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Communication

Rotational-echo double resonance of uniformly labeled ^{13}C clusters

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Abstract

The use of rotational-echo double resonance NMR to measure distances from an observed tightly coupled cluster of ^{13}C spins to a distant ^{15}N, ^{31}P, or ^{19}F is practical if ^{13}C chemical shifts and homonuclear ^{13}C–^{13}C isotropic J interactions are refocused by a combination of rotor-synchronized ^{13}C π and $\pi/2$ pulses. This scheme is illustrated by experiments performed on diluted and recrystallized L-[$^{13}C_3$,^{15}N]alanine and L-[$^{13}C_6$,α-^{15}N]histidine.

1. Introduction

The information content of rotational-echo double resonance (REDOR) experiments could be improved if more than just the two spins of an isolated pair were involved. Clusters of ^{13}C labels coupled to some heterospin are of particular interest. Unfortunately, the use of REDOR to measure distances from an *observed* tightly coupled cluster of two or more ^{13}C labels (in a uniformly labeled residue of a protein, for example) to a distant ^{15}N, ^{31}P, or ^{19}F is compromised by homonuclear dephasing of the ^{13}C echo train. Long-range distance determinations by REDOR require that rotational echo trains persist for times of the order of 30 ms [1]. Even though the 5–10 kHz magic-angle spinning speeds most commonly used in solid-state NMR experiments essentially eliminates the homonuclear dephasing effects of the 2-kHz dipolar coupling between directly bonded ^{13}C–^{13}C pairs, spinning has no effect on ^{13}C–^{13}C isotropic J couplings, which are typically 50–100 Hz for directly bonded carbons and as much as 10 Hz for carbons separated by two bonds [2]. The result is that ^{13}C echo trains for coupled carbons are attenuated and lose phase coherence after about 15 ms (Fig. 1).

One approach to eliminate the effect of homonuclear ^{13}C isotropic J coupling, at least in selected regions of the spectrum, is to apply a tailored, frequency-selective ^{13}C π pulse to a single ^{13}C spin [3,4]. The selected spin then exhibits normal heteronuclear coupling to a distant ^{15}N, for example, while all other ^{15}N–^{13}C, and all ^{13}C–^{13}C isotropic J couplings involving the selected spin, are suppressed. This approach has been used successfully to measure selected ^{15}N–^{13}C couplings within ^{13}C clusters in both peptides [4] and proteins [5]. Frequency selectivity can also be achieved for the full ^{13}C spectrum using ^{13}C–^{15}N coherence transfers and J-filtering in two-dimensional versions of the experiment [6].

A second approach is to combine ^{13}C π pulses (to refocus chemical shifts in Hahn echoes) with ^{13}C $\pi/2$ pulses (to refocus J couplings in solid echoes). For an isolated ^{13}C–^{13}C pair, ^{13}C π pulses at T_r and $3T_r$, together with a $\pi/2$ pulse at $2T_r$, result in full refocusing at $4T_r$. For more than two coupled spins, however, the refocusing is incomplete. Nevertheless, the notion of combining Hahn echoes and solid echoes can be retained using just slightly more complicated pulse sequences, one of which is shown in Fig. 2. This sequence (called RDX for REDOR of $^{13}C_x$) has four π pulses and four $\pi/2$ pulses in $8T_r$. (The π pulse centered in the last two rotor cycles produces a Hahn echo that avoids beginning data acquisition coincident with a pulse; this pulse is not an integral part of the RDX sequence.) Reduction of the number of $\pi/2$ pulses to 2–3 resulted in significantly less refocusing.

Under the RDX pulse sequence, the difference and full-echo $^{13}C\{^{15}N\}$ spectra of L-[$^{13}C_6$,α-^{15}N]histidine (Isotec, Miamisburg, OH) are all in phase (Fig. 3). The labeled histidine was diluted ten-fold by natural abun-

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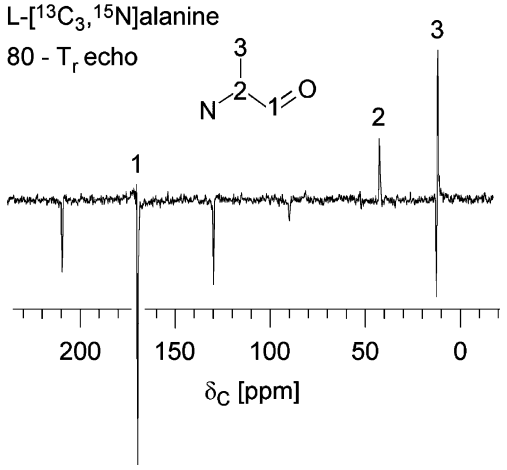

Fig. 1. Rotor-synchronized, 80-T_r Hahn echo for L-[$^{13}C_3$,^{15}N]alanine diluted and recrystallized with natural-abundance alanine. The ^{13}C–^{13}C isotropic J couplings for alanine are $^1J_{12} = 54$ Hz, $^1J_{23} = 35$ Hz, and $^2J_{13} = 2$ Hz. Magic-angle spinning was at 9091 Hz.

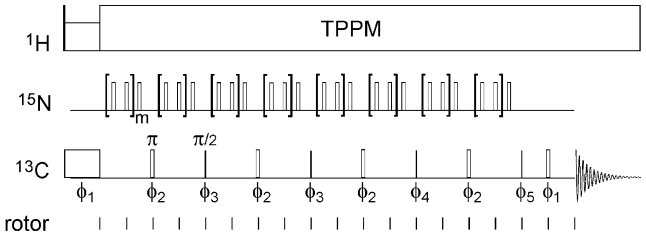

Fig. 2. Pulse sequence for $^{13}C\{^{15}N\}$ REDOR of ^{13}C clusters (RDX) using 8 ^{13}C π and $\pi/2$ refocusing pulses. The last ^{13}C π pulse results in a Hahn echo after two rotor cycles and is not part of the RDX sequence proper. All dephasing pulses are on the ^{15}N channel with $m = 1, 2, 3, \ldots$ corresponding to dipolar evolution for $16T_r$, $24T_r$, $32T_r \ldots$, respectively. The phase cycling for ^{15}N is $xy{-}8$. The phase cycling for ^{13}C has a 16-scan period: $\varphi_1 = (x,y,x,y)_4$; $\varphi_2 = (x,y,-x,-y)_4$; $\varphi_3 = (x,y,-x,-y,-x,-y,x,y)_2$; $\varphi_4 = (-x,-y,x,y,x,y,-x,-y)_2$; $\varphi_5 = (x,y,-x,-y,-x,-y,x,y,-x,-y,x,y,x,y,-x,-y)$.

dance material and recrystallized in the free base form from water. Anti-phase components that evolve due to homonuclear scalar couplings and are not completely refocused are small for RDX [7], and with the sort of line widths typical in solid-state NMR spectra, are not a problem (Fig. 4). Multiple ^{15}N–^{13}C couplings are measured by RDX in the same one-dimensional experiment. This approach therefore has a potential advantage over the use of frequency-selective π pulses that keep only regions of the spectrum in phase and require multiple experiments for multiple couplings, or with time-consuming two-dimensional experiments that have no refocusing of isotropic J couplings.

A disadvantage of RDX is that the total refocusing and dephasing are reduced relative to that obtained by REDOR (for an isolated pair), or by frequency-selected REDOR (for one pair within a cluster). For example, the observed dephasing of the carbonyl-carbon signal in

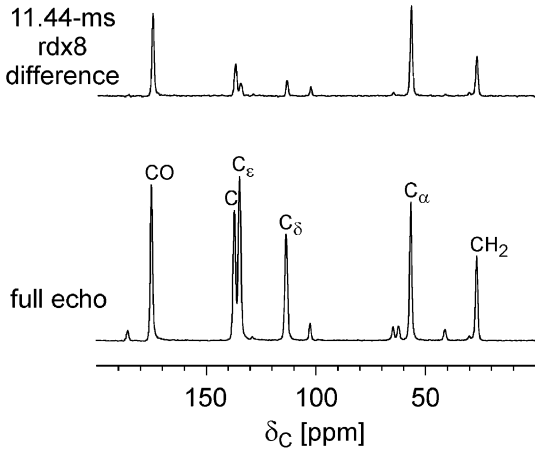

Fig. 3. The 125-MHz $^{13}C\{^{15}N\}$ RDX spectra of L-[$^{13}C_6$,α-^{15}N]histidine (free base), diluted with natural-abundance histidine (1:9), and recrystallized from water, obtained using the pulse sequence of Fig. 2 with 104 rotor cycles of dipolar evolution. The difference spectrum (S_0–S, where S and S_0 are the spectra obtained with and without ^{15}N dephasing pulses, respectively) is shown at the top of the figure and the full-echo spectrum (S_0) at the bottom. The scale is in ppm from external TMS. Magic-angle spinning was at 9091 Hz.

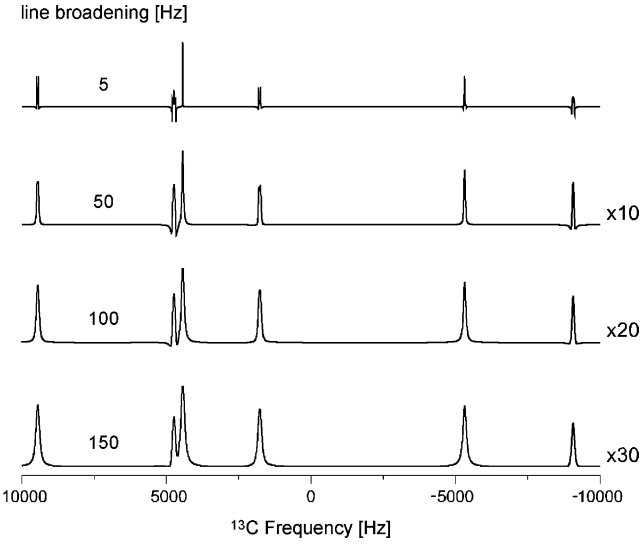

Fig. 4. Calculated RDX full-echo spectra as a function of line broadening using SIMPSON for the cluster-labeled histidine of Fig. 3 with an evolution period of 25.752 ms.

diluted and recrystallized L-[1,3-$^{13}C_2$,^{15}N]alanine and L-[$^{13}C_3$,^{15}N]alanine is only 50 and 35%, respectively, after 10 ms of dipolar evolution using RDX. Normal REDOR dephasing for a 2-bond ^{15}N-^{13}C coupling in an isolated pair on the other hand is complete after 10 ms [8]. Frequency-selective REDOR has better sensitivity for pairs of ^{13}C labels, while RDX has better sensitivity for clusters of four or more ^{13}C labels. The two methods are roughly equivalent for clusters of three ^{13}C labels. The recoupling method of choice for a cluster of ^{13}C labels therefore varies with the size of the cluster.

Multi-spin calculations (the $^{13}C_x$ cluster and ^{15}N) using SIMPSON [7] show that the relative ^{15}N-^{13}C couplings in cluster-labeled alanine (Fig. 5) and histidine (Fig. 6) are consistent with the observed relative dephasing. The absolute distances are determined with 10% accuracy. The similar ^{15}N–^{13}C dipolar coupling for the δ and ε carbons of histidine give rise to substantially different dephasing. There is no obvious physical picture for this difference, which arises from differences in homonuclear couplings for the two carbons. This result illustrates the fact that there is no universal dephasing behavior for RDX that depends only on heteroatom distances.

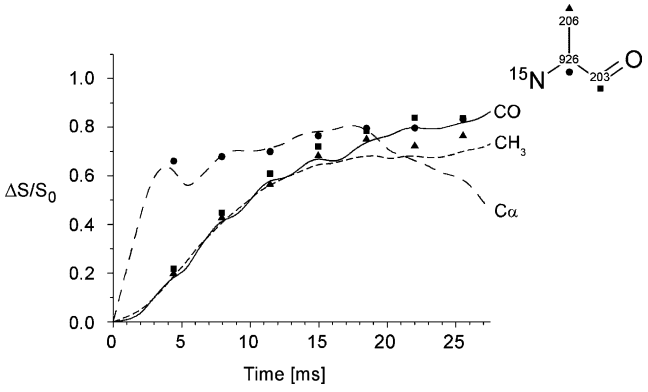

Fig. 5. RDX dephasing ($\Delta S/S_0$) as a function of dipolar evolution time for cluster-labeled alanine. Symbols show experimental values using the pulse sequence of Fig. 2, and solid, dashed, and dotted lines the values calculated using SIMPSON. The numbers in the structure (inset) give the theoretical rigid-lattice ^{15}N–^{13}C dipolar couplings to the ^{15}N label.

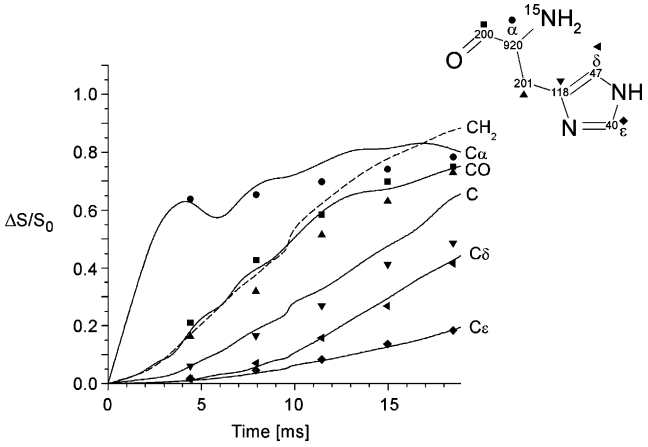

Fig. 6. RDX dephasing ($\Delta S/S_0$) as a function of dipolar evolution time for the L-[$^{13}C_6$,α-^{15}N]histidine (free base) diluted with natural-abundance histidine (1:9) and recrystallized from water. Symbols show experimental values using the pulse sequence of Fig. 2, and solid and dashed lines the values calculated using SIMPSON. The numbers in the structure (inset) give the theoretical rigid-lattice ^{15}N-^{13}C dipolar couplings to the histidine α-^{15}N label. The ring nitrogens are not labeled.

The RDX simulations for histidine included carbon–carbon homonuclear and carbon–nitrogen heteronuclear dipolar couplings, calculated from the amino-acid crystal structure [9], and homonuclear isotropic J couplings [10]. The isotropic J couplings that were used are $^1J_{CO-C\alpha} = 60$ Hz, $^1J_{C\alpha}$–$CH_2 = 35$ Hz, $^1J_{CH_2-C} = 51$ Hz, $^1J_{C-C\delta} = 75$ Hz, $^2J_{CH_2-C\delta} = 6$ Hz, and $^3J_{CH_2-C\varepsilon} = 3$ Hz. RDX dephasing is not strongly dependent on values of the shift tensors. No carbon shift tensors were included to speed the already lengthy simulations. The heteronuclear couplings are shown in the insert to Fig. 5. The parameters used for the alanine simulations appear in a companion paper in this Journal [11].

The distance information from multiple couplings can be combined to infer orientation, and this sort of application has been used recently in characterizing vancomycin binding in bacterial cell walls [11]. The RDX sequence is likely to work even better with higher spinning speeds. Some of the oscillations in dephasing for long evolution times seen in the calculations for cluster-labeled alanine spinning at 9 kHz (Fig. 5) disappear in calculations for spinning at 30 kHz. One-dimensional RDX should be easily extended to multiple dephasers by using frequency-selected pulses on the dephasing channel. We believe that RDX provides reliable distances from ^{13}C clusters of labels simultaneously and this sensitivity advantage extends the utility of REDOR.

2. Experimental details

RDX was performed using a 6-frequency transmission line probe having a 12-mm long, 6-mm inside-diameter analytical coil and a Chemagnetics/Varian ceramic spinning module. Powdered samples (typically 100 mg) were contained in thin-wall Chemagnetics/Varian 5-mm outside diameter zirconia rotors. The rotors were spun at 9091 Hz with the speed under active control to within ±2 Hz. The spectrometer was controlled by a Tecmag pulse programmer. The ^{13}C and ^{15}N radiofrequency pulses were produced by 1- and 2-kW American Microwave Technology power amplifiers, respectively. The ^1H radiofrequency pulses were generated by a 1-kW Creative Electronics tube amplifier driven by 50-watt American Microwave Technology power amplifier. A 12-T static magnetic field was provided by an 89-mm bore Magnex superconducting solenoid. Proton-carbon cross-polarization transfers were made in 2 ms at 62.5 kHz. Proton dipolar decoupling was 100 kHz during data acquisition. Two-phase pulse modulation [12] was used during both dipolar evolution and acquisition periods.

Acknowledgments

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