Identification of Partially Disordered Peptide Intermediates through Residue−Specific NMR Diffusion Measurements

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · SEPTEMBER 2005

Impact Factor: 12.11 · DOI: 10.1021/ja052801d · Source: PubMed

J|A|C|S
COMMUNICATIONS

Published on Web 07/09/2005

Identification of Partially Disordered Peptide Intermediates through Residue-Specific NMR Diffusion Measurements

Yingjie Li,[‡] Seho Kim,[‡] Barbara Brodsky,[§] and Jean Baum*,[‡]

Department of Chemistry, Rutgers University, Piscataway, New Jersey 08854, and Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

Received April 29, 2005; E-mail: baum@rutchem.rutgers.edu

It is technically challenging to detect the low population of partially disordered species that are in equilibrium with the folded and unfolded states. Characterization of these states is critical to understand protein-folding processes and equilibrium properties. The ability of NMR to probe the exchange dynamics of interconverting species of proteins at specific residues allows the design of sophisticated experiments to approach the identification of these intermediates. Recently, relaxation dispersion NMR was used to define folding intermediates in equilibrium with its unfolded and fully folded states for an SH3 domain.[1] Here, residue-specific translational diffusion coefficients are used to detect equilibrium intermediates in self-associating systems. This methodology is applied to equilibrium solutions of related triple helical peptides that model a small region of type I collagen with and without a mutation known to cause osteogenesis imperfecta.[2a] The peptides are composed of interconverting trimer and monomer species, where the trimer folded form has significantly different hydrodynamic parameters from the unfolded state, allowing residue-specific diffusion coefficients to be sensitive to the existence of partially disordered intermediates.

Triple helical peptides of varying lengths and sequences have been used to model folding, stability, and binding regions of collagen.[2] NMR spectra of such peptides always show distinct monomer and trimer resonances, indicating slow interconversion of these two populations on the NMR time scale.[3] A peptide (T1−892c) designed to model type I collagen, with a collagen G−X−Y sequence (α1(I) chain residues 892−909) and a $(GPO)_4$ sequence on the C terminus was found to have trimer and monomer peaks at four labeled positions along the chain, and relaxation studies indicated rigid trimers are in equilibrium with flexible monomers.[3a,4] Similar results were found for a related peptide of identical sequence but with the $(GPO)_4$ sequence located at the N terminus (T1−892n).[3b] A peptide homologous to T1−892c with a G-to-A change modeling a known OI mutation site (T1−892c[G10A]) showed trimer peaks only C terminal to the mutation site at A10, suggesting the mutation prevented complete folding and resulted in a partially folded molecule that is triple helical C terminal to the mutation site and disordered at the N-terminal end.[3c]

Translational diffusion coefficients have been largely used to investigate hydrodynamic properties of folded and unfolded globular proteins as well as having been applied to monitor kinetic folding processes in a globular protein and in collagen model peptides.[5,6] NMR pulsed field gradient diffusion measurements were obtained for peptides T1−892c, T1−892n, and T1−892c[G10A]. Specific ^{15}N labeling of individual residues within each peptide chain allows residue- and position-specific diffusion measurements to be obtained using the CCLED-HSQC pulse sequence (Supporting Information, Figure S1).[6] Translational diffusion coefficients were obtained by single-exponential fitting for monomer and trimer resonances of all labeled positions within the three peptides at 10 and 40 °C (Supporting Information, Figure S2B).

At 40 °C, where the three peptides exist as unfolded monomer species, the corrected diffusion coefficients to 10 °C (denoted as $D_M^{40°C}$) for all labeled positions within a given peptide are similar to one another ($9.88-10.87 \times 10^{-7}$ cm^2/s) (see Supporting Information). The uniformity for residues at different positions is consistent with sampling a similar averaged denatured shape. Comparison among the three peptides shows comparable $D_M^{40°C}$, reflecting the similar molecular weights and hydrodynamic properties. At 10 °C, the monomer is in equilibrium with the trimer and the diffusion coefficients of monomer peaks (denoted as $D_M^{10°C}$) as well as trimer peaks (denoted as $D_T^{10°C}$) can be measured within a single experiment. The $D_T^{10°C}$ ($5.23-6.43 \times 10^{-7}$ cm^2/s) are significantly smaller than $D_M^{10°C}$ and $D_M^{40°C}$ as expected for the 3-fold increase in molecular weight and the acquisition of the rodlike shape.

The relationship between the $D_M^{10°C}$ and $D_M^{40°C}$ differs among the three peptides (Figure 1). In T1−892n the $D_M^{10°C}$ for all labeled residues (G7, A18, G25) are similar to the corrected $D_M^{40°C}$ values ($10.13-10.88 \times 10^{-7}$ cm^2/s) (Figure 1A). For T1−892n, the slow interconversion between monomer and trimer species and the uniform diffusion coefficients for all residues in each state support a simple two-state equilibrium of unfolded monomer and fully triple-helical trimer at low temperature (Figure 2A).

For T1−892c and T1−892c[G10A], nonuniform diffusion coefficients are observed along the peptide chain for the monomer and trimer resonances. For T1−892c[G10A], the $D_M^{10°C}$ show higher values at the C terminus, in contrast to the uniform $D_M^{40°C}$ values for all residues (Figure 1B). In particular, the two N-terminal monomer positions (A6, A10) show very low $D_M^{10°C}$ values ($D_M^{10°C} = 5.93, 5.97 \times 10^{-7}$cm^2/s, respectively) that are similar to the trimer $D_T^{10°C}$ values for residues G13 and G25 ($D_T^{10°C} = 5.58, 5.95 \times 10^{-7}$cm^2/s, respectively). No trimer resonances are seen for A6 and A10 since the native state of T1−892c[G10A] is a partially folded conformation with no triple-helix N-terminal to the G10A position. To interpret the nonuniform diffusion coefficients of the monomer resonances, the populations, chemical shifts, and interconversion rates of the partially disordered trimer and the unfolded state need to be considered.[7] Previous NMR data indicate that the partially disordered T1−892c[G10A] trimer is highly populated and that the monomer resonances N terminal to A10 arise primarily from the disordered regions of the partially disordered final state.[3c] Interconversion rates for T1−892c[G10A] between monomer and partially disordered trimer are slow compared to the NMR diffusion experiment time scale resulting in distinct diffusion coefficients for both species.[3] The nonuniform diffusion coefficients of the monomer resonances can therefore be explained by the chemical shift overlap of the pure monomer and the partially disordered trimer; the low N-terminal monomer diffusion coefficients reflect

[‡] Rutgers University.
[§] UMDNJ-Robert Wood Johnson Medical School.

10.1021/ja052801d CCC: $30.25

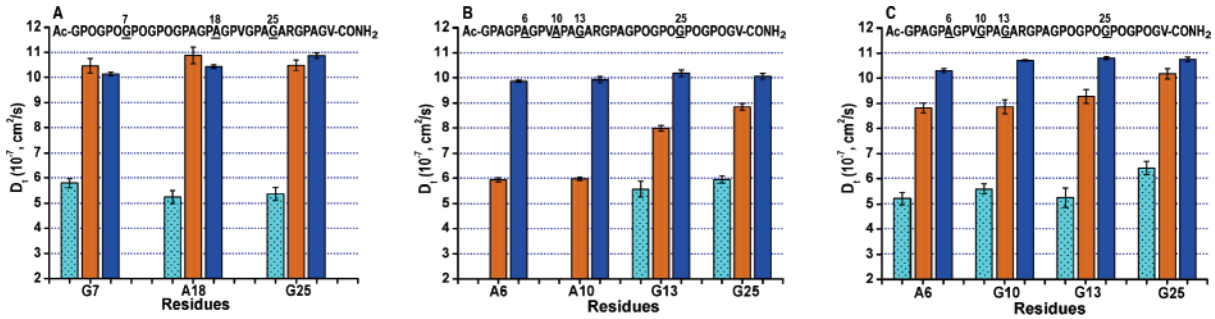

Figure 1. Histogram of the residue-specific diffusion coefficients versus ^{15}N-labeled residues for (A) T1−892n, (B) T1−892c[G10A], and (C) T1−892c. $D_T^{10°C}$, $D_M^{10°C}$, and $D_M^{40°C}$ are shown in dotted cyan, orange, and blue, respectively.

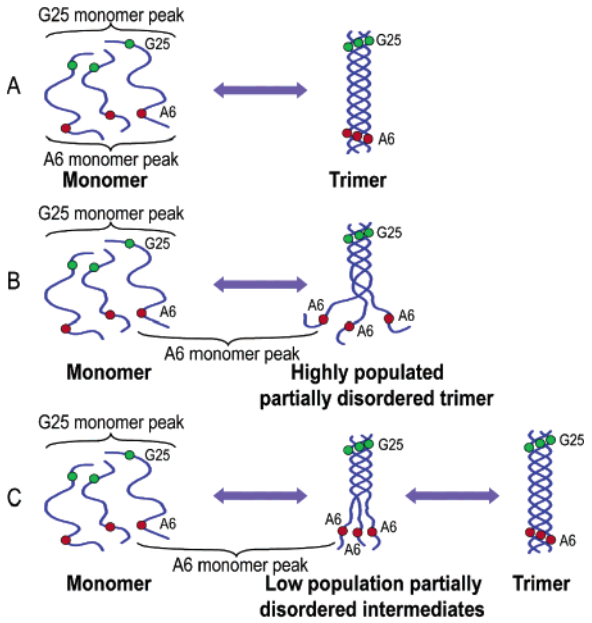

Figure 2. Schematic representation of the equilibrium states of (A) T1−892n, (B) T1−892c[G10A], and (C) T1−892c. The green circle and red circle represent the position of G25 and A6, respectively.

a superposition of a low population of pure monomer species and the highly populated partially disordered trimers (Figure 2B).

For T1−892c, a two-state model of native trimer and unfolded monomer cannot explain the differences among the $D_M^{10°C}$ values for different residues. The $D_M^{10°C}$ value for G25 (10.16×10^{-7} cm^2/s) is similar to the G25 $D_M^{40°C}$ value, while $D_M^{10°C}$ for A6, G10, and G13 are smaller than $D_M^{40°C}$ values. As opposed to T1−892c[G10A], the final folded form of T1−892c has been shown to be a rigid, fully folded triple helix along the entire chain; therefore, the different $D_M^{10°C}$ values here need to be interpreted in terms of the existence of low populations of partially folded species. The similarity of the $D_M^{10°C}$ and $D_M^{40°C}$ of G25 (Figure 1C) indicates the G25 $D_M^{10°C}$ is not modulated by species of molecular weight higher than the monomer, and thus there is no significant population of intermediates with partial unfolding at the C terminus. The decreased values for A6, G10, and G13 compared with the value for $D_M^{40°C}$ indicate sampling by additional species of higher-molecular weight. It is probable that the lower monomer diffusion coefficients reflect the presence of trimer species that are partially disordered at the N terminus, in equilibrium with the fully folded trimer and the unfolded monomer. In this way, the diffusion coefficient of G25 reflects pure monomer species, whereas the diffusion coefficients for the more N-terminal residues reflect a population average of monomer species and partially disordered trimers at the N-terminal end (Figure 2C). Assuming slow interconversion between equilibrium intermediates and monomer species, biexponential fits to the data allow estimates of the relative population of partially disordered species to monomer population (Supporting Information, Table S1). Values of P_{pd}/P_m range from 0.13 (G13) to 0.26 (A6).

The existence and the nature of the equilibrium intermediates derived from the residue-specific diffusion experiments described above may be related to folding pathways of triple helical peptides. Previous kinetic real-time NMR folding experiments have shown that C-to-N-terminal directional folding is observed in peptides where the $(GPO)_4$ nucleation domain is at the C terminus, e.g., T1−892c.[6,8] Here residue-specific diffusion experiments allow identification of a species which is partially disordered at the N-terminus, as expected for this zipper-like directional kinetic pathway.

In summary, the appearance of nonuniform diffusion coefficients within a given peptide suggests that the method can be used to detect the presence and define the conformation of partially disordered species that are in equilibrium with the folded and unfolded states. This method allows discrimination between a simple two-state model and more complex multistate models. Thus, the use of residue-specific diffusion coefficients offers an approach for detection of low population intermediates in self-associating systems. Such experiments may be applicable to aggregation as found in protein-folding diseases.

Acknowledgment. This work was supported by NIH Grants GM45302 (to J.B.) and GM60048 (to B.B.).

Supporting Information Available: Experimental details, Figures S1 and S2, and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Korzhnev, D. M.; Salvatella, X.; Vendruscolo, M.; Di Nardo, A. A.; Davidson, A. R.; Dobson, C. M.; Kay, L. E. *Nature* **2004**, *430*, 586−590.

(2) (a) Baum, J.; Brodsky, B. *Curr. Opin. Struct. Biol.* **1999**, 9, 122−128. (b) Fields, G. B.; Prockop, D. J. *Biopolymers* **1996**, *40*, 345−357.

(3) (a) Liu, X.; Kim, S.; Dai, Q. H.; Brodsky, B.; Baum, J. *Biochemistry* **1998**, *37*, 15528−15533. (b) Buevich, A. V.; Silva, T.; Brodsky, B.; Baum, J. *J. Biol Chem.* **2004**, *279*, 46890−46895. (c) Bhate, M.; Wang, X.; Baum, J.; Brodsky, B. *Biochemistry* **2002**, *41*, 6539−6547.

(4) O is a single letter code for hydroxyproline.

(5) (a) Jones, J. A.; Wilkins, D. K.; Smith, L. J.; Dobson, C. M. *J. Biomol. NMR* **1997**, *10*, 199−203. (b) Wilkins, D. K.; Grimshaw, S. B.; Receveur, V.; Dobson, C. M.; Jones, J. A.; Smith, L. J. *Biochemistry* **1999**, *38*, 16424−16431. (c) Mansfield, S. L.; Jayawickrama, D. A.; Timmons, J. S.; Larive, C. K. *Biochim. Biophys. Acta* **1998**, *1382*, 257−265. (d) Balbach, J. *J. Am. Chem. Soc.* **2000**, *122*, 5887−5888.

(6) Buevich, A. V.; Baum, J. *J Am Chem Soc*. **2002**, *124*, 7156−7162.

(7) Johnson, C. S. *J. Magn. Reson. A* **1993**, *102*, 214−218.

(8) Buevich, A. V.; Dai, Q. H.; Liu, X.; Brodsky, B.; Baum, J. *Biochemistry* **2000**, *39*, 4299−4308.

JA052801D