolecular distance that occurs with only 12.5% probability is omitted from the simulation (dash−dotted line), then a clear discrepancy with the experimental data is observed. This indicates that long C−H distances are accurately detected by this technique. Finally, the calculated C−H REDOR curves for 4.5 Å (- - -) and 6.0 Å (• • •) show that significant dephasing can be readily detected even for these long distances.

The ^{15}N-detected C−H REDOR experiment introduced here should be applied routinely whenever $^{13}C-^{15}N$ REDOR is used to characterize a biomolecular structure. It promises to provide particularly useful information on $CO-H^N$ hydrogen bonding. The new approach can be further developed in many ways, such as incorporating ^1H or ^{13}C chemical shift evolution, or using ^{15}N-induced dephasing and ^{13}C detection in uniformly labeled peptides and proteins.

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