

Characterization of the Steady-State Folding Intermediates of Cytochrome c with Fourier Transform Infrared Spectroscopy

M. Chance, Q. He, A. Adzic, and N. Marinkovic (Albert Einstein College of Medicine)

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The role of the heme group in the folding of cytochrome c has been extensively studied by Dr. Denis Rousseau and others. However, much less work has been done on probing the secondary structure of the protein during the folding process. Using synchrotron FTIR, we are probing the secondary structure of cytochrome c during the folding process on a sub-millisecond time scale. The deconvolution of the Amide I band (1600 - 1700 cm^{-1}), provides details of the percent of the protein structure that is α -helical, β -sheet, β -turn, or extended coil. Before examining the folding intermediates in a time-resolved fashion with the rapid mixer (see Subproject 30), we have determined the equilibrium folding and unfolding conditions. Cytochrome-c was examined in its native form (pH~9), unfolded (pH~2, adjusted by HCl), and molten globular form (refolded, by addition of salt). In our experiments, 1 mM cytochrome-c in D₂O is completely unfolded at pH 2 and refolded by adding 200 mM KCl. It was determined that Amide I band can be deconvoluted into three bands representing native α -helix, solvated α -helix and coil. By plotting the IR intensities of the deconvoluted individual bands with the concentration of KCl it was found that all three bands follow a Hill function. Folding process increases the relative contribution of native α -helix on the expense of the other two bands. By plotting the loss of relative percentage of coil and solvated α -helix versus time and fitting the resulting curve with a first-order exponential decay function, one obtains the time constant for the cytochrome-c folding to be around 30 ms, which is in agreement with literature data.