Folding and Unfolding Mechanism of the Metalloprotein Rubredoxin: a Single Molecule Force Spectroscopy Perspective

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Metal ions play important roles in a range of biological processes. In metalloproteins, metal centers serve both as the active site for a range of functional purposes, as well as important structural elements to facilitate protein folding and assembly. The release and incorporation of metal ions from/into metalloproteins have important biological consequences and are intimately connected to the unfolding and folding of metalloproteins. However, it is challenging to directly observe the reversible unfolding and folding of metalloproteins due to loss or decomposition of the metal center. Here, we combine single molecule atomic force microscopy and protein engineering techniques to investigate the unfolding and refolding of a small iron sulfur protein rubredoxin at the single molecule level. Our results reveal that the unfolding of rubredoxin and its subsequent mechanical rupture of the iron center is stochastic and follows multiple, complex pathways that include concurrent rupture of multiple ferric-thiolate bonds as well as sequential rupture of ferric-thiolate bonds that leads to the formation of intermediate species. After unfolding, iron can remain attached to the CXXC iron chelation motif when rubredoxin is completely unfolded. Upon relaxation, the unfolded rubredoxin can refold into its holo-native state with the reconstituted iron-sulfur center, demonstrating the reversible nature of the unfolding-refolding rubredoxin. The loss of iron from the unfolded rubredoxin prevents the protein from refolding into its holo-native state. Our results clearly demonstrate the unfolding and refolding mechanism of rubredoxin, and provide supporting evidence for the iron-priming mechanism for the folding of rubredoxin.