7.6: Multisite Redox Enzymes (Part 2)

3. Iron Hydrogenases

The iron hydrogenases[189a] generally have higher activities than the NiFe enzymes, with turnover numbers approaching 10^6 min^{-1}. Iron hydrogenases from four genera of anaerobic bacteria have been isolated: *Desulfovibrio*,[190] *Megasphaera*,[188] *Clostridium*,[191] and *Acetobacterium*.[192] Of these, the enzymes from *Desulfovibrio vulgaris*, *Megasphaera elsdenii*, *Clostridium pasteurianum* (which contains two different hydrogenases), and *Acetobacterium woodii*[192] have been well-characterized (especially the *D. vulgaris* and *C. pasteurianum* enzymes). Although *Acetobacterium* and *Clostridium* are closely related, the other organisms are only distant cousins.[193] Nevertheless, their hydrogenases display significant similarities; all contain two different types of iron-sulfur cluster, called F and H clusters,[194] and carbon monoxide is a potent inhibitor (although this has not been reported for the *M. elsdenii* enzyme). The F clusters are thought to be of the $Fe_4S_4^{+/2+}$ thiocubane type, and give $S = \frac{1}{2}$ EPR signals when the enzyme is in the reduced form. On the other hand, the H cluster, which is thought to be the hydrogen-activating site, gives an EPR signal only when the enzyme is in the oxidized form. The H-cluster EPR signals of all the enzymes are quite similar (g = 2.09, 2.04, 2.00), and are quite unlike the signals from other oxidized iron-sulfide clusters (such as Fe_3S_4 clusters and HiPIPs), in that they are observable at relatively high temperatures (> 100 K). Inhibition of the *D. vulgaris* and both *C. pasteurianum* enzymes by carbon-monoxide yields a photosensitive species that has a modified H-cluster EPR signal.[195,196]

The two different hydrogenases of *C. pasteurianum*, called hydrogenase I and II, have both been quite extensively studied, and can be regarded as prototypical iron-only hydrogenases. Hydrogenase I is active in catalyzing both H_2 oxidation and H_2 evolution, whereas hydrogenase II preferentially catalyzes H_2 oxidation.[188] The two enzymes differ in their iron contents: hydrogenase I contains about 20 iron atoms, 16 of which are thought to be involved in four F clusters,[194] while the remainder presumably constitute the H cluster, which may contain six Fe atoms.[194] Hydrogenase II contains about 14 iron atoms as two F clusters and one H cluster.[194] These estimates of iron content result from a recent reappraisal of the metal contents (based on amino-acid analysis) that indicated a rather higher Fe content than previously realized.[188,194] It is important to note that much of the spectroscopic work, which will be discussed below, was initially interpreted on the basis of the earlier, erroneous, iron analysis. Of particular interest is the possibility (first suggested[197] for the *D. vulgaris* enzyme) that the H cluster contains six iron atoms.

Carbon-monoxide treatment of the *D. vulgaris* and both *C. pasteurianum* enzymes yields a photosensitive species that has a modified H cluster EPR signal.[195,196] Interestingly, the *C. pasteurianum* enzymes also form complexes with O_2, in a process that is distinguishable from the deactivation of hydrogenase by O_2, which results from a much more prolonged exposure to O_2 than that required to form the O_2 complex. The O_2 complexes have (photosensitive) EPR signals much like those of the CO complex.[196] It is important to note that although CO, when in excess, is a potent inhibitor of the enzymes, the hydrogenase I-CO complex is actually quite active.[196] With hydrogenase II, the CO complex is dissociated on exposure to H_2, restoring the "active" enzyme.[196] The EPR spectrum of reduced hydrogenase I is typical of (interacting) $Fe_4S_4^+$ clusters, and integrates to 3 or 4 spins/protein.[194,198] Electrochemical studies[199] show that these clusters possess indistinguishable reduction potentials. Recently, MCD and EPR spectroscopies have been used to demonstrate the presence of significant quantities of an $S = \frac{3}{2}$ species in reduced hydrogenase I. This signal apparently integrates to about one spin per molecule, and probably originates from an $S = \frac{3}{2}$ state of an Fe_4S_4 cluster.[198] No information is yet available on the reduction potential of the $S = \frac{3}{2}$ species. However, based on analogy with the nitrogenase iron protein,[200] we might expect the $S = \frac{3}{2}$ form to have electrochemistry indistinguishable from the $S = \frac{1}{2}$ form. EPR signals with high g values (g = 6.1 and 5.0) have also been observed in *C. pasteurianum* hydrogenase I, and in the *D. vulgaris* enzyme.[198,201] Since there is some uncertainty about the nature and origin of these signals,[198] we will not discuss them further. The F clusters of hydrogenase II, on the other hand, give two different EPR signals that integrate to one spin each per protein molecule, and that correspond to sites with different redox potentials.[194,198,199] This suggests that hydrogenase II contains two different F clusters, called F and F' (note that the presence of F' was in fact first suggested by Mössbauer spectroscopy[202]). The EPR spectrum from the F' cluster is unusually broad. The H-cluster EPR signals of active hydrogenase I and II are quite similar and have essentially identical redox potentials.[199]

The redox behavior of the F and H centers in hydrogenases I and II is nicely consistent with their respective modes of function. As shown in Figure 7.22, the F clusters are presumed to transfer electrons intermolecularly with the external electron carrier and intramolecularly with the H center. In the reversible hydrogenase I, the F and H centers have the same redox potentials (about -400 mV at pH 8), similar to that of the hydrogen electrode (-480 mV at pH 8). Thus, electrons may flow in either direction when a mediator such as methyl viologen ($E^{\circ\prime}$ = -440 mV at pH 8) is used as the external electron acceptor (methyl viologen is the 4,4'-bipyridinium ion). On the other hand, for hydrogenase II, the F clusters have $E^{\circ\prime}$ (pH 8) = -180 mV and $E^{\circ\prime}$ > -300 mV for F and F',

respectively. In hydrogenase II, therefore, electrons can only move favorably from H_2 to the H cluster, through F' and F, and then to a lower-potential acceptor, such as methylene blue [for which $E^{0'}$ (pH 8) = 11 mV].

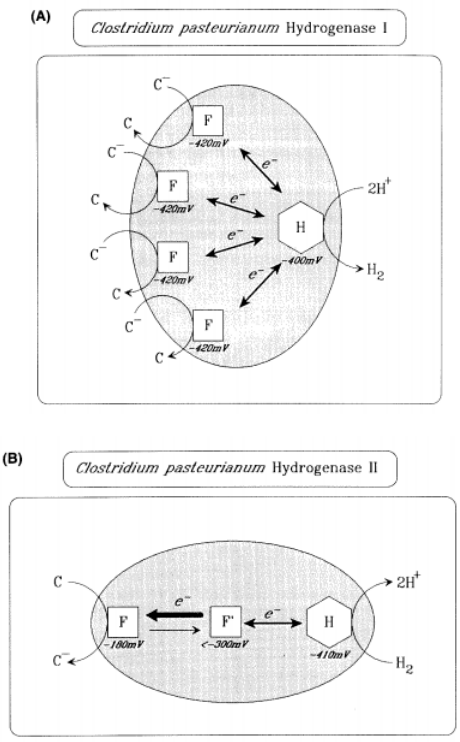


Figure 7.22 - Redox schemes illustrating proposed action of Fe hydrogenases: (A) Clostridium pasteurianum hydrogenase I; (B) Clostridium pasteurianum hydrogenase II.[189a,199]

Mössbauer spectroscopic studies of both hydrogenase I and II have been reported.[202,203] Our discussion focuses primarily on the H cluster. The results are similar for the two enzymes, but better defined for hydrogenase II because of the smaller number of clusters. The H cluster apparently contains only two types of iron, in the ratio of 2:1, with quadrupole splittings reminiscent of Fe_3S_4 clusters. The oxidized cluster is confirmed to be an $S = \frac{1}{2}$ system, also reminiscent of Fe_3S_4 clusters, and the reduced H cluster is an S = 0 system; this contrasts with reduced Fe_3S_4 clusters, which have S = 2. In agreement with the Mössbauer studies, ENDOR spectroscopy of ^{57}Fe-enriched protein indicates at least two different types of iron in the H cluster, with metal hyperfine couplings of about 18 and 7 MHz in hydrogenase II. Rather-less-intense ENDOR features were also observed at frequencies corresponding to couplings of about 11 and 15 MHz.[204] The H-cluster EPR signals of hydrogenase II and hydrogenase I change on binding carbon monoxide. Although the signals of the uncomplexed enzymes are quite similar, the signals of the CO-bound species are very different (note, however, that *C. pasteurianum* CO-bound hydrogenase I has EPR similar to that of the CO-bound *D. vulgaris* enzyme), When produced with ^{13}C-enriched CO, the EPR signal of hydrogenase II shows resolved ^{13}C hyperfine coupling (A_{av} = 33 MHz) to a single ^{13}C nucleus, indicating that only a single CO is bound, presumably as a metal carbonyl. A slightly smaller coupling of 20 MHz was obtained using ENDOR spectroscopy for the corresponding species of hydrogenase I.[205]

Recent ESEEM spectroscopy of hydrogenase I indicates the presence of a nearby nitrogen, which may be a nitrogen ligand to the H-cluster. This nitrogen possesses an unusually large nuclear electric quadrupole coupling and a rather novel structure, involving an amide amino-acid side chain connected to an H-cluster sulfide via a bridging proton ligand, has been suggested for it.[206] Although the nitrogen in question must come from a chemically novel species, the proposed proton bridge might be expected to be exchangeable with solvent water. The ENDOR-derived result[205] that there are no strongly coupled exchangeable protons in the oxidized H cluster may argue against such a structure.

Rather weak MCD[198,207] and resonance Raman spectra[208] have also been reported for iron hydrogenases. The lack of an intense MCD spectrum[198,207] contrasts markedly with results for other biological FeS clusters. The resonance Raman spectra of hydrogenase I resemble, in some respects, spectra from Fe_2S_2 sites.[208] These results further emphasize that the hydrogenase H clusters are a unique class of iron-sulfur clusters. Perhaps most tantalizing of all are the recent EXAFS results on hydrogenase II.[209] It is important to remember that for enzymes with multiple sites, the EXAFS represents the sum of all sites present (i.e., the iron of the F, F', and H clusters). Despite this complication, useful information is often forthcoming from these experiments. The EXAFS

of oxidized hydrogenase I showed both iron-sulfur and iron-iron interactions; the latter, at about 2.7 Å, is at a distance typical of Fe_2S_2, Fe_3S_4, and Fe_4S_4 clusters, and thus is not unexpected. The reduced enzyme, however, gave an additional, long Fe-Fe interaction at 3.3 Å. This Fe-Fe separation is not found in any of the FeS model compounds reported to date.[209] The appearance of the 3.3-Å interaction indicates a change in structure on reduction of the H cluster, again revealing a cluster of unique structure and reactivity. The large structural change of this H cluster on H_2 reduction is likely to have significant mechanistic implications.

4. Nickel-iron Hydrogenases

The presence of nickel in hydrogenases has only been recognized relatively recently. Purified preparations of the active enzymes were the subject of quite intensive studies for years before the Ni content was discovered by nutritional studies (see Reference 189 for a history). Some workers even tried (in vain) to purify out "impurity" EPR signals that were later found to be from the Ni. In contrast to the Fe hydrogenases discussed in the previous section, the Ni enzymes possess a variety of compositions, molecular weights, activation behavior, and redox potentials.[189,210,211] As Table 7.5 shows, some of the Ni hydrogenases contain selenium, likely in the form of selenocysteine, some contain flavin (FMN or FAD), and all contain iron-sulfur centers, but in amounts ranging from 4 to 14 iron atoms per Ni atom.

Among the different Ni hydrogenases there is a common pattern of protein composition, to which many, but not all, seem to conform (especially those enzymes originating from purple eubacteria). There are two protein subunits, of approximate molecular masses 30 and 60 kDa, with the nickel probably residing in the latter subunit. The hydrogenase of the sulfate-reducing bacterium *Desulfovibrio gigas* is among the best investigated, and we will concentrate primarily on this enzyme. *D. gigas* hydrogenase contains a single Ni, two Fe_4S_4 clusters, and one Fe_3S_4 cluster. Of primary interest is the Ni site, which is thought to be the site of H_2 activation.[189,210,211]

EPR signals attributable to mononuclear Ni [as shown by enrichment with ^{61}Ni ($I = \frac{3}{2}$)] have been used in numerous investigations of the role of Ni in hydrogenases.[189,210,211] Three major Ni EPR signals are known, which are called Ni-A, Ni-B, and Ni-C. The principal g values of these signals are: 2.32, 2.24, and 2.01 for Ni-A; 2.35, 2.16, and 2.01 for Ni-B; and 2.19, 2.15, and 2.01 for Ni-C. Of these, Ni-C is thought to be associated with the most active form of the enzyme (called active); the other two are thought to originate from less-active enzyme forms.[189,210]

In the enzyme as prepared (aerobically) the Ni-A EPR is characteristically observed. On hydrogen reduction the Ni-A EPR signal disappears, and the enzyme is converted into a higher-activity form (Ni-B arises from reoxidation of this form). Further progressive reduction of the enzyme gives rise to the Ni-C EPR signal, which also finally disappears. These redox properties show that Ni-C arises from an intermediate enzyme oxidation state. Although the Ni-A and Ni-B EPR signals[189,210-212] almost certainly originate from low-spin Ni(III), the formal oxidation state of Ni-C is rather less certain. Both an Ni(I) site[189,210] and an Ni(III) hydride[213] have been suggested, with the former alternative currently favored because of the apparent absence of the strong proton hyperfine coupling expected for the latter. In the fully reduced enzyme, Ni-C is converted to an EPR-silent species. This has variously been suggested to be Ni(0), Ni(II), or an Ni(II) hydride.[214] One possible reaction cycle[189,210] is shown in Figure 7.23.

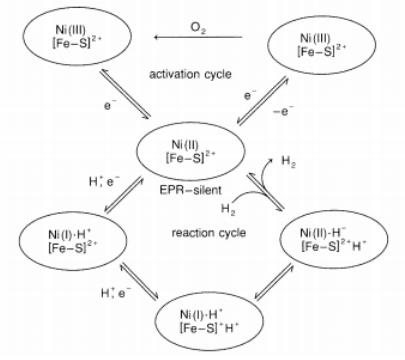


Figure 7.23 - Proposed activation/reactivity scheme for Ni hydrogenases.[189]

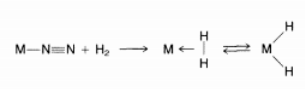
Information on the coordination environment of the nickel has been obtained from both x-ray absorption spectroscopy and EPR spectroscopy. The Ni K-edge EXAFS of several different hydrogenases,[215-218] and EPR spectroscopy of ^{33}S enriched *Wolinella succinogenes* hydrogenase,[219] clearly indicate the presence of sulfur coordination to nickel. A recent x-ray absorption spectroscopic investigation of the selenium-containing *D. baculatus* hydrogenase, using both Ni and Se EXAFS, suggests seienocysteine coordination to Ni.[216]

ESEEM spectroscopy of the Ni-A and Ni-C EPR signals[220,221] indicate the presence of ^{14}N coupling, which probably arises from a histidine ligand to Ni. Interestingly, Ni-C, but not Ni-A, shows coupling to a proton that is exchangeable with solvent water. Although this coupling is too small to suggest a nickel-hydride (consistent with conclusions drawn from EPR), the proton involved could be close enough to the Ni to playa mechanistic role.

Despite the extensive studies reported to date, there are still many unanswered questions about the mechanism of the NiFe hydrogenases, which remain as exciting topics for future research. Despite our lack of detailed knowledge of enzyme mechanism, it is nevertheless not premature to seek guidance from inorganic chemistry.

5. Insights from Inorganic Chemistry

Recent years have brought insights into the way dihydrogen can be bound at a transition-metal site. Unexpectedly, it has been shown that molecular H_2 forms simple complexes with many kinds of transition-metal sites.[222-224] This finding contrasts with the classical situation, in which H_2 interacts with a transition-metal site by oxidative addition to form a dihydride complex.[23]The H—H bond is largely maintained in the new/nonclassical structures. The dihydrogen and dihydride complexes can exist in simple equilibrium,[224] as in Equation (7.9).



(7.9)

The bonding of dihydrogen to a metal occurs via the σ^b orbital of the H—H bond acting as a donor, with the σ^* level of H_2 acting as a weak acceptor. If the back donation is too strong, sufficient electron density will build up in the $sigma^*$ level to cause cleavage of the H—H bond, leading to the formation of a dihydride. Dihydrogen complexes therefore require a delicate balance, in which the metal coordination sphere facilitates some back-bonding, but not too much.

The proclivity of a metal center to form H_2 complexes can be judged by the stretching frequency of the corresponding N_2 complexes: N_2 can usually displace H_2 from the H_2 complex to form an N_2 complex without changing the remainder of the coordination sphere. If ν(N≡N) is between 2060 and 2160 cm^{-1}, H_2 complexes form upon replacement of N_2. If ν(N≡N) is less than 2060 cm^{-1}, indicative of electron back-donation from the metal center, a dihydride complex should form. For example, ν(N≡N) = 1950 cm^{-1} in $MoN_2(PCy_3)_5$ and $MoH_2(PCy_3)_5$ is a dihydride complex, but ν(N≡N) = 2090 cm^{-1} in $Mo(Ph_2PCH_2CH_2PPh_2)CO(N_2)$ and $Mo(Ph_2PCH_2CH_2PPh_2)H_2(CO)_2$ is a dihydrogen complex. By comparison, $Mo(Et_2PCH_2CH_2PEt_2)_2(CO)(N_2)$ has ν(N≡N) = 2050 cm^{-1} and forms a dihydride complex, $Mo(Et_2PCH_2CH_2.PEt_2)_2H_2(CO)$. The correlation between ν(N≡N) and the type of hydrogen complex formed seems quite useful.

Since Fe-S and Ni-S sites are implied for hydrogenase, the reactivity of transition-metal/sulfide systems with H_2 may also be relevant. Interestingly, H_2 can react with metal-sulfide systems at S instead of at the metal site. For example,[225] $(Cp')_2Mo_2S_4$ reacts with H_2 to form $(Cp')_2Mo_2(SH)_4$. Here the dihydrogen is cleaved without any evidence for direct interaction with the metal center, and the resulting complex contains bridging SH groups and no direct metal-H bonding.[225] In recent work,[226] the binuclear rhodium-sulfur complex $\{RhS[P(C_6H_5)_2CH_2CH_2]_3CH\}_2$ was reported to react with two equivalents of dihydrogen to yield the complex $\{Rh(H)(SH)[P(C_6H_5)_2CH_2CH_2]_3CH\}_2$, in which two SH groups bridge the two Rh centers, each of which contains a single hydrido ligand. Figure 7.24 illustrates the possibilities for hydrogen activation. Each of these types of reactivity must be considered as possibilities for the hydrogen activation process of hydrogenase.

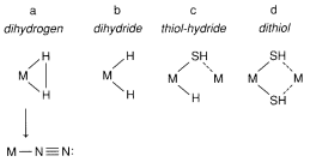


Figure 7.24 - Possible modes of H_2/H bonding at a transition-metal sulfide site.

Recently, a great deal of attention has been given to the chemistry of nickel-sulfur systems, inspired in part by the results showing that many hydrogenases are nickel-sulfur proteins.[227-230] A particularly interesting finding is that Ni thiolates can react with O_2 to produce sulfinate complexes.[231,232] The oxygenated thiolate can be regenerated, thus providing a potential model for the O_2 inactivation of Ni hydrogenases.