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Early Events in the Refolding of Cytochrome-c Evaluated by Time-Resolved FTIR Microscopy

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ABSTRACT: How an unfolded protein searches through conformational space to form the MG state in fractions of a second is of fundamental importance to the protein-folding problem. Two different models, the hydrophobic collapse model and the framework model, have been proposed to account for the rapid formation of the MG state. We are investigating how structure develops during the formation of MG cytochrome-c by refolding the acid-denatured protein using high concentrations of KCl. By using time-resolved FTIR microscopy in conjunction with our rapid mixing continuous flow cell, changes in cytochrome-c structure after KCl exposure were evaluated by monitoring the amide I band profile ($1600-1700\text{ cm}^{-1}$) as a function of refolding time. Progressive development of solvated and buried alpha helix at the expense of unordered and turn structure was observed from 0.3-9.0 ms. Data analysis in the single-millisecond regime has revealed that within 2-3 ms of the reaction nearly 90% of unordered structure disappears, while an amount of solvated helix exceeding that of the MG-state is already formed. The majority of solvated helix appears as early as 500 microseconds. A portion (28%) of buried alpha-helix begins to form by 2 ms but the majority appears in the millisecond timescale (80% by 7 ms). At later timescales ($> 10\text{ ms}$), the remaining fraction of buried MG alpha-helix appears at the expense of the excess solvated helix formed in the initial burst phase of the reaction. Examination of the reaction down to timescales prior to 300 microseconds is underway using our new "T" cell prototype.