Assembly and Evolution of Complex Fe-S Clusters as Revealed by X-ray Crystallography

Complex Fe-S cluster-containing enzymes are ubiquitous in nature where they are involved in a number of fundamental reactions for life including carbon dioxide fixation, nitrogen fixation, and hydrogen metabolism. One of the more complex and unusual biological clusters is found in the [FeFe]-hydrogenase. The active-site H-cluster in these enzymes has a [4Fe-4S] subcluster bridged via a cysteine thiolate to a 2Fe subcluster, which in turn is coordinated by CO and CN^- ligands and a bridging dithiolate ligand (*1*). The biologically unique CO and CN^- ligands finely tune the 2Fe subcluster of the H-cluster making it able to efficiently catalyze the activation of molecular H_2 through the reversible reaction $H_2 \leftrightarrows 2H^+ + 2e^-$. How this complex metallocluster is assembled in nature is intriguing and the precise mechanism by which various enzymes, scaffolds, and carriers carry out H-cluster maturation is unknown.

We have determined the x-ray crystal structure of an intermediate form of [FeFe]-hydrogenase ($HydA^{\Delta EFG}$) from the green algae *Chlamydomonas reinhardtii* expressed in *Escherichia coli* in a genetic background devoid of the hydrogenase genes required for maturation. The x-ray crystal structure of $HydA^{\Delta EFG}$ was refined to 1.97 Å resolution from data collected at Beam Lines 9-1 and 9-2 at SSRL. Insights from the structure establish unifying themes for the assembly of complex Fe-S clusters in nature and reveal fundamental features of metalloenzyme evolution by providing clues as to how these clusters have evolved in complexity through time. Also, the research, by revealing key insights into the biosynthesis of a biological catalyst (the H-cluster) that promotes hydrogen production, provides clues into approaches for biomimetic catalysts for hydrogen as a renewable energy source.

The crystal structure of $HydA^{\Delta EFG}$ reveals that the [4Fe-4S] cluster is the only Fe-S cluster present at the active site and the 2Fe subcluster of the H-cluster is absent (Figure 1). The overall structure of $HydA^{\Delta EFG}$ reveals the formation of a positively charged channel from the protein surface linked to the [4Fe-4S] cluster site (Figure 2). Adjacent to the [4Fe-4S] cluster is an open cavity, which is poised for insertion of the 2Fe subcluster. Presumably, insertion of the 2Fe subcluster occurs through the positively charged channel that collapses following incorporation through conformational changes two loop regions

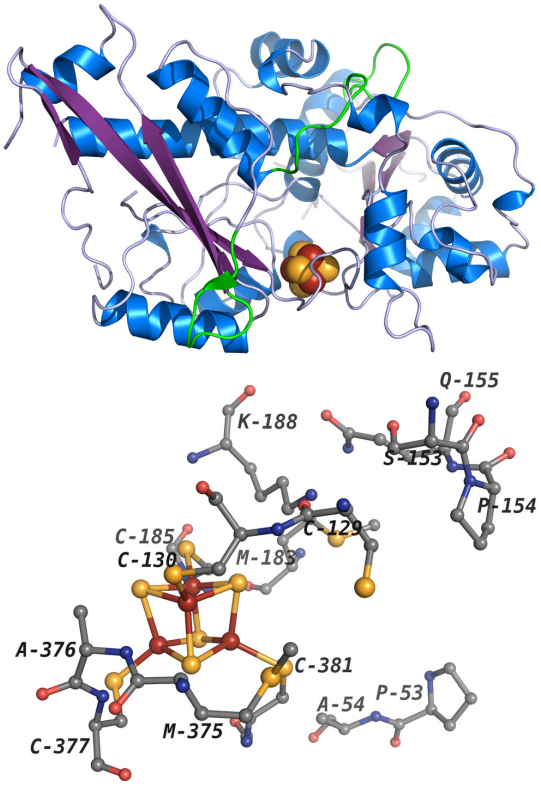

Figure 1. Top: overall ribbon and surface representation of the x-ray crystal structure of [FeFe]-hydrogenase $HydA^{\Delta EFG}$. Loop regions important for the cluster insertion process are colored green. Bottom: Ball and stick representation of the active site of $HydA^{\Delta EFG}$ at which a [4Fe-4S] cluster is present. Coloring scheme: rust, iron; orange, sulfur; gray, carbon; red, oxygen; blue, nitrogen.

(Figure 2). These observations suggest a stepwise mechanism for H-cluster biosynthesis and that the [4Fe-4S] subcluster is synthesized and inserted first by general Fe-S cluster maturation machinery followed by the synthesis and insertion of the 2Fe subcluster by specialized hydrogenase maturation machinery. Interestingly, a sequence alignment of a diversity of HydA indicates that the two loop regions implicated to be involved in the cluster insertion process are highly conserved and a phylogenetic analysis suggests that HydA emerged within bacteria most likely from a Nar1p-like ancestor lacking the 2Fe subcluster, followed by acquisition in several lower order eukaryotes.

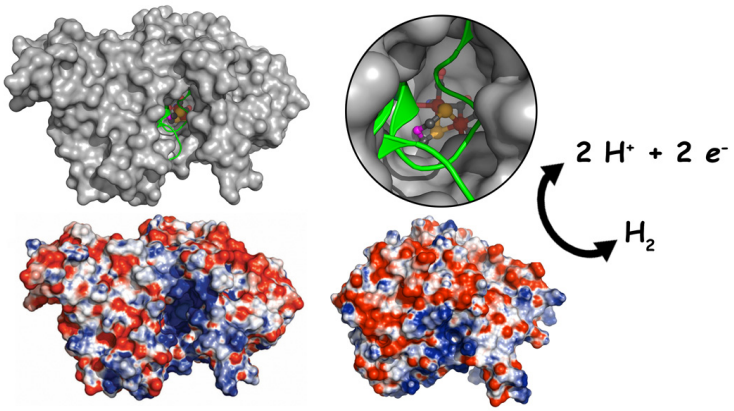

Figure 2. Pathway for Fe-S cluster insertion into [FeFe]-hydrogenase during complex Fe-S cluster assembly. The surface representation of HydA$^{\Delta EFG}$ is gray (top) and loop regions important for cluster insertion are overlayed from the active [FeFe]-hydrogenase (HydA). Below are electrostatic surface representations of HydA$^{\Delta EFG}$ (left) and HydA (right).

The structure of HydA$^{\Delta EFG}$ also establishes a parallel to the cluster assembly process for nitrogenase and indicates that the cluster insertion mechanism is conserved between these two evolutionary distinct enzymes. For nitrogenase, structural determination of a FeMo-co deficient form of the enzyme reveals major structural rearrangement in the domain involved in FeMo-co binding, resulting in a positively charged channel linked to the FeMo-co site (*2*). The structural rearrangements resulting in the formation of a positively charged channel in both [FeFe]-hydrogenase and nitrogenase indicate that the process for complex Fe-S cluster insertion into proteins may be conserved and that the evolution of functional [FeFe]-hydrogenase may have occurred stepwise, a feature which is consistent with the evolution of nitrogenase and possibly Fe-S enzymes in general. Also, like [FeFe]-hydrogenase, different Fe-S cluster components of nitrogenase are targets of different maturation machinery—general and specialized nitrogenase maturation machinery. These parallels suggest that there are unifying themes by which biology constructs complex Fe-S clusters and gives us insight into which components are likely to be the youngest and which might be the oldest in the quest to trace their evolutionary origin.

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References

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