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Unraveling the Biosynthesis of Nature's Fastest Hydrogenase

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Cofactors are very special because they reveal the needs of catalysts that cannot be satisfied by the myriad abilities of proteins. Cofactors are often the most easily replicated components of a biocatalyst. Of course, cofactors are important because they shed light on biosynthetic pathways that often reveal novel reaction mechanisms. Some of the most spectacular scientific achievements are the result of the enduring affinity of chemists for cofactors, for example, the B₁₂ story. It is intriguing that cofactors are potential keys in this new era of energy-driven research: How exactly does nature design catalysts that process important gases, such as H₂, CO, and CO₂?^[1] The answers are being found, but it has been hard work.

The hydrogenases have proven to be a particularly rich source of cofactors. The active site of the fastest hydrogenases, the [FeFe]-hydrogenases, consists *solely* of cofactors—the diiron core is coordinated to seven cofactors: an 4Fe–4S cluster, an aminedithiolate cofactor,^[2] three CO ligands, and two cyanide ligands (Scheme 1). The biosyntheses of the ligands, aside from the 4Fe–4S cluster, are unknown. This situation is changing rapidly now with the elucidation of the pathway to the cyanide ligands.^[3,4]

Cyanide is widely considered by inorganic chemists as the ultimate “strong ligand”. It is featured in the first synthetic coordination compound, Prussian Blue. As a ligand, cyanide is at or near the top of many lists, notably for the stability constants of its complexes and the spectrochemical series. For related reasons, it is a potent poison which disables key respiratory metalloenzymes.

Industrial routes to cyanide begin with the platinum catalyzed reaction of ammonia–methane–oxygen mixtures. In the main variant of this process, the aza analogue of steam reforming, oxygen is excluded, and H₂ is also generated. By these two processes, more than a billion kilograms of HCN are produced annually.^[5]

Many routes are known to metal cyanides,^[6,7] not surprisingly, since such complexes are highly stable. Typically, metal cyanides are made from cyanide salts. Metal–cyano complexes also arise from many CN-containing precursors: hydrogen cyanide, cyanogen, cyanogen halides and thiocyanates, and even nitriles^[8] and isonitriles.^[9] More interesting

and more obscure routes to metal cyanides do not start with a preformed C N bond. For example, $[\text{Fe}(\text{CO})_5]$ reacts with ammonia (and its equivalents^[10]) to give cyano complexes. Such reactions appear to occur via carbamoyl intermediates.^[7,11] Metal complexes of methylamines are susceptible to oxidative dehydrogenation to the corresponding cyanide complexes (Scheme 2).^[12]

In plants and arthropods, cyanide is produced from any of several amino acids, but not from glycine. The oxidation of the amine is catalyzed by two different cytochrome P450 enzymes and results in α -hydroxynitriles. These compounds or their derivatives are utilized by their hosts as precursors to HCN as a defensive antifeedant response.^[13] Bacteria, on the other hand, commonly generate cyanide from glycine [Eq. (1)].



The biosynthetic precursor of the cyanide in the [NiFe] hydrogenases is the metabolite carbamoyl phosphate ($\text{H}_2\text{NC}(\text{O})\text{OPO}_3\text{H}^-$). In the Böck pathway, the $\text{C}(\text{O})\text{NH}_2$ unit is transferred to the C-terminal cysteinyl thiol of the protein HypE.^[14] The dehydration of HypE- $\text{SC}(\text{O})\text{NH}_2$ requires adenosine triphosphate hydrolysis and affords HypE-SCN, which is the precursor to at least one and probably both of the Fe-CN subunits of this hydrogenase. [NiFe]- and [FeFe]-hydrogenases are, however, unrelated genetically,^[15] so the biosynthesis of the cyanide ligands in the [FeFe] enzyme was expected to differ.

Following the development of a heterologous expression system for the *Chlamydomonas reinhardtii* [FeFe]-hydrogenase, three proteins, HydE, HydF, and HydG, were implicated in the biosynthesis of the CO, cyanide, and dithiolate ligands of the H cluster.^[16] HydE and HydG are members of the radical *S*-adenosylmethionine (SAM) family,^[16] the class of enzymes that are responsible for generating the C-S bonds in lipoic acid and biotin. By using a cell-free approach, Roach and co-workers demonstrated that HydG converts tyrosine into the cyanide needed for the biosynthesis of the H cluster.^[4] In a further illustration of the special capabilities of radical SAM enzymes, tyrosine was degraded into *p*-cresol and dehydroglycine, which released CN^- into the medium, where it was detected by conversion into a fluorescent derivative. Dehydroglycine is also involved in the formation of thiamin in some bacteria, where it is also generated by radical SAM enzymes (Scheme 3). In *Escherichia coli*, the enzyme is called ThiH and shares significant sequence homology with HydG.^[3,17]

The details of the process by which cyanide becomes attached to the diiron center remain unknown. Being a promiscuous ligand, CN^- probably needs to be guided to the targeted Fe_2 center, not simply released in its vicinity. The Fe center that receives the cyanide is bound to a scaffold protein,^[18] which may solve this problem. For the coordination chemist, the role of the dehydroglycine is especially intriguing. Several metal complexes of dehydroglycine are known; they are mainly prepared by oxidative dehydrogenation of glycinate complexes (Scheme 4).^[19] Thus, it is reasonable to hypothesize that cyanide is formed by oxidative degradation of an iron-dehydroglycinate complex. The conversion would be reminiscent of ethylene biosynthesis, which occurs through the iron(II/III)-mediated release of HCN from

aminocyclopropanecarboxylic acid.^[20] Glyoxalic acid, obtained upon the hydrolysis of dehydroglycine, is a potential source of CO. In contrast to this unfolding (and still unconfirmed) biosynthetic scenario, the CO and CN⁻ ligands in the [NiFe] hydrogenase do not originate from the same precursor.^[21]

Regarding the biosynthesis of the dithiolate cofactor, Pilet et al. have suggested that this subunit arises through the condensation of two equivalents of dehydroglycine with a 2Fe–2S center.^[3] This route is reminiscent of the known aminomethylation of iron sulfides (Scheme 5).^[22]

The elucidation of the routes to the dithiolate as well as the three CO ligands will form the basis of exciting further research. Overall, the hydrogenases have proven to be a particularly rich source of novel chemistry, not only in the realm of catalysis, but also for new synthetic routes that might be more broadly applicable.^[23]

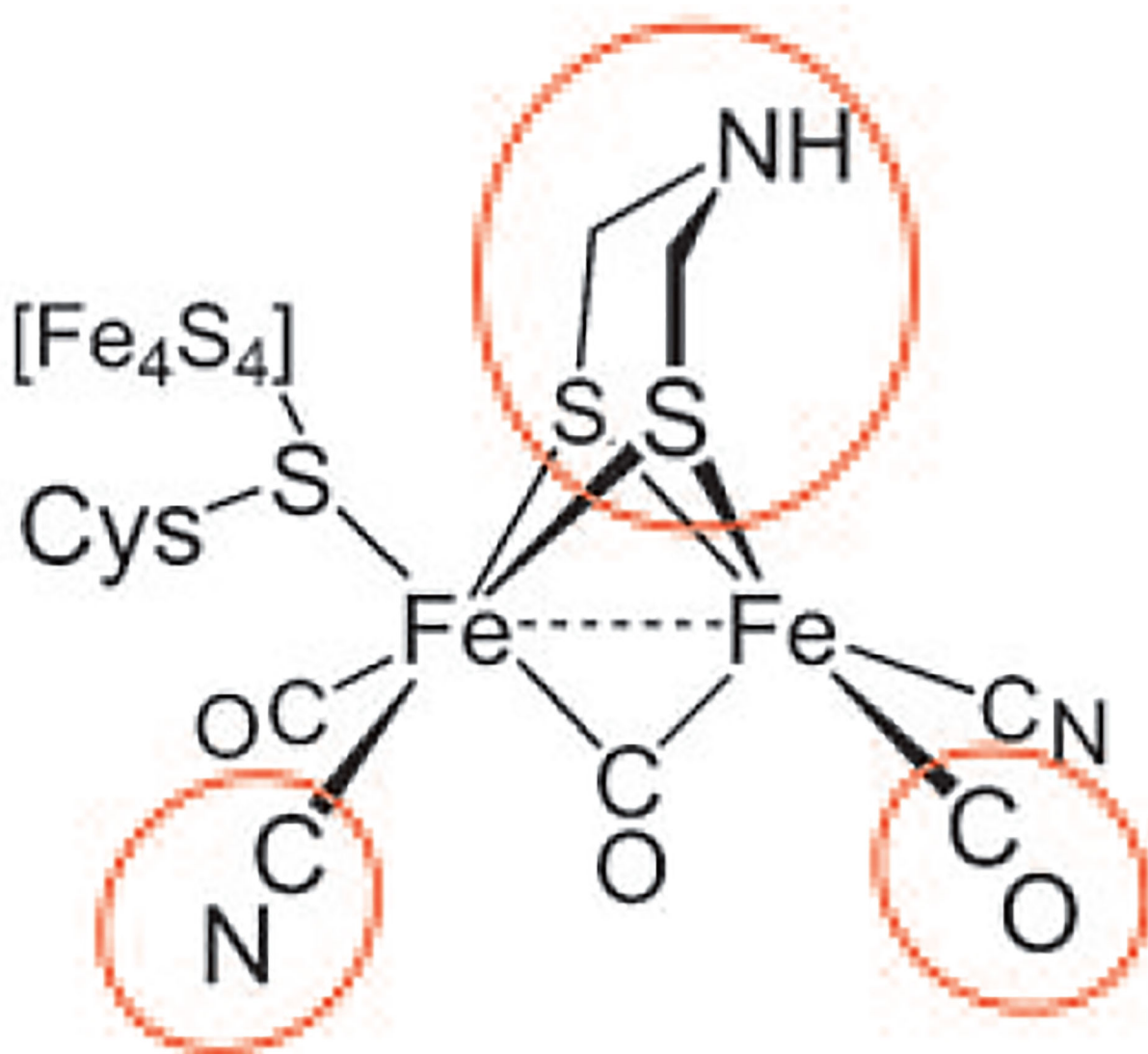
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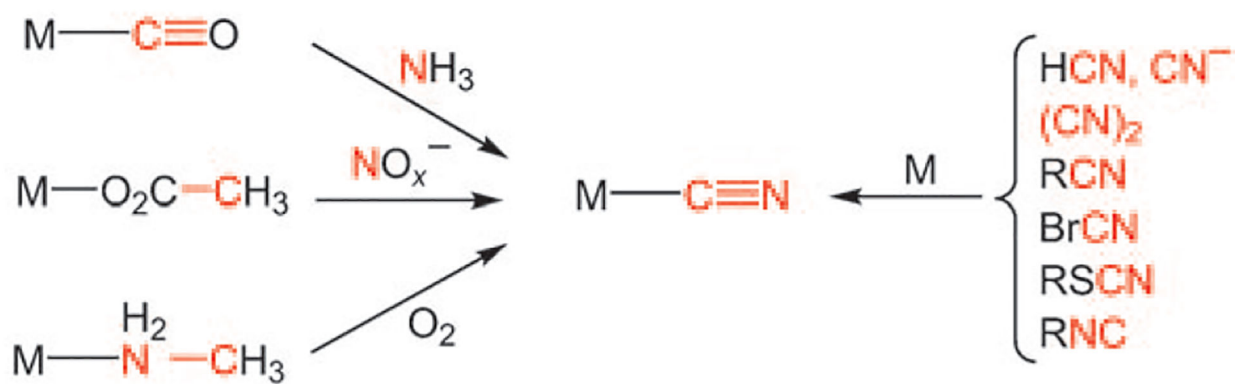
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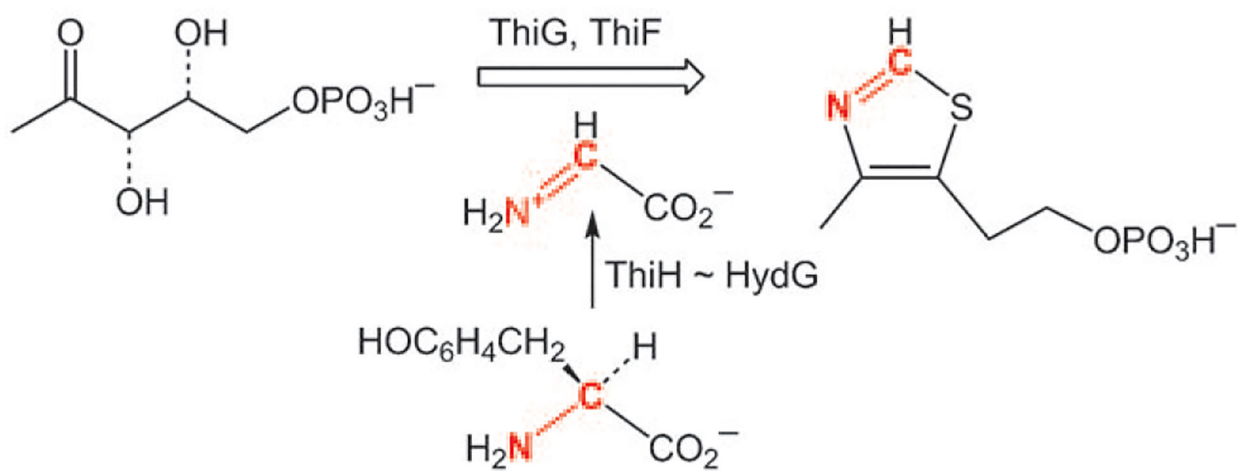
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**Scheme 1.**

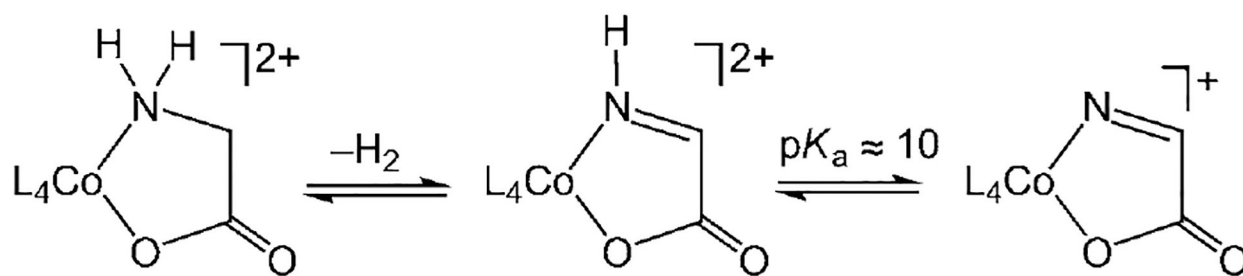
Active site (“H cluster”) of the [FeFe]-hydrogenases. The three types of cofactors of unknown origin are highlighted. Roach and co-workers recently showed that at least one cyanide ligand is derived from tyrosine via the intermediacy of dehydroglycine.^[4]



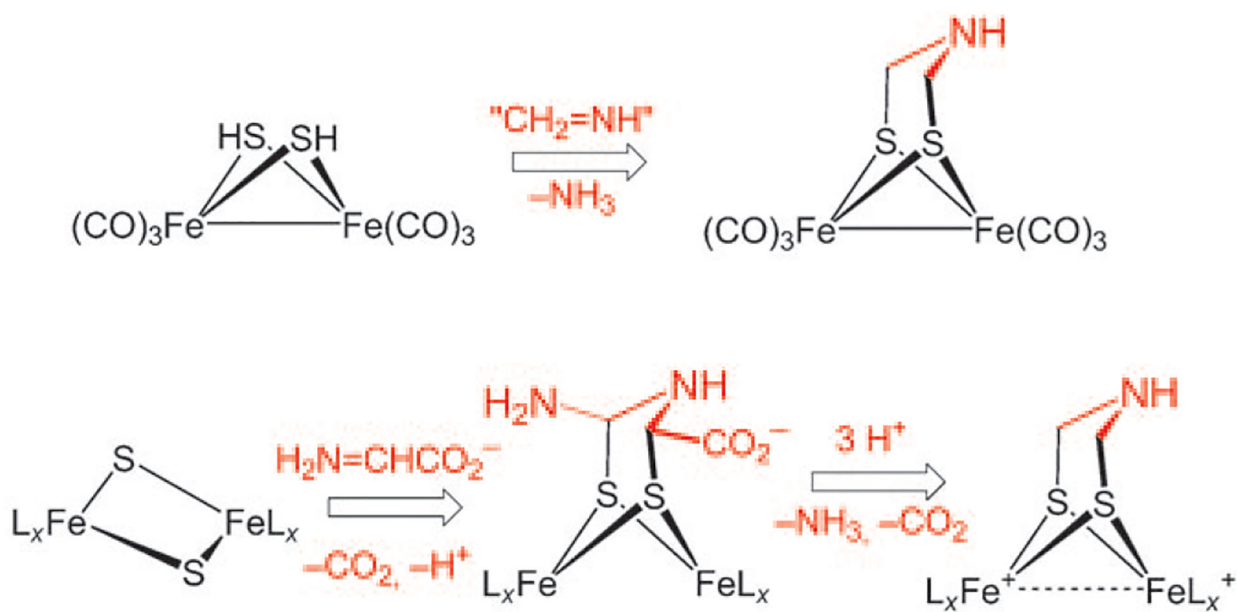
Scheme 2.
Routes to metal cyanides.

**Scheme 3.**

Role of dehydroglycine in the biosynthesis of the thiazole in thiamin.

**Scheme 4.**

Formation and acid–base reaction of a cobalt(III) complex of dehydroglycine.



Scheme 5.
Organometallic route to the azadithiolate cofactor and the pathway proposed by Pilet et al.^[3]