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Review

Hydrogenase enzymes: Recent structural studies and active site models

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ABSTRACT

Recent developments in active site structure determination of the three types of hydrogenase enzymes are described. Aspects of recent studies using model complexes relevant to the structure and function of the enzymes are reviewed.

Contents

1. Introduction

The current energy infrastructure is dominated by fossil fuel production and combustion, which is causing massive emission of greenhouse gases. Hydrogen is often suggested as an alternative fuel, indeed hydrogen is sometimes touted as "*the fuel of the future*". This statement has been uttered with conviction for at least a generation, usually with greater fervor during times of high petroleum prices. Hydrogen is not a fuel, since it is derived from fuels such as methane only with energy input. It is more correct to call hydrogen an energy currency. As an alternative clean energy currency, hydrogen has great potential [1].

It is likely that the hydrogen economy will remain hypothetical unless several fundamental problems can be solved. Realization of these possibilities depends upon significant progress in hydrogen generation, storage, transportation and utilization. It is particularly important to develop new ways to generate hydrogen that do not use hydrocarbons.

Technology for hydrogen utilization is highly advanced, but scale up remains an issue. Although hydrogen can be efficiently used in fuel cells, widespread adoption of this technology will be limited by the high cost and limited availability of platinum group metals which are required as catalysts [2].

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Biomimetic approaches to these problems are a promising avenue for research [3]. The long term goal of this work is to use insights gained from biology to develop new catalysts for hydrogen production/utilization that do not require platinum group metals [4]. This article presents a brief review of recent results on the structures of the hydrogenase enzymes and of some of the active site model studies that have developed from these insights. A summary of the biological and structural research on the enzymes is provided in the recent reviews by Fontecilla-Camps [5] and by Vignais and Billoud [6]. Aspects of model chemistry have been reviewed by Darensbourg et al. in 2000 [7], Schröder and coworkers in 2001 [8], Pickett and coworkers in 2002 [9], Fernandez and coworkers in 2007 [10], Fontecave and coworkers in 2008 [11], Schollhammer and coworkers in 2008 [12] and by Rauchfuss and Gloaguen in 2009 [13]. Research concerning hydrogenase enzymes has been discussed by Kubas as part of a larger review of dihydrogen complexation [14].

2. Hydrogenase active site structures

In nature, hydrogen is utilized/produced with impressive efficiency by hydrogenase enzymes, which are metalloenzymes employing iron and/or nickel. Hydrogenases efficiently and reversibly catalyze the conversion of hydrogen into two electrons and two protons:

$$H_2 \leftrightharpoons 2H^+ + 2e^-$$

This enzymatic activity was first reported in 1931, when it was noted that *Escherichia coli* evolves hydrogen in the course of growth under certain conditions. Hydrogen can be utilized to reduce a variety of substrates [15]. Activity can be assayed by hydrogen production in the presence of various donors, by reduction with H_2 of synthetic dyes such as methylviologen and by H/D exchange reactions [16].

The efficient catalysis of this reaction makes available the strong reducing power of dihydrogen, ultimately harnessing it in kinetically useful ways for the reduction of a variety of substrates. Hydrogenases have been found in a wide range of microorganisms, from prokaryotic microbes to eukaryotic protozoa and fungi [17]. The hydrogenase enzymes catalyze both uptake and production of hydrogen, depending upon the conditions. Since the solubility of hydrogen in water is very low, hydrogenases must have a very high affinity for hydrogen. Many species utilize H_2 arising from fermentation or other hydrogen generating processes by coupling hydrogen oxidation to energy generation, thus recapturing "lost" reducing ability. In complex microbial communities such as those found in the mammalian gut, it has recently been demonstrated that pathogenic bacteria such as *Helicobacter pylori* are able to utilize hydrogen as an energy source [18].

The two electron potential for proton reduction at pH 7 and a reasonable "natural abundance" of $P(H_2) = 10^{-7}$ atm is *ca.* −208 mV. At higher hydrogen pressures this potential increases to the one atmosphere value of −414 mV. The reduction potentials for cytochrome c3 (−290 mV) and NAD^+/NADH (−320 mV) are between these limiting values. As pointed out by Collman [19], the local concentration of H_2 must increase above natural levels in order to reduce these substrates. Thus, fluctuations in the pressure of hydrogen may be used in a feedback mechanism to direct the flow of electrons toward hydrogen uptake or production of hydrogen in the reversible hydrogenases [20].

3. NiFe hydrogenases

All hydrogenases are metalloenzymes, containing nickel and iron or iron only. It was long thought that the Ni center in the NiFe enzymes was the site of hydrogen activation. The discovery of dihydrogen adducts of various transition metals led Crabtree to propose the intermediacy of a nickel dihydrogen complex in hydrogen activation by hydrogenase enzymes [21]. With an emphasis on the Ni site, functional models were pursued using various Ni complexes for the electrocatalytic reduction of protons to dihydrogen [22]. In terms of structural and reactivity models, this line of investigation has been limited by the paucity of well characterized nickel hydrides and the complete absence of examples of nickel dihydrogen complexes [23].

In 1995, crystallographic studies on the NiFe hydrogenase from the bacterium *Desulfovibrio gigas* showed that the hydrogen activating site contains one Ni and one Fe atom [24]. The coordination sphere of the Fe atom includes three diatomic ligands which could not be conclusively identified from the crystallographic data. Infrared spectroscopy combined with isotopic labeling ($^{13}C/^{15}N$) has been used to establish that the iron atom is ligated by two cyanide and one carbon monoxide ligands. These studies were carried out on an enzyme isolated from *Chromatium vinosum*. Overall similarities in the infrared spectra of the two NiFe hydrogenases leave no doubt that this result is generally applicable [25]. The coordination environment around the iron is completed by two bridging thiolate groups from cysteine residues and a hydroxo or oxo bridge in the oxidized form. With the infrared spectral data in hand, detailed examination of the active site environment in the hydrogenase from *D. gigas* allows a tentative assignment of the CO versus CN^- ligands. This assignment is based on apparent hydrogen bonding interactions between the cyanide ligands and adjacent residues of the protein, while the third diatomic ligand is in a more hydrophobic environment and is assigned as the carbonyl group [26,27]. Infrared spectroscopy was also employed in conjunction with EPR spectroscopy and hydrogenase activity assays to assign infrared spectral features to each of the various redox states of the enzyme. It was suggested that redox activity is centered at nickel, with Ni^{II} (S = 0) oxidized to Ni^{III} (S = ½) at −250 mV [28].

Based on this and other spectroscopic data, detailed mechanisms for hydrogen activation involving hydrides of both Ni and Fe have been proposed [29,30]. Several computational studies at various levels of theory have been reported [31] (see Fig. 1).

4. FeFe hydrogenases

In some Fe hydrogenases, an infrared signature similar to the NiFe hydrogenases has been reported [32], suggesting that hydrogen activation requires an iron center with carbonyl and cyanide ligands. Fe hydrogenases are usually about two orders of magnitude more active with artificial electron donors and acceptors than the Ni/Fe enzymes, and bind hydrogen more strongly.

The structure of the so-called H cluster in these enzymes has been studied by various methods. This cluster is quite similar in all enzymes studied and was surmised to contain 4–6 iron atoms. In the oxidized state it contains two types of magnetically distinct Fe atoms, has an S = 1/2 spin state and is characterized by an unusual rhombic EPR signal [33]. A pulsed EPR investigation reported evidence for an unusual non-protein N containing ligand, which was thought to be cyanide. This study also suggested that inactivation of the enzyme may be due to binding of an imidazole moiety at the active site [34]. Hydrogenase enzymes isolated in air are inactive, but can be activated by reduction with hydrogen or other reducing agents. The reaction with hydrogen is in general reversible. Reductive titration of the oxidized form of these enzymes is complicated by the onset of H_2 production at the expense of reduced titrant [35], leading to some uncertainty in the reported reduction potential values [36]. A novel feature of these enzymes is the presence of low spin Fe(II) centers. Low spin ferrous iron is

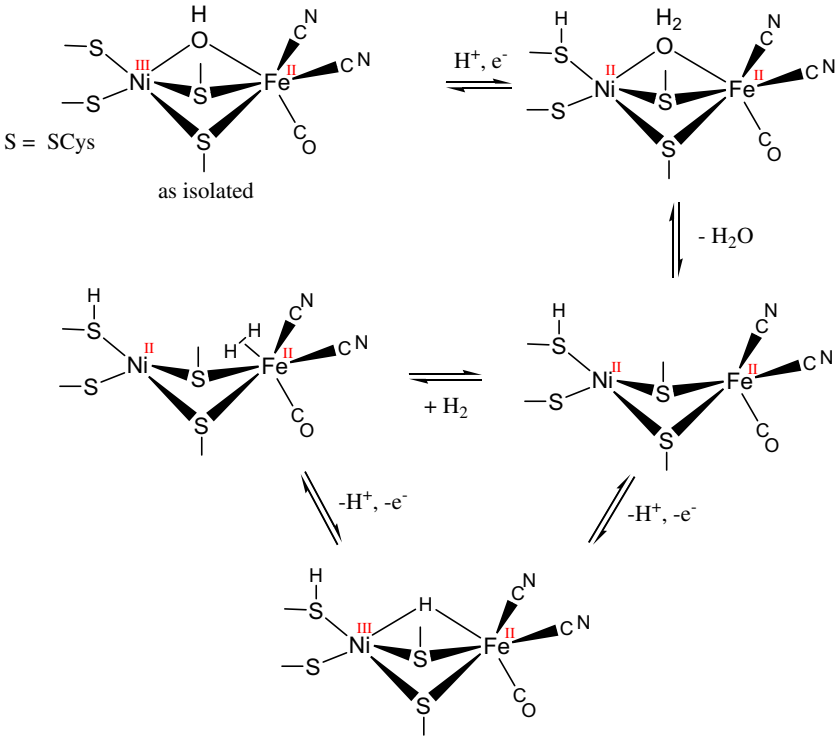


Fig. 1. Active site structure of NiFe hydrogenase and one proposed mechanism of hydrogen activation.

uncommon in non-heme environments, but the presence of strong field ligands such as carbon monoxide and cyanide is consistent with this spin state.

Two crystallographic studies of the FeFe hydrogenase active site appeared early in 1999. Peters and coworkers reported the structure (1.8 Å resolution) of the FeFe hydrogenase from *Clostridium pasteurianum* (CpI) [37]. The structure of the active site reveals a novel dinuclear Fe species, which is connected to the protein only by a single cysteinyl ligand (Cys 503). One of the iron centers has a bound ligand (likely water) occupying the most likely site for activation of hydrogen. In this structure, the bridging group connecting the two Fe centers was refined as a carbonyl group. A computational study (DFT) based on this structure was reported soon after the crystallographic work was published [38]. A very high quality (1.6 Å resolution) structure has been reported by Fontecilla-Camps and coworkers for the Fe hydrogenase from *Desulfovibrio desulfuricans* (Dd) [39]. In this structure, the two bridging sulfide ligands are thought to be linked by a three carbon bridge. Since the S–S distances in the two structures are quite similar, it seems possible that there is little real difference in the groups bridging the S atoms.

Another difference between the two structures is in the nature of the group bridging the two Fe centers. In the Dd structure, a bridging oxo (or hydroxyl) group was refined, although a more recent interpretation of this data postulates a mixture of terminal and bridging carbonyl groups [40]. Most interestingly, one of the Fe centers in the Dd active site has a vacant coordination site. The overall geometry at this iron center is a slightly distorted square pyramid. It is possible that this site contains a crystallographically undetectable hydride (or dihydrogen) ligand. These differences in the refined active site structures may be attributed at least in part to differences in the conditions under which the crystals were grown. The CpI crystals were obtained in the presence of dithionite (reducing conditions), but the authors noted that over the course of crystallization, a reducing environment may not have been maintained. The Dd crystallization was carried out under a H_2/N_2 atmosphere, and therefore is likely to represent a more reduced state of the enzyme. More recent crystallographic and IR studies of Dd have led to the view that the three atom group bridging the two sulfur atoms may be a dithiomethylamine moiety (S–CH_2–NH–CH_2–S) [41]. This insight has led to new directions in model studies and computational work.

Prior to the crystallographic results, Albracht and coworkers used infrared spectroscopy on the Fe hydrogenase from *Desulovfibrio vulgaris* to show that the ligand set is a mixture of CO and CN^- ligands [42]. Since this report preceded the crystallographic

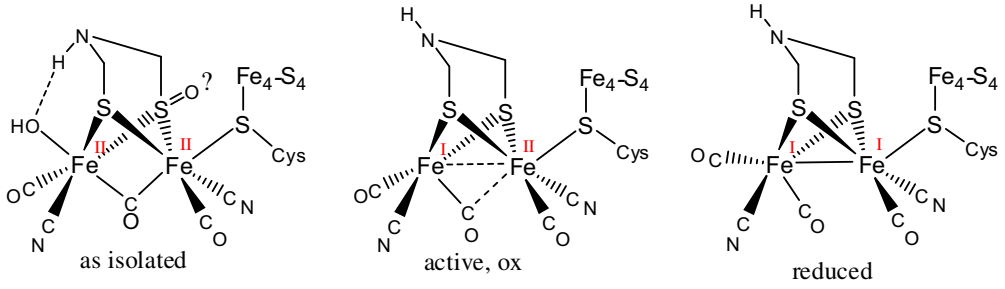
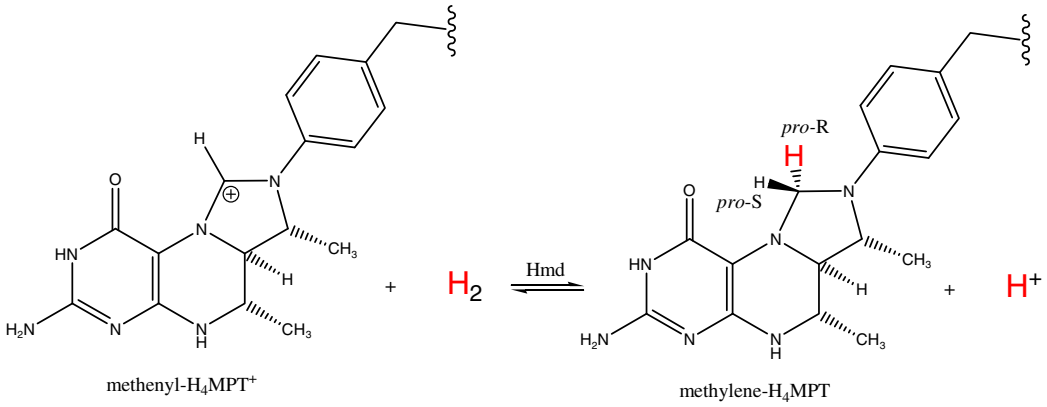


Fig. 2. Proposed structures for the FeFe hydrogenase active site.

results, the working hypothesis for the active site included two Fe atoms, but it was postulated that only one CO and one CN^- ligand were present, which were believed to be attached to the same Fe atom. Striking among the data reported is that *two* CN stretches differing by up to 37 cm^{-1} were observed in all redox states of the enzyme. In view of the crystallographic results, which indicate that each Fe atom has two diatomic ligands, it is very reasonable to conclude based on this infrared data that one cyanide ligand is bound to each of the two Fe centers. The large differences in the observed stretching frequencies presumably arise from differences in the coligands bound to the two iron centers. Similarly, at least two terminal CO stretches are present in each of the redox states of the enzyme and presumably arise from isolated CO groups on Fe centers with different ligand sets.

sulfur clusters. The apoenzyme has been purified, along with a low MW cofactor tightly associated with Hmd and essential for activity [48]. The great difficulty encountered in purification of this enzyme is due in part to the thermal and photo lability of this cofactor. The Hmd hydrogenase from *Methanothermobacter marburgensis* is the most thoroughly studied variant, but sequence homologies suggest that all known Hmd enzymes are similar. Hmd plays a key role in CO_2 reduction to methane in methanogenic archaea. Early work in this area has been summarized by Thauer et al. [49].

In contrast to the NiFe and FeFe hydrogenases, Hmd does not catalyze the reduction of dyes such as methylviologen with hydrogen. Hmd facilitates the stereoselective transfer of a hydride from H_2 to a pterin substrate.



It has long been known that CO is an inhibitor of hydrogenase activity. Peters and Lemon have reported the crystal structure of the CO inhibited form of CpI, where the ligated water has been replaced with CO, with essentially no other changes in the structure. This suggests that hydrogen activation occurs at the distal iron atom by displacement of bound water [43]. Computational studies have used experimental infrared spectra to model the various redox states for the active site. From these and other studies, a consensus mechanism for hydrogen activation has emerged [44]. DFT studies suggest that the three experimentally observed redox levels correspond to $Fe^{II}Fe^{II}$, $Fe^{I}Fe^{II}$ and $Fe^{I}Fe^{I}$. The heterolytic mechanism for H_2 activation was confirmed by Hall and Cao, who postulate a mechanism where H_2 binding at the distal iron leads to a terminal hydride upon deprotonation of the bound H_2 by the adjacent bridgehead amine [45,46]. Structures proposed for the various redox levels are depicted below (see Fig. 2).

5. Hmd hydrogenase

The H_2 forming methylene-tetrahydromethanopterin dehydrogenase (Hmd) is sometimes referred to as an iron–sulfur cluster-free hydrogenase. Hmd was first reported by Thauer and coworkers in 1990 [47]. Hmd is phylogenetically unrelated to the NiFe and FeFe hydrogenases, has MW of *ca.* 76 kD, and is a homodimer comprised of two identical subunits, with two Fe atoms per homodimer and no acid labile sulfur, indicating the absence of iron

Hmd was long thought to be free of metal ions in the active site, but this view has changed with recent discoveries by Thauer and coworkers, who have shown that the active site of this enzyme contains one iron atom [50]. Spectroscopic data for the Hmd active site suggests that the iron center has two CO ligands in a *cis* disposition, one S ligand and two N/O ligands [51]. It has been shown that treatment with CO leads to a tricarbonyl complex, presumably by displacement of a weakly bound solvent molecule. Mössbauer spectroscopy suggests that the active site is diamagnetic under a range of conditions [52]. The active site ligation was confirmed by an XAFS study [53]. The iron in the Hmd active site appears to be redox inactive and is EPR silent under all conditions studied. Nuclear resonance vibrational spectroscopy (NRVS) was used to study and further define the vibrational modes of the Fe bound CO ligands [54].

In 2008, the crystal structure of the Hmd active site was reported by Thauer and coworkers, confirming the presence of two carbonyl ligands [55]. Subsequent studies have suggested that a previously unidentified ligand may be an acyl moiety derived from the pendant acid group of the pyridine ring [56] (see Fig. 3).

The hydrogenase active sites have been revealed by crystallography and other methods to be metalloenzymes with very unusual active site structures. The presence of CO and cyanide as ligands to iron in a functioning enzyme is without precedent in biology. These discoveries have led to intense activity in synthetic model studies, which are discussed below.

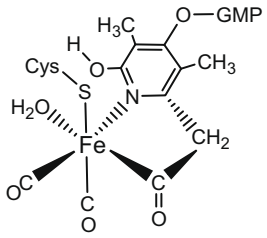


Fig. 3. Structure of the Hmd active site (GMP = guanidine monophospate).

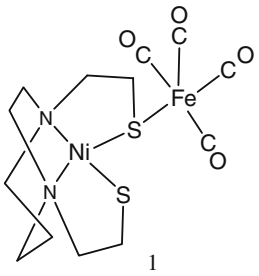
6. Model studies

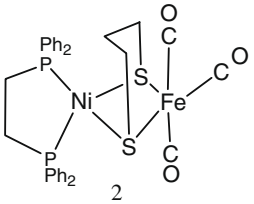
6.1. NiFe hydrogenase models

Early work in this area predates the report on the active site structure, focusing on preparation, structure and reactivity of Ni complexes with sulfur ligation. This work has been reviewed [57]. Subsequent to the active site structure report, there has been vigorous activity in model studies, with considerable progress in spectroscopic and structural models, but more modest achievements in functional models. The preparation of model systems for this active site is challenging on several levels. Heterobimetallic systems are inherently difficult to prepare, the facial arrangement of two cyanide and one CO ligand at the iron center was unprecedented, and the coordination geometry at the nickel center is unusual. A synopsis of these model systems will be presented here, with an emphasis on heterodinuclear Ni/Fe complexes which have carbonyl and/or cyanide ligands at Fe and S donors at Ni.

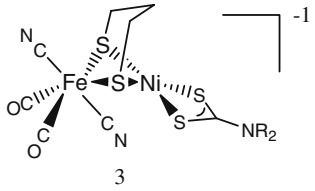
A surprisingly good structural and spectroscopic model for the Fe site is provided by the organometallic iron complex $[(\eta^5\text{-cyclopentadienyl})Fe(CO)(CN)_2]^-$ [58]. When the infrared spectra of this cyclopentadienyl complex in various oxidation states were carefully studied (including ^{13}C and ^{15}N labels), excellent agreement with the IR spectra of the Ni/Fe enzyme active site was observed [59]. An iron phosphine trithiolate complex with CN^- and CO ligands which exhibits a reversible Fe(II)/Fe(III) redox couple at −476 mV has been suggested as a model for the iron moiety in the Ni/Fe active site [60]. Based on the 100 cm^{-1} shifts in CO stretching frequencies observed in this model complex upon oxidation from Fe(II) to Fe(III), it is suggested that the oxidation state of the iron in the Ni/Fe enzymes remains unchanged upon redox cycling of the enzyme.

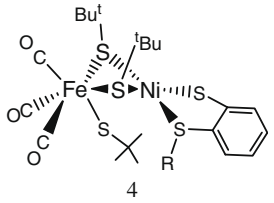
Soon after the elucidation of the NiFe active site structure, Darensbourg and coworkers [61] reported the preparation of complex **1**, with a Ni–Fe distance of 3.76 Å, considerably longer than the Ni–Fe distance in the enzyme of about 2.5–2.6 Å (reduced) or 2.9 Å (oxidized). Schroder and coworkers have prepared a dinuclear NiFe complex (**2**) by reacting a mononuclear Ni(II) precursor, $[Ni(SCH_2CH_2CH_2S)(dppe)]$, with $Fe_3(CO)_{12}$ [62]. The Ni–Fe distance in **2** is 2.47 Å, similar to that found in the reduced enzyme.





The most plausible structural models for the NiFe active site have been reported by Tatsumi and coworkers. Complex **3** was prepared by reaction of $[Fe(CO)_2(CN)_2(pdt)]^{2-}$ with a Ni(II) precursor [63]. Complex **4** was prepared from $Fe(CO)_4Br_2$ and a Ni(II) precursor in the presence of thiolate [64].

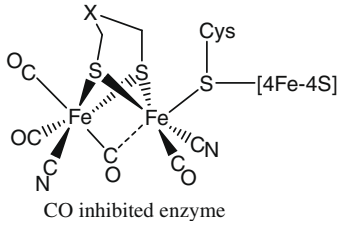


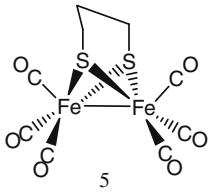


While complexes such as **3** and **4** represent reasonable structural models for the NiFe active site, *functional* models remain elusive. It is generally believed that hydrogen activation occurs at the Ni site, but very few stable nickel hydrides are known with any ligand set. Although hundreds of transition metal dihydrogen complexes have been reported, there are no examples for nickel. DuBois and coworkers have made some progress in catalytic hydrogen activation using nickel centers, but the ligands employed are primarily phosphorous donors [65]. This area of investigation has been recently reviewed [66].

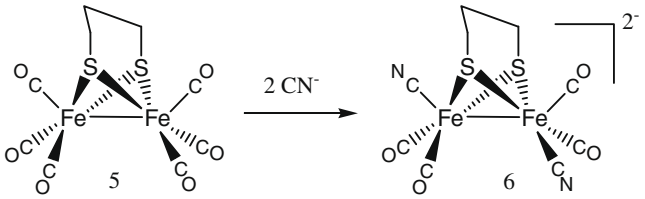
6.2. FeFe hydrogenase model studies

The elucidation of the active site structure by the groups of Peters and Fonticella-Camps made it clear that this active site resembles molecular species long known to inorganic chemists. Indeed, several routes to iron carbonyl thiolate complexes have been developed from simple starting materials which may have been available in the primordial ocean [67].





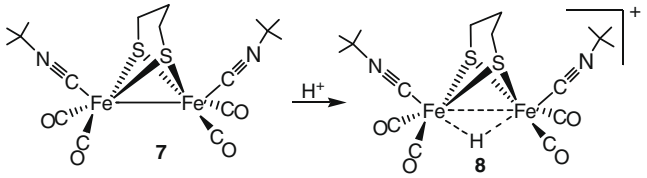
The crystallographic results led to rapid developments in the preparation of model complexes for this active site. The synthetic chemistry of a wide range of related derivatives has been reviewed by Song [68]. In particular, complex **5** has a Fe–Fe distance of 2.510 Å, similar to that reported for the enzyme active site. This structural similarity was pointed out early in the development of model systems by Darensbourg and coworkers, who also were the first to report the preparation from **5** of the dianionic dicyanide derivative [69]. Independently, the groups of Rauchfuss and coworkers [70] and Pickett and coworkers [71] had utilized ligand substitution on **5** with cyanide to prepare the dianionic dicyano derivatives. All three groups report the same dicyano complex (based on IR spectroscopy), but various isomers were postulated. The isomer of **6** depicted here is the one identified by Rauchfuss and coworkers using X-ray crystallography.



The dicyano dianion resembles the FeFe hydrogenase active site, and reaction with acid generates substoichiometric quantities of H_2. The iron containing product of this reaction is described by Rauchfuss as an insoluble polymeric species of unknown structure. Darensbourg and coworkers report that protonation of **6** affords a transient bridging hydride species, which was characterized spectroscopically but could not be isolated [72].

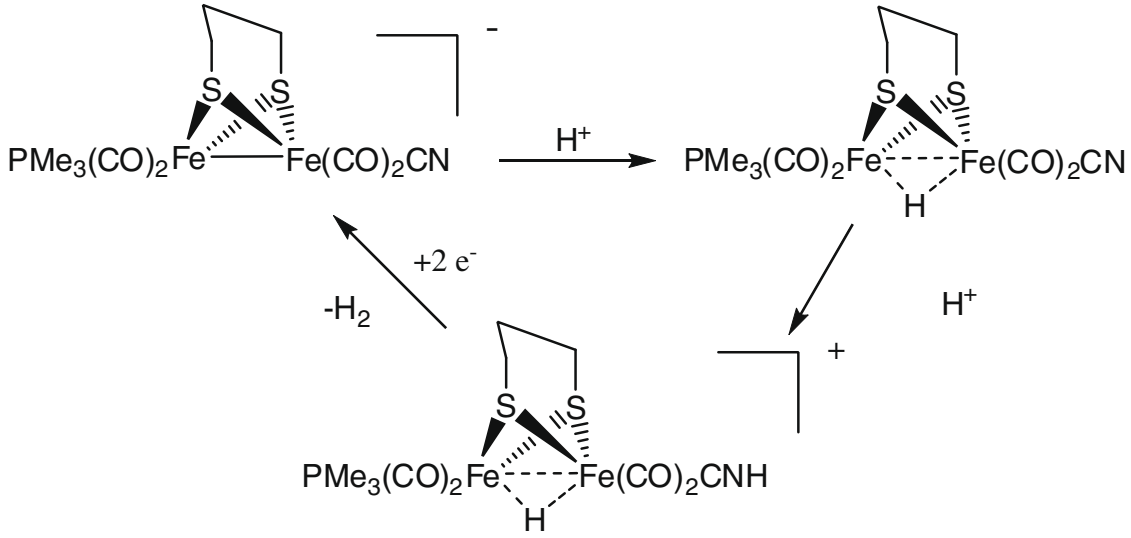
In contrast, Rauchfuss and coworkers found that a monoanionc FeFe derivative with one phosphine and one CN^- ligand reacts with acid to give a stable dinuclear bridging hydride species [73]. Catalysis of proton reduction was demonstrated using cyclic voltammetry in the presence of varying acid concentrations.

Replacement of carbonyl ligands with isonitrile ligands rather than cyanide ligands leads to *neutral* disubstituted species, which can subsequently be protonated [75].



Similar to the results reported by Darensbourg, complex **8** undergoes isotope exchange with D_2 upon photolysis. These results are significant, since very few well characterized model complexes react with hydrogen.

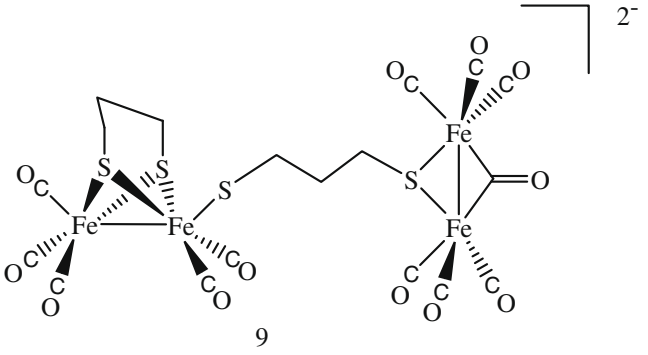
Several reports have described the reduction chemistry of **5**, with somewhat conflicting results. A reversible two electron reduction of complex **5** in DMF under CO was previously reported based on CV studies [76]. A recent report by Darensbourg and coworkers using CV and bulk electrolysis studies in CO-saturated



Similar investigations by Darensbourg and coworkers employed a bis-PMe_3 complex [74]. Again, protonation gives a stable bridging hydride species. Interestingly, this cationic complex facilitates D_2/H^+ isotope exchange, but only upon illumination [72].

CH_3CN is consistent with an initial one-electron reduction. A further one-electron reduction to a dianion occurs only at highly reducing potentials. Darensbourg and coworkers reported that the reduction of **5** led to electrocatalysis of H_2 evolution in the presence of acetic acid [77]. Pickett and coworkers demonstrated that H_2 evolution from protic media catalyzed by complex **5** under reducing conditions is comprised of two processes with different onset potentials [78]. Also reported was the detailed study of a one-electron reduction product and the subsequent chemistry of this complex, which includes a proposed disproportionation/ligand redistribution reaction that affords a dimeric species containing four Fe atoms. The structure of this molecule depicted below as com-

plex **9** was proposed based on limited spectroscopic data because the complex could not be isolated [79]. This material was subsequently prepared by chemical reduction and characterized by crystallography [80].



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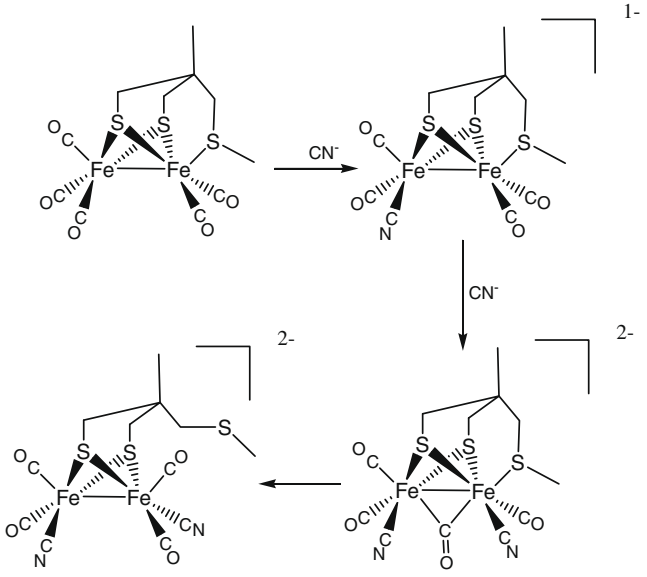
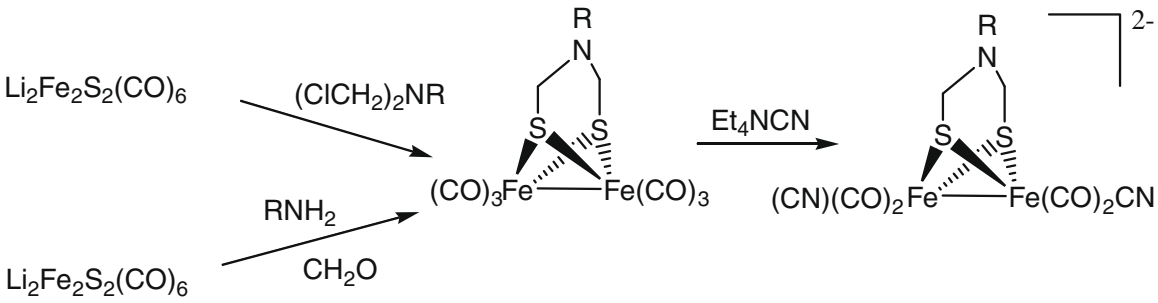


This observation points out a common difficulty in studies of electrocatalytic proton reduction using hydrogenase model complexes. As summarized recently by Pickett and coworkers, the identity of the actual active catalyst is not always well defined [81]. A general review of the electrochemistry of diiron dithiolate complexes relevant to hydrogenase models has recently appeared [82].

A very promising Fe_2S_3 model system that mimics the active site with a pendant thioether moiety attached to the central C atom of a propanedithiolate bridging ligand has been reported by Pickett and coworkers. Interestingly, with this ligand system, a transient bridging CO species is formed upon reaction with cyanide [83]. This is a very significant observation because the existence of a bridging CO ligand in some redox states of the enzyme has been clearly demonstrated by IR spectroscopy.

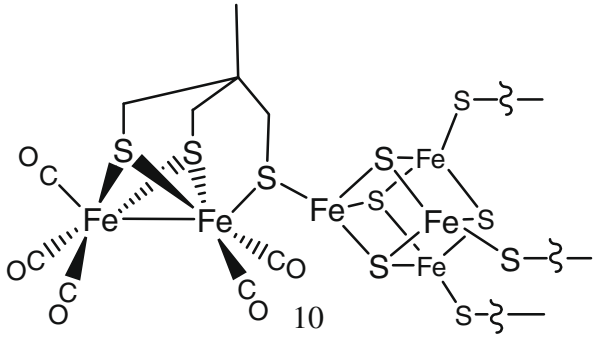
Crystallographic results suggested the presence of a N atom central in the three atom bridge linking the two thiolate sulfur atoms in the active site of the FeFe hydrogenases [41]. The basic N atom is thought to facilitate heterolysis of dihydrogen bound at the adjacent iron center. Computational studies have suggested that the presence of this internal base may be essential to catalytic activity [45]. The possibility that the central atom in the bridge is actually an oxygen atom has also been raised by DFT calculations [84].

Based on these observations, Rauchfuss and coworkers have developed fascinating model complexes involving N atoms in the bridge [85].



Subsequently, it was demonstrated that condensation of formaldehyde with an amine and a dimeric Fe sulfide could be used to prepare these complexes [86]. Recent studies of phosphine containing model complexes containing an azadithiolate linker establish that the N atom in the bridge can serve as a proton relay [87].

A very sophisticated model of the FeFe active site resulted from the work of Pickett and coworkers, who have constructed a Fe_2 center with three S ligands and an attached Fe_4 cluster [88].

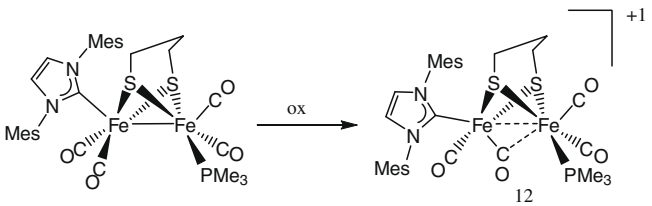


10

Complex **10** is the most complete structural model for the FeFe active site reported to date. Mössbauer spectroscopy of this complex reveals similar parameters to those reported for the reduced form of the hydrogenase from *Clostridia pasteurianum* [89]. Complex **10** facilitates efficient electrocatalysis of proton reduction, confirming the .functional relevance of this model compound. Related complexes featuring a $Fe_4(SR)_6$ core have been reported to be very effective electrocatalysts for proton reduction [90].

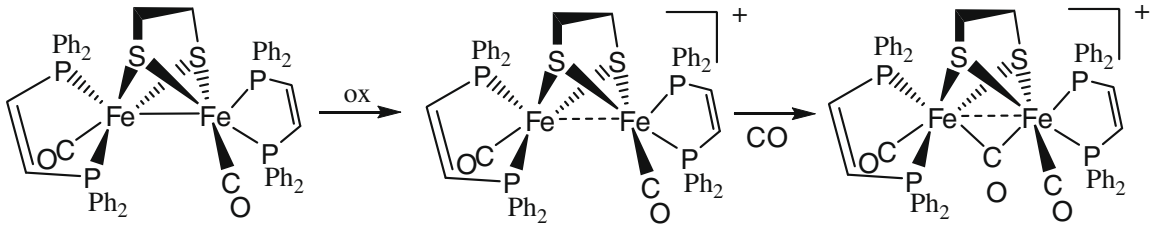
The $Fe^{II}Fe^{I}$ redox level is a characteristic feature of the FeFe hydrogenases that has proven difficult to model. exhibits a rhombic S = ½ signal in the EPR spectrum with g values comparable to those of the CO inhibited form of the FeFe hydrogenase from *C. pasteurianum* reported by Adams [92].

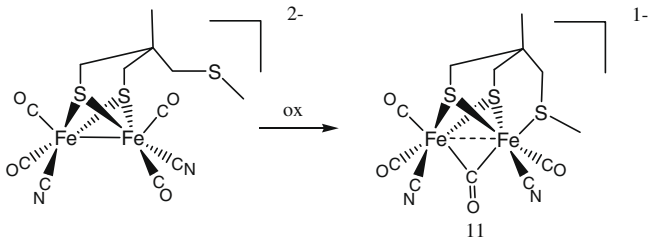
Greater stability in the mixed valence state has been achieved by the adoption of ligands not found in biology. The preparation and isolation of mixed valence complexes such as complex **12** was reported by Darensbourg and Liu [93].



12

The infrared spectrum and the EPR spectrum of complex **12** is a good match for the FeFe hydrogenase from *D. desulfuricans*. The Fe–Fe distance in complex **12** is 2.566 Å, only slightly longer than in the precursor complex. Darensbourg and coworkers have reported that the mixed valence structure with a semibridging CO ligand is stablilized by steric bulk in the propanedithiolate bridge [94]. In related studies, Rauchfuss and coworkers have prepared a compelling model for the oxidized active site using a phosphine complex [95]. A recent contribution by Rauchfuss summarizes redox and structural properties of mixed valence model complexes [96].





11

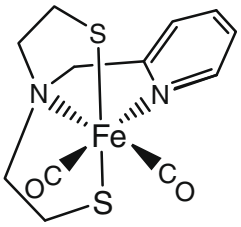
Pickett, Best and coworkers demonstrated that complex **11** can be prepared by oxidation of a dicyano dianion precursor [91]. Complex **11** is only transiently observable, but has an IR spectrum similar to the $Fe^{I}Fe^{II}$ state of the CO inhibited enzyme. Complex **11**

While the mixed valence $Fe^{I}Fe^{II}$ species are rare, there are several reported model complexes which feature $Fe^{II}Fe^{II}$ or "diferrous" motifs. Rauchfuss and coworkers have shown that substitution of CO in complexes such as **5** by more electron donating ligands allows access to stable diferrous dithiolate carbonyl complexes. These diferrous dithiolate carbonyl complexes can be synthesized by oxidative decarbonylation of **5** in the presence of trapping ligands, leading to a series of complexes with bridging carbonyls. Interestingly, all diferreous complexes which have been characterized are stabilized by a bridging or semibridging carbonyl, a feature also observed in the enzyme active site [97].

6.3. Hmd model chemistry

The crystal structure of the actives site was reported only in 2008. No functional models have been reported to date, but

the coordination chemistry of the pyridone cofactor has been explored using 6-(carboxymethyl)-4-methyl-2-hydroxypyridine as a surrogate [98]. Prior to the report of the crystal structure of the active site, Pickett and coworkers reported the iron complex **13**, which exhibits infrared spectra similar to that of the enzyme active site [99].

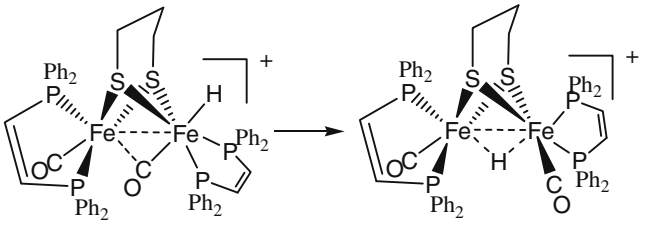


13

6.4. Evidence for hydride or dihydrogen ligands

There is much speculation but little direct evidence for the involvement of hydride or dihydrogen ligands at hydrogenase active sites. ENDOR and ESEEM experiments have suggested the presence of a bridging hydride ligand in the NiFe hydrogenase from *Ralstonia eutropha* [100].

Model studies provide indirect evidence for the involvement of hydride ligands in the active site of the FeFe hydrogenases. For example, Rauchfuss and coworkers have reported the preparation of complex **14a** by a low temperature protonation reaction. Based on infrared spectroscopy, complex **14a** is a good model for the reduced state of the hydrogenase from *D. desulfurican*. At higher temperatures, facile intramolecular isomerization occurs to give the bridging hydride complex **14b**. Both complexes catalyze proton reduction, but the bridging hydride isomer **14b** requires a potential more reducing by 200 mV [101].



14a
low temperature

14b
high temperature

Direct activation of dihydrogen by an apparent oxidative addition pathway has recently been reported in a photochemical reaction using a phosphine complex [102].

6.5. Immobilized enzymes as electrocatalysts

Rather than seek insights from the preparation of active site models, an entirely different approach utilizes isolated hydrogenase enzymes immobilized on electrode surfaces. This area was recently reviewed by Armstrong and coworkers [103]. One example of an exciting development in this area is provided by the report that the NiFe hydrogenase from *Allochromatium vinosum* can retain activity when adsorbed on a graphite electrode. When hydrogen transport is controlled by rapid rotation of the electrode, it is found that hydrogen oxidation is catalyzed at a rate controlled by diffusion and comparable to rates for electrodes coated with platinum [104]. Catalysis by hydrogenases of hydrogen oxidation by O_2 is difficult to demonstrate, due to the acute oxygen sensitivity of most hydrogenases. Some progress has been made toward an enzyme based fuel cell using the more oxygen tolerant NiFe hydrogenase from the Knallgas bacteria *R. eutropha*, which is able to maintain some activity in H_2/O_2 mixtures [105]. In a very demanding test of this idea, the NiFe hydrogenase from *Ralstonia metallidurans* was used as a catalyst to oxidize H_2 present at the level of 3% in ambient air [106].

In a very exciting development, light driven hydrogen production has been recently demonstrated. Combining a NiFeSe hydrogenase with a Ru bipyridyl photosensitizer on TiO_2 allows for the production of H_2 from neutral water using visible light [107].

7. Outlook and prospects

This article has outlined progress made toward the long term goal of using biomimetic chemistry to develop new catalysts based on inexpensive metals for the efficient production and utilization of hydrogen. Future progress in this area will require the development of ligand sets which allow for suitable redox tuning as well as fostering water solubility and water stability. Traditional inorganic/organometallic chemistry of transition metal hydride and dihydrogen complexes has been largely confined to the study of diamagnetic complexes linked by two electron redox processes. In contrast, enzymes process hydrogen in a series of one electron steps. This suggests that exciting new areas of chemistry can be developed, but that chemists must develop strategies for characterizing very reactive species which may have limited thermal stability.

New approaches involving hydrogenase enzymes attached to electrode surfaces for electrocatalytic and photochemical production of hydrogen appear to be very promising, but issues of long term catalyst stability in the presence of oxygen must be addressed.

Acknowledgment

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