Published in final edited form as:

Angew Chem Int Ed Engl. 2013 December 16; 52(51): 13518–13520. doi:10.1002/anie.201307132.

And the Winner is...Azadithiolate: An Amine Proton Relay in the [FeFe] Hydrogenases

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Keywords

cofactors; enzymology; hydrogen; hydrogenases; iron

In an age in which we turn to new energy technologies, an increasingly prominent energy vector—molecular hydrogen—is taking center stage.^[1] Whereas many consider a “hydrogen economy” to be a thing of the future, for certain bacteria and archaea, their “hydrogen economy” dates back to a time when Earth’s atmosphere was rich in this very lightest of gases. Microorganisms metabolize hydrogen by expressing hydrogenases (H₂ases), metalloenzymes that have, in light of the above, garnered much recent interest.^[2] Their active sites feature three central components—iron, thiolate groups, and carbon monoxide—also present in CO-processing enzymes (although surprisingly absent from any commercial catalysts). Depending on the metals at their active sites, H₂ases are classified as [Fe], [NiFe], or [FeFe] H₂ase. Examples from each of these classes have been crystallized, and in the latter two cases, the ligands at the active site have been fully identified.

Despite similarities between the H₂ase active sites, that of the [FeFe] H₂ases stands out as largely consisting of non-proteic ligands (Scheme 1, right), as it is covalently anchored to the backbone at only a single cysteinate residue. Whereas X-ray crystal-structure analysis indicated that an [Fe₄S₄] cluster is attached to the [2Fe] subsite through a S^{Cys} atom (the ensemble is referred to as the H-cluster), owing to the similar scattering of C, N, and O, crystallography proved less useful for distinguishing the two CN[−] and CO cofactors shown to be present by IR spectroscopy.^[3] One of each of these ligands, rarely present in active enzymes, occupies a basal site at each of the two iron residues; the stereochemical structure of the cluster is deduced from the polarity of the protein pockets and the respective hydrophobicity and hydrophilicity of the Fe–CO and Fe–CN groups. An additional CO ligand that bridges the atoms Fe^P (“proximal” to [Fe₄S₄]) and Fe^d (“distal”) was located by a combination of X-ray crystallography and IR spectroscopy. This CO ligand is key to the organization of Fe^d in such a way that an apical site is vacant for substrate binding.

Following the identification of the first coordination sphere of the diiron moiety, attention turned to the dithiolate ligand, which after some early ambiguity was found to be of the

form $^{-}\text{SCH}_2\text{XCH}_2\text{S}^{-}$. The properties of C, N, and O once more posed a problem: the identity of X could not be determined crystallographically, although it was narrowed down to CH_2 , NH, and O. In any case, the respective propane-, aza-, or oxadithiolate ($\text{pdt}^{2-}/\text{adt}^{2-}/\text{odt}^{2-}$) would be unprecedented in biology. Many, starting with Fontecilla-Camps and co-workers, favored adt^{2-} ,^[4] owing to a short contact (3.1 Å) from X to a neighboring S^{Cys} atom (reflecting H bonding), as well as mechanistic considerations (see below). Spectroscopy (notably HYSORE) has implicated adt^{2-} ,^[5] but the evidence is not one-sided: some counter examples suggest odt^{2-} .

Although indirect, lessons from synthetic modeling also pointed to a native adt^{2-} proton relay. For example, the H^+ -reduction catalyst $[\text{Fe}_2(\text{adt})(\text{CO})_2(\text{dppv})_2]$ (**4**) is 10^4 times more active than its pdt^{2-} congener.^[6] Could the dithiolate ligand hold the answer to the remarkable activity of $[\text{FeFe}] \text{H}_2\text{ase}$, an enzyme that catalyzes the reaction $\text{H}_2 \rightleftharpoons 2\text{H}^+ + 2\text{e}^-$ at rates of up to 28000 s^{-1} ? The first coordination sphere was understood: strong-field ligands enforce a low-spin, unsaturated diiron core. However, beyond steric and electronic effects, the second coordination sphere, in particular the dithiolate—the cofactor that, along with Fe^{d} , actually does the H_2 splitting—was unresolved. Fontecave et al., armed with synthetic mimics, a $[\text{FeFe}] \text{H}_2\text{ase}$ and its maturase, largely put the dithiolate debate to rest.^[7] Modeling again comes to the fore with their use of $[\text{Fe}_2(\text{SCH}_2\text{XCH}_2\text{S})(\text{CO})_4(\text{CN})_2]^{2-}$ (**1–3**; Scheme 1, left), as these species differ in the nature of the dithiolate, the cofactor very much in question.

Earlier maturation studies had shown a simple $[\text{Fe}_2\text{S}_2]$ cluster to assemble into a $[2\text{Fe}]$ subsite precursor on a scaffold protein denoted HydF, radical *S*-adenosylmethionine enzymes being responsible for the stepwise installation of $^{-}\text{SCH}_2\text{XCH}_2\text{S}^{-}$ and CO/CN^{-} . HydF then transfers $[2\text{Fe}]$ to apo-HydA, the H_2ase bearing an $[\text{Fe}_4\text{S}_4]$ center but lacking the diiron core. Mimicking the latter half of this maturation, Fontecave et al. loaded HydF (in this case from *Thermotoga maritima* expressed in *Escherichia coli*) with each of **1–3** to afford **1HydF**, **2HydF**, and **3HydF**, respectively (Scheme 1, center). The IR spectra of the adducts corresponded to those of *Clostridium acetobutylicum* HydF, a maturase known to possess $[\text{Fe}_4\text{S}_4]$ and $[2\text{Fe}]$ moieties. Nuclear spin coherence transfer revealed each to feature CN^{-} -bridged clusters. Thus, the study was not only a feat of synthesis, with the incorporation of three exogenous species, but also one of careful spectroscopy. The success of Fontecave et al. is significant given that native $[2\text{Fe}]$ is structurally undefined, although this evidence suggests that it is most likely a cluster similar, if not identical to, those in **xHydF**.

Although **1**–**3**HydF each contain an [Fe₄S₄] redox cofactor and a [2Fe] subsite, they do not mediate H₂ production. This observation reflects the importance of the first coordination sphere: **1**–**3**HydF do not possess the bridging CO ligand and vacant Fe^d site for catalysis, which consequently does not occur, regardless of the dithiolate ligand. Of course, the function of HydF is rather the construction and transfer of the [2Fe] subsite to apo-HydA. The researchers explored whether the loaded HydF proteins would enable the maturation of apo-HydA (in this case apo-HydA1 from *Chlamydomonas reinhardtii* algae expressed in *E. coli*). Reconstitution was not trivial, as the proteins were native to different species, and **1**–**3**HydF were possibly quite distinct from native HydF evolved to install the HydA H-cluster. Moreover, from a coordination chemistry standpoint many steps had to occur: [2Fe] dissociation from HydF, CN[−] linkage (re)isomerism, S^{Cys} binding to Fe_p, and CO loss.

Given the intricacies of [FeFe] H₂ase maturation,^[8] one can appreciate how remarkable it is that the treatment of apo-HydA1 with **1**–**3**HydF gave complete proteins **1**–**3**HydA1. IR spectra in each case featured ν(CO) and ν(CN[−]) bands, which confirmed [2Fe] transfer; importantly, they also matched that of HydA1. Maturation revealed the configuration of the diiron complex, which could not be determined for **1**–**3** and **1**–**3**HydF: the basic S^{Cys} atom was located *trans* to the bridging CO ligand, and the latter was also *trans* to the vacant Fe^d site. The chemistry of **1**–**3**HydA1 contrasts with that of substitutionally inert **1**–**3**, indicative of lability conferred on these dianions once they enter the enzyme pocket and H bonds are formed between the Fe–CN groups and nearby Lys and Ser residues. Indeed, the interactions of the second coordination sphere dictate the configuration of the first coordination sphere, and as Fontecave et al. emphatically demonstrated, they are also essential to H₂ase function: the activity of the adt^{2−}-containing biohybrid approximated that of functional HydA1, whereas that of the pdt^{2−} and odt^{2−} species was nonexistent. In fact, **2**HydA1 is probably not a biohybrid at all, but precisely native HydA1. For synthetic chemists, it is satisfying that **2** had been prepared more than a decade ago, in anticipation of its relevance to this remarkable active site.^[9]

The dithiolate cofactor, which with confidence can now be said to include X = NH, is key to relaying H⁺ to the active site for H₂ production. The basicity of the amine is presumably tuned to both protonate and deprotonate Fe–H; the oxygen atom in odt^{2−} is insufficiently basic for these tasks, and pdt^{2−} has no acid/base chemistry to speak of. The replication of diiron ligation is not enough, as although acid–base reactions are often considered to be instantaneous, they are not instantaneous for the protonation of sterically congested metals, a problem nature overcomes by setting the amine above the binding site. Beyond the second coordination sphere, the protection afforded by the surrounding proteic mass of >50 kDa is also nontrivial (contrast the high activity of reconstituted **2**HydA1 with the instability of free **2** in acid solution).

Despite the challenges in incorporating nature's design into synthetic molecules, several homogeneous electrocatalysts for H₂ evolution (and, more recently, H₂ oxidation) have been reported. These electrocatalysts range from “biomimetic” models exemplified by **4** to “biologically inspired” systems, such as **5** (Bn = benzyl),^[10] which share less in common with the H₂ases. Essential to the operation of both is the positioning of an amine near an unsaturated electro-active metal—a frustrated Lewis pair poised for action. Although

bidirectional catalysts that operate at high rates and low overpotentials have yet to be prepared, the work of Fontecave et al. is encouraging, as it validates efforts chemists have made towards this goal.

Having unambiguously implicated a role for azadithiolate, Fontecave et al. also hint at the nature of maturase HydF and its [2Fe] core. What is the nature of its precursors upstream in the maturation process? And will this artificial maturation process be compatible with combinatorial methods to enable the discovery of more efficient and more practical catalysts for the evolution and oxidation of H₂.

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Scheme 1.

[FeFe] H₂ase maturation with three synthetic diiron cofactors: biologically active **2** and inactive **1** and **3**.