Enzyme Mimics

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The Third Hydrogenase: A Ferracyclic Carbamoyl with Close Structural Analogy to the Active Site of Hmd**

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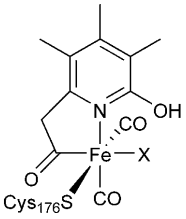
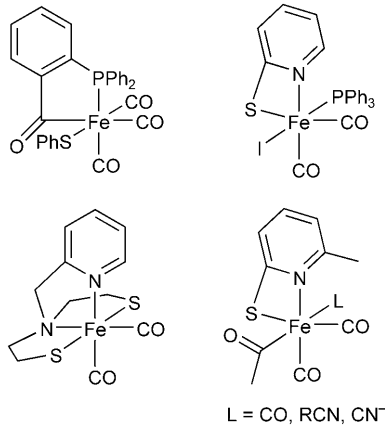
Cleavage of the dihydrogen molecule to give a bound proton and hydride, its oxidation to yield protons and electrons, and the converse of these two processes, are intrinsic to the use of molecular hydrogen as a potential energy vector for clean energy storage and transduction ("hydrogen economy"). Nature has its own hydrogen economy. Communities of bacterial or archael microbes perform these same interconversions at high rates using three phylogenetically distinct hydrogenase enzymes, each with a unique metallo-center at the heart of their catalytic machinery.[1] The de novo syntheses of free-standing analogues of the enzyme active sites are major scientific challenges, not least to provide both a deeper understanding of structure and function of the natural systems and new catalytic or electrocatalytic materials for dihydrogen use which are based on earth-abundant elements rather than precious metals.[2–5] Here we describe the synthesis of a close structural analogue of the active site of perhaps the least understood and characterized of the hydrogenase enzymes—the "third hydrogenase", H_2-forming methylene-tetrahydromethanopterin dehydrogenase (Hmd), also known as [Fe]-hydrogenase.[6]

Hmd was discovered in 1990 and initially thought to be metal-free.[7] Subsequently it was found to contain an iron center coordinated by two *cis*-carbonyl ligands, with a preliminary crystal structure of a reconstituted apoenzyme of *Methanocaldococcus jannaschii* appearing in 2008.[8] Soon after, informed by a higher resolution structure of a mutant enzyme possessing an exogenous dithiothreitol ligand, the structure of the "native" enzyme was re-refined.[9] The revised structure shows that the iron is ligated by two carbonyl ligands, a protein-bound sulfur and a pyridinone-containing cofactor. This cofactor binds to the metal through the nitrogen atom of the ring and through an acyl group forming a metallocycle unprecedented in biological systems.



Hitherto, structurally characterized mimics of the [Fe]-hydrogenase active site have mainly been confined to systems bearing phosphine ligands (Scheme 1).[10–13] The reported phosphine-free model compounds either include two sulfur donors,[14] or a tight bite angle chelating N,S-ligand.[15] While all these models have provided useful information about the likely binding motifs in the [Fe]-hydrogenase active site, none possess the ferracyclic structure nor the deployment of ligands with bond angles close to those of the active site.



Scheme 1. Literature models for the active site of the [Fe]-hydrogenase. Clockwise from top-left: Ref. [12], [11], [15], and [14].

We noted that certain rhenium and ruthenium carbonyl complexes formed five-membered carbamoyl ring systems upon reaction with amines[16] and questioned whether Fe^{II} carbonyls might similarly form ferracyclic rings. We show that this is the case and that it provides an entry for the synthesis of an active-site analogue which matches the geometry of the enzymatic core to an unprecedented degree.

Reaction of $[Fe(CO)_4Br_2]$ with 2-aminopyridine in dichloromethane rapidly produced a yellow precipitate accompanied by evolution of carbon monoxide (Scheme 2). The precipitate dissolved in acetonitrile to give an orange solution which on concentration and cooling afforded a mass of homogenous X-ray quality crystals of **1**·MeCN (Figure 1, top). The reaction between the pendant amino group of the pyridine and a metal-bound carbonyl ligand leads to the formation of a coordinated carbamoyl unit with loss of HBr. Two carbonyl ligands are retained with relative *cis* geometry: one is located *trans* to the pyridine nitrogen and the second is *trans* to the anionic ligand, the same arrangement as extant in the [Fe]-hydrogenase subsite. The coordination sphere in **1** is

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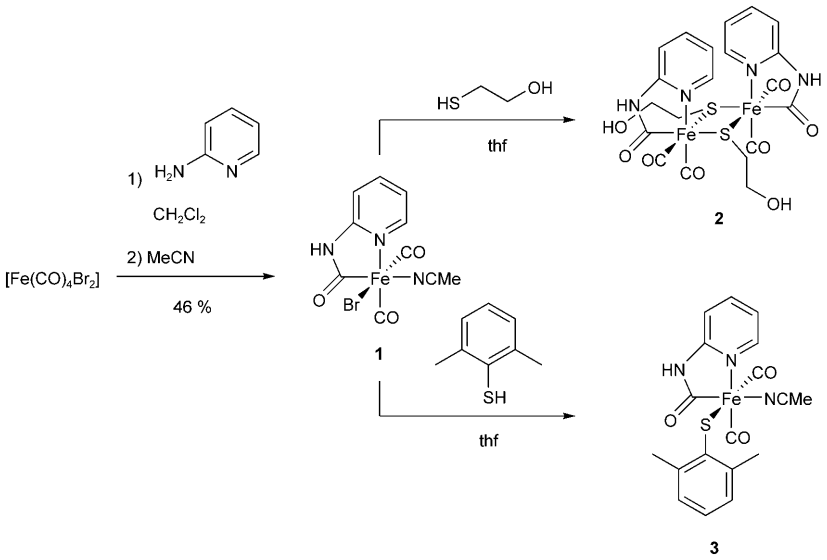


Scheme 2. Synthesis of model compounds.

completed by the retained halide and a bound acetonitrile, which occupy the positions that in the natural system bind the anionic thiolate and neutral unknown ligands, respectively.

The solid-state infrared spectrum of **1** (Figure 2, bottom) shows the two bands for a *cis* dicarbonyl (1984 cm^{-1} and 2046 cm^{-1}) in agreement with the X-ray structure. Spectra of **1** in both acetonitrile and thf show evidence for multiple geometric isomers in solution. In thf the carbonyl stretches fall at 1967 cm^{-1}, 1986 cm^{-1}, 1994 cm^{-1}, and 2046 cm^{-1}, with very similar values in acetonitrile (1964 cm^{-1}, 1987 cm^{-1}, 1998 cm^{-1}, and 2052 cm^{-1}). Density function theory (DFT) simulation of the infrared spectra of a series of isomers indicates that the solution spectra are consistent with the presence of three distinct geometric isomers. The simulated spectra show two absorptions in the carbonyl region with good agreement between the experimental data for three of the isomers calculated (for full details see Supporting Information).

Exposure of the thf solution to an atmosphere of carbon monoxide for 20 min resulted in new signals at 2108 cm^{-1}, 2097 cm^{-1}, 2047 cm^{-1}, and 2016 cm^{-1}. This process could be reversed by purging the solution with nitrogen, which fully regenerated the original spectrum, suggesting that the sixth coordination site on the metal is highly labile.

To fully reproduce the ligand set of the natural system, the bromide in **1** needed to be replaced by a thiolate group. At the same time, the sixth coordination site on the metal was a potential candidate for chelation by a bidentate thiol bearing a second, weaker, donor. One clear candidate for this role was 2-mercaptoethanol (Scheme 2). Treatment of **1** in thf with the thiol gave no color change but did result in the appearance of new infrared absorbances at 2044 cm^{-1}, 2029 cm^{-1}, 1978 cm^{-1}, and 1973 cm^{-1} in the carbonyl region. Crystallization of this new material revealed a dimeric structure, in which the thiolate ligand acts as a bridging donor (Figure 1, middle).

In the asymmetric unit there are three iron subunits, all with the same coordination geometry and similar metrical parameters. The asymmetric unit also contains one half of a highly disordered non-coordinated thf molecule. Two of the subunits form a dimer with no symmetry relationship between the two ends, while the third iron subunit forms a dimer with a symmetry-related group. The arrangement of the ligand set in **2** reflects that in **1**, with the carbonyl and pyridine-carbamoyl ligands remaining in place. The thiolate ligand replaces both the bromide and solvent mol-

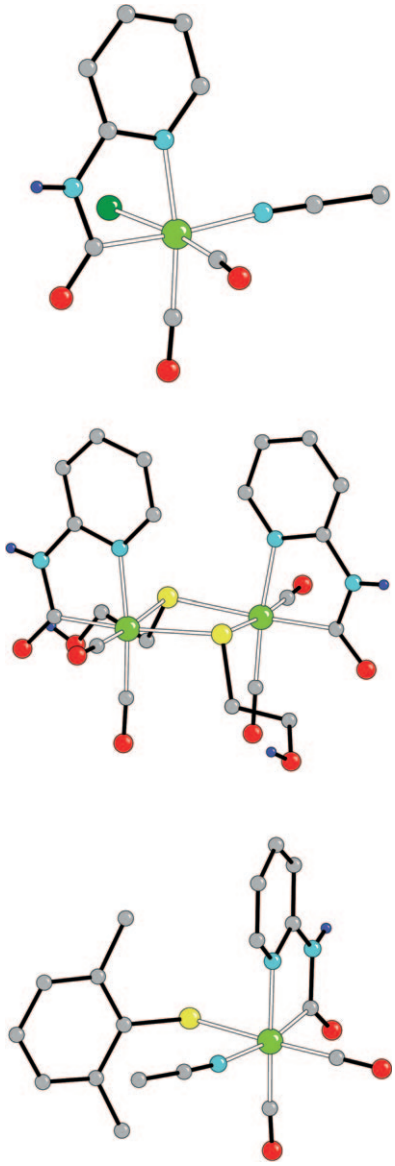


Figure 1. CAMERON representations of the structures of **1**·MeCN (top), **2**·$^1/_3$(thf) (middle) and **3** (bottom) showing spheres of arbitrary size; hydrogen atoms other than those bound to nitrogen and oxygen have been removed for clarity. A non-coordinated molecule of MeCN has been omitted from the structure of **1**. In the structure of **2**·$^1/_3$(thf), only the discrete dimer in the asymmetric unit is shown. Color scheme: Br (dark green), C (gray), H (dark blue), Fe (light green), N (light blue), O (red), S (yellow). For selected bond lengths and angles see the Supporting Information.

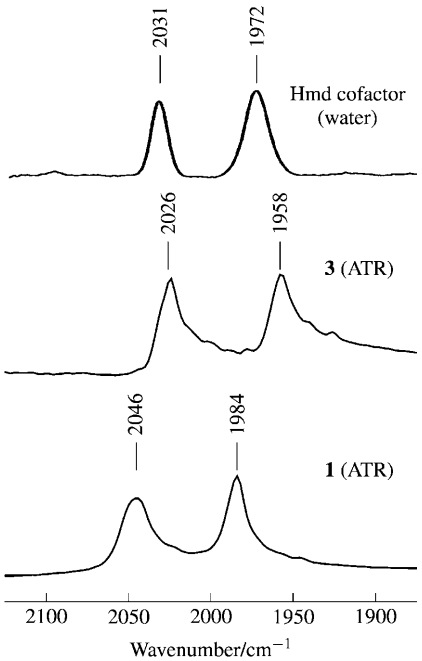


Figure 2. IR Spectra for **1**, **3**, and enzyme cofactor. Bottom: **1** (ATR); middle: **3** (ATR); top: Hmd cofactor (water).[17] ATR = attenuated total reflectance.

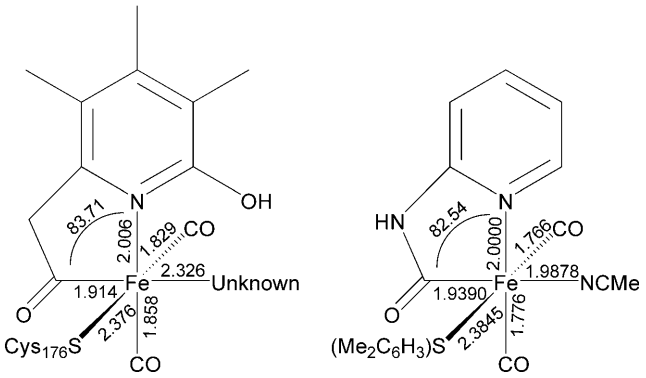
ecule from **1**, leaving the alcohol group free (and somewhat disordered). The two iron and two sulfur atoms of the dimer lie approximately in the same plane: the maximum deviation of the atoms from the mean plane is around 0.09 Å.

In an attempt to break up the dimer in **2**, the synthesis was repeated by first saturating the solution of **1** with carbon monoxide to form the CO adduct. Infrared spectra showed that the formation of **2** was much more sluggish under these conditions, but that no other products were formed by the reaction. Reaction of **2** with triphenylphosphine or $[NEt_4][CN]$ as good donors for iron also failed to break open the dimeric unit.

An alternative approach to synthesizing a monomeric iron system was to increase the bulk of the thiolate to prevent bridging occurring at all. Reaction of **1** with the bulky thiol 2,6-dimethylbenzenethiol in thf (Scheme 2) led to loss of the starting material stretches in the IR. The product could be crystallized from a concentrated solution after dilution with acetonitrile, after which an X-ray diffraction study revealed that this generated the desired monomeric complex **3** (Figure 1, bottom). As anticipated, the sulfur unit replaces the bromide of **1**, with the solvent site remaining occupied by a bound acetonitrile. This acetonitrile both in the bromide **1** and this thiolate derivative can be displaced by CO. Binding of carbon monoxide is reversible: flushing with nitrogen restores the parent system.

In the solid state, the IR spectrum of this new compound again confirmed the presence of *cis* carbonyl groups (Figure 2, middle), with maxima at 1958 cm^{-1} and 2026 cm^{-1}.

Complex **3** features a five-membered cyclic N,CO ligand with stereochemistry exactly as that observed in the natural subsite, i.e. sulfur *trans* to carbon monoxide, nitrogen *trans* to carbon monoxide, and an acyl-like ligand *trans* to a weakly coordinating solvent molecule (Scheme 3). Comparing the metrical data for **3** with that of the wild-type enzyme, it is apparent that the **3** is also a good model for the [Fe]-subsite



Scheme 3. Metrical data for the wild-type enzyme of *M. jannaschii* reconstituted with FeGP-cofactor (left)[9] and compound **3** (right): distances in Å, angles in °, standard uncertainties have been omitted for clarity.

when considering the detail of the active-site geometry. The binding of the bidentate ligand and sulfur group is very similar in the two cases, while the carbon monoxide ligands in **3** are only slightly closer to the metal than is observed in the enzymatic system. The nature of the ligand at the sixth coordination site in the enzyme crystal structure is not clear from the available diffraction data, and it has been modeled using a weakly bound water.[8,9] In **3** the solvent molecule MeCN is bound at a normal distance.

Comparison of the infra-red data for **1** and **3** with that for the wild-type enzyme (Figure 2) shows that in all three cases the pattern of a *cis* pair of carbonyl ligands is clear. The difference between the positions of the two bands in **1** (62 cm^{-1}) and **3** (68 cm^{-1}) is greater than that for the nature subsite (59 cm^{-1}). This may be accounted for by the use of the carbamoyl ligand in the models, or may be due to the nature of the ligand in the sixth position at the metal (the unknown ligand in the enzyme structure). However, the systems are sufficiently similar that both **1** and **3** may be regarded as good models for the active site. The carbamoyl stretches observed here fall within the region that in the natural system is covered by the enzyme backbone, and it is reasonable to expect that the acyl ligand in the subsite will be at a similar wavenumber and so obscured by the protein stretches.

In summary, we have shown that from readily available materials the access of **3** requires only three steps: synthesis of $[Fe(CO)_4Br_2]$, the amidation reaction to give **1**, and substitution by a thiol ligand. Nature most probably constructs the five membered ferracyclic ring to poise the 2-hydroxy pyridine substituent in a position to assist the heterolytic cleavage of dihydrogen, preventing the formation of an otherwise favored N,O chelate.[11] The demonstrable accessibility of the molecules **1** and **3** should now open up chemistry for probing this and exploring dihydrogen and carbocation chemistry.

Experimental Section

Preparation of $[FeBr(C_6H_5N_2O)(CO)_2(MeCN)]$ (**1**): Solid 2-aminopyridine (718 mg, 7.63 mmol) was added to a solution of $[Fe(CO)_4Br_2]$ (2.51 g, 7.65 mmol) in dichloromethane (150 mL). Gas was evolved, and a yellow precipitate formed over a few minutes. Once precipitation was complete the solid was recovered by filtration and washed with hexane (80 mL). The residue was recrystallized from acetonitrile giving **1**·MeCN as orange crystals suitable for an X-ray diffraction study (1.40 g, 46%). M.p. 108 °C. Found C 36.32, H 2.73, N 14.12%; $C_9H_8BrFeN_3O_3{\cdot}C_2H_2N$ requires C 36.49, H 2.81, N 14.18%. ν_{max} (thf) 2046, 1994, 1986, 1967, 1665, 1621 cm^{-1}. *m/z* (ESI) 353.8 $[M^+]$.

Preparation of $[\{Fe(C_6H_5N_2O)(\mu^2\text{-}SCH_2CH_2OH)(CO)_2\}_2]$ (**2**): 2-Mercaptoethanol (0.030 mL, 0.43 mmol) was added to a solution of **1**·MeCN (160 mg, 0.46 mmol) in thf (15 mL), and the mixture stirred for 1 h. The solution volume was then reduced in vacuo to approximately 1 mL, giving a syrup-like solution. After standing for 16 h large crystals of **2**·$^1/_3$(thf) were formed, suitable for an X-ray diffraction study. Found C 39.15, H 3.69, N 8.62%; $C_{20}H_{20}Fe_2N_4O_8S_2$ requires C 38.72, H 3.55, N 8.70%. ν_{max} (thf) 1973, 1978, 2029, 2044 cm^{-1}. *m/z* (ESI) 642.9 $[M^+ + Na, 50\%]$, 621.0 $[M^+ + H, 100\%]$.

Preparation of $[Fe(C_6H_5N_2O)(SC_6H_3Me_2)(CO)_2]$ (**3**): 2,6-Dimethylbenzenethiol (0.060 mL, 0.45 mmol) was added to a solution of **1**·MeCN (170 mg, 0.43 mmol) in thf (20 mL), and the mixture stirred for 2 h. It was then concentrated to ca. 4 mL and diluted with MeCN (1 mL). Cooling of this solution afforded crystals of **3** suitable for X-ray diffraction studies. Found C 52.71, H 4.09, N 10.31%; $C_{18}H_{17}FeN_3O_3S$ requires C 52.57, H 4.17, N 10.22%. ν_{max} (MeCN) 2052, 2032, 1971, 1620 cm^{-1}. *m/z* (ESI) 315.1 $[M^+]$.

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