$^1H-^{13}C$ Dipole−Dipole Cross-Correlated Spin Relaxation As a Probe of Dynamics in Unfolded Proteins: Application to the DrkN SH3 Domain

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A complete understanding of protein folding requires characterization of the structures and dynamics of all states along the folding pathway. NMR spectroscopy can be a powerful technique for studying unfolded or partially unfolded molecules via $^1H-^2H$ exchange or measurement of chemical shifts, scalar coupling constants, and NOEs, for example.[1,2] ^{15}N spin relaxation studies have also provided insights into the structures of disorded proteins, indicating that while these molecules are significantly more dynamic than their folded counterparts they can be quite compact.[3−6] One example is the N-terminal SH3 domain from the *Drosophila* protein drk (drkN SH3), which exists in equilibrium between folded (F_{exch}) and unfolded (U_{exch}) states in aqueous buffer and near neutral pH.[7] Studies of the drkN SH3 domain in 2M guanidinium chloride have established a correlation between the hydrophilicity of residues and backbone ^{15}N dynamics, with regions of the molecule enriched in hydrophobic amino acids less mobile.[4] To obtain more quantitative information about dynamics in unfolded protein states we have measured $^1H^\beta-^{13}C^\beta$ dipole−dipole cross-correlated spin relaxation[8] in both the F_{exch} and U_{exch} states of the drkN SH3 domain. Most, but not all, of the aromatic side chains in the U_{exch} state have reduced mobility on a ps−ns time scale, while other hydrophobic side chains such as Leu have much less restriction of motion. These results suggest that aromatic residues may be involved in interactions in the U_{exch} state of the drkN SH3 domain.

Cross-correlated dipole−dipole spin relaxation has long been used as a probe of molecular dynamics[9,10] and more recently of solution structural properties as well.[11,12] In cases where the relative orientation of the two interacting dipoles is known, the cross-correlation rate provides information directly on the dynamics of the interacting spins. Consider, for example, a $^{13}CH_2$ spin system. In the absence of cross-correlation a 1:2:1 multiplet structure is observed in the carbon spectrum. Applications to proteins and unfolded states in particular are limited by resolution, and cross-correlation rates at the $^{13}C^\beta$ side chain position are most readily obtained from CBCA(CO)NNH-type spectra where correlations of the form $(\omega[^{13}C^\beta] + k\pi J_{HC},\omega[^{15}N],\omega[^1HN])$ with $k = \pm 2, 0$ are observed.[8] In this case, the dipole−dipole cross-correlation relaxation rate derived from the two $^1H-^{13}C$ dipoles in the CH_2 group, $\Gamma_{HC1,HC2}$, is given by the relation,

$$\Gamma_{HC1,HC2} = -(0.25/T)\ln\{4 \times I_U(T) \times I_D(T)/I_C(T)^2\} \quad (1)$$

where I_U, I_D and I_C are the intensities of the $^{13}C^\beta$ multiplet components for $k = -2$, 2, and 0, respectively, and T is the duration of the ^{13}C constant time evolution period during which cross-correlated spin relaxation evolves.[8] $\Gamma_{HC1,HC2}$ can, in turn, be written in terms of spectral densities which are evaluated in the context of a particular motional model.[13] In the case of applications to unfolded proteins a quantitative analysis of cross-correlation data is difficult. Nevertheless, a simple comparison of $\Gamma_{HC1,HC2}$ values in the U_{exch} and F_{exch} states allows an evaluation of which residues in the unfolded state have restricted dynamics on a ps−ns time scale. Because $\Gamma_{HC1,HC2}$ is sensitive to motions which modulate the position of the $C^\beta-H^\beta$ bond vectors, including backbone and side chain (rotation about $\chi1$, for example) dynamics, $\Gamma_{HC1,HC2}$ values provide insights that are not available from ^{15}N relaxation studies of unfolded proteins.

Figure 1 illustrates ^{13}C traces for F9 and Y37 of the drkN SH3 domain in 50 mM sodium phosphate, pH 6.0, 5 °C (U_{exch}, F_{exch}), and in 2 M guanidinium chloride, pH 6.0, 5 °C (U_{Gdn}). For both F9 and Y37 in the folded (F_{exch}) state significant deviations from 1:2:1 multiplet components are observed, corresponding to $\Gamma_{HC1,HC2}$ values of -25.4 ± 0.8 s^{-1} (F9) and -21.8 ± 1.5 s^{-1} (Y37). Note that in the case of complete restriction of motion a value of $\Gamma_{HC1,HC2} \approx -26$ s^{-1} is estimated for the F_{exch} state of the drkN SH3 domain (correlation time of 8.8 ± 0.4 ns as established by ^{15}N spin relaxation[14]). F9 in both the U_{Gdn} and U_{exch} states has significantly more mobility than in the folded molecule, with $\Gamma_{HC1,HC2}$ values of -5.1 ± 0.1 s^{-1} and -5.6 ± 0.1 s^{-1}, respectively. Less negative values of the cross-correlation relaxation rate may be due to significant ps−ns time scale dynamics involving either backbone or side chain degrees of freedom (or both). In contrast, increased values of $|\Gamma_{HC1,HC2}|$ imply restricted motion (ns−ps) for both backbone and side chain positions. As an example, Y37 is more rigid than F9 in both the U_{Gdn} ($\Gamma_{HC1,HC2} = -11.3 \pm 0.1$ s^{-1}) and U_{exch} ($\Gamma_{HC1,HC2} = -15.2 \pm 1.2$ s^{-1}) states. In general, residues of the drkN SH3 domain which have restricted mobility (ps−ns) in both U_{Gdn} and U_{exch} states show increased mobility when the domain is dissolved in 2 M guanidine (U_{Gdn}). This is consistent with backbone ^{15}N relaxation data which indicate that the U_{exch} state is somewhat more compact than the U_{Gdn} state.[4] However, the presence of some $\Gamma_{HC1,HC2}$ values in the U_{Gdn} state that are more negative than the corresponding values in the U_{exch} state (Supporting Information) indicates that guanidine stabilizes different conformers within the unfolded state ensemble.

Figure 2 compares $\Gamma_{HC1,HC2}$ values for the hydrophobic residues in the U_{exch}, U_{Gdn}, and F_{exch} states of the drkN SH3 domain. L41 and L47 have significantly reduced values of $|\Gamma_{HC1,HC2}|$ in the

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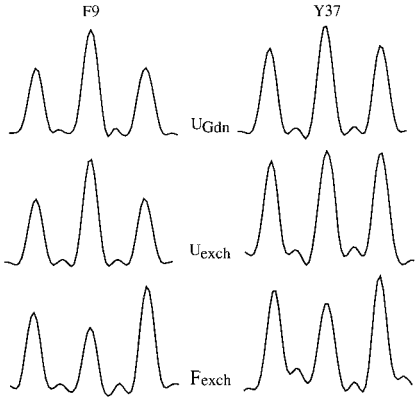

Figure 1. ^{13}C traces from CBCA(CO)NNH spectra recorded by using the pulse scheme and experimental parameters described by Yang et al.[8] (see Figure 1b of ref 8). Samples of ^{15}N,^{13}C labeled drkN SH3 domain (1.5 mM), 0.05 M sodium phosphate, 90% H_2O/10% D_2O, pH 6.0, 5 °C either without (U_{exch}/F_{exch}) or with (U_{Gdn}) guanidinium chloride (2 M) were prepared as described previously.[3,4] Spectra were recorded on a Varian Inova 600 MHz spectrometer.

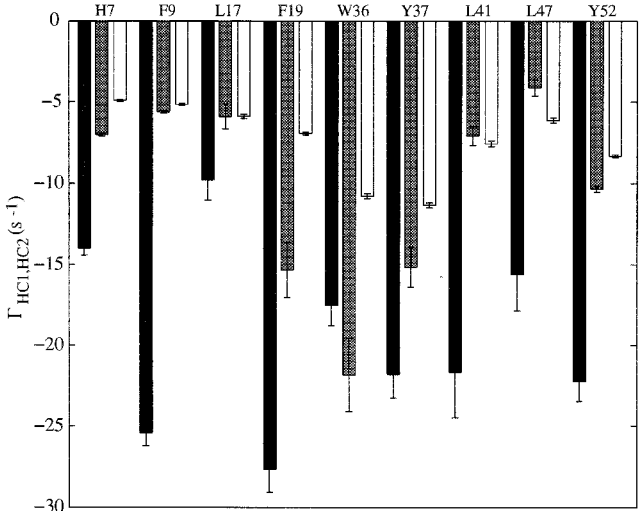

Figure 2. Comparison of $\Gamma_{HC1,HC2}$ values for hydrophobic residues in the F_{exch} (black), U_{exch} (shaded), and U_{Gdn} (white) states for which data from all states are available.

U_{exch} and U_{Gdn} states relative to the folded molecule indicating increased ps−ns time scale dynamics in these states. In contrast, reasonably large values of $|\Gamma_{HC1,HC2}|$ are measured for many of the aromatics in the U_{exch} state. The reduced mobility of these amino acids may be due to their involvement in tertiary contacts which stabilize the turn-like structures which are present in the unfolded drkN SH3 domain.[7,15] The larger $|\Gamma_{HC1,HC2}|$ value observed for W36(U_{exch}) than for W36(F_{exch}) is particularly noteworthy in lieu of recent results from other studies of this domain.[15] First, stopped-flow fluorescence measurements have suggested that W36 is more buried in the U_{exch} state than in the F_{exch} state; second, several long-range NH−NH NOEs (separated by as many as 28 residues) are observed between the side chain NH of W36 and proximal backbone amides in the drkN SH3 domain U_{exch} state; third, structural studies of the drkN SH3 domain based on NOEs obtained in a fully deuterated protein show a solvent accessibility of 6% for W36 in the U_{exch} state compared to 30% in the folded state.[15] The data presented here are supportive of a number of NOE and fluorescence experiments suggesting that aromatic residues, particularly tryptophans, are involved in hydrophobic clusters in molten globule and folding intermediate states in a variety of proteins.[16−19]

While the cross-correlation results described above provide information about the restriction of fast time scale motion (ps−ns) it is of considerable interest to study the dynamics on slower time scales as well. Many $^1H-^{15}N$ correlations in spectra of the U_{exch} state show considerable broadening at 5 °C, including a region extending from Q23-L28 where very weak or no signals are found.[20] Line broadening is a signature of conformational fluctuations on a ms−μs time scale and in the case of the U_{exch} state likely reflects the presence of interactions which partially stabilize a subset of conformations. Scalar coupling constants provide motional information spanning a wide range of time scales, and $^3J_{C'C\gamma}$, $^3J_{NC\gamma}$ coupling values have been measured for both U_{exch} and F_{exch} states.[21,22] For side chains in well-defined canonical $\chi1$ conformations $^3J_{C'C\gamma}$ values >3 Hz or <1.5 Hz are observed while $|^3J_{NC\gamma}|$ values of ≈2 or <0.5 Hz are measured. In contrast, in the case of significant averaging about $\chi1$, values on the order of 2 Hz and −1 are obtained for $^3J_{C'C\gamma}$ and $^3J_{NC\gamma}$, respectively. The coupling data for the drkN SH3 domain indicate that extensive averaging about $\chi1$ occurs for most of the residues in the U_{exch} state. In particular, the ($^3J_{C'C\gamma}$,$^3J_{NC\gamma}$) values change from (4.3,≈0) and (3.3,−0.3) for W36 and Y37 of the F_{exch} state to (2.3,−1.1) and (2.0,−1.3) in the U_{exch} state. Although the $C^\beta-H^\beta$ cross-correlation data for W36 and Y37 of the U_{exch} state indicate that large amplitude rotation about $\chi1$ does not occur on a ps−ns time scale, the coupling data suggest that slower averaging of a significant amplitude is present. Because this rotation has little influence on $\Gamma_{HC1,HC2}$ the time scale for the motion must be greater than ≈100 ns.[23]

In summary, we have presented a $C^\beta-H^\beta$ cross-correlated spin relaxation study of the drkN SH3 domain. A significant fraction of the aromatic side chains in the U_{exch} ensemble have restricted mobility on a ps−ns time scale, consistent with their involvement in interactions leading to a compact unfolded state. Differences in $\Gamma_{HC1,HC2}$ values in the U_{exch} and U_{Gdn} states indicate that guanidine affects the relative populations of different conformers within the unfolded state ensemble and does not merely disrupt interactions.

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Supporting Information Available: Table of $\Gamma_{HC1,HC2}$ values for the F_{exch}, U_{exch}, and U_{Gdn} states of the drkN SH3 domain, table of ($^3J_{C'C\gamma}$,$^3J_{NC\gamma}$) values for the aromatic residues in F_{exch} and U_{exch} states, and a figure illustrating all of the $\Gamma_{HC1,HC2}$ values (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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