Unraveling Folding Dynamics of Heme Proteins with Fluorescence Energy Transfer Kinetics

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We have explored the folding mechanisms of cytochrome *c* and cytochrome *c*' through analysis of fluorescence energy transfer (FET) kinetics. Multiple protein variants, with a dansyl fluorophore attached at different sites, have been examined. The dansyl-to-heme FET kinetics have revealed distance distributions in the unfolded state and during a folding reaction. Both proteins, when chemically denatured with guanidine hydrochloride, do not behave as simple random coils. The denatured ensemble is highly heterogeneous and possesses non-native compact structures. These compact structures, which may result from interactions between non-polar protein residues and the hydrophobic heme group, likely play a role in forming a protein hydrophobic core during folding. Folding is triggered by conventional stopped-flow dilution of a denatured protein and by rapid photoinduced reduction. Analysis of cytochrome *c* FET kinetics indicates considerable structural heterogeneity at various points during folding. The experimental distributions argue against global collapse: both compact and extended structures constitute the transient species. Fluctuations among populations of compact and extended structures are prominent throughout the folding process, indicating that non-native collapsed structures are not substantially more stable than extended structures. A more hydrophobic cytochrome *c'* has unusually slow and highly heterogeneous folding kinetics, which may be caused by collapsed folding intermediates. We aim to characterize the transient folding species in cytochrome *c'* in work now in progress.