

Structure-energy relations in hen egg white lysozyme observed during refolding from a quenched unfolded state

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We use infrared spectroscopy to study the evolution of 10 protein folding intermediate structures on arbitrarily slow time scales by rapidly quenching thermally unfolded hen egg white lysozyme in a glassy matrix, followed by reheating of the protein to refold; upon comparison with differential scanning calorimetric experiments, low-temperature 15 structural changes that precede the formation of energetic native contacts are revealed.

The folding of proteins into their functional state is one of nature's most ubiquitous phenomena. Understanding the 20 mechanisms underlying protein folding is important for the rational modification and design of novel proteins, the understanding of human degenerative diseases that are tied to protein misfolding and/or aggregation^{1,2}, and the development of improved formulations to prevent protein degradation during 25 storage. An important objective in unraveling the mechanisms of protein folding is to characterize intermediate or partially folded states in terms of their structural, kinetic and thermodynamic properties.

Current optical methods for studying folding 30 mechanisms with sub- μs resolution do not probe folding from a fully denatured state^{3,4}. Standard stopped-flow techniques, on the other hand, are able to prompt folding from fully-denatured samples, but have a time resolution on the order of 1 ms^{3,4}. Consequently, important events in the refolding of fully 35 denatured states that occur on the μs time scale are not observable, which creates an important gap in fundamental understanding. Recent developments in microfluidics have allowed denaturant dilution in less than 20 μs , offering the prospect of vastly improved time resolution using stopped-flow 40 approaches^{3,4}. Such promising developments notwithstanding, the experimental study of early-stage (μs) folding events from a fully denatured state remains a frontier area of research. Here we present an experimental method that is capable of detecting the changes in intermediate structure during the folding process on 45 arbitrarily slow (arrested) time scales, and starting from the fully thermally-denatured state.

To address the challenge of capturing the structural intermediates as a protein refolds, a recently introduced quench-and-refold approach⁵ was adapted to spectroscopic scrutiny. The 50 approach consists of the following steps: (1) thermally unfold / reversibly denature the protein, (2) trap the unfolded protein into a glassy matrix by quenching the sample in liquid nitrogen (LN₂), and (3) refold the protein by slowly heating the sample across the glass transition temperature using a temperature-controlled unit. 55 At this stage, infrared spectroscopy (IR) is used to collect spectra of the protein at various temperatures. Intermediates are observed and captured by taking advantage of the very slow kinetics in the viscous liquid state near the glass temperature (T_g)⁶.