Carbon-Proton Dipolar Decoupling in REDOR

Anil K. Mehta,* Donald J. Hirsh,* Nathan Oyler,† Gary P. Drobny,† and Jacob Schaefer*

*Department of Chemistry, Washington University, St. Louis, Missouri 63130; and †Department of Chemistry,
University of Washington, Seattle, Washington 98195

Dipolar decoupling of protons with radiofrequency field amplitudes comparable to those used for the rare-spin refocusing and dephasing π pulses results in accurate, high-sensitivity determinations of internuclear distances in rotational-echo double-resonance experiments and simulations performed on ^{13}C and ^{15}N-labeled L-alanine. © 2000 Academic Press

Rotational-echo double-resonance (REDOR) NMR of an I–S spin pair is a magic-angle spinning experiment that involves the observation of S-spin rotational echo amplitudes under the influence of rotor-synchronized I-spin dephasing pulses (*1*). The ratio of the echo amplitude with dephasing (S) to that without dephasing (S_0) has a simple interpretation that depends on the length of the rotor period, the number of rotor periods used for the dephasing, and the I–S dipolar coupling. REDOR is becoming widely used in solid-state NMR and applications in materials science (*2*) and structural biology (*3*) have recently been reported.

Even though the name of the REDOR experiment suggests that only two spins and therefore two radiofrequencies are present, typical applications may use three or even four frequencies. The most common application requires decoupling of I and S rare spins from interactions with abundant protons. Some reports have indicated that ^1H decoupling fields with amplitudes at least three times that used for the S-spin pulses are essential for REDOR to limit signal loss during dipolar evolution or mixing (*4*), avoid cross-relaxation during S-spin pulses (*5*), and optimize the observed total S-spin dephasing (*6*). In this Communication, we show by experiment, and by 5-spin simulations which explicitly include the ^1H radiofrequency field amplitude, that practical distance determinations by REDOR ($0.2 < S/S_0 < 0.9$) are essentially independent of the level of proton decoupling for values between 50 and 100 kHz (one to two times the S-spin radiofrequency field amplitude).

REDOR experiments were performed on L-[1,3-$^{13}C_2$, ^{15}N]alanine and L-[2-^{13}C, ^{15}N]alanine, each diluted 10-fold with natural-abundance alanine and recrystallized. Magic-angle spinning was at 5 kHz ($T_r = 200$ μs). The $^{13}C\{^{15}N\}$ S/S_0 for the methyl carbon is shown in Fig. 1 as a function of the level of the continuous-wave ^1H decoupling field. The results are

indistinguishable for decoupling fields with values close to the 50 kHz of the ^{13}C π pulses to values more than double the carbon radiofrequency field amplitude. Comparable results were observed for the carbonyl carbon. The homogeneous linewidth ($1/\pi T_2$) for the methyl and carbonyl carbons (as measured by the S_0 dependence on evolution time) decreased by less than 20% upon increasing the decoupling field from 53 to 113 kHz. The integrated intensities of S and S_0 were independent of the choice of decoupling field strength for both methyl and carbonyl carbons.

The homogeneous linewidth for the protonated methine carbon had a stronger dependence on decoupling, decreasing from 98 to 64 Hz upon increasing the decoupling field from 50 to 100 kHz. Nevertheless, the S/S_0 ratios ($0.2 < S/S_0 < 0.9$) are unaffected by the level of decoupling (Fig. 2). The integrated intensities for the methine–carbon S and S_0 were reduced by factors of 1.2 and 2.0 after 4 T_r and 34 T_r, respectively, for 50-kHz decoupling relative to 100-kHz decoupling. About 85% of the lost signal after 34 T_r is due to T_2 decay. We attribute the remaining 15% to ^1H–^{13}C contact during the single Hahn-echo refocusing pulse.

Numerical simulations were performed for 5 spins, the observed ^{13}C and its coupled ^{15}N, together with three nearby protons. The three protons included the α proton, a single proton bonded to the methyl carbon, and a single proton bonded to the nitrogen. The bond lengths and angles for the three carbons, the α proton, and the nitrogen of alanine were based on the crystal structure of L-alanine (*7*). Single protons were assumed to be directly bonded to the methyl and nitrogen atoms; these protons were placed along the respective C_3 axes with effective CH and NH bond lengths of 1 Å. All spins were modeled as fully dipolar coupled to each other. Additionally, the three protons were modeled as coupled to a proton bath by applying a phenomenological decay to their single-quantum transitions at each propagation step in the full density matrix simulations. No other phenomenological decay was applied. Each rotor period of evolution was divided into 68 propagation steps. A full five-spin simulation with 64 rotor periods of REDOR evolution with finite pulses was calculated in about 8 h using a 400-MHz Pentium processor.

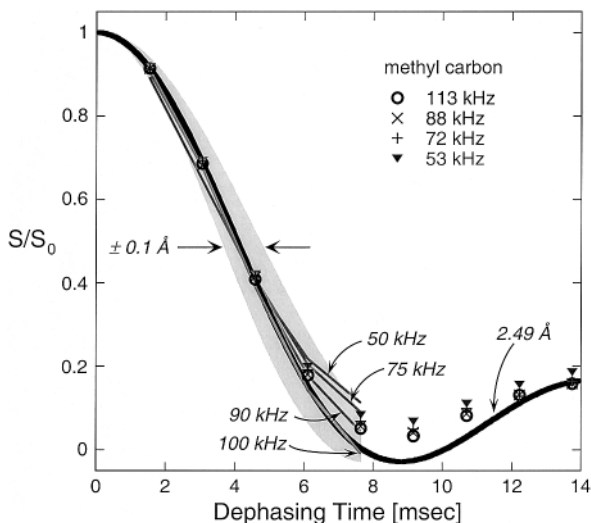The agreement between experiments and simulations of

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FIG. 1. 50.3-MHz $^{13}C\{^{15}N\}$ REDOR dephasing (S/S_0) for the methyl carbon of L-[1,3-$^{13}C_2$, ^{15}N]alanine diluted 10-fold with natural-abundance alanine and recrystallized. Equal numbers of dephasing π pulses were used on both ^{13}C and ^{15}N channels with XY8 phase cycling. Dephasing is shown as a function of the level of the continuous-wave ^{1}H decoupling field. The ^{13}C radiofrequency field amplitude was 50 kHz and the magic-angle spinning speed was 5 kHz. The heavy solid line is the dephasing calculated for an isolated ^{13}C–^{15}N two-spin system with an internuclear separation of 2.49 Å. The shading indicates calculated dephasing ranges for ± 0.1 Å variations of the C–N distance. These variations far exceed the experimental uncertainty, which is represented by the height of the symbols. The light lines show the dephasing calculated using a 5-spin simulation model as a function of the ^{1}H radiofrequency field amplitude.

Figs. 1 and 2 emphasizes the simplicity of REDOR for practical applications. Accurate dipolar couplings can be determined by S/S_0 ratios with values between 0.9 and 0.2. Extraordinarily large decoupling fields are not necessary and the observed dephasing (S/S_0) is clearly not a function of the level of decoupling. We believe that the minor deviations between observed and calculated two-spin REDOR dephasing between about 8 and 11 ms in Fig. 1 are due to the effects of ^{13}C–^{13}C couplings, which were not included in the calculation. The 5-spin modeling is clearly inadequate to describe longer evolution times and misrepresents the observed S/S_0 oscillations. However, the details of the spin physics in this regime are not important for practical distance determinations of I–S spin pairs, which is the focus of this Communication.

Only minor line broadening due to incomplete decoupling is observed for the methyl and nonprotonated carbonyl carbons. In addition, integrated signal intensities for both S and S_0 are independent of the decoupling amplitudes, which means there is no significant leakage of ^{13}C polarization to the ^{1}H reservoir either during REDOR evolution or during the ^{13}C pulse periods. However, the integrated signal intensity for the protonated methine carbon is a modest function of the decoupling field. About half the signal observed after 34 T_r with 100-kHz decoupling is lost when 50-kHz decoupling is used. Thus, the signal-to-noise sensitivity of the REDOR experiment is com-

promised by weak decoupling (although as shown in Fig. 2, not the accuracy of the experiment).

We have also performed $^{13}C\{^{15}N\}$ REDOR experiments on Ac–Phe–[1-^{13}C]MeA–MeA–MeA–Val–[^{15}N]Gly–Leu–MeA–MeA–O–Bzl (emerimicin 1–9), the benzyl ester of the amino terminal portion of the peptide antibiotic, emerimicin. The REDOR dephasing over a 20-ms dipolar evolution time ($0.4 < S/S_0 < 1$) was independent of proton decoupling levels between 53 and 113 kHz (data not shown). The experimentally determined ^{13}C–^{15}N distance was 4.1 Å, consistent with previous X-ray and NMR determinations (*8*). This result shows that the conclusions about the insensitivity of REDOR dephasing to proton decoupling levels based on Figs. 1 and 2 for relatively strong dipolar couplings and short distances extend to weaker couplings and longer distances.

Obviously, it is better to use strong decoupling in REDOR if possible, which is the established practice for other types of cross-polarization magic-angle spinning echo experiments (*9*). Strong dipolar decoupling avoids signal losses due to homogeneous decay during evolution and acquisition periods. Moreover, if inadvertent ^{1}H–^{13}C contact is to be avoided because strong decoupling fields are not available, then it is adviseable to select a version of REDOR in which only a single ^{13}C refocusing π pulse is used (*10*). Efficient S_0 refocusing for methylene carbons
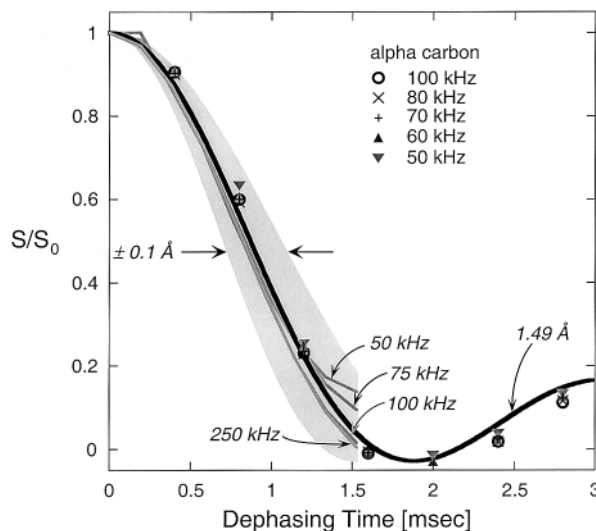

FIG. 2. 50.3-MHz $^{13}C\{^{15}N\}$ REDOR dephasing (S/S_0) for the methine carbon of L-[2-^{13}C, ^{15}N]alanine diluted 10-fold with natural-abundance alanine and recrystallized. Only a single refocusing pulse was used on the ^{13}C channel with all dephasing pulses on the ^{15}N channel. Phases of the ^{15}N π pulses followed the XY8 scheme. Dephasing is shown as a function of the level of the continuous-wave ^{1}H decoupling field. The ^{13}C radiofrequency field was 50 kHz and the magic-angle spinning speed was 5 kHz. The heavy solid line is the dephasing calculated for an isolated ^{13}C–^{15}N two-spin system with an internuclear separation of 1.49 Å. The shading indicates calculated dephasing ranges for ± 0.1 Å variations of the C–N distance. These variations far exceed the experimental uncertainty, which is represented by the height of the symbols. The light lines show the dephasing calculated using a 5-spin simulation model as a function of the ^{1}H radiofrequency field amplitude.

in such experiments has been observed following REDOR evolution for times as long as 35 ms (*11*). Perhaps the best strategy for REDOR of protonated carbons when only weak decoupling fields are available is to use a proton-decoupling modulation scheme like two-pulse phase modulation (*12*) during the evolution and acquisition periods, together with a single *S*-spin refocusing π pulse. All the dephasing pulses can then be shifted to the *I*-spin channel and effectively modulated by the XY8 phase-alternation scheme (*13*).

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