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COMMUNICATION

Monitoring conformational dynamics with solid-state $R_{1\rho}$ experiments

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Abstract A new application of solid-state rotating frame ($R_{1\rho}$) relaxation experiments to observe conformational dynamics is presented. Studies on a model compound, dimethyl sulfone (DMS), show that $R_{1\rho}$ relaxation due to reorientation of a chemical shift anisotropy (CSA) tensor undergoing chemical exchange can be used to monitor slow-to-intermediate timescale conformational exchange processes. Control experiments used d_6-DMS and alanine to confirm that the technique is monitoring reorientation of the CSA tensor rather than dipolar interactions or methyl group rotation. The application of this method to proteins could represent a new site-specific probe of conformational dynamics.

Keywords Rotating frame relaxation · Chemical shift anisotropy · Molecular dynamics · Chemical exchange · Dimethyl sulfone

Knowledge of both structure and dynamics is important for understanding protein function. In recent years, nuclear magnetic resonance (NMR) spectroscopy has developed into a powerful method for monitoring fluctuations in molecular conformation, providing important insight into protein function (Palmer et al. 2001; Krushelnitsky and Reichert 2005; Boehr et al. 2006). $R_{1\rho}$ relaxation experiments probe the microsecond to millisecond timescale, which is an important timescale in protein dynamics, including processes such as enzyme catalysis, domain motions, and protein folding. $R_{1\rho}$ is the rate constant for the decay of magnetization parallel to the effective field in the rotating frame:

$$R_{1\rho} = R_1 \cos^2\theta + R_2^\circ \sin^2\theta + R_{ex} \sin^2\theta \quad (1)$$

where R_1 is longitudinal relaxation, R_2° is transverse relaxation in the absence of chemical exchange, R_{ex} is transverse relaxation due to chemical exchange, and θ is the tilt angle between the static magnetic field and effective field in the rotating frame. For intermediate exchange processes, the exchange contribution is often distinguished from the transverse and longitudinal relaxation terms due to its characteristic field strength dependence. For solution NMR studies, there is a field strength dependent expression relating R_{ex} to isotropic chemical shift differences between species in exchange, populations, rates and other spectroscopic parameters, allowing for site-specific, quantitative measurement of rates of chemical exchange in proteins and even revealing structural details of minor species (Mulder et al. 1999; Korzhnev et al. 2003; Lundström and Akke 2005; Massi et al. 2004; Loria et al. 2008). In contrast, solid state $R_{1\rho}$ studies have been used primarily to monitor relaxation due to dipolar interactions (Krushelnitsky et al. 2002; Farès et al. 2005). Because the chemical shift anisotropy (CSA) is not averaged in solids, there is an opportunity to monitor $R_{1\rho}$ relaxation due to reorientation of the CSA tensor as well, as has been discussed theoretically for fast limit processes (Farès et al. 2005). Monitoring the $R_{1\rho}$ relaxation due to reorientation of a CSA tensor during magic angle spinning (MAS), and quantitative interpretation of the data could represent a site-specific probe of intermediate exchange motion in

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non-soluble polymers, including systems for which the isotropic shift is insensitive to the motion.

To characterize the sensitivity of $R_{1\rho}$ relaxation to modulation of a CSA tensor, the conformational exchange process of solid dimethyl sulfone (DMS) was studied computationally (Fig. 1) and experimentally (Fig. 2). Solid DMS has a well-characterized motion and has been frequently studied by solid-state NMR methods (Solum et al. 1983; Brown et al. 1996; Gérardy-Montouillout et al. 1996; deAzevedo et al. 2000). The dynamics of the two chemically equivalent carbon atoms in this molecule can be modeled as a two-site chemical exchange process in which the atoms undergo a 180° reorientation around the molecule's C_2 axis. Simulations of on-resonance $R_{1\rho}$ relaxation due to chemical exchange of the carbon CSA tensor of DMS were performed using a variety of exchange rates and an MAS rate of 10 kHz (Fig. 1a). The most notable feature of these simulations is the dramatic increase in relaxation rate observed as the power of the applied spin-lock approaches the first and second rotary resonance conditions ($\omega_1 = n\omega_R$). The dispersion curve, or dependence of the relaxation rate on the field strength used in the spin lock, is complicated in magic angle spinning experiments as compared with solution NMR experiments. In addition to the dynamic effects of interest, which have a number of spectral density terms (Farès et al. 2005), rotary resonance phenomena also occur (Gan and Grant 1990), namely crystallite dependent chemical shifts that occur on matching conditions and also lead to dephasing. The presence of multiple dephasing processes near this matching condition leads to complex, multi-exponential behavior, which complicates the analysis and will be further analyzed in a subsequent paper. At present, we have chosen to use spin-lock powers above the second rotary resonance condition, where the decay of magnetization is expected to be primarily due to dynamical effects, and experimentally displays behavior closer to single-exponential.

On-resonance $R_{1\rho}$ measurements were performed on DMS over a range of spin-lock powers above the $\omega_1 = 2\omega_R$ condition. Peak integrals as a function of spin-lock length were fit to single exponentials to extract $R_{1\rho}$. A dispersion curve, $R_{1\rho}$ as a function of applied field strength, is presented in Fig. 2, which exhibits qualitative features consistent with the simulations in Fig. 1b. Specifically, for elevated temperatures, where the motion is thermally activated, the relaxation rate $R_{1\rho}$ increases dramatically as the field strength approaches the $\omega_1 = 2\omega_R$ condition. In addition, in data that will be presented in a forthcoming publication, the rate is strongly dependent upon applied magnetic field strength, as expected.

A concern regarding quantitative interpretation of these data is that dipolar couplings of the carbon to neighboring protons, and conformational exchange that modulate this coupling, could also contribute to the rotating frame relaxation. Indeed, previous measurements of $R_{1\rho}$ relaxation due to methyl rotation suggest that when no proton decoupling is applied, this contribution may be significant (Akasaka et al. 1983). Residual heteronuclear dipolar interactions during proton decoupling could also contribute to $R_{1\rho}$, since conformational dynamics that modulate the heteronuclear coupling strength can interfere with the efficiency of decoupling (Long et al. 1994; Chevelkov et al. 2007). To clarify whether dipolar effects are likely to be significant for these data, a number of control experiments were performed. The 30°C DMS experiments, with 30 kHz spin-lock power, were repeated using a range of decoupling powers during the spin-lock, and very little difference was observed among the data sets, suggesting that the ^{13}C–^{1}H coupling is not likely to be a significant contribution. (For example, $R_{1\rho} = 13.0 \pm 0.2\ s^{-1}$ for 125 kHz proton decoupling, but

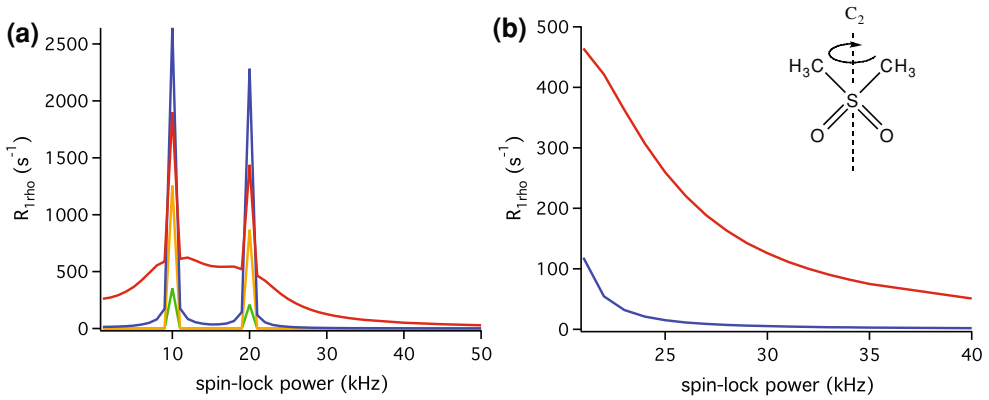

Fig. 1 Simulations of DMS $R_{1\rho}$ relaxation, showing the dependence of $R_{1\rho}$ on the timescale of dynamics **a**: $k_{ex} = 1$ Hz (*green*), 1 kHz (*blue*), 30 kHz (*red*), infinitely fast (*yellow*). **b** Expansion of intermediate exchange simulations in the region of 20–40 kHz applied spin-lock power. (*inset*) Structure and motional mode of DMS. Parameters to model the reorientation of the DMS CSA tensor were $\delta = -36.77$ ppm, $\eta = 0.0625$, $\beta = 109°$ (Gérardy-Montouillout et al. 1996). Simulations were performed using Spinevolution (Veshtort and Griffin 2006)

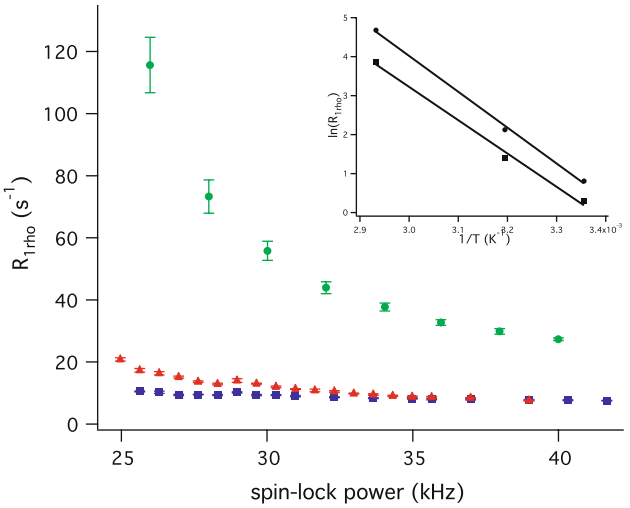

Fig. 2 Experimental $R_{1\rho}$ values for the methyl carbon of DMS as a function of on-resonance spin-lock powers, at 15 (■), 30 (▲), and 58°C (●) collected with 100–125 kHz proton decoupling during the spin-lock. (*Inset*) The natural log of the decay rate is plotted as a function of inverse temperature for spin lock field strengths of 25 and 30 kHz (*upper* and *lower traces*, respectively). The $R_{1\rho}$ values were measured by extracting the area under the peak and fitting to a single-exponential. Error in the fit to a single exponential is indicated with *error bars*. NMR experiments were performed on a Varian 400 Infinity Plus spectrometer using a 4 mm probe at an MAS rate of 10 kHz (±3 Hz). The pulse sequence used for the on-resonance $R_{1\rho}$ experiments is shown in Supplementary Fig. 1. The spin-lock on the carbon channel was applied at fields of 25–40 kHz with continuous wave decoupling of 86–125 kHz on the proton channel during the spin-lock. For each scan, a 768 point FID was collected with a rotor-synchronized 100 μs dwell time. $R_{1\rho}$ data was collected at temperatures of 15, 30, and 58°C for DMS and 30°C for d_6-DMS and alanine. The temperatures were measured at the end of the variable gas flow line. Sample temperatures are expected to be ∼10° higher due to magic angle spinning

$R_{1\rho} = 12.7 \pm 0.4\ s^{-1}$ for 86 kHz decoupling. The rate was dramatically faster without application of decoupling as expected: $R_{1\rho} = 330 \pm 10\ s^{-1}$.) Control experiments were also performed with deuterated DMS to observe the contribution of dipolar interactions to $R_{1\rho}$. Figure 3(b) shows dispersion curves contrasting deuterated and protonated DMS as a function of applied spin-lock field. The essential features in the dispersion curve as the rotary resonance condition is approached appear to be largely independent of the presence of absence of proton couplings, confirming that the major contribution to $R_{1\rho}$ in these experiments is the chemical exchange of the CSA tensor. We have also confirmed that the features are, as expected, dependent on the presence and rate of chemical exchange. For this purpose, the β-methyl group of alanine was chosen as a static control system. The β-carbon of alanine has dipolar couplings to methyl protons and the methyl group undergoes fast rotation, analogous to DMS, but there is no chemical exchange process and thus no reorientation of the carbon CSA tensor occurs. Figure 3a displays $R_{1\rho}$ for DMS and alanine as a function of applied spin-lock power. $R_{1\rho}$ for alanine has no dependence on the applied field in this regime, leading to the conclusion that the feature above $\omega_1 = 2\omega_R$ in the $R_{1\rho}$ dispersion curve for DMS is due to reorientation of the carbon CSA tensor, not primarily dipolar couplings to protons or fast rotation of the methyl group. Analogously, at low temperature, the chemical exchange process for DMS is suppressed (although while the methyl rotation and couplings to the protons are still present); it is notable that the elevated relaxation above the rotary resonance condition is suppressed as expected.

Field strength independent $R_2°$ processes are evident in the dispersion curves, although extracting them quantitatively with this limited spin lock power variation may be fraught. For protonated DMS, these processes appear to contribute a magnitude of $\sim 8\ s^{-1}$ to $R_{1\rho}$ values of DMS at 15 and 30°C. A much lower value is observed for deuterated DMS, consistent with the hypothesis that proton motions in the methyl rotation are a dominant contribution to $R_2°$. For alanine these processes appear to be considerably more efficient than for DMS, probably because of a different (longer) methyl three-site-hop correlation time.

The dependence of the dispersion curve on motion rate (or temperature) clearly illustrates that $R_{1\rho}$ is sensitive to the timescale of dynamics in a model exchange system, suggesting that the reorientation of a CSA tensor can be used to observe conformational exchange. A plot of $\ln(R_{1\rho})$

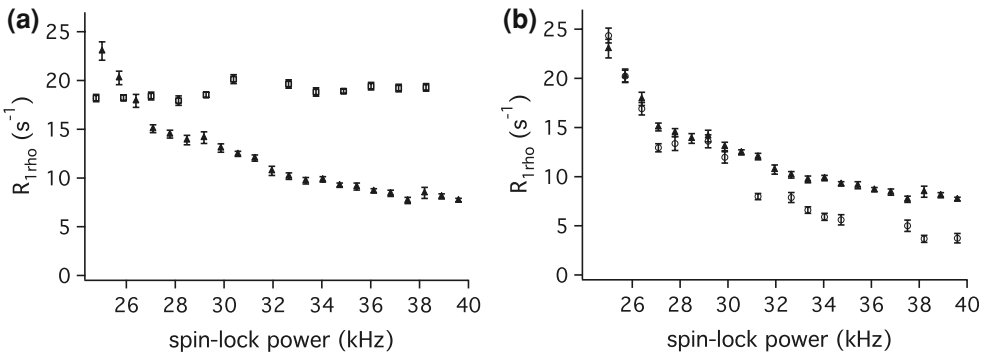

Fig. 3 **a** Experimental dispersion curves, $R_{1\rho}$ values as a function of spin lock strength, for DMS (▲) and alanine (□) at 30°C with 100 kHz proton decoupling applied during the spin-lock. **b** Experimental dispersion curves for protonated (▲) and deuterated (○) DMS at 30°C. Both sets of experiments in (**b**) utilized 86 kHz proton decoupling during the spin-lock to ensure equal sample heating

as a function of inverse temperature gives a slope of the order of 75 kJ/mol (see Fig. 2 inset), which is consistent with prior studies (Brown et al. 1996).

In conclusion, a new application of solid-state $R_{1\rho}$ experiments to monitor conformational exchange processes is presented. These experiments are sensitive to dynamics on the microsecond-to-millisecond timescale as illustrated by the effect of thermal activation of the motion of DMS. Detailed quantitative comparisons of experimental $R_{1\rho}$ data with simulations may in future studies allow for the quantitative measurement of exchange rate constants, hop angles, and other characteristics of a chemical exchange process.

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