Hydrogenases

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New Iron–Sulfur Clusters Help Hydrogenases Tolerate Oxygen**

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Hydrogenase enzymes have received attention for a number of reasons. First, they catalyze the interconversion of protons and H_2—a carbon-free energy-storing molecule. Unlike most synthetic catalysts for H_2 formation, hydrogenases are composed of Earth-abundant materials, are biodegradable, and are extremely rapid catalysts.[1] Second, hydrogenases show how natural systems perform multielectron catalysis while avoiding unstable intermediates. Third, hydrogenases have metal–carbonyl cofactors that link classical organometallic chemistry (which usually uses strong-field ligands like CO and CN^-) and bioinorganic chemistry (which is dominated by weak-field ligands). Now, recent back-to-back crystallographic papers in *Nature* by Fritsch, Scheerer et al. and by Shomura et al. describe another surprising discovery in hydrogenases: a new kind of iron–sulfide cluster.[2] These new results have important implications for electron control in enzymes, and will help chemists to understand the structural basis of O_2 tolerance in one class of hydrogenases.

A difficulty with handling hydrogenases has been their oxygen sensitivity. The majority of known hydrogenases are poisoned by even small amounts of O_2 to give inactive forms, only some of which can be re-activated upon reduction with H_2.[3] Interestingly, membrane-bound hydrogenases (MBH) in the bacteria *Ralstonia eutropha*, *Ralstonia metallidurans*, *Hydrogenovibrio marinus*, and *Aquifex aeolicus* tolerate limited amounts of oxygen (though they can be overwhelmed by high enough amounts of O_2).[4] However, the question of how the MBH enzymes can resist O_2 has been difficult to answer. All hydrogenases have iron–sulfide clusters that serve as a relay to transfer electrons to and from the active site, which in the MBH enzymes is a heterobimetallic bridged nickel–iron cofactor. Both the nickel–iron active site and the iron–sulfide clusters are inherently O_2-sensitive, and so it is surprising that the MBH enzymes resist the potentially destructive effects of O_2. Initial hypotheses focused on the iron–nickel active site, but recent spectroscopic studies and the new crystal structures show that the Ni–Fe bimetallic active sites of the MBH enzymes have no significant differences from those in O_2-sensitive nickel–iron hydrogenases.[5] Another hypothesis was that the width of the H_2-transporting channel in MBH enzymes is narrow enough to exclude O_2, but the recent crystal structures do not support this idea.[1a,4,5b,6]

The most distinctive and relevant feature of the MBH enzymes has turned out to be in one of the iron–sulfide clusters. Iron–sulfide clusters are widespread electron-transfer sites in metalloproteins, and their constitution is typically specified through delineation of the number of iron atoms *n* and sulfides *m* in the core as [*n*Fe-*m*S]. The air-sensitive nickel–iron hydrogenases have a chain of three clusters, and the proximal one (closest to the Ni–Fe site) is a normal [4Fe-4S] cuboidal cluster (Figure 1 A). However, the new crystal structures of the O_2-tolerant MBH in its reduced form show that the proximal iron–sulfur cluster is [4Fe-3S] where one of the sulfides is replaced with a bridging thiolate from a cysteine residue (Figure 1 B). Another iron atom has an additional terminal cysteine that replaces a site vacated by the missing sulfide. As a result of replacing one triply bridging sulfide with a bridging cysteine thiolate and a terminal cysteine thiolate, the cluster coordinates a total of six cysteine side-chains rather than the usual four. This unique structure explains the unusual spectroscopic features of the MBH enzymes.[7] This cluster shape has not yet been observed in small-molecule iron–sulfide compounds, and will pose an exciting challenge to the synthetic modeling community.

How does the unusual [4Fe-3S] cluster contribute to the oxygen tolerance of the MBH enzymes? The answer is provided by one of the new crystallographic studies, which shows changes in the cluster coordination at different redox potentials.[2b] Under oxidizing conditions, the structure shifts to a form in which the backbone amide of Cys26 of the smaller subunit becomes deprotonated and displaces the bridging cysteine from one of the iron atoms; as a result, this thiolate is attached in the more typical terminal binding mode (Figure 1 C, indicated in red). The additional negative charge from the deprotonated amide stabilizes the oxidized form (i.e., lowers the redox potential). As a result, the [4Fe-3S] cluster can transfer two electrons in a narrow potential window of only 0.2 V, and is stable at three different oxidation levels.[7b] It is proposed that the ability to transfer multiple electrons increases MBH's oxygen tolerance by enabling the

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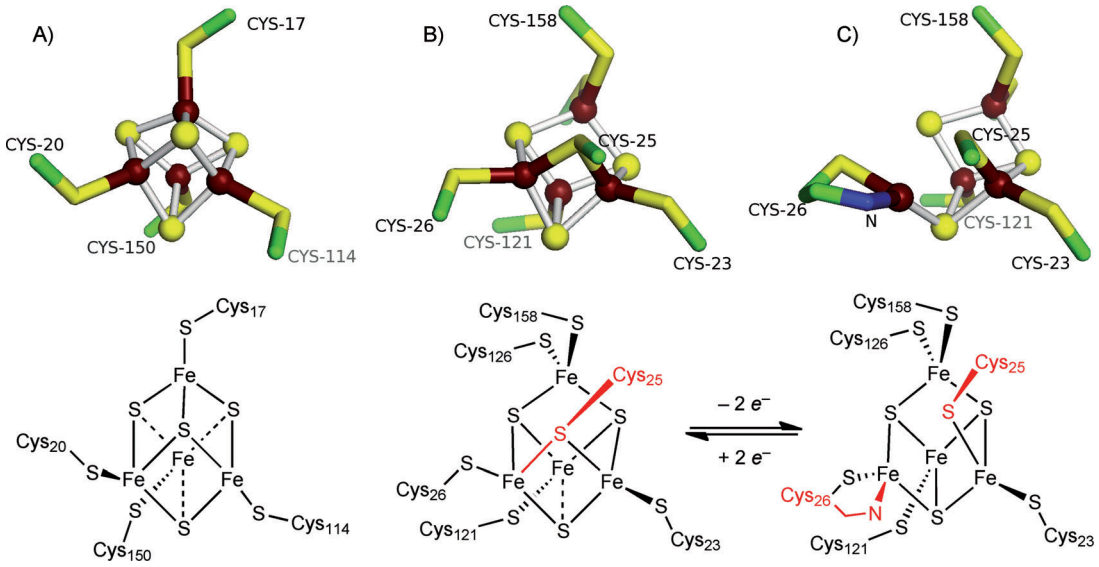

Figure 1. Comparison of traditional [4Fe-4S] cluster to new [4Fe-3S] clusters. Each is shown as a perspective view of the crystal structure on the top and as a picture of the bonding below. Changes are highlighted in red. A) Normal [4Fe-4S] cluster (PDB 1H2R);[1b] B) reduced [4Fe-3S] cluster from MBH (PDB 3AYX);[2b] C) oxidized [4Fe-3S] cluster from MBH (PDB 3AYZ).[2b]

enzyme to reduce O_2 rapidly, converting the dioxygen into harmless water before it can damage the protein.[5b,8]

The synergism of amide deprotonation and cluster oxidation is an example of proton-coupled electron transfer (PCET), in which protons and electrons are moved simultaneously in order to avoid a high activation barrier from charge buildup. No detailed studies of proton-coupled electron transfer with iron–sulfide clusters have been reported, and the new structures will certainly inspire future work in both protein and small-molecule chemistry. Additionally, the amide coordination in the oxidized form of the new [4Fe-3S] cluster may act as a "redox switch" between the two reactivity modes of the enzyme, oxidation of H_2 and reduction of O_2. The redox-dependent conformational change in the [4Fe-3S] cluster is reminiscent of previously characterized changes in the "P cluster" of nitrogenase (Figure 2). Coordination of the amide in the oxidized cluster enables multielectron redox processes.[9] Thus, the new crystal structures may point toward a general strategy for rapid electron-transfer cascades in multielectron redox enzymes. In this way, it is anticipated that further study of "shape-shifting" iron–sulfide clusters will help chemists to better understand how electron flow is controlled in proteins.

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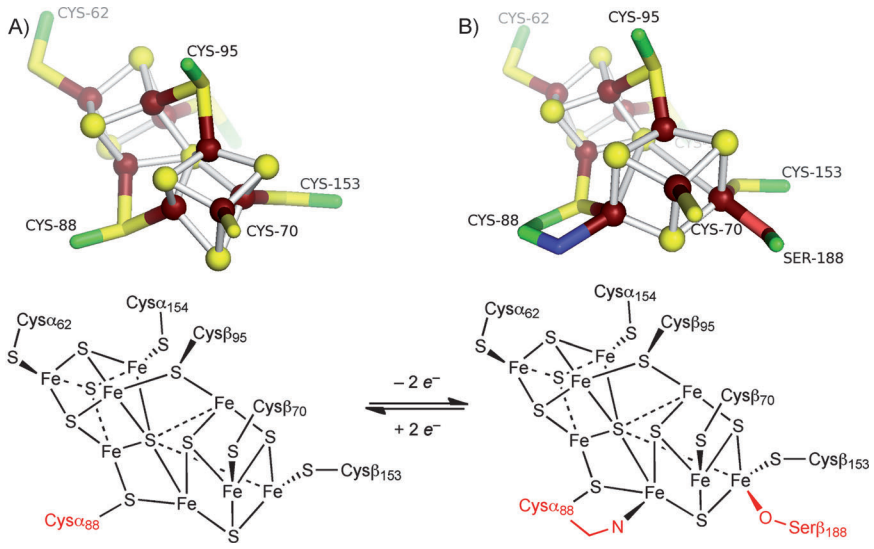

Figure 2. P clusters of iron–molybdenum nitrogenase. Each is shown as a perspective view of the crystal structure on the top and as a picture of the bonding below. A) reduced P cluster from nitrogenase (PDB 3U7Q);[9] B) oxidized P cluster from nitrogenase (PDB 2MIN).[9]

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