Site-specific Reactivity of [4Fe-4S] Clusters in Disulfide Reductases and Radical SAM Enzymes

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New types of site-specific [4Fe-4S] chemistry have recently been identified in ferredoxin:thioredoxin reductase (FTR), an Fe-S containing disulfide reductase, and in MOCS1A, a radical-SAM enzyme involved with the first step in molybdenum cofactor biosynthesis. The active site of FTR comprises a [4Fe-4S] cluster and nearby disulfide and site-specific cluster chemistry has been identified in each of the three accessible redox states using the combination of resonance Raman and Mössbauer spectroscopies. In the oxidized state, the active-site disulfide interacts weakly with a unique Fe site to yield a $[4Fe\text{-}4S]^{2+}$ cluster with one valence-delocalized and one partially valence-localized $Fe^{3+}Fe^{2+}$ pair. In stable analogs of the one-electron-reduced intermediate, one of the cysteines of the cleaved active-site disulfide is coordinated at the unique Fe site to yield a $[4Fe\text{-}4S]^{3+}$ cluster with five cysteinate ligands. In the two-electron-reduced state, one of the free thiols of the active-site disulfide is anchored to the cluster via a strong H-bonding interaction involving S of a coordinating cysteine residue to yield an unprecedented type of electron-rich $[4Fe\text{-}4S]^{2+}$ cluster comprising both valence-delocalized and valence-localized $Fe^{2+}Fe^{3+}$ pairs. The mechanistic implications of the site-specific cluster chemistry will be discussed.

Spectroscopic and crystallographic studies of MOCS1A have revealed two [4Fe-4S] clusters, each with only three cysteine ligands. As in other radical-SAM enzymes, one [4Fe-4S] cluster binds SAM at a unique Fe site in order to facilitate reductive cleavage and generation and of a 5′-deoxyadenosyl radical. EPR and resonance Raman studies indicate that the other [4Fe-4S] cluster binds the 5′-GTP substrate at a unique Fe site in order to position or activate the substrate for interaction with the radical. Recent spectroscopic, analytical and mutagenesis studies have also identified a second [4Fe-4S] cluster coordinated by only three cysteines in MiaB, a radical-SAM enzyme that catalyzes the thiomethylation of tRNAs, and the role of this cluster will also be discussed.