Spectroscopic Investigations of Iron-Containing Metalloenzymes: New Intermediates and Novel Mechanisms

Abstract:

Metal-containing enzymes (metalloenzymes) play essential roles in numerous metabolic pathways in all kingdoms of life. They catalyze key chemical steps for biosynthesis of natural products, for bioenergy transduction, and for gene/protein expression and regulations. A fundamental understanding of their catalytic reaction mechanisms has far-reaching impact on human health and global energy sustainability.

In our laboratory, a multi-faceted biophysical approach consisting of spectroscopic techniques (Mössbauer, EPR, and X-ray techniques) and computational tools (MD and QM calculations) is utilized to explore intricate reaction mechanisms of iron-containing metalloenzymes by trapping and characterizing reactive intermediates and exploring their detailed electronic structures in order to derive the structure-reactivity correlations of these transient species. In this talk, I will present two recent studies on non-heme mononuclear iron-containing (NHM-Fe) enzymes and iron-sulfur-cluster-containing (FeS) enzymes. In NHM-Fe enzymes, a common high-valent iron intermediate, the high-spin (S = 2) oxoferryl (Fe(IV)-oxo, or Fe(IV)=O) species, is used as the key reactive species to enable C-H bond activation. But what is not known is whether intermediate spin (S = 1) Fe(IV)=O intermediates exist in NHM-Fe enzyme reactions and if so, how they compare with the S = 2 congeners in terms of chemical reactivity. In the first project, I will discuss our recent discovery of the first S = 1 Fe(IV) intermediate found in a NHM-Fe enzyme that catalyzes a novel oxidative carbon-sulfur bond formation between histidine and cysteine. The implications of our study will also be discussed in the context of general reaction mechanisms of NHM-Fe enzymes. In the second project, a study on a [4Fe4S]-containing enzyme, Ferredoxin:Thioredoxin Reductase from *Methanosarcina acetivorans* (*Ma*AFTR), will be discussed, where a novel S = 7/2 reduced [4Fe4S] ($[Fe_4S_4]^+$) cluster is identified via biochemical and spectroscopic investigation. The S = 7/2 ground spin state of this cluster is in stark contrast to the canonical S = ½ spin ground state found in almost all the protein bound $[Fe_4S_4]^+$ clusters. We further show that the S = 7/2 $[Fe_4S_4]^+$ state is stabilized by a protonation on one of the bridging sulfides of the cluster core, thus suggesting that this unique cluster state may function as a proton-coupled electron transfer agent in *Ma*AFTR. The broader implication of this discovery to other FeS enzymes will be discussed.[1,23]

Most Relevant Publications:

1. Cheng, R. *et al.* OvoAMtht from Methyloversatilis thermotolerans ovothiol biosynthesis is a bifunction enzyme: thiol oxygenase and sulfoxide synthase activities. *Chem. Sci.* **13**, 3589–3598 (2022).
2. Paris, J. C. *et al.* An S =1 Iron(IV) Intermediate Revealed in a Non-Heme Iron Enzyme-Catalyzed Oxidative C–S Bond Formation. *Angew. Chemie Int. Ed.* **62**, (2023).
3. Prakash, D. *et al.* Catalytic Activity of the Archetype from Group 4 of the FTR-like Ferredoxin:Thioredoxin Reductase Family Is Regulated by Unique S = 7/2 and S = 1/2 [4Fe–4S] Clusters. *Biochemistry* **63**, 1588–1598 (2024).