Measurement of ^{13}C−^{15}N Distances in Uniformly ^{13}C Labeled Biomolecules: *J*-Decoupled REDOR

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Measurement of distances between pairs of heteronuclei is important for constraining the conformation of biomolecules in the solid state. In particular, rotational-echo double-resonance (REDOR)[1,2] has been extensively applied to problems of biological interest.[3−7] Most of these experiments have been performed on isolated heteronuclear spin pairs, where the interpretation of experimental results is relatively straightforward. It is preferable to perform REDOR in multispin systems, because of the possibility of measuring several distances in a single sample. However, in REDOR experiments on multiply ^{13}C labeled samples the dephasing profiles are complicated by additional interactions. While placing all REDOR dephasing pulses on the ^{15}N channel avoids the recoupling of ^{13}C spins by the π-pulse train,[8] the homonuclear ^{13}C−^{13}C dipole and *J* couplings can still contribute to the dephasing of ^{13}C coherences. Here we discuss an approach to ^{13}C observe REDOR, which relies on the selective excitation of the ^{13}C spectrum that removes the coherent evolution of the spin system under homonuclear ^{13}C−^{13}C *J* couplings.

Several recently proposed techniques address the problem of heteronuclear distance measurements in spin systems consisting of multiple ^{13}C and ^{15}N nuclei.[9−11] The multiple pulse decoupled REDOR sequence[11] was designed to attenuate the effects of residual homonuclear ^{13}C−^{13}C dipole couplings on the dephasing of ^{13}C coherences. However, it does not explicitly account for ^{13}C−^{13}C *J* couplings, which compromise the accurate measurement of weak heteronuclear dipole couplings. In peptides, *J* couplings between directly bonded ^{13}C nuclei are ∼30−60 Hz, and the most informative ^{13}C−^{15}N dipolar couplings are often of similar magnitude (e.g., 25 Hz for a 5.0 Å C−N distance).

For a spin system consisting of *n* ^{13}C nuclei and a single ^{15}N spin, the effective Hamiltonian for the REDOR pulse sequence (Figure 1a) in the rapid spinning regime is:

$$H = \sum_{i=1}^{n} \Phi_{C_iN} 2C_{iz}N_z + \sum_{i<j} \pi J_{C_iC_j} 2C_{iz}C_{jz} \qquad (1)$$

Here $J_{C_iC_j}$ is the C_i−C_j scalar coupling constant, $\Phi_{C_iN} = -(\sqrt{2}/\pi)b_{C_iN}\sin(2\beta)\sin(\gamma)$ is the effective C_i−N dipolar coupling, with the coupling constant $b_{C_iN} \propto r_{C_iN}^{-3}$ (r_{C_iN} is the internuclear distance), and the Euler angles β and γ relate the principal axis system of the interaction to the rotor-fixed reference frame. Equation 1 assumes that (i) *J* couplings can be treated in the weak coupling limit[12] and (ii) coherent evolution of ^{13}C signals under ∼2 kHz dipolar couplings between directly bonded ^{13}C nuclei is refocused by rapid spinning ($\omega_r/2\pi \sim 10$ kHz) for integer multiples of the rotor period. For a coupled three-spin system (C$_1$−C$_2$−N) the initial density operator $\rho(0) = C_{1x} + C_{2x}$ evolves under the effective Hamiltonian into observable coherences $C_{1x}\cos(\Phi_{C_1N}\tau)\cos(\pi J_{C_1C_2}\tau)$ and $C_{2x}\cos(\Phi_{C_2N}\tau)\cos(\pi J_{C_1C_2}\tau)$, and antiphase coherences $2C_{1y}C_{2z}\cos(\Phi_{C_1N}\tau)\sin(\pi J_{C_1C_2}\tau)$ and $2C_{1z}C_{2y}\cos(\Phi_{C_2N}\tau)\sin(\pi J_{C_1C_2}\tau)$. The antiphase coherences can evolve into observable magnetization under $J_{C_1C_2}$ during the detection period, leading to phase-twisted spectra (Figure 2a). This problem can be overcome by filtering the coherence prior to detection (see Figure 1 caption). Figure 2a shows slices from spin−echo experiments for [1,2-^{13}C,^{15}N]glycine for 8.4 ms of J_{CC} evolution acquired with and without the coherence filter. The spectrum obtained with a simple spin−echo experiment displays phase-twisted line shapes. In contrast, the spin−echo experiment followed by the coherence filter results in purely absorptive signals.

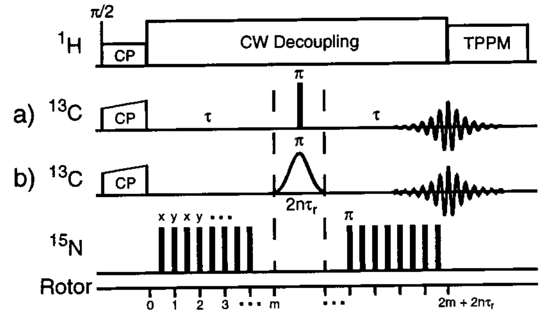

Figure 1. Pulse sequences for conventional REDOR (a) and *J*-decoupled REDOR (b). The ^{15}N π pulse length was 10 μs and the pulses were phased according to the *xy*-16 scheme.[18] For the sequence in part a, the ^{13}C π pulse length was 10 μs, and for experiments on uniformly ^{13}C labeled samples, the coherence filter ($\pi/2$)−*z*-filter−($\pi/2$) was inserted prior to signal acquisition. For *J*-decoupled REDOR (b) the Gaussian π pulse parameters were $\tau_{Gauss} = 0.6$ ms, 64 increments, and 5% truncation. CW ^{1}H decoupling at 100 kHz was applied during the evolution period, and 83 kHz TPPM[16] was used during acquisition. The phase cycle used, $\phi_{Gauss} = xy\bar{x}\bar{y}$ and $\phi_{rec'r} = x\bar{x}x\bar{x}$, ensures that the only ^{13}C spins contributing to the observable signal are those inverted by the selective pulse.

The problem of coherent evolution under ^{13}C−^{13}C *J* couplings during dipolar dephasing (*S*) and reference (S_0) experiments is addressed by replacing the hard π pulse with a rotor-synchronized, frequency-selective Gaussian π pulse applied to one ^{13}C spin (Figure 1b). For all evolution times the dipole interaction between this ^{13}C and the ^{15}N spin is retained as in conventional REDOR, while the *J* couplings to the remaining ^{13}C nuclei are refocused (the signs of all spin terms having the form $2C_{iz}C_{jz}$; $j = 1, 2, ..., n \neq i$, are reversed following the selective inversion of the C_i spin). This type of homonuclear *J*-decoupling was used previously to enhance resolution in two-dimensional solution[13] and solid-state[14] spectra. With the assumption that the selective pulse on

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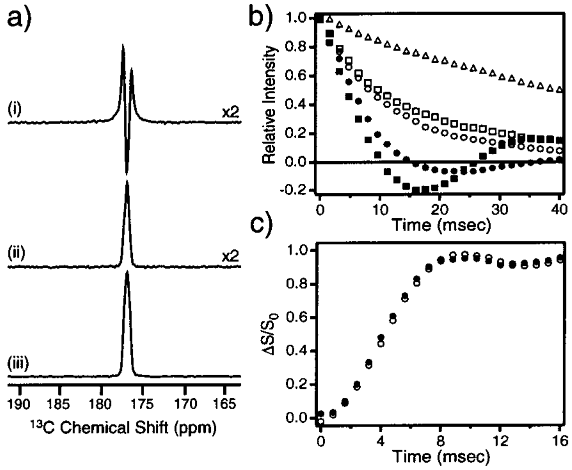

Figure 2. Comparison of conventional REDOR and *J*-decoupled REDOR for samples with single and multiple ^{13}C labels. (a) Slices through S_0 curves acquired for [1,2-^{13}C,^{15}N]glycine (only C′ resonance is shown) with the following pulse sequences on ^{13}C: (i) CP−τ−π−τ−acquire; (ii) CP−τ−π−τ−coherence filter−acquire; (iii) CP−τ−π (Gauss)−τ−acquire; $\tau = 4.2$ ms. (b) S_0 curves for the C′ resonance in [1,2-^{13}C,^{15}N]-glycine (■ = conventional REDOR; □ = *J*-decoupled REDOR) and the C$^{\gamma}$ resonance in [U-^{13}C,^{15}N]threonine (● = conventional REDOR; ○ = *J*-decoupled REDOR). Also shown is the S_0 curve for [1-^{13}C,^{15}N]glycine (△). (c) $\Delta S/S_0$ curves obtained with conventional REDOR for C′ resonance in [1-^{13}C,^{15}N]glycine (○) and *J*-decoupled REDOR in [1,2-^{13}C,^{15}N]glycine (●). All experiments were performed at 500 MHz ^{1}H frequency and $\omega_r/2\pi = 10.0$ kHz (±5 Hz).

C_i can be treated as an ideal pulse, the effective Hamiltonian for the pulse sequence in Figure 1b is given by the two-spin REDOR Hamiltonian, $H = \Phi_{C_iN} 2C_{iz}N_z$, and the observable signal averaged over the crystallite ensemble is then $s(\tau) = \int\int d\beta \sin(\beta) d\gamma \cos(\Phi_{C_iN}\tau)$.[2] Since the selective pulse removes all ^{13}C−^{13}C *J* couplings to the spin of interest, the coherence filter is not necessary to obtain in-phase spectra (Figure 2a). Although the experimental Gaussian pulse is not ideal, it is a *constant-time* element present in all *S* and S_0 experiments and any effects due to the pulse can be taken into account by calculating $(S_0 - S)/S_0 = \Delta S/S_0$ curves.[2] For [1-^{13}C,^{15}N]glycine at $\omega_r/2\pi = 10.0$ kHz and 500 MHz ^{1}H frequency (data not shown), *S* and S_0 curves experience only an overall scaling (∼25% loss in signal intensity) for a 0.6 ms Gaussian pulse relative to a hard pulse.

Figure 2b compares S_0 curves for [1,2-^{13}C,^{15}N]glycine and [U-^{13}C,^{15}N]threonine obtained with conventional and *J*-decoupled REDOR. The selective pulse applied to the C′ and C$^{\gamma}$ spins in glycine and threonine, respectively, removes the coherent evolution due to ∼50 Hz C′−C$^{\alpha}$ and ∼30 Hz C$^{\beta}$−C$^{\gamma}$ *J* couplings, resulting in S_0 curves of positive intensity for all evolution times. For comparison with a spin-pair sample, the S_0 curve for [1-^{13}C,^{15}N]glycine is also included. Although the selective pulse refocuses the ^{13}C−^{13}C *J* couplings, residual ^{13}C−^{13}C dipole interactions may still be present at 10 kHz spinning, and the 100 kHz ^{1}H CW decoupling used here is not sufficient to provide identical dephasing profiles for the isolated C′ spin and the C′ with residual dipole coupling to the C$^{\alpha}$ spin.[15] Figure 2c shows that matching $\Delta S/S_0$ curves are obtained for the C′−N coupling in [1-^{13}C,^{15}N]- and [1,2-^{13}C,^{15}N]glycine with conventional and *J*-decoupled REDOR sequences, respectively.

In principle, the $\Delta S/S_0$ analysis for conventional REDOR accounts for the evolution under ^{13}C−^{13}C *J* couplings. However, for multiply ^{13}C labeled systems the quality of experimental data

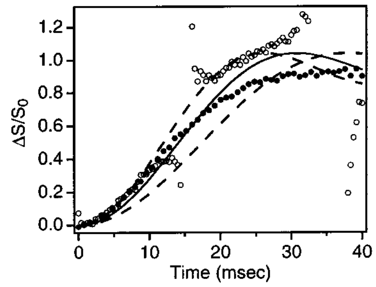

Figure 3. $\Delta S/S_0$ curves for the C$^{\gamma}$ resonance in [U-^{13}C,^{15}N]threonine obtained with conventional REDOR (○) and *J*-decoupled REDOR (●). $\Delta S/S_0$ curve (—) simulated according to the analytical expression (see text) for the C$^{\gamma}$−N coupling of 54 Hz obtained from the neutron diffraction structure and curves (---) for 44 and 64 Hz couplings are shown for comparison.

is compromised due to multiple zero-crossings and low signal intensities in *S* and S_0 curves. This is demonstrated in Figure 3 for the case of ∼50 Hz C$^{\gamma}$−N dipole coupling in [U-^{13}C,^{15}N]-threonine, where $\Delta S/S_0$ curves obtained with conventional and *J*-decoupled REDOR are compared. Accurate determination of weak couplings requires evolution times on the order of 30 ms.[11] However in the presence of homonuclear *J* couplings, conventional REDOR $\Delta S/S_0$ curves can account for *J* coupling effects only up to ∼10 ms (depending on the exact value of J_{CC}). On the other hand, *J*-decoupled REDOR has the ability to provide useful experimental data for the entire evolution period because the S_0 curve has a simple exponential decay profile. As a result, the quality of the data appears to be mainly limited by residual ^{13}C−^{13}C dipole couplings, insufficient ^{1}H decoupling, and ^{15}N pulse imperfections. The simulated $\Delta S/S_0$ curve for the neutron diffraction[17] C$^{\gamma}$−N coupling of 54 Hz and curves for 44 and 64 Hz dipole couplings are included for comparison. Reasonably good agreement between the 54 Hz simulation and experiment is obtained up to ∼20 ms, and it is evident that experimental data in the 10−20 ms range are important for the accurate determination of weak dipole couplings. For longer evolution times the observed dipolar dephasing is less than that predicted by the simulation, possibly due to the problems noted above.

In summary, we have described a REDOR experiment, which refocuses ^{13}C−^{13}C *J* couplings and enables accurate distance measurements in spin systems consisting of a heteronucleus interacting with a tightly coupled cluster of ^{13}C spins. The technique is expected to be particularly useful for weak ^{13}C−^{15}N dipolar couplings, because in the presence of ^{13}C−^{13}C *J* couplings the REDOR experiment designed for spin pairs does not provide reliable data for evolution times greater than ∼10 ms.

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