Local Conformational Similarity Between Native and Denatured States

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Traditionally, the denatured state of a protein has been viewed as unfolded, having little to no secondary or tertiary structure. Recently, however, residual dipolar couplings have been used to demonstrate the presence of residual structure under denaturing conditions in a number of protein systems [1]. Furthermore, recent computational work has demonstrated that random coil statistics can be produced by allowing only a small fraction of a protein's phi-psi angles to vary [2].

In the present study we seek to experimentally validate this notion that on a local scale (8-12 residues) primary conformations of the native state are represented as significant conformations in the denatured state. The denatured state of staphylococcal nuclease is modeled as an 11 residue peptide corresponding in sequence to a loop region in the parent protein suggested to adopt multiple conformations in the native state. Monoclonal antibodies raised against the peptide and screened for tight binding to the parent protein serve as conformational probes. Isothermal titration calorimetry yields thermodynamic binding parameters from which the relative populations of the bound conformation in the native and denatured states can be obtained. Crystal structures of the peptide and protein bound complexes serve to verify the bound conformations and inform the analysis of the ITC data. Here, we report the structure of the 11-mer peptide in complex with an Fab fragment.

[1] Shortle D., Ackerman M.S., *Sci.*, 2001, **293**, 487. [2] Fitzkee N.C., Rose G.D., *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 12497.

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