

Site-specific chemistry in the $[\text{Fe}_4\text{S}_4]$ cluster of FTR revealed by Mössbauer spectroscopy

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Light regulation in oxygenic photosynthesis is mediated by ferredoxin:thioredoxin reductase (FTR), a novel class of disulfide reductase with an active-site comprising a $[\text{Fe}_4\text{S}_4]^{2+}$ cluster and an adjacent disulfide, that catalyzes reduction of the thioredoxin disulfide in two sequential one-electron steps using a $[\text{Fe}_2\text{S}_2]^{2+/+}$ ferredoxin as the electron donor. To study the chemical reaction involved in the FTR catalytic process, we have characterized the active site of FTR in a variety of different forms: wild-type, variants involving point mutations of the active-site disulfide cysteines, and chemically modified forms in which a sulfur of the active site disulfide is alkylated with *N*-ethylmaleimide or covalently attached to a cysteine of thioredoxin *m*.

Mössbauer spectroscopy provides compelling evidence that the enzyme in its native state, as well as in the one and two-electron reduced states, possesses a $[\text{Fe}_4\text{S}_4]$ cluster with a unique, electron-rich iron site. Our studies suggest that this iron is in interaction with the active site disulfide and participates directly in the catalysis. The figure on the right shows a proposed catalytic mechanism consistent with these findings.

