Site-specific Fe_4S_4 Chemistry in Ferredoxin:Thioredoxin Reductase

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Ferredoxin:thioredoxin reductase (FTR) represents a distinct class of disulfide reductases that catalyze the reduction of the disulfide in thioredoxin (Trx) in two one-electron steps using a Fe_2S_2-ferredoxin as the electron donor. It contains a unique active site that comprises a Fe_4S_4 cluster with an adjacent redox-active disulfide. It plays a key role in the light-regulated activation of chloroplast enzymes involved in the Calvin cycle by transforming the light signal received by photosystem I in the thylakoid membrane into a disulfide/dithiol interchange redox-signal between Trx and the target enzymes. We have used a combination of spectroscopic methods (Mössbauer, EPR, resonance Raman, and variable temperature MCD), and EPR redox-titration technique to characterize the active site of FTR in various forms of the enzyme, including wild-type FTR, point-mutation variants at each of the active-site cysteine residues, stable analogs of the one-electron-reduced FTR-Trx heterodisulfide intermediate, and methyl viologen-reduced FTR. The results reveal novel site-specific Fe_4S_4-cluster chemistry in all forms of FTR under investigation. In the resting enzyme, a weak interaction between the Fe_4S_4 cluster and the active-site disulfide promotes charge buildup at a unique Fe site, and primes the active site to accept an electron (from ferredoxin) to break the disulfide bond. In the one electron-reduced analogs, cleavage of the active-site disulfide is accompanied by coordination of one of the cysteine residues that form the disulfide to the unique Fe site, resulting in an unusual $[Fe_4S_4]^{3+}$ cluster with a five-coordinate FeS_5 site and a $[Fe_4S_4]^{3+/2+}$ redox potential that is more than 500 mV lower than that of a high-potential iron protein (HiPIP). The other cysteine residue is free to attack the disulfide of Trx. Most interestingly, the methyl viologen-reduced FTR, in which the disulfide is reduced to a dithiol, was found to contain an unprecedented electron-rich $[Fe_4S_4]^{2+}$ cluster composed of both a valence-delocalized and a valence-localized $Fe^{2+}Fe^{3+}$ pair, with the unique Fe site being the valence-localized high-spin Fe^{2+} site. These results provide molecular level insights into FTR mechanism and suggest two possible catalytic mechanisms for FTR.