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Hydrogenases

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The Modular Assembly of Clusters Is the Natural Synthetic Strategy for the Active Site of [FeFe] Hydrogenase[**]

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The recognition of the presence of iron–sulfur clusters and their electron shuttling roles in redox-active enzymes is one of the giant steps in a march of almost 80 years towards understanding the enzymes that control hydrogen metabolism in microorganisms of ancient origin: hydrogenases.[1] The versatility of the Fe/S/SR combination in structural and physical properties has been well-established through synergistic studies evolving from the laboratories of chemists, biochemists, and biophysicists.[2,3] The remarkable coincidences of FeS cluster reactivities in vitro (using synthetic analogues) and in vivo (in FeS cluster-containing proteins) impress regarding the minor role of the protein in determining the existence of the FeS clusters, and have led to proposals that small chunks of iron sulfide minerals might have been the first catalysts on planet earth.[4] Their eventual incorporation into proteins led to such sophisticated constructs as are found in the inorganic/organometallic natural products shown in Scheme 1.[2,5–8]

Clearly, the presence and alignment of multiple FeS clusters observed in the protein crystal structures of [NiFe] and [FeFe] hydrogenases (H_2ases) can only be interpreted as the electron-transfer routes that connect the active sites to the electron-donor or -acceptor unit docked into the exterior of the protein.[9] In the [FeFe] H_2ase active site (Scheme 1 e), one typical 4Fe4S cluster is "hard-wired" or directly attached to an unusual 2Fe subsite through a cysteinyl bridge.[6] In this way, the composition of the "H-cluster", the hydrogen-producing cluster of [FeFe] H_2ase, resembles that of sulfite reductase (Scheme 1 g) or acetyl-CoA synthase (Scheme 1 f); the 4Fe4S cluster has been called upon to serve as a redox-variable metallothiolate ligand via its cysteinyl sulfur that bridges to the 2Fe subsite. That the 2Fe portion of the H-cluster is a genuine organometallic species, replete with carbon monoxide, cyanide, and a previously biologically unknown dithiolate cofactor, coupled with the impressive rate of the [FeFe] H_2ase catalysis of H_2 production from mild potential electrons and water as proton source,[7] has brought global attention of chemists in search of an optimal synthetic analogue of the active site, without protein, as prospective molecular electrocatalysts for hydrogen production.

An engaging and difficult challenge has been biosynthesis issues: How does nature generate and manage CN^- and CO, known to poison metal sites if uncontrolled? How is the azadithiolate that connects the irons within the 2Fe subsite made? How is the H-cluster assembled? Does a 6Fe supercluster precede and extrude the 2Fe subsite, or is the assembly modular? Insights into the first two questions have been gained in recent discoveries of gene products utilizing radical SAM (*S*-adenosyl methionine) pathways that result in degradation of tyrosine into *p*-cresol and the diatomic ligands, CO and CN^-, the latter presumably though a glycyl radical.[10–12] Guidance to answers to the latter two questions is the focus of this Highlight on a structural report from Mulder, Peters, Broderick et al., and additional biosynthetic and spectroscopic results on the nature of the 2Fe2S subsite precursor.[13,14]

As to the question "How is the H-cluster assembled?", the trivial answer of "very carefully" is without a doubt correct. Mulder et al. have been able to obtain the [FeFe] H_2ase (also known as HydA) protein as expressed in the absence of the HydE, HydF, and HydG proteins required for the synthesis of the 2Fe subsite and the maturation of the enzyme into active form.[13] The immature protein, produced without the accessory proteins and known as $HydA^{\Delta EFG}$, was derived from the *Chlamydomonas reinhardtii* green alga and expressed in *E. coli*; its X-ray crystal structure was determined and compared to those of the holoprotein crystallized from *C. pasteurianum* and *Desulfovibrio desulfuricans*. Whereas both latter structures show the full H-cluster in the form of cysteine-bridged subsites, that is, 4Fe4S(μ-SCys)2Fe, only the 4Fe4S cluster is found in $HydA^{\Delta EFG}$. The structure of $HydA^{\Delta EFG}$ shows the already-present 4Fe4S cluster resides in a cavity at the end of a channel (8–15 Å wide and 25 Å long; Figure 1). Overlays of the structures of the immature or apoprotein that lacks the 2Fe subsite with the complete or

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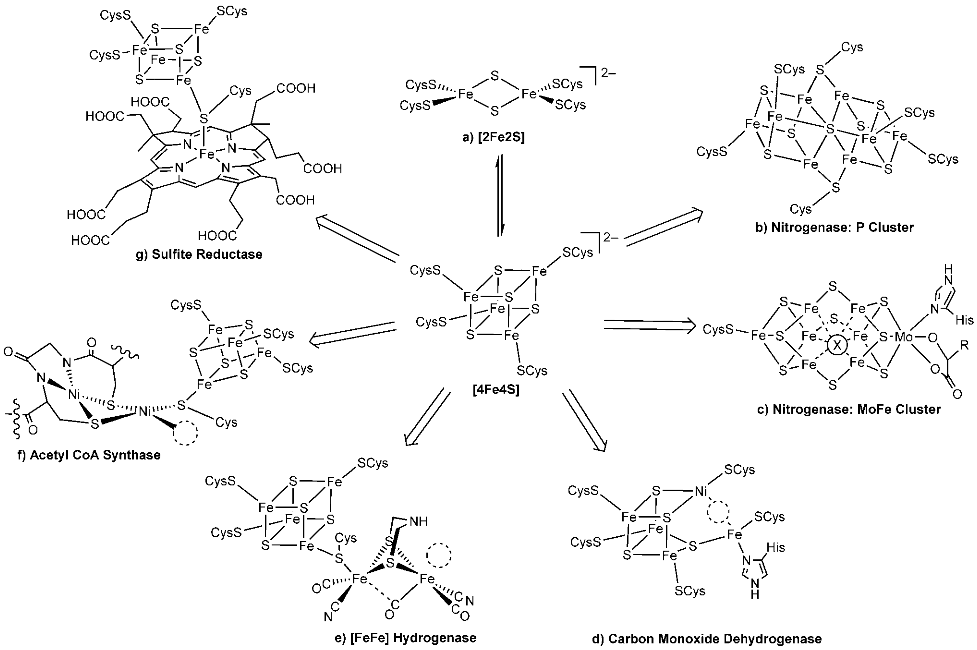

Scheme 1. A selection of protein-bound iron–sulfur clusters. (Structures are taken from crystallographic databases or from the references as noted in the text.)

holoprotein show that the channel has closed in the latter, thus wrapping up the completed active site.

Analysis of the channel composition in HydA$^{\Delta EFG}$ indicates that positive amino acid residues (an arginine and two lysines) flank the entrance, thus most likely attracting the 2Fe subcluster with its negatively charged cyanides; another lysine within the channel is purported to form hydrogen bonds to the 2Fe subsite once it is in place as a constituent of the H-cluster.[13] Yet another clue as to the guidance mechanism is a cysteine near the end of the cavity, the sulfur side chain of which is exposed and might be expected to swap out with a labile ligand on the 2Fe unit, becoming the bridge between the 4Fe4S and the 2Fe subunits.

So, what delivers the 2Fe subcluster to HydA$^{\Delta EFG}$? Recent evidence suggests that HydF is a scaffold protein; the 2Fe subcluster or its precursor is assembled on this protein while the other two accessory proteins HydE and HydG manage the syntheses of CO, CN^{-}, and the SCH$_2$NHCH$_2$S linker via the afore-mentioned radical S-adenosylmethionine (SAM) process.[14] The current working hypothesis is that HydF orchestrates the Fe–CO and Fe–CN bond forming processes, presumably on a 2Fe2S cluster analogous to that shown in Scheme 1 a. HydF is also expected to serve as a carrier for the subcluster insertion into the channel of apo-HydA. To date, the structure of HydF has not been determined. Whether the 2Fe subcluster on HydF is in its final structural form as is found in the fully formed H-cluster (Figure 1) is not known. Early spectroscopic data suggest the presence of 2 CN^{-} and 2 or 3 CO molecules in a reduced state, as the ν(CO) positions match those of (μ-SRS)[Fe^{I}(CO)$_2$CN]$_2^{2-}$ or (μ-SRS)-[Fe$_2^{I}$(CO)$_3$(PR$_3$)$_3$].[15,16]

The 2Fe subsite in the Fe^{II}Fe^{I} redox level within the completed and as-isolated H$_{ox}$ cluster is found to be in an unusual form, described by synthetic chemists as rotated

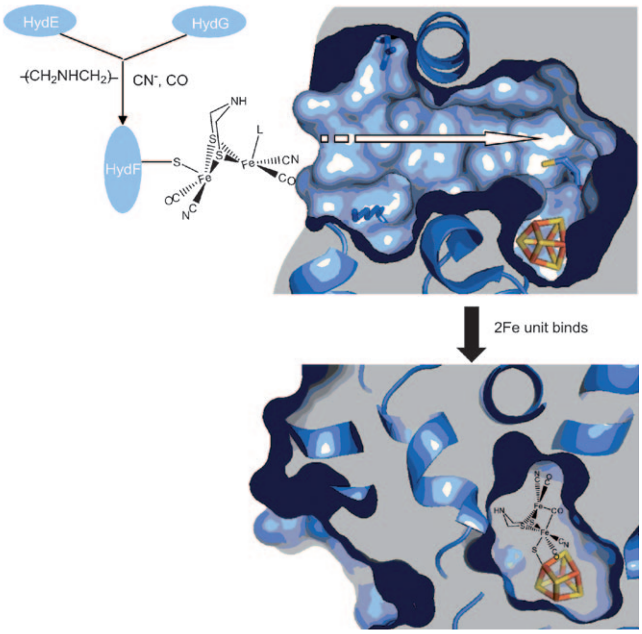

Figure 1. The preformed 2Fe organometallic unit insertion into apo-HydA. Positively charged residues help direct the precursor unit to the already present 4Fe4S, possibly with assistance of a cysteine sulfur atom within the channel. When the 2Fe units bind to the 4Fe4S cluster at the base of the channel, completing the H-cluster, the cavity collapses, burying the active site within the protein. Enzyme cavity surfaces for HydA and HydA$^{\Delta EFG}$ with relevant amino acid residues and metal clusters were visualized using PyMOL (W. L. DeLano, "The PyMOL molecular graphics system", 2002), with structural coordinates obtained from Refs [13, 14]. Figures of the cavities were then overlayed on the ribbon diagram representation of HydA and HydA$^{\Delta EFG}$ (also visualized with PyMOL) to illustrate changes in the overall protein structure.

relative to the well-known edge-bridged square pyramids of the reduced dinuclear species (μ-SRS)$[Fe^I(CO)_3]_2$ and (μ-SRS)$[Fe(CO)_2CN]_2^{2-}$. We speculate that the insertion process and lodging of the 2Fe subcluster, cysteine-bridged to the 4Fe4S cluster and with concomitant hydrogen bonding requirements of the cyanide ligands, proceeds with oxidation and rotation, which initiates the conformational changes of the protein loops that close the channel.

The implications of this stepwise assembly mechanism are profound for biologists and for chemists. Mulder et al. note a similarity between the structural features described above and the nitrogenase protein that lacks the FeMo cofactor (Scheme 1 c) and the holoprotein.[13] This could signal that the ability of such proteins to incorporate a pre-existing abiotic catalyst could be a wide spread motif in early metalloenzyme development.

For chemists, the speculation that the original 2Fe subsite was a standalone ancient catalyst, recognized by evolving microorganisms as beneficial to their growth and prosperity, reinvigorates designs to use synthetic ligands to reproduce the electronic environment about the prebiotic iron sulfide catalyst. However, already some 300 such diiron models have been reported; none perform the $2H^+ + 2e^- \rightarrow H_2$ catalysis as does the H-cluster. Is there a requirement for the 4Fe4S cluster to be attached? This feat has been demonstrated to be feasible in an elegant synthetic analogue of the full 6Fe cluster,[17] however with no greater catalytic success. Thus it indeed appears to be the 2Fe subsite that must be strategically tuned. The natural approach to the synthesis begins with oxidized iron in the 2Fe2S clusters, whereas the synthetic analogues typically use reduced iron in (μ-SRS)$[Fe^I(CO)_3]_2$ as precursor, achieving stable rotated forms in few cases,[18] and these return to the unrotated form on reduction. This suggests that the chemists' efforts might profitably be directed in pursuit of 1) alternative FeFe precursors and 2) supramolecular constructs that will collapse around the 2Fe synthetic analogue and maintain it in the rotated form throughout its electrocatalytic cycle. This appears to be the structural biosynthetic story.

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