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CSA-Enabled Spin Diffusion Leads to MAS Rate-Dependent T_1's at High Field

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ABSTRACT: A surprisingly strong spin rate dependence of ^{15}N and ^{13}C NMR T_1 times in magic angle spinning experiments on solid peptides is demonstrated. Using a variety of isotopomers, the phenomenon is shown to be the result of chemical shift anisotropy-mediated spin diffusion. This effect has the potential to be used to detect long-range distance constraints in macromolecular systems.

Dynamics in macromolecular assemblies very often determines function as much as amino acid sequence or tertiary structure. Recognition of this has led to the development of a variety of NMR relaxation methods[1] to characterize protein dynamics, and to link them to specific biochemical functions. In the crystalline state overall rotational diffusion is quenched, making solid state NMR (ssNMR) attractive for selectively observing internal dynamics.[2−4] Immobilization in the solid state is also potentially useful for paramagnetic relaxation enhancement (PRE) by spin labels,[5] a powerful approach to obtain long range distance constraints in proteins.[6] In the solid state, the naturally smaller ^{15}N relaxation rates ($R_1 = 1/T_1$) permit more modest PREs to be observed, facilitating the detection of even longer range distances.

To convincingly interpret ssNMR relaxation data in these applications it is important to understand the many factors that can affect relaxation rates. Uniform ^{15}N enrichment used in protein NMR increases the possibility that ^{15}N spin diffusion will confound ssNMR data analysis.[7,8] Since amide to amide ^{15}N dipolar couplings are <45 Hz, this is usually assumed effectively quenched by rapid magic angle spinning (MAS). Measurements of ^{15}N spin diffusion between amides[7] do in fact seem to support this assumption. Another possible pathway is spin diffusion between ^{15}N amides and mobile ^{15}N amines. Since the latter have inherently fast relaxation rates, they have the potential to serve as efficient relaxation sinks.

We report here on the surprising observation that ^{15}N amide T_1's abruptly increase over 20-fold when the MAS frequency ν_R passes a critical threshold value in the presence of such a relaxation sink. It is shown that ^{15}N spin diffusion enabled by the significant amide chemical shift anisotropy (CSA) provides the underlying mechanism. Due to this effect, it is not safe to assume ^{15}N spin diffusion has been quenched unless ν_R exceeds twice the frequency separation $\Delta\nu$ between the amide and lysine ^{15}N resonances in proteins. This observation also suggests that the MAS dependence of R_1's could be used to obtain long-range distance constraints in suitable cases.

Glycyl-alanyl-leucine·3H_2O (GAL) was prepared by solid-phase synthesis in several isotopic compositions and crystals were grown in H_2O or HOD. ^{15}N spectroscopy was performed on a Varian instrument operating with a proton frequency of 800 MHz with a home-built 2.5 mm MAS probe[9] at ∼8 °C. T_1's were determined using saturation recovery, and experimental signal decays were fitted to single exponential curves.

The MAS dependence of the ^{15}N T_1's in uniformly ^{15}N-enriched GAL crystallized from HOD is shown in Figure 1. The T_1 for the gly amine ^{15}N is ∼0.8 s regardless of ν_R. In contrast, the ala and leu amide T_1's are ∼40 s until ν_R exceeds 9 kHz, at which point they abruptly increase and then level off to a limiting value of ∼1000 s. One might infer this is a result of the rapidly relaxing amine acting as a relaxation sink for the amides, with ^{15}N−^{15}N proton-driven spin diffusion[10,11] (PSD) moderating the exchange. Following this line of reasoning the abrupt rise in amide T_1 occurs because PSD is quenched when ν_R exceeds the approximately 11 kHz amide ^{15}N−^{1}H dipolar coupling.

Figure 1, however, demonstrates that PSD cannot mediate the cross relaxation, as the effect persists when the amides are deuterated. The ^{15}N−^{1}H and ^{15}N−D peaks are well resolved due to a significant secondary isotopic chemical shift, enabling simultaneous measurement of T_1 for each isotopomer. Confirmation that the glycyl-^{15}N is in fact a relaxation sink is provided by a sample where the gly amine is not ^{15}N-enriched. In this instance the amide ^{15}N T_1's are largely ν_R independent.

Early in the history of MAS, Andrew and co-workers[13] observed a related phenomenon where a slowly relaxing spin takes on the T_1 of a rapidly relaxing partner under conditions of "rotational resonance"[14] (R^2). This occurs when the separation $\Delta\nu$ between the two lines is a multiple of ν_R. While the amide T_1's are shortened at the R^2 conditions, these effects are small in comparison to the sudden increase in T_1 at high spin rates. The observation that the amide relaxation has distinct slow spinning and fast spinning regimes when R^2 conditions are avoided, and that the ^{15}N spin diffusion is not mediated by ^{15}N−^{1}H dipolar couplings, is unprecedented.

A simple rate matrix formulation[15] for the relaxation of a single amine (A) and amide (B) ^{15}N pair describes the underlying physics. In terms of the departure of the z-magnetizations from equilibrium,

$$\frac{d}{dt}\begin{pmatrix} M_{z_A} - M^{\infty}_{z_A} \\ M_{z_B} - M^{\infty}_{z_B} \end{pmatrix} = -\begin{pmatrix} R_{1A} - R_{AB} & R_{AB} \\ R_{AB} & R_{1B} - R_{AB} \end{pmatrix} \cdot \begin{pmatrix} M_{z_A} - M^{\infty}_{z_A} \\ M_{z_B} - M^{\infty}_{z_B} \end{pmatrix}$$

For solid samples the cross-relaxation rate R_{AB} is inherently <0 and can be equated to the spin diffusion rate.[7,15] The auto rates

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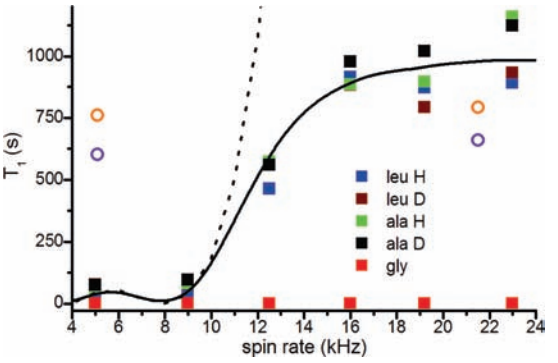

Figure 1. (■) ^{15}N T_1's for u-^{15}N-enriched GAL crystallized from HOD. (···) Estimate of $1/|R_{AB}|$ from calculation of f_{AB}. (—) Calculated ν_R dependence of amide T_{1B}. f_{AB} computed using MAS sideband intensities from SIMPSON[12] with default amide CSA and N−H bond orientation. Line width w/o ^{1}H decoupling for amine = 400 Hz, amide = 2500 Hz. ^{15}N T_1's for (○ orange) ala, (○ purple) leu, in 2,3-^{15}N-enriched GAL crystallized from H_2O. These are slightly shorter as the ^{1}H density is higher with crystallization from H_2O.

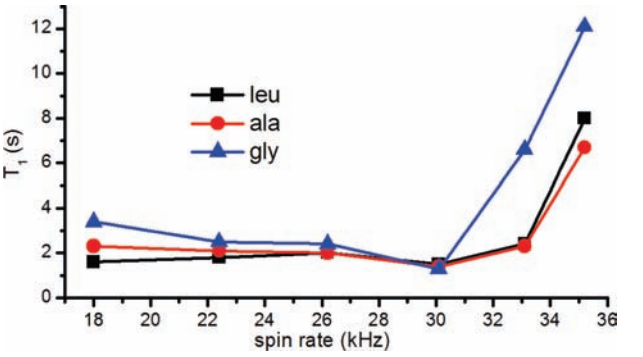

Figure 2. ^{13}C T_1's of carbonyl groups in uniformly ^{13}C-enriched GAL vs ν_R at 18.8 T. The frequency difference between the carbonyl and methyl groups is ∼30 kHz. When repeated at 7.05 T, the onset of the steep increase in T_1 scales with B_o and is observed at 11.2 kHz as expected (data not shown).

are the sum of cross-relaxation independent terms (R_{1A} and R_{1B}) and $-R_{AB}$. When the amine $R_{1A} \gg |R_{AB}|, R_{1B}$, each spin relaxes as a single exponential with T_1's given by

$$\frac{1}{T_{1A}} \approx R_{1A} + R_{AB} \text{ and } \frac{1}{T_{1B}} = |R_{AB}| + R_{1B}$$

Our data show that under slow MAS the amide spin B relaxes at essentially the cross-relaxation rate $|R_{AB}|$. Once ν_R crosses a threshold value, R_{AB} rapidly goes to zero, and $1/T_{1B}$ asymptotically approaches R_{1B}.

All descriptions[10,11,16] of the spin diffusion rate R_{ij} between a pair of spins ij reduce to expressions involving their dipolar coupling d_{ij} (s^{-1}) and a line shape function f_{ij} ($s \cdot rad^{-1}$).

$$R_{ij} = -\frac{\pi}{2} d_{ij}^2 f_{ij} = -\frac{\pi}{2}\left(\frac{\mu_o}{4\pi}\frac{\gamma_i\gamma_j\hbar}{2r_{ij}^3}(1 - 3\cos^2\theta_{ij})\right)^2 f_{ij}$$

In the present application a powder sum MAS average of $\langle \pi d_{ij}^2/2\rangle = \mu_o^2\gamma_N^4\hbar^2/160\pi r_{ij}^6$ is appropriate. The function f_{ij} provides a measure of the mutual spectral overlap. The notion is that spin exchange is most efficient if the spins have the same frequency, otherwise their frequency separation $\Delta\nu$ truncates the dipolar interaction. Under MAS the most effective Hamiltonian terms for untruncating the dipolar interaction are time-dependent at ν_R or $2\nu_R$. Following Kubo and McDowell,[16] we approximate f_{AB} as the overlap of the amide spinning side bands with the single ∼400 Hz wide Lorentzian observed for the amine in the ^{15}N spectrum without ^{1}H decoupling. Whether ^{1}H, D or no dipolar couplings to the amide ^{15}N are included has little effect on the f_{AB} profile calculated in this manner. This is because the sideband patterns reflect the fact that the dipolar field either adds to or subtracts[17] from the ^{15}N CSA to produce an effective anisotropy that depends on the spin state $|m\rangle$ of the ^{1}H or D. Since there is a $-m$ for every $+m$, the average of any sideband intensity over m when the CSA is large is approximately the same as if the dipolar coupling were set to zero. For this reason this chemical shift anisotropy enabled spin diffusion or CSD is largely unaffected by deuteration.

The dependence of f_{AB} on ν_R is then largely a function of how the CSA shapes the spinning sideband intensities and the amide−amine resonance offset $\Delta\nu$. At slow rates there is always an amide sideband close to the amine resonance, and if R^2 conditions are avoided, f_{AB} is fairly constant. However, once ν_R exceeds $\nu_{ala} - \nu_{gly}$ = 7592 Hz, there are no more side bands between the two peaks. As ν_R is further increased, f_{AB} precipitously drops, CSD is quenched, and R_{AB} goes to zero.

Since $\nu_{leu} - \nu_{ala}$ is only 439 Hz, center band overlap alone gives an amide−amide spin exchange time ≤50 s, and their T_1's are then essentially the same at all ν_R. Using the 3.6 Å ala amide to gly amine distance, $\langle \pi d_{AB}^2/2\rangle \approx 8700\ s^{-2}$. With this value, and assuming an effective amide line width of 2500 Hz, we obtain $1/|R_{AB}|$ as plotted in Figure 1. The ν_R dependence of T_{1B} computed from this is seen to agree very well with the observed average behavior.

CSD can be a dominant factor in high-field MAS NMR spin dynamics whenever uniform isotopic enrichment is used. CSD to efficient relaxation sinks cannot be ruled out by the apparent independence of T_1 vs ν_R, or the absence of cross peaks in two-dimensional spin exchange measurements. Fortunately, as long as the MAS rate is greater than 2 × the resonance offset between a potential relaxation sink and a spin of interest, CSD will be efficiently suppressed. Differential relaxation can then be more confidently interpreted in dynamics or PRE experiments.

The same physics will apply to ^{13}C T_1 relaxation of backbone ^{13}CO groups (which have significant ^{13}C CSA) by ^{13}CH$_3$ relaxation sinks in proteins.[18,19] The ^{13}CO T_1 should be fairly independent of ν_R until the frequency separation of the ^{13}CO and ^{13}CH$_3$ resonances is surpassed. At 18.78 T we expect this transition to occur at ∼30 kHz, and Figure 2 depicts just this behavior for the CO ^{13}C T_1's in uniformly ^{13}C-enriched GAL.

Work is ongoing to determine whether the manipulation of T_1 relaxation by CSD to a sink can be used for detecting long distances in proteins. The difference between slow and fast MAS T_1's for different ^{13}CO groups in a protein with a single ^{13}CH$_3$ sink in principle measures the relative ^{13}CO to ^{13}CH$_3$ spin diffusion rate constants, and thus $r_{CO-CH_3}^6/r'^6_{CO-CH_3}$. This method would be similar in spirit to using spin labels to measure electron−electron distances. With inherently long ^{13}CO T_1 times at high fields, it is plausible that ^{13}C−^{13}C distances beyond 10 Å could be detected in this manner with selective labeling. Since measuring T_1 is a relatively simple experiment, this approach has the potential to be developed into a robust method that could be applied to complex biochemical and materials systems and that could be accessible to the nonexpert.

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REFERENCES

(1) Palmer, A. G.; Kroenke, C. D.; Loria, J. P. *Methods Enzymol.* **2001**, *339*, 204–238.
(2) Agarwal, V.; Xue, Y.; Reif, B.; Skrynnikov, N. R. *J. Am. Chem. Soc.* **2008**, *130* (49), 16611–16621.
(3) Giraud, N.; Blackledge, M.; Goldman, M.; Bockmann, A.; Lesage, A.; Penin, F.; Emsley, L. *J. Am. Chem. Soc.* **2005**, *127* (51), 18190–18201.
(4) Krushelnitsky, A.; deAzevedo, E.; Linser, R.; Reif, B.; Saalwachter, K.; Reichert, D. *J. Am. Chem. Soc.* **2009**, *131* (34), 12097–12099.
(5) Nadaud, P. S.; Helmus, J. J.; Kall, S. L.; Jaroniec, C. P. *J. Am. Chem. Soc.* **2009**, *131* (23), 8108–8120.
(6) Liang, B. Y.; Bushweller, J. H.; Tamm, L. K. *J. Am. Chem. Soc.* **2006**, *128* (13), 4389–4397.
(7) Giraud, N.; Blackledge, M.; Bockmann, A.; Emsley, L. *J. Magn. Reson.* **2007**, *184* (1), 51–61.
(8) Krushelnitsky, A.; Brauniger, T.; Reichert, D. *J. Magn. Reson.* **2006**, *182* (2), 339–342.
(9) Martin, R. W.; Paulson, E. K.; Zilm, K. W. *Rev. Sci. Instrum.* **2003**, *74* (6), 3045–3061.
(10) Henrichs, P. M.; Linder, M.; Hewitt, J. M. *J. Chem. Phys.* **1986**, *85* (12), 7077–7086.
(11) Suter, D.; Ernst, R. R. *Phys. Rev. B* **1985**, *32* (9), 5608–5627.
(12) Bak, M.; Rasmussen, J. T.; Nielsen, N. C. *J. Magn. Reson.* **2000**, *147* (2), 296–330.
(13) Andrew, E. R.; Bradbury, A.; Eades, R. G.; Wynn, V. T. *Phys. Lett.* **1963**, *4* (2), 99–100.
(14) Raleigh, D. P.; Levitt, M. H.; Griffin, R. G. *Chem. Phys. Lett.* **1988**, *146* (1−2), 71–76.
(15) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41* (1), 95–117.
(16) Kubo, A.; McDowell, C. A. *J. Chem. Soc., Faraday Trans.* **1988**, *84*, 3713–3730.
(17) Zilm, K. W.; Grant, D. M. *J. Am. Chem. Soc.* **1981**, *103* (11), 2913–2922.
(18) Anderson, J. E.; Slichter, W. P. *J. Phys. Chem.* **1965**, *69* (9), 3099–3104.
(19) White, J. L. *Solid State Nucl. Magn. Reson.* **1997**, *10* (1−2), 79–88.