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Protein Side-Chain Dynamics Observed by Solution- and Solid-State NMR: Comparative Analysis of Methyl ^{2}H Relaxation Data

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It is well-known that protein structures in solution are generally very similar to those found in hydrated crystals. Relatively little has been done, however, to compare the internal dynamics of proteins in solution and in solids.[1−4] The progress in this area has been hampered by the lack of high-resolution solid-state NMR techniques. With recent advances in solid-state spectroscopy, it became possible to obtain dynamic information on a per-residue basis using uniformly labeled protein samples.[5,6] In this communication we demonstrate how solid-state relaxation data collected in this manner can be analyzed jointly with solution-state data.

Side-chain methyl groups that dominate the protein hydrophobic core are among the most interesting dynamic entities in the protein. A convenient probe of methyl dynamics is provided by deuterium relaxation. ^{2}H relaxes via a quadrupolar mechanism, with the quadrupolar tensor essentially invariant among different methyl sites.[7] The relaxation is driven mainly by the fast spinning of the methyl groups, so that the T_1^{-1} rates are approximately proportional to the corresponding correlation times, τ_f^{Me} (Figure S1, Supporting Information (SI)). The rates vary substantially from one site to another since τ_f^{Me} are sensitive to the details of the van der Waals environment.[8−10]

To compare methyl dynamics in solid and solution NMR samples we conducted a series of relaxation measurements on the SH3 domain from chicken α-spectrin. Protein was expressed in *E. coli* by growing cells in 100% D_2O, using 3-[60%-^{2}H,^{13}C]-labeled pyruvate as the sole carbon source.[11] Pulse sequences used to measure solution-state ^{2}H T_1, $T_{1\rho}$, T_{1zz} and ^{15}N T_1, $T_{1\rho}$, NOE were adapted, with minor alterations, from the literature.[12,13] A newly developed pulse sequence for solid-state ^{2}H T_1 measurements is shown in Figure S2. The data were collected at 10 °C, 600 MHz, 24 kHz MAS frequency.

Two additional samples, u(^{2}H,^{13}C,^{15}N) and u(^{13}C,^{15}N),50%-^{2}H, were prepared for solid and solution experiments, respectively, using glucose as a carbon source. All measurements were repeated with these samples; in the case of solids, the recently reported ^{2}H T_1 pulse sequence was used.[6] The quality of the spectra for uniformly ^{13}C-labeled material was somewhat lower; therefore, only the data from Ala and Ile-δ methyls (which are poorly labeled in the pyruvate-based sample) were retained from this data set.

The representative relaxation curves from methyl ^{2}H T_1 measurements are shown in Figure S3 and the correlation between the solid- and solution-state rates is presented in Figure 1a. While Figure 1a establishes a useful point of reference, one should bear in mind that the solution T_1^{-1} rates contain substantial contribution from the overall tumbling. To deal with this contribution, we determined the rotational diffusion tensor of α-spc-SH3 using ^{15}N relaxation data.[13,14] We further interpreted the set of solution-state methyl ^{2}H

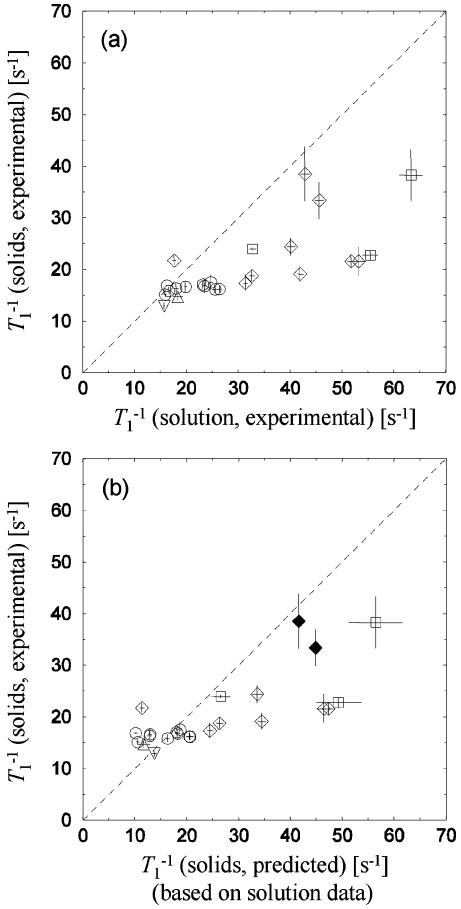

Figure 1. ^{2}H T_1^{-1} relaxation rates for 24 methyl sites in α-spectrin SH3 domain. Solid-state rates are plotted against (a) respective solution-state rates, and (b) predicted solid-state rates, where the prediction is based on the analyses of solution data. Methyls are labeled as □ (Ala), ◊ (Val), △ (Ile-γ), ▽ (Ile-δ), and ○ (Leu). Two Val-23 sites are indicated by filled symbols. The correlation coefficient for the data in panel b is $r = 0.76$.

rates, T_1^{-1}, $T_{1\rho}^{-1}$, and T_{1zz}^{-1}, in terms of the Lipari−Szabo model[15,16]

$$J(\omega) = \left(\frac{1}{9}\right)S^2\frac{\tau_R}{1+\omega^2\tau_R^2} + \left(1-\left(\frac{1}{9}\right)S^2\right)\frac{\tau}{1+\omega^2\tau^2}$$

$$\tau^{-1} = \tau_f^{-1} + \tau_R^{-1} \quad (1)$$

The fast-motion correlation time, τ_f, and its associated order parameter, $(^1/_9)S^2$, were treated as fitting variables, whereas τ_R was fixed according to ^{15}N data, $\tau_R = 6.0$ ns.[17] The time τ_f is mainly determined by the methyl rotation, τ_f^{Me}, but also reflects backbone and side-chain librations as well as fast rotameric jumps (provided that these jumps connect substantially populated rotameric states).[18−20]

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In using the two-parameter Lipari−Szabo model we neglected the possible effect of slower (∼1 to 10 ns) rotameric transitions in methyl-bearing side chains. It has been previously demonstrated that these transitions play a role only for a small fraction of all residues.[21,22] Spectral density mapping[23] confirmed that eq 1 adequately describes all methyls with a notable exception of Val-23 (see Figure S4). Our previous crystallographic studies and ^{2}H MAS line shape analyses showed that the side chain of Val-23 samples multiple conformations in solids.[6,24]

The best-fit S^2 and τ_f values obtained from the analysis of the solution-state data were used to calculate rotation-free spectral densities

$$J(\omega) = \left(1 - \left(\frac{1}{9}\right)S^2\right)\frac{\tau_f}{1 + \omega^2\tau_f^2} \qquad (2)$$

and subsequently predict solid-state ^{2}H T_1^{-1} relaxation rates. Those predicted rates are correlated with the experimental solid-state rates in Figure 1b.

Figure 1b demonstrates a substantial degree of similarity between methyl dynamics in solids and solutions. The solid-state rates, however, tend to be more homogeneous and lower than expected. We attribute this effect to ^{2}H−^{2}H spin diffusion which occurs under the conditions of the MAS experiment.[25] In brief, the interchange of magnetization between different ^{2}H sites tends to equalize the observable relaxation rates. In particular, partial averaging takes place between rapidly relaxing methyls and slowly relaxing "rigid" sites. The resulting trend toward lower and more uniform apparent rates is especially visible for several Ala and Val methyls where the expected rates are higher than average (points on the right side of the plot).

On the basis of the formalism by Gan and Robyr,[26] we conducted a series of numerical simulations to evaluate the effects of ^{2}H spin diffusion on the measured solid-state relaxation rates (see SI for details). It has been estimated, for instance, that the coupling between 3-^{2}H$^\gamma$ and ^{2}H$^\beta$ in the valine side chain typically causes a drop of 0−4 s^{-1} in the measurable methyl relaxation rate (depending on chemical shift offset between the two spins and on methyl τ_f). At the same time, this coupling increases the effective relaxation rate of ^{2}H$^\beta$, in agreement with our previous data.[27] The methyls play, therefore, a familiar role of "relaxation sinks".[28] We also simulated spin diffusion between two proximal methyl groups belonging to different residues. The changes in apparent relaxation rates up to 4 s^{-1} have been found in these simulations. Although accurate analysis of spin diffusion in the extended spin network is not feasible, our simulations clearly account for the trends observed in Figure 1b.

After making an allowance for the spin diffusion, our data suggest that there is a high degree of similarity between methyl dynamics in solid and in solution. Indeed, in small globular proteins such as the SH3 domain the hydrophobic core is encapsulated in a fairly rigid scaffold. In this sequestered environment, side chain motion (and particularly the rotation of methyl groups) does not depend on whether the sample is classified as liquid or solid, so long as the protein remains in contact with a "thermal bath" represented by a large pool of fluid water.

Since methyl ^{2}H T_1^{-1} relaxation rates are controlled by rapid methyl spinning, these data are well suited to demonstrate the similarity between solution- and solid-state dynamics. Once the similarity is established, it opens up some interesting possibilities for future studies. In backbone, for example, fast local dynamics (τ_f) is relatively inefficient in causing relaxation so that slow forms of internal motion (τ_s) can play a significant role.[29] Of special interest is the situation where solution data are sensitive to τ_f and τ_R, while solid-state data are sensitive to τ_f and τ_s. In this case, the combination of the two methods can be particularly useful, providing valuable information about slow collective motions.[30,31]

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Supporting Information Available: MD simulations of methyl ^{2}H T_1 data; solid-state pulse sequence for measuring ^{2}H T_1 relaxation; solid- and solution-state relaxation curves; spectral density profiles; table of S^2 and τ_f values; simulations of ^{2}H−^{2}H spin diffusion in MAS experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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