NMR Structural Characterization of the 6 M Urea-unfolded Ensemble of an Ultrafast Folding Mini-protein

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Whereas detailed structural information is readily available for the native states of proteins, experimentally determined residue-specific information on kinetic intermediates and, in particular, on the unfolded states of proteins remains relatively sparse due to their conformational heterogeneity and dynamic nature. The 'Trp-Cage' molecule (TC5b) is a small, 20-residue mini-protein (NLYIQ WLKDG GPSSG RPPPS) which exhibits a well-defined hydrophobic core together with pronounced secondary and tertiary interactions in its native state [1]. Also, TC5b is known to have one of the fastest kinetic refolding rates ever observed and, because of its small size, has been adopted as a 'de facto' benchmark for computational folding studies and molecular dynamics (MD) simulations. Following our recent observation of hydrophobic cluster formation in the 6 M urea-denatured state of TC5b obtained from photo-CIDNP NOE pulse-labelling experiments [2], we have further elucidated the structural properties of the unfolded state of this highly unusual biomolecule using multidimensional nuclear magnetic resonance (NMR) spectroscopy and heteronuclear backbone relaxation studies (R_1, R_2, and het. NOE) on a uniformly ^{15}N-labelled TC5b construct. Even though residual elements of secondary structure seem to be absent in unfolded TC5b, the results permit a clear identification of sequence-remote contact interactions between aliphatic and aromatic ^{1}H nuclei for those regions of the peptide whose amino acid side chains also exhibit significant cross-polarization in the pulse-labelling experiment [3]. Moreover, an NOE-restrained calculation of an ensemble of unfolded TC5b molecules makes the direct assignment of aromatic tryptophan side chain ^{1}H nuclei as individual sources of cross-polarization feasible thereby further clarifying the CIDNP NOE data. Our results corroborate previous findings of a 'pre-existing' hydrophobic cluster in the unfolded state of TC5b comprising both native and non-native contact interactions. These interactions appear to be crucial prerequisites for the ultrafast folding kinetics of the peptide as suggested both by molecular dynamics (MD) simulation studies as well as independently performed tryptophan fluorescence quenching experiments on the unfolded ensembles of TC5b [4,5].

[1] Neidigh, J. W., Fesinmeyer, R. M., Andersen, N. H., *Nat. Struct. Biol.*, **2002**, *9*, 245-430. [2] Mok, K. H., Kuhn, L. T., Goez, M., Day, I. J., Lin, J. C., Andersen, N. H., Hore, P. J., *Nature*, **2007**, *447*, 106-109. [3] Kuhn, L. T., *D. Phil. Thesis*, **2008**, University of Oxford. [4] Snow, C. D., Zagrovic, B., Pande, V. S., *J. Am. Chem. Soc.*, **2003**, *124*, 14548-14549. [5] Neuweiler, H., Doose, S., Sauer, M., *Proc. Natl. Acad. Sci.*, **2005**, *102*, 16650-16655.

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