Probing the Mechanism of Iron-Sulfur Cluster Biosynthesis with Assembly Intermediate Structures and Metalloprotein Design

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Eukaryotes have evolved highly conserved sets of proteins to chaperone and regulate iron-sulfur (Fe-S) cluster assembly, thereby controlling the toxicity and indiscriminant reactivity of "free" iron and sulfide. Defects in this Fe-S biosynthetic machinery are associated with neurodegenerative ataxia and ataxia-susceptibility, suggesting an in-depth understanding of Fe-S assembly will not only have an impact in the bioinorganic community, but also in human health and disease. Fe-S clusters are found in many different protein environments, from completely buried to solvent-exposed, and exhibit diverse reactivity and electronic properties. Our objectives are to address the following questions: (i) what are the protein-based intermediates and mechanism for building and transferring Fe-S clusters into target scaffolds, (ii) what are the structural elements in the diverse set of target proteins that are recognized by this biosynthetic machinery, and (iii) how does the protein environment control Fe-S cluster electromagnetic properties and reactivity? Towards these goals, we have initiated crystallographic trials for enzymes and intermediates in iron-sulfur assembly, as well as design experiments (in collaboration with Prof. Hellinga at Duke University Medical Center) to transplant Rieske and ferredoxin [Fe_2S_2] binding motifs into different regions of the green fluorescent protein (GFP) protein scaffold. GFP was chosen for its high protein yields and tolerance to site-directed mutations, reproducible crystal growth of mutant proteins that diffract to ultra-high resolution, and its natural reporter system, which allows rapid assessment of protein folding. In addition, designed proteins in which iron-sulfur cluster binding perturbs GFP fluorescent properties can be subjected to random mutagenesis and screening methodologies to probe local recognition elements for *in vivo* [Fe_2S_2] assembly. Here we present our experimental design strategy and results, including crystallographic structures of Fe-S maturation intermediates.