

Orientalional Information in Solids from REDOR Sidebands

Jon M. Goetz and Jacob Schaefer

Department of Chemistry, Washington University, St. Louis, Missouri 63130

Received July 29, 1997

In the past decade, a number of solid-state nuclear magnetic resonance experiments have been developed to measure heteronuclear dipole–dipole couplings in specifically labeled compounds (1–6). In a rigid solid, these couplings give an accurate measure of the distance separating a pair of spins. In addition to distances, second-rank dipole–dipole and chemical-shift interactions can be used to measure the relative orientations of chemical groups in unoriented, powder samples. This information is valuable for structural studies including, for example, the measurement of torsion angles in peptides. An impressive number of two-dimensional techniques have been developed to make this measurement (7–16). In this Communication, we propose a simple method for obtaining orientational information using a one-dimensional experiment. The sidebands of REDOR-dephased echo spectrum are a strong function of the relative orientation of the dipolar and chemical-shift tensors. A comparison of experimental and calculated sideband intensities of the dephased-echo spectrum gives distance and orientational information simultaneously.

The integrated intensity of REDOR-dephased echo relative to the full echo is a function of the heteronuclear dipolar coupling alone. However, the distribution of intensities in the sidebands is strongly dependent upon the relative orientation of the chemical shift tensor and the internuclear vector as shown in Fig. 1. The strategy for measuring the relative orientation of a chemical-shift tensor principal-axis frame and an internuclear vector is straight forward. First, find the values of the chemical-shift anisotropy tensor (σ_{xx} , σ_{yy} , σ_{zz}) from the full-echo spectrum using a Herzfield–Berger analysis or similar fitting routine (17). Next, simulate how sideband intensities evolve during a REDOR experiment as a function of the two Euler angles (Θ , Ψ) which describe the relative orientation of the internuclear vector and the principal axis of the chemical-shift tensor (18). Finally, use some form of least-squares-fit analysis to compare calculated and observed REDOR-dephased sideband intensities to obtain the orientation.

In alanine, the molecular structure and relative orientation of the principal axis of the carbonyl chemical-shift tensor are known from single-crystal NMR and neutron diffraction studies. The carbonyl chemical-shift eigenvalues are $\sigma_{xx} =$

-352 Hz, $\sigma_{yy} = -3220$ Hz, $\sigma_{zz} = 3572$ Hz for carbons resonating at 50,323,528 Hz (19). Relative to this chemical-shift principal-axis system, the orientation of the C–N intramolecular vector is given by polar and azimuthal Euler angles of Θ , $\Psi = 79^\circ$, -67° (20).

The experimental REDOR spectra presented in Fig. 2 were acquired from L-[1,3- $^{13}\text{C}_2$, ^{15}N] alanine which was diluted 1:10 in natural abundance L-alanine by recrystallization. The carbon-observed nitrogen-dephased REDOR experiment began with a 1-ms, 50-kHz matched proton–carbon

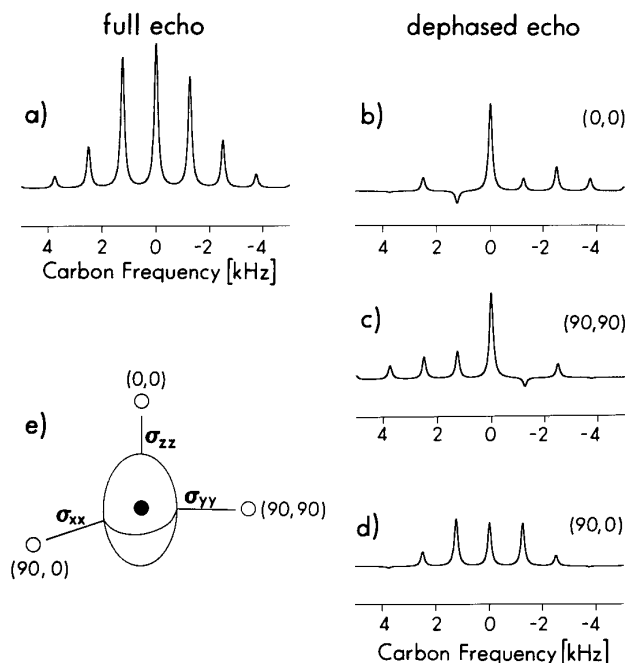


FIG. 1. The full-echo spectrum for a carbonyl carbon with chemical-shift anisotropy eigenvalues of $\sigma_{xx} = -216$ Hz, $\sigma_{yy} = -3140$ Hz, $\sigma_{zz} = 3356$ Hz is simulated in (a) assuming a spinning speed of 1250 Hz. After 4.8 ms of REDOR dephasing by a single ^{15}N spin 2.5 Å away, the integrated intensity of the dephased echo is 33% that of the full echo. Although the total intensity of the dephased echo only depends on the ^{13}C – ^{15}N distance, individual sideband intensities depend on the orientation of the ^{13}C – ^{15}N internuclear vector and the principal axis system of the carbonyl chemical-shift tensor. Dephased echos are simulated for Eulerian polar and azimuthal angles (b) Θ , $\Psi = 0^\circ$, 0° , (c) Θ , $\Psi = 90^\circ$, 90° , and (d) Θ , $\Psi = 90^\circ$, 0° .

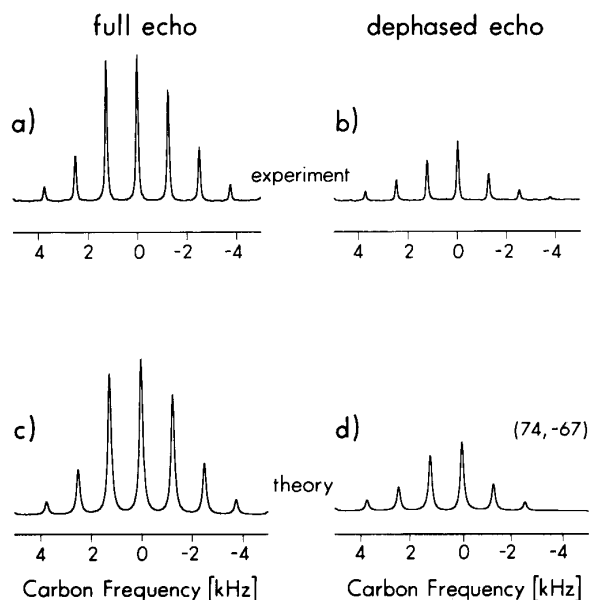


FIG. 2. Experimental REDOR full-echo (a) and dephased-echo (b) spectra for the carbonyl carbon in diluted L-[1,3- $^{13}\text{C}_2$, ^{15}N] alanine. The REDOR experiment was run for six rotor cycles at 1250 Hz. The full-echo results are simulated in (c) using chemical-shift anisotropy eigenvalues of $\sigma_{xx} = -216$ Hz, $\sigma_{yy} = -3140$ Hz, $\sigma_{zz} = 3356$ Hz. The dephased echo is simulated in (d) using the same chemical-shift anisotropy values and a ^{13}C – ^{15}N dipolar coupling of 189 Hz with the C–N internuclear vector aligned relative to the shift tensor of Θ , $\Psi = 74^\circ, -67^\circ$.

spin-lock polarization transfer. Protons were decoupled for the rest of the experiment using 110 kHz of radiofrequency irradiation. The ^{13}C π pulses were placed at the end of every rotor period to refocus isotropic chemical shifts. All π pulses were phase alternated using an (XY) phase-cycling scheme. Dephasing ^{15}N π pulses were applied in the middle of the rotor period. The carbon spectra acquired with application of 50-kHz ^{15}N π pulses (dephased echo) and without the ^{15}N pulses (full echo) are presented in Fig. 2. The REDOR data were acquired with six rotor cycles of REDOR evolution averaging 240 scans with a repetition rate of 1 Hz.

By subtracting the 10% of the signal from natural abundance carbons, the spectrum presented in Fig. 2 is carbon signals from labeled alanine alone. The simulation of the full echo used chemical-shift eigenvalues within 200 Hz of those in the literature (18). After six rotor cycles of nitrogen-dephased REDOR at 1250 Hz, the dephased carbon echo has 33% of the intensity of the full echo. This measurement of the ratio of the intensities of the echos with and without dephasing gives a ^{13}C – ^{15}N coupling of 189 Hz. This is 5% smaller than the ^{13}C – ^{15}N coupling predicted by the neutron diffraction distance of 2.473 Å (19), a diminution probably due to librational motions at room temperature (1).

The simulation of the dephased spectrum, shown in Fig. 2, which gave the best match to the observed spectrum was

found by simple trial and error to be $\Theta, \Psi = 74^\circ, -67^\circ$. We are comfortable citing an error in this determination of at most $\pm 5^\circ$ because small changes in Θ and Ψ have sizeable effects on the sideband (n_i) and centerband (n_0) intensities. For example, a $\pm 10^\circ$ variation in Θ changes the n_1/n_0 ratio from 1.08 to 0.56, and the n_{-1}/n_0 ratio from 0.85 to 0.28, while a $\pm 10^\circ$ variation in Ψ changes n_1/n_0 from 0.56 to 0.91 and n_{-1}/n_0 from 0.26 to 0.78.

We conclude that monitoring the sidebands of a REDOR-dephased spectrum can give useful orientational information for rigid moieties in molecules. The combination of ^{13}C and ^{15}N observed REDOR sideband intensities may aid in structural studies such as the determination of Ramachandran angles in polypeptides.

ACKNOWLEDGMENTS

We thank Simon Vega for the use of his spin-dynamics simulation code. This work was supported by NIH Grant GM51554.

REFERENCES

1. T. Gullion and J. Schaefer, *J. Magn. Reson. A* **81**, 196 (1988).
2. M. H. Levitt, D. P. Raleigh, F. Creuzet, and R. G. Griffin, *J. Chem. Phys.* **92**, 6347 (1990).
3. R. Tycho and G. Dabbagh, *Chem. Phys. Lett.* **173**, 461 (1990).
4. T. Gullion and S. Vega, *Chem. Phys. Lett.* **194**, 423 (1992).
5. A. W. Hing, S. Vega, and J. Schaefer, *J. Magn. Reson. A* **103**, 151 (1993).
6. D. M. Gregory, D. J. Mitchell, J. A. Stringer, S. Kiihne, J. C. Shields, J. Callahan, M. A. Mehta, and G. P. Drobny, *Chem. Phys. Lett.* **246**, 654 (1995).
7. M. G. Munowitz, R. G. Griffin, G. Bodenhausen, and T. H. Huang, *J. Am. Chem. Soc.* **103**, 2529 (1981).
8. R. A. Haberorn, R. E. Stark, H. van Willigen, and R. G. Griffin, *J. Am. Chem. Soc.* **103**, 2534 (1981).
9. A. P. M. Kentgens, E. de Boer, and W. S. Veeman, *J. Chem. Phys.* **87**, 6859 (1987).
10. Z. Luz, H. W. Spiess, and J. J. Titman, *Isr. J. Chem.* **32**, 145 (1992).
11. X. Feng, Y. K. Lee, D. Sandstrom, M. Eden, H. Maisel, A. Sebald, and M. H. Levitt, *Chem. Phys. Lett.* **257**, 314 (1996).
12. R. Tycko, D. P. Weliky, and A. E. Berger, *J. Chem. Phys.* **105**, 7915 (1996).
13. K. Schmidt-Rohr, *J. Am. Chem. Soc.* **118**, 7601 (1996).
14. Y. Ishii, T. Terao, and M. Kainosho, *Chem. Phys. Lett.* **256**, 133 (1996).
15. T. Fujiwara, T. Shimomura, and H. Akutsu, *J. Mag. Reson.* **124**, 147 (1997).
16. D. M. Gregory, M. A. Mehta, J. C. Shiels, and G. P. Drobny, *J. Chem. Phys.* **107**, 28 (1997).
17. J. Herzfeld and A. E. Berger, *J. Chem. Phys.* **73**, 6021 (1980).
18. A. Schmidt and S. Vega, *Isr. J. Chem.* **32**, 215 (1992).
19. A. Naito, S. Ganapathy, K. Akasaka, and C. A. McDowell, *J. Chem. Phys.* **74**, 3190 (1981).
20. M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *J. Am. Chem. Soc.* **94**, 2657 (1972).