MAPPING THE CYTOCHROME *C* FOLDING LANDSCAPE

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Abstract

The solution to the riddle of how a protein folds is encoded in the conformational energy landscape for the constituent polypeptide. Employing fluorescence energy transfer kinetics, we have mapped the *S. cerevisiae* iso-1 cytochrome *c* landscape by monitoring the distance between a C-terminal fluorophore and the heme during folding. Within 1 ms after denaturant dilution to native conditions, unfolded protein molecules have evolved into two distinct and rapidly equilibrating populations: a collection of collapsed structures with an average fluorophore-heme distance ($\bar{r}$) of 27 Å and a roughly equal population of extended polypeptides with $\bar{r}$ > 50 Å. Molecules with the native fold appear on a timescale regulated by heme ligation events (~300 ms, pH 7). The experimentally derived landscape for folding has a narrow central funnel with a flat upper rim on which collapsed and extended polypeptides interchange rapidly in a search for the native structure.

Nonnative states of proteins are involved in a variety of cellular processes, including translocation of proteins across membranes and formation of amyloid fibrils. Probes that report on the structural heterogeneity of a polypeptide ensemble could resolve ambiguities in the classification of these states. We have shown that added anions shift the equilibrium between the compact and extended polypeptide structures that are present during refolding of *Saccaromyces cerevisiae* iso-1 cytochrome *c*. Specifically, at high salt concentrations (≥ 700 mM), all the polypeptides are compact with a mean C-terminal fluorophore-heme separation quite close to that in the native protein (25 Å). Addition of chemical detaturants, on the other hand, tends to shift the equilibrium towards unfolded structures.

Folding of modified Fe(II) cyt *c* was probed by fluorescence in presence of imidazole with NADH as photochemical sensitizer. At very high imidazole concentrations (400 mM),

protein was still found to fold but the rate that coincides with Met80 ligation was slowed down significantly.

Reductive flash-quench/scavenge experiments, in which ascorbic acid was used to scavenge $MeODMA^{\bullet+}$, were shown to keep ferrocyt *c* reduced for up to 500 μs. Electron injection into unfolded modified yeast Fe(III)cyt *c* was fast in comparison to injection using NADH as photochemical sensitizer. The overall electron transfer process was reversible. This photoreduction system could be used to trigger folding of Fe(II) cyt *c* to monitor the changes in dansyl fluorescence intensity on μs time scales.

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To

my son David Gregory Shane

and

my entire family