

The catalytic machinery

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In [Chapter 2](#) we saw that hydrogenases of the three basic types are made by organisms that have existed over billions of years. In [Chapter 6](#), the structures of the proteins were laid out in three dimensions. In [Chapter 7](#) we saw that the metal centres of the protein could exist in particular chemical states. We can now begin to understand how the hydrogenases catalyse their reactions with such extraordinary efficiency. Furthermore we ask, can similar catalysts be constructed artificially?

8.1. Significant features of the active sites of hydrogenases

There must be something special about the hydrogenase active site, that allows it to react easily with H_2 . It is safe to assume that the nickel–iron centre in the [NiFe] hydrogenases, and the H cluster in the [Fe] hydrogenases, are the active sites at which hydrogen is produced and consumed. They are unique to hydrogenases, whereas the other iron–sulfur clusters are similar to those found in many other proteins which cannot produce hydrogen. Most iron–sulfur proteins, although they can achieve low redox potentials, do not react with or produce H_2 . An exception is the active centre in nitrogenase, which produces hydrogen as it fixes N_2 to ammonia (Section 3.2 in [Chapter 10](#)).

Nickel occurs in many hydrogenase active sites, though not all. This element is used in relatively few other enzymes (Hausinger 1993; Cammack and van Vliet 1999; Maroney 1999). In order to use it, the bacterial cell requires complex and energetically costly systems to take up and store nickel ([Chapter 3](#)). What are the advantages of the [NiFe] centre over the [Fe] centre? We note that the nickel-containing hydrogenases tend to be less sensitive than the iron-only hydrogenases, to inhibition by carbon monoxide and oxygen. The methanogens, which grow in the most extreme anaerobic conditions, have been found to contain only [NiFe] hydrogenases. CO is a common metabolite in anaerobic environments. Strict aerobes, such as *Ralstonia eutropha*, also use [NiFe] hydrogenases, which may be related to the resistance to O_2 .

Free coordination site. A significant characteristic of the structures of the nickel–iron site in [NiFe] hydrogenases and the H cluster in [Fe] hydrogenases, is a vacant, or potentially vacant, position. The two ions in the dinuclear centre each have a free coordination site. In the reduced NiFe hydrogenase, there is a position that can accommodate a third bridging ligand; in the oxidized state this site is occupied by oxygen or sulfur. In the [Fe] hydrogenases, the position is occupied by a bridging carbonyl and there is a free terminal coordination site. Such an open site is probably

the key to efficient binding of H₂. It is difficult to create in synthetic homogeneous catalysts, because ligands and solvent have a strong tendency to bind to any vacant site.

Diatomic ligands. The diatomic ligands -CN and -CO are likely to be an essential feature. Both the iron-only and nickel-iron hydrogenases contain at least one iron ion with cyanide and carbonyl ligands, bridged by thiolates to a second metal ion (iron or nickel) which can be oxidized and reduced. Hydrogen-bonded cyanide has σ -donor properties, similar to those of a phosphine. The combination of sulfur, CO, and CN ligands tend to make the metal site 'softer', so that it behaves more like the zero-valent metal in the platinum electrode. The dinuclear sites in the hydrogenases are beautifully adapted for the binding of H₂ or hydride.

We do not know exactly where the hydrogen binds at the active site. We would not expect it to be detectable by X-ray diffraction, even at 0.1 nm resolution. EPR (Van der Zwaan *et al.* 1985), ENDOR (Fan *et al.* 1991b) and electron spin-echo envelope modulation (ESEEM) (Chapman *et al.* 1988) spectroscopy have detected hyperfine interactions with exchangeable hydrons in the NiC state of the [NiFe] hydrogenase, but have not so far located the hydron. It could bind to one or both metal ions, either as a hydride or H₂ complex. Transition-metal chemistry provides many examples of hydrides and H₂ complexes (see, for example, Bender *et al.* 1997). These are mostly with higher-mass elements such as osmium or ruthenium, but iron can form them too. In order to stabilize the compounds, carbonyl and phosphine ligands are commonly used (Section 6).

The spectroscopic evidence (Chapter 7), notably the very weak ⁵⁷Fe hyperfine coupling observed in all the EPR-detectable states of the [NiFe] hydrogenase, indicates that the iron stays in the diamagnetic Fe(II) state.

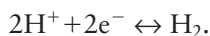
In summary, the active site has control over the following features, which are difficult to replicate in a chemical catalyst:

- 1 access to water hydrons, but not water itself;
- 2 electron transfer to specific donor and acceptor molecules;
- 3 free ligand site on each ion for binding of hydride and H₂;
- 4 an iron atom with unusual diatomic ligands which favours the formation of a hydride; and
- 5 protection of the active site from reaction with oxygen.

8.2. Connections to the active site

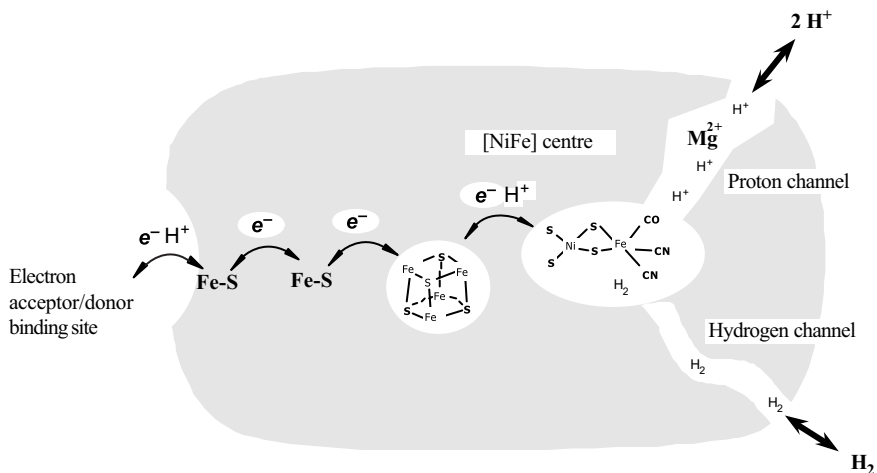
Compared to most enzymes, which often undergo considerable conformational changes on binding of the substrates, hydrogenases are rather rigid proteins. The substrates are small and mobile, and can penetrate to the active site. Probably the only parts of the enzyme that move significantly are amino acid side-chains and bound water molecules involved in transfer of hydrons to the active site (Fig. 8.1).

The structures of the metal-containing hydrogenases (Chapter 6) reflect, in an unexpectedly literal way, the different components of the reaction:



There are three pathways for hydrons, electrons and hydrogen molecules leading into the enzyme to the catalytic site.

A. [Ni-Fe] hydrogenase



B. [Fe] hydrogenase

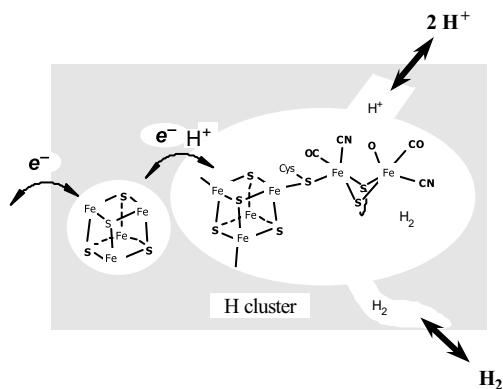


Figure 8.1 Diagrams of the catalytic machinery of [NiFe] and [Fe] hydrogenases.

The structures of the [NiFe] and [Fe] hydrogenases have been refined by evolution over billions of years. The relative positions of the metal atoms in the catalytic site, the electron-carrying iron–sulfur clusters, and the hydron and hydrogen channels (Fig. 6.11), all appear to be optimized to facilitate the reaction cycle. This provides clues to the movement of H₂, hydrons and electrons during the catalytic cycle. Cavity calculations, performed on a model of the [NiFe] hydrogenase from which all active site atoms are removed, showed that the internal hydrophobic channel network is directly connected to the one vacant nickel coordination site that is observed in the crystal structures of the oxidized enzymes. The electron-transfer pathway is directed toward the nickel site in the [NiFe] hydrogenases, and the Fe-[4Fe-4S] group in the [Fe] hydrogenases.

8.2.1. Hydron channels, and sites of hydron binding at the active site

The features of a putative hydron channel have been described by Volbeda *et al.* (See Section 3 in [Chapter 6](#)). Hydrons, like electrons, can tunnel from one site to another, but only over very limited distances (less than 0.1 nm). Therefore, the movement of hydrons must rely on the movement of transferring groups. These are probably the only moving parts in the machine. They comprise groups that can exchange hydrons (water, carboxyl, amino, amido and hydroxyl groups) in a chain from the protein surface. In *D. gigas* hydrogenase the chain incorporates water molecules coordinated to the magnesium ion. The motion of these groups is very rapid (on the nanosecond timescale) so that proton transfer is unlikely to restrict the rate of the reaction.

The next question is, where the protons go to in the active site during the catalytic cycle. For the base, there are too many possibilities to be certain. Groups near to the dinuclear cluster that can accept or exchange protons include the side chains of the amino acids arginine and histidine, thiolate ligands to the cluster, and peptide NH groups.

In addition there are water molecules. In *D. gigas* hydrogenase, no water molecules are seen in the active site by crystallography, but there are plenty of them in cavities leading through the protein to the surface, the nearest being 0.68 nm from the nickel. Hydrons can readily move between these sites by diffusion. So it is possible that multiple hydrogen-binding sites are involved in the reaction.

8.2.2. The electrical connection

Electron-transfer proteins have a mechanism that is quite different from the conduction of electrons through a metal electrode or wire. Whereas the metal uses a continuous conduction band for transferring electrons to the centre of catalysis, proteins employ a series of discrete electron-transferring centres, separated by distances of 1.0–1.5 nm. It has been shown that electrons can transfer rapidly over such distances from one centre to another, within proteins (Page *et al.* 1999). This is sometimes described as quantum-mechanical tunnelling, a process that depends on the overlap of wave functions for the two centres. Because electrons can tunnel out of proteins over these distances, a fairly thick insulating layer of protein is required, to prevent unwanted reduction of other cellular components. This is apparently the reason that the active sites of the hydrogenases are hidden away from the surface.

Electron acceptors and donors for hydrogenases, such as cytochromes, ferredoxins or NAD, bind to an electron-transfer site, which is usually close to the surface of the protein. This site binds the specific electron-transfer molecules, like a socket for an electrical plug. Other molecules that can accept or donate electrons to hydrogenase, such as methyl viologen, may bind at the same or different sites. In the structures of the hydrogenases, we can see the iron–sulfur clusters, neatly arranged in a chain, leading from the catalytic site to the electron-transfer site (see [Fig. 6.8](#)). The number and types of iron–sulfur clusters vary from one type of hydrogenase to another. In the [NiFe] hydrogenases, the clusters are in a different subunit from the [NiFe] centre, and there is considerable variation in this part of the molecule, sometimes involving several ancillary protein subunits.

The redox potentials of the electron-carrying groups have been the subject of much debate. In order to transfer electrons efficiently, one would expect that the electron-carrying groups along the chain should have progressively more positive midpoint redox potentials. In many hydrogenases this appears not to be the case. For example in some [NiFe] hydrogenases, such as those from *D. gigas* and *D. fructosovorans*, the middle iron–sulfur cluster is a [3Fe-4S] cluster that has a midpoint redox potential about 300 mV more positive than the [4Fe-4S] clusters on either side. This means that a reducing electron in this centre would be trapped in an energy minimum, and it is difficult to see how it could transfer to the next [4Fe-4S] cluster. Nevertheless, it appears to work. Electrons can transfer down the chain so rapidly that they do not hinder the hydrogenase reaction at all.

Would the hydrogenase be improved by having a more even distribution of redox potentials along the chain? Rousset *et al.* (1998) examined this possibility. By means of some difficult genetic engineering, Rousset mutated a proline to a cysteine in *D. fructosovorans* hydrogenase, and thus artificially converted the [3Fe-4S] cluster into the [4Fe-4S] type. However the enzyme with this smoother electron pathway did not perform any better than the native protein; moreover, it was more sensitive to oxygen damage. One way to interpret this result is that the reduced [3Fe-4S] cluster is good at conducting electrons by tunnelling, even if it is almost fully charged with an electron.

Electrostatic neutrality: Hydrons go with the electrons

In the hydrogenase active site, the effective dielectric constant is low, which means that there is a considerable energetic premium on introducing an electrostatic charge. In general, the addition of each electron is compensated by the introduction of a hydron. We know this, at least in *D. gigas* hydrogenase, because the pH dependence of the redox potentials has been determined for the nickel centre (Cammack *et al.* 1987). This is also true for the [4Fe-4S] clusters which, unusually, show pH-dependent redox potentials. The distal [4Fe-4S] cluster has a histidine ligand near the surface, which could acquire a hydron from the solution on reduction. The proximal cluster is distant from the surface but there is a possible channel for hydrons, leading to the NiFe centre, and thence to the surface (see Fig. 8.2). Thus an electron and a hydron can be transferred from the NiFe centre to the cluster during hydrogen oxidation. The [3Fe-4S] cluster does not transfer hydrons on reduction and this channel does not seem to extend right through the protein.

It is worth noting here that from the point of view of biological evolution, some of the subunits of hydrogenases are homologous, in their amino-acid sequences, to the proton-pumping NADH:ubiquinone reductases of present-day mitochondria (see Section 3.3 in Chapter 2). Molecular mechanisms that have evolved in one type of protein are often found in proteins with different functions. Proton channels are a feature of the enzymes that pump protons across membranes in order to produce a ‘proton motive force’ which is used to generate ATP (see, e.g., Puustinen and Wikstrom 1999; Luecke *et al.* 1999). It seems likely that hydrogenases were essential in some of the earliest organisms on Earth. Their pathways for proton transfers, required to maintain electrical neutrality at the active site, may have been the precursors of other enzymes that generate transmembrane electrochemical gradients.

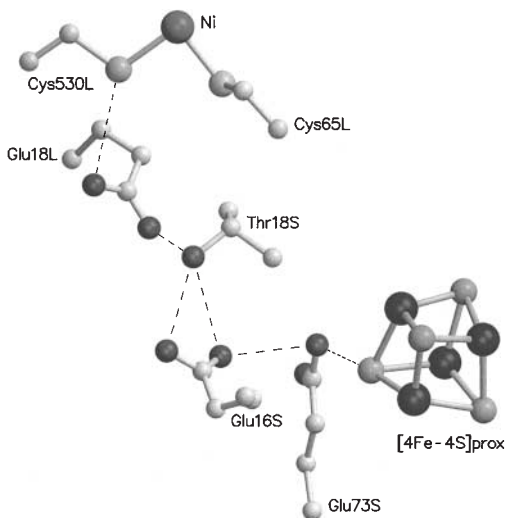


Figure 8.2 One of the possible pathways for hydron transfer between the [NiFe] centre and the proximal [4Fe-4S] cluster in *D. gigas* hydrogenase (A.Volbeda, unpublished).

8.2.3. How H_2 gets to the active site

Since the active site is deeply buried in the protein molecule, the reactants, H_2 and hydrons, have to diffuse to the catalytic centre. The first conjecture for hydrogenase was that H_2 diffused through the protein (Cammack 1995), just as oxygen is believed to diffuse into the oxygen-carrier molecule of the blood, hemoglobin. However, the heme in hemoglobin is more accessible to the surface than the hydrogenase active site. Moreover hemoglobin oxygenation does not have to be particularly fast; it has only to take up each oxygen molecule once in the lungs and release it once in the tissues. By contrast hydrogenase, as an enzyme, turns over its substrates very rapidly, so H_2 transfer might become a limiting factor. In retrospect, it is no surprise that hydrogenases have specific channels for transfer of H_2 to the active site.

Topological analyses of hydrogenase crystallographic models, along with X-ray diffraction studies of the diffusion of xenon within crystals and molecular dynamics calculations, first suggested that molecular hydrogen exchanges are mediated by large hydrophobic internal cavities interconnected by narrow channels (Fig. 6.8). One end of the channel network points to the active site nickel and several other ends lead out into the external medium (Montet *et al.* 1997; see Section 3 in Chapter 6). The major cavities and channels are conserved in each of the four structurally characterized NiFe hydrogenases, including the *Desulfomicrobium baculatum* enzyme which shows a much lower sequence identity to *D. gigas* hydrogenase than the others. Site-directed mutagenesis experiments to further investigate the role of the ‘gas channels’ are in progress.

The number of atoms of Xe or H_2 in the putative gas channels represents a higher solubility for H_2 in hydrogenase, than in water. The channels appear to concentrate the gas. It is interesting that one of the ways that has been discovered for storing H_2

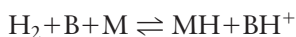
is in carbon nanotubes, designed to be of a size such that H₂ gas molecules can line up inside (Dillon *et al.* 1997). These can achieve very high densities of H₂.

8.3. What happens at the active site

In the years since the discovery of nickel and iron in the catalytic centres, numerous different descriptions of the catalytic cycle of hydrogenase have been proposed. These were based on different oxidation states of the metal centres, and different sequences of transfer of electrons and hydrons. Although the reaction cycle has not been definitively resolved, the spectroscopic evidence places constraints on possible models that should be considered.

8.3.1. Hydride formation

Hydrogenases belong to a select class of oxidoreductases that can convert two hydrogen reducing equivalents to electron equivalents. The active sites of such enzymes all have the property that they can accept one or two electrons at a time. From the hydrogen isotope exchange experiments, we know that the metal-containing hydrogenases operate by heterolytic cleavage of H₂ into a hydride and a hydron. To bind a hydride, we need a suitable electron-deficient metal centre, M, and to hold a hydron we need a base, B, thus



(Note that, by convention in organometallic chemistry, the hydride is written MH and not MH⁻). The hydride represents two reducing equivalents, equivalent to two electrons, or two hydrogen atoms. Now, it is very difficult to withdraw the electrons one at a time from a hydride or H₂ molecule, because it would be energetically unfavourable to create hydrogen in the intermediate state H[•], a hydrogen atom. Therefore, one or both of the metal ions in the active site *must accept two electrons simultaneously* from the hydride MH. The electrons are then transferred, *one at a time*, to the iron–sulfur clusters, which are normally one-electron acceptors. That is the trick that the hydrogenase active centre is specially designed to do.

The reaction mechanism can be likened to the cycle of an internal combustion engine, going through a series of steps (fuel and air induction, ignition, exhaust). When it is running, we only see a blurred view of the engine. It can be stopped and turned into various positions. However, some of the crucial steps (such as ignition of the fuel) are transient and it is not possible to stop the engine just then. The states we can observe are energy minima. The situation with the hydrogenase active site is analogous. As described in [Chapters 5](#) and [7](#), hydrogenases can be isolated or frozen in various states of oxidation and reduction. Examination of the structures and spectroscopic properties of these states can provide clues to the course of the enzyme-catalysed reaction. When we gradually decrease the redox potential, we are applying an increasing ‘electron pressure’ which forces the centres to lower redox states, rather like turning over the engine. For the hydrogenase it is more complicated than looking at a single engine, because we are dealing with a solution containing many

molecules. What we observe by spectroscopy, etc., is the macroscopic state of the system, which is the average of many molecules, each in a particular microscopic state. The states that can be frozen are stable ones; the mechanism is the way in which the enzyme converts between these states.

We should also remember that not all of the states that we see when freezing the enzyme (Section 7.4) are necessarily part of the mechanism. The most stable enzyme molecule is a dead one, so we must be aware that some of the spectroscopic signals represent damaged molecules. In the [NiFe] hydrogenases, the NiA and NiB states probably are not involved in the catalytic cycle, because they react slowly, if at all, with H_2 . In the mechanism shown in Fig. 8.3, it is assumed that the relevant active states are NiSR, NiA and NiR.

In the NiFe centre, EPR spectroscopy has shown that the nickel is redox active, being reduced from Ni(III) to Ni(II) before formation of the hydride. A fraction of the electron density is distributed the thiolate ligands, as predicted by DFT calculations (Section 7.15). The next steps in the reaction cycle involve the transfer of the two electrons from the hydride to the iron–sulfur clusters. We propose that the nickel is oxidized to Ni(III); then one electron from the hydride reduces the nickel, while the other is transferred to the proximal iron–sulfur cluster. During this process the nickel can be transiently reduced by both electrons, Ni(III) \rightarrow Ni(I). The Ni(I) state, though paramagnetic, would be very short-lived and probably undetectable by EPR spectroscopy. In the Fe hydrogenases, we can argue by analogy, that the iron atom that is

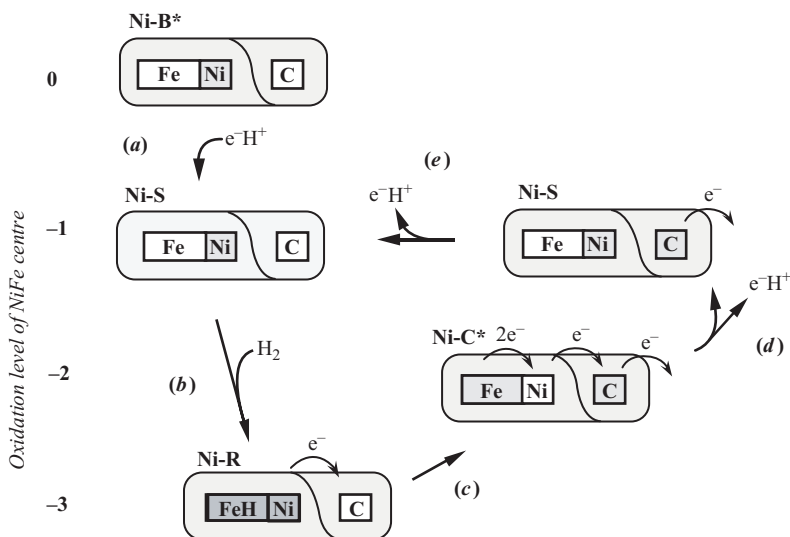


Figure 8.3 Outline reaction cycle of NiFe hydrogenase. The ‘minimal hydrogenase’ is depicted, consisting of the [NiFe] centre in the large subunit, and the proximal [4Fe-4S] cluster (C) in the small subunit. The reaction is written in the direction of the oxidation of H_2 . Electrons are transferred out through the other iron–sulfur clusters to an acceptor protein (not shown). The equivalent states of the NiFe centre B, SR, R and C are indicated. Reduced centres are shaded. Electron transfers are accompanied by transfers of hydrons (not shown).

reduced in the H cluster, is the one that is linked to the [4Fe-4S] cluster. Like nickel, this would be able to accept two electrons transiently.

A reaction cycle that is consistent with most of the available evidence for the NiFe hydrogenases is illustrated in Fig. 8.3. The reaction is drawn in the direction of hydrogen uptake, starting from the oxidized ready state, NiB.

- Activation: the Ni ion is reduced by an external donor.
- The metal ions are in the Ni(II) and Fe(II) oxidation state (reaction of H₂ with Ni(III) or Fe(III) is unlikely on chemical grounds). H₂ reacts with the centre, forming a bound H₂ or hydride on one of the metal ions, here assumed to be Fe(II).
- The Ni(II) is oxidized to Ni(III), by transfer of an electron to the proximal [4Fe-4S] cluster. To maintain charge neutrality, a hydron released from the H₂ is transferred to a base adjacent to the [4Fe-4S] cluster, leaving a hydride on the iron.
- The proximal cluster donates an electron to the external acceptor. Two electrons are transferred from the hydride, at first reducing the nickel from Ni(III) to Ni(I). An electron is transferred from the nickel to the proximal cluster.
- The second electron is transferred from the cluster to the acceptor protein, leaving the enzyme in the NiSR state. Meanwhile two hydrons are transferred to the solution.
- The production of H₂ comprises the same steps in the opposite direction, with electrons coming from a donor protein.

A similar reaction can be written for the [Fe] hydrogenases with a Fe-[4Fe-4S] complex replacing the nickel. Note that the nickel atom in the NiFe cluster, and the Fe-[4Fe-4S] sites are nearest to the electron carrier [4Fe-4S] clusters, indicating that electron transfer occurs through these atoms. The other atom in each of the centres is an iron atom with -CN and -CO ligands, and it seems likely that this is a binding site for hydride (Fig. 8.1).

This mechanism easily accounts for the other reactions catalysed by hydrogenase. Exchange of the hydron and/or hydride with another hydron from the water, and reversal of step 1 would explain the ¹H – ²H exchange reactions of hydrogenase (Chapter 5).

8.4. The metal-free hydrogenase from methanogenic archaea

Gerrit Buurman, Christina Afting and Rudolf K. Thauer

So far, the hydrogenases described have all used transition-metal ion clusters to react with H₂, and transfer electrons. However, it appears that there is another mechanism by which enzymes can catalyse reactions with H₂, without transition metal ions. This exception is the H₂-forming methylenetetrahydromethanopterin dehydrogenase from methanogenic archaea which contains neither nickel nor iron (Thauer *et al.* 1996). This unusual metal-free hydrogenase, abbreviated Hmd, catalyses the reversible reaction of N⁵,N¹⁰-methenyltetrahydromethanopterin (methenyl-H₄MPT⁺) with H₂ to N⁵,N¹⁰-methylenetetrahydromethanopterin (methylene-H₄MPT) and a proton ($\Delta G^{\circ} = -5.5$ kJ/mol). This reaction is involved in the pathway of methane formation from CO₂ and H₂ (Afting *et al.* 1998; Thauer 1998). Tetrahydromethanopterin (H₄MPT)

is an analogue of tetrahydrofolate (H_4F). However, the redox potential of the methenyl- H_4MPT^+ /methylene- H_4MPT couple ($E^{\circ'} = -390$ mV) is more negative than that of the methenyl- H_4F^+ /methylene- H_4F couple ($E^{\circ'} = -300$ mV); it is close to that of the H_2 electrode at pH 7.0 ($E^{\circ'} = -414$ mV).

Hmd is composed of only one type of subunit with an apparent molecular mass of approximately 40 kDa and has a specific activity of above 1,000 U/mg (Zirngibl *et al.* 1992). Evidence for the absence of nickel and iron in the metal-free hydrogenase is based on several findings (Thauer *et al.* 1996): (i) the purified active enzyme contains nickel and iron only in substoichiometric amounts (< 0.1 mol/mol) that do not correlate with activity; (ii) the primary structure of the enzyme shows no binding motifs for nickel or iron and is not sequence similar to Ni/Fe and Fe-only hydrogenases; (iii) the specific activity of the enzyme increases rather than decreases in cells growing under conditions of nickel limitation (Afting *et al.* 2000); and (iv) the activity is not inhibited by CO, NO or acetylene which inhibit Ni/Fe and Fe-only hydrogenases most likely by binding to the transition metal in the active site.

With respect to the catalytic mechanism the following findings are of importance (Thauer *et al.* 1996): (i) in contrast to Ni/Fe and Fe-only hydrogenases, the enzyme *per se* neither catalyses an exchange between H_2 and the protons of water nor the conversion of *para*- H_2 to *ortho*- H_2 ; however, in the presence of methenyl- H_4MPT^+ the enzyme does catalyse both exchange reactions; (ii) the enzyme catalyses a direct hydride transfer from H_2 into the pro-R position of methylene- H_4MPT , the rate of incorporation being almost identical for H_2 and D_2 ; and most importantly (iii) the enzyme catalyses a direct exchange of the pro-R hydrogen of methylene- H_4MPT with protons of water.

The results indicate that in methenyl- H_4MPT^+ reduction with H_2 , the methenyl group is activated by the metal-free hydrogenase in a way that it can directly react with H_2 . They further indicate that in methylene- H_4MPT dehydrogenation, the pro-R hydrogen of the methylene group is activated such that it can directly react with a proton. Thus in case of the metal-free hydrogenase the hydrogen donor/acceptor rather than H_2 is activated. How this could be achieved is depicted in Fig 8.4. The proposed catalytic mechanism is similar to the one assumed for the reversible reaction of carbocations with H_2 under superacidic conditions (Berkessel and Thauer 1995). At present only this mechanism explains the reversible exchange of the pro-R hydrogen of methylene- H_4MPT with protons of water catalysed by the metal-free hydrogenase (Thauer *et al.* 1996).

In Fig. 8.4 it is assumed that methenyl- H_4MPT^+ undergoes a conformational change upon binding to the enzyme. The conjugational stabilization of the planar formamidinium ion is thus eliminated and a cationic centre at C14a is created which in its properties corresponds to a carbocation normally generated only under superacidic conditions. The activated carbocation is able to reversibly react with H_2 , a hydride being incorporated into the pro-R position of the methylene group and a proton being released into the solvent. Methylene- H_4MPT is generated in a constraint conformation which relaxes upon dissociation from the enzyme as deduced from the stereochemical course of the reaction and the reversed conformation of methylene- H_4MPT free in solution (Geierstanger *et al.* 1998; Bartoschek *et al.* 2001). The mechanism is supported by *ab initio* molecular orbital calculations (Cioslowski and Boche 1997; Scott *et al.* 1998; Teles *et al.* 1998).

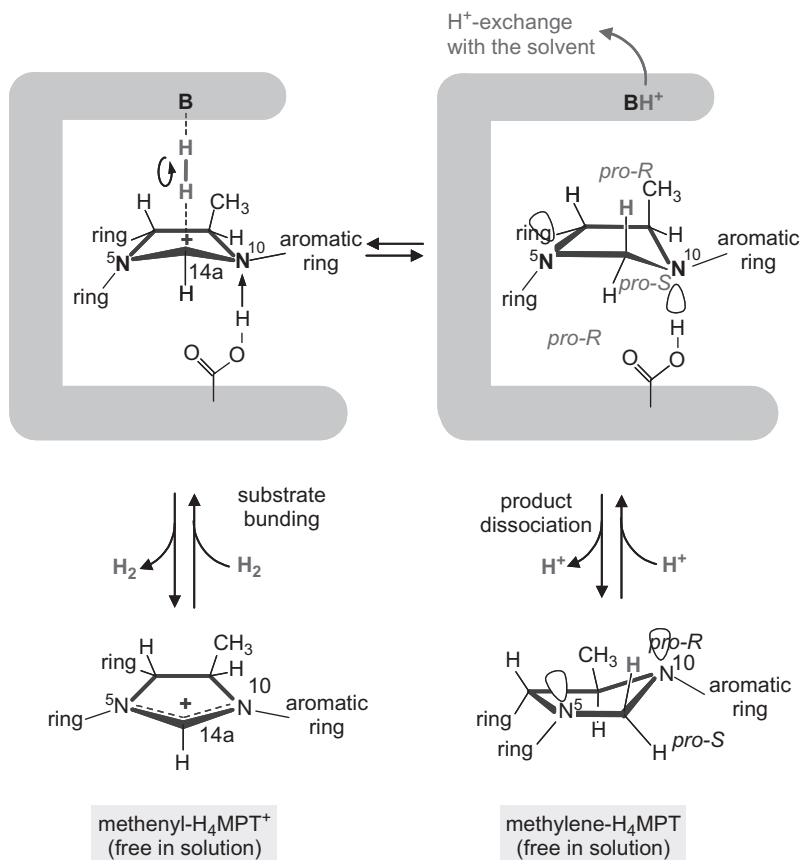


Figure 8.4 Proposed mechanism for the reversible reaction of N^5 , N^{10} -methenyltetrahydromethanopterin (methenyl- H_4MPT^+) with H_2 to N^5 , N^{10} -methylene tetrahydromethanopterin (methylene- H_4MPT) and a proton catalysed by the metal-free hydrogenase from methanogenic archaea. For complete structures of methenyl- H_4MPT^+ and methylene- H_4MPT , see reference Thauer *et al* (1996). Methylene- H_4MPT free in solution is in a conformation in which the methylene *pro-R* C-H bond is synclinal to the lone electron pair of N^{10} . Upon binding to the enzyme the imidazolidine ring of methylene- H_4MPT is forced into an activated conformation in which the *pro-R* C-H bond is antiperiplanar to the lone electron pair of N^{10} resulting in the preformation of the leaving hydride (Bartoschek *et al.* 2001).

Very recently evidence was provided that Hmd contains a low-molecular-mass, thermolabile cofactor that is tightly bound to the enzyme but could be released upon enzyme denaturation in urea or guanidinium chloride (Buurman *et al.* 2000). No indications were found that the cofactor contains a redox-active transition metal. Further studies are needed to determine the structure of the cofactor and its putative role in the catalytic mechanism.

8.5. Can we mimic nature, or even improve on it?

8.5.1. Hydrogenase biomimetics as substitutes for platinum

A worldwide transition from fossil energy to renewable energy sources such as solar energy seems inescapable. Research on hydrogenases and biomimetic model compounds is highly relevant. One of the major long-term goals of the research on hydrogenases is to synthesize a stable and cheap catalyst for interconversion of electricity and H₂. Such a catalyst would be essential for processes such as the production of H₂ by solar energy (electrolysis of water) and the conversion of H₂ into electricity (fuel cells). Present-day systems still make use of platinum as a catalyst, which prevents such systems from becoming economically viable for large-scale applications (Appleby 1999). Although there have been improvements, using platinum in the form of small particles, the supplies of this precious metal would not be sufficient for a worldwide hydrogen economy (Berger 1999). An alternative catalyst is needed for the reversible and rapid activation of molecular hydrogen, preferably at normal temperature and pH. However, the catalyst would also have to be more durable. The discovery of the structure and mechanism of the hydrogenases is a considerable step forward, which should help to guide further spectroscopic, mechanistic and biomimetic studies on hydrogenases.

It has already been shown that a very efficient catalyst can be produced by adsorbing the enzyme onto a carbon electrode (see Section 9 in [Chapter 5](#)). It produces H₂ at high rates. This can be considered as the biological equivalent of platinum. Though stable, by biological standards, it cannot compare with metallic catalysts. Nature tends to use such catalysts as disposable, and regenerates them as necessary. We have learned much about the intricate processes involved in assembling the hydrogenase molecule ([Chapter 4](#)), and it is clear that, in order to construct and maintain these catalysts, all the resources of a living cell are required. One possible approach would be to use bacterial cells as 'cell factories'. The catalytic properties of the enzymes could be refined either by targeted mutagenesis of parts of the structure, or by random mutation with evolutionary selection, or, most probably a combination of these approaches.

The alternative approach is to use the knowledge gained about the enzymes, to construct chemical catalysts. Here the field is in its infancy, since the construction of structural models could only start when the structure of the proteins began to emerge, in 1995. Previously, many heterogeneous catalysts had been known, for example for hydrogenation processes. But most of these used heavier, and scarcer metals.

With these possible applications in mind, we should review the significant characteristics of hydrogenase as a catalyst. Compared with most chemical catalysts, hydrogenases are large molecules. The protein has been selected by evolution, from an almost infinite number of possible structures. The whole protein is part of the machinery. Therefore even minor tampering with the protein, for example by site-directed mutagenesis, is likely to lead to unexpected changes in the properties of the enzyme.

Hydrogenases, like most enzymes, are extraordinary in their specificity. They catalyse a specific reaction with a particular set of substrates, and produce particular products. The reaction can go backwards and forwards many times without producing unwanted by-products. They do not, for example, reduce CO₂ to CO, which would

poison most of them (there are other enzymes that do catalyse this reaction, and interestingly, they also contain nickel and iron–sulfur clusters). Chemical catalysts, by contrast, are plagued by unwanted side reactions. This is because in a chemical catalyst such as platinum, the reactions take place at the surface. In an enzyme the catalytic centre is protected from molecules in the solution by a layer of protein, and access to the catalytic site is controlled.

The side reaction that most hydrogenases find difficult to avoid is the reduction of oxygen. In the presence of oxygen, low-potential catalysts tend to generate oxygen radicals such as superoxide and hydroxyl radical, which are damaging to cells as well as the enzyme itself. However, most hydrogenases from organisms that are exposed to oxygen have some sort of protective mechanism, usually to switch them off in the presence of oxidants. The hydrogenases of hydrogen-oxidizing bacteria such as *R. eutropha*, and the sulfhydrogenase of *Pyrococcus furiosus*, have a specially protected active site that allows them to reduce compounds such as NAD, even in the presence of oxygen (see Chapter 7). Under these conditions, platinum would catalyse the wasteful reaction between oxygen and hydrogen. Thus oxygen is less of a problem with hydrogenase than it is with chemical catalysts.

The mechanism of action, and organization of the catalytic sites, in hydrogenases are different from a solid catalyst such as platinum. For a start, the reaction of H₂ with hydrogenase involves heterolytic cleavage into a hydron and a hydride. This contrasts with the reaction of H₂ at the surface of a metal such as platinum, which is usually considered to involve the homolytic cleavage into two hydrogen atoms. Moreover in the enzyme, the catalyst is a cluster of metal ions (with oxidation states +2 or +3) rather than the metal (oxidation state 0).

8.6. Synthetic model compounds – how chemists mimic nature

Arnd Müller

From the point of view of an inorganic chemist, the metal sites in metalloproteins can be defined as classic coordination compounds, also called complex compounds, with exceptionally large ligands (the proteins), because their chemistry cannot be separated from that of the corresponding metals. Most of the structural, physical, spectroscopic, and chemical properties of coordination compounds are determined by the composition of the immediate centre consisting of the metal ion(s) and all directly bound, surrounding atoms, that are referred to as ligands. Complex compounds, which resemble the biological originals in important properties, are then termed ‘model compounds’. These are usually low-molecular-mass compounds, in which the large protein is replaced by smaller biomimetic ligands and that can be characterized and manipulated more readily than the metalloproteins.

An ideal model compound should correspond to the biological metal centre in terms of structure, composition as well as coordination and oxidation states of the individual metal ions, and also possess comparable spectroscopic and chemical properties. However, real model compounds rarely meet all these requirements at once. Usually, only special aspects of a metal centre are modelled, such as the structure, magnetic or electronic properties (spectroscopy), or the reactivity (function), and the

corresponding model compounds are accordingly called ‘structural’, ‘spectroscopic’ or ‘functional’ models, respectively. Synthetic model compounds yield only information on the intrinsic properties of a metal centre, as the influence of the surrounding protein is not included. They provide information that is necessary for the understanding of basic principles and is difficult to derive from investigations on the complex biological systems. The concept of synthetic model compounds is not restricted to the replicative modelling of structural, spectroscopic and functional properties of already known metal centres, but can also be utilized to check the validity of hypotheses, that are based on spectroscopic studies. Important parameters necessary for the interpretation of spectroscopic data of metalloenzymes can only be obtained and calibrated by measurements on suitable model compounds. Synergic studies, that include the progressive improvement of the model compounds, are required in order to develop significant theories. The following paragraphs should demonstrate the principles outlined above with some examples for model compounds relevant to hydrogenases, but by no means attempt to give a comprehensive review of all the excellent and extensive work done in this field so far.

A textbook example for synthetic model compounds is provided by the thermodynamically stable iron–sulfur cluster compounds, that were designed to resemble the biological metal centres found in the iron–sulfur proteins (e.g. Holm 1977). These proteins range from electron transport proteins of low molecular mass (ferredoxins) to highly complex enzymes comprising several subunits, such as the hydrogenases, in which they are believed to form electron-transfer pathways through the protein matrix. Synthetic compounds such as the ‘cubane’-type clusters $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ in Fig. 8.5 (1) are readily formed in solution by the reaction of Fe^{3+} ions with hydro-sulfide anions (HS^-) or elemental sulfur in the presence of thiolates with small hydrocarbon residues (RS^- , with R = methyl, ethyl, benzene, etc.), which are the biomimetic equivalent of cysteine (i.e. they have the same functional group, called thiolate) (Herskovitz *et al.* 1972). It is believed that similar spontaneous self-assembly reactions might also be involved in the *in vivo* synthesis of iron–sulfur centres, and it was even speculated that related reactions might have taken place at the origin of life (Hall *et al.* 1971). In fact, the cluster cores of [4Fe-4S] or [2Fe-2S] centres are stable enough to be removed intact from a protein under denaturing conditions, or (re)-inserted into an apo-protein.

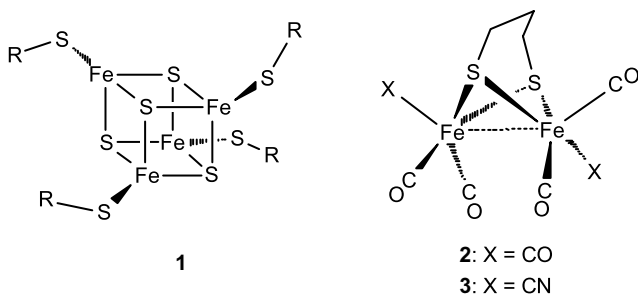


Figure 8.5 (1–3) Structures of chemical models for the active sites of hydrogenases. For references, see text.

The ability to synthesize reasonable amounts of model compounds, not only identical to the biological originals, but also suitably modified, has helped substantially with the investigation and the understanding of structure and function of biological iron–sulfur centres during the last thirty years. Of special importance are the high-precision structures, that were obtained by single-crystal X-ray diffraction on model compounds and are routinely used for the structure solution of metalloproteins, when only data sets of less than atomic resolution are available (see [Chapter 6](#)). For small molecules such as model compounds, X-ray diffraction usually provides important structural parameters such as bond distances and angles as accurate as 0.1 pm and 0.1°, respectively, while the accuracy determined in protein crystallography is rarely within 10 pm or several degrees for bond distances or angles, respectively. Another important piece of information was learned from electrochemical studies on model compounds: the [4Fe-4S] cluster can be reversibly oxidized twice, which explained the extremely wide range of electrochemical potentials covered by ferredoxins. Model compounds were also employed to probe the complex electronic and magnetic properties of iron–sulfur clusters.

Although reported procedures for synthesis of inorganic coordination complexes typically sound straightforward, they are mostly the result of extensive studies undertaken to overcome the unique problems encountered in transition-metal chemistry. First, complex compounds differ from the majority of other molecules in that they normally are kinetically unstable, i.e. they exist in equilibrium with other species of comparable thermodynamic stability in solution. Hence, the preparative task is not just restricted to synthesizing the desired compound, but also to find the appropriate conditions to stabilize and isolate it as the main product. It should preferably be in crystalline form, because X-ray diffraction is usually the only method to determine its structure unequivocally. A second problem characteristic of nickel-thiolate chemistry is that nickel ions in oxidation states higher than +2 have under most circumstances enough oxidizing potential to convert thiolate molecules to the corresponding disulfides. Moreover, nickel ions can catalyse reactions of the ligands involving the breakage or formation of covalent bonds, respectively, which can lead for example in the unexpected appearance of sulfide ions in the isolated product (e.g. Krüger *et al.* 1989; Müller and Henkel 1996), or can lead to the formation of sulfoxy groups in the presence of oxygen (Kumar *et al.* 1989; Farmer *et al.* 1992). Although many of the model compounds reported do not seem to be related to the biological centre at all, they help us to understand what is chemically possible, and what chemical obstacles the process of biological centre assembly has to overcome.

8.6.1. Models of the hydrogenase active sites

The structure of the dimetallic [2Fe] moiety of the unprecedented ‘H cluster’ found at the active site of ‘iron-only’ hydrogenases has only been known for a short time (Chapter 6, and Peters *et al.* 1998; Nicolet *et al.* 1999), but has already led to the construction of many new model compounds (Darensbourg *et al.* 2000). The chemistry of iron complexes is well investigated, and there are a large number of known complexes with mixed carbonyl and thiolate ligands which provided a good starting point. The complex compound $[(\mu\text{-SCH}_2\text{CH}_2\text{CH}_2\text{S})\text{Fe}_2(\text{CO})_6]$ in [Fig. 8.5\(2\)](#) has been known for over twenty years as a member of the comprehensively studied family of

$[(\mu\text{-SR})_2\text{Fe}_2(\text{CO})_{6-x}\text{L}_x]$ compounds (e.g. Seyferth *et al.* 1982). These stable complexes are thermodynamic sinks in low-valent iron-thiolate carbonyl chemistry. By using well-established chemical reactions, $[(\mu\text{-SCH}_2\text{CH}_2\text{CH}_2\text{S})\text{Fe}_2(\text{CO})_6]$ in Fig. 8.5(2) could be readily converted to the complex $[(\mu\text{-SCH}_2\text{CH}_2\text{CH}_2\text{S})\text{Fe}_2(\text{CO})_4(\text{CN})_2]^{2-}$ in Fig. 8.5(3), which was shown to be a good structural and spectroscopic (IR) model for the dimetallic [2Fe] moiety (Lyon *et al.* 1999; Schmidt *et al.* 1999). This compound could only be oxidized irreversibly; the logical next step is to connect it via a sulfur bridge to a [4Fe-4S] cluster. The significance of this second moiety of the ‘H-cluster’ may be to serve as an oxidation level buffer in the enzyme.

The history of model compounds for the heterodimetallic nickel–iron centre of [NiFe] hydrogenase is an excellent example of the synergistic process in bioinorganic research (Darensbourg *et al.* 2000). In fact, the extended field of nickel-thiolate chemistry known today principally originates from the interest triggered by the discovery of nickel–sulfur bonds in the [NiFe] hydrogenases from *D. gigas* and *Methanothermobacter marburgensis* by EXAFS spectroscopy (Scott *et al.* 1984; Lindahl *et al.* 1984). Hence, the first synthetic goals were mononuclear nickel compounds with complete sulfur ligation, which preferably should show EPR signals similar to the hydrogenase. Attempts to use spontaneous self-assembly reactions, similar to the ones that work so well for iron–sulfur complexes, did not produce the desired model compounds, but did reveal some important general principles of nickel-thiolate chemistry. The nickel compounds formed using thiolate ligands with saturated hydrocarbon residues, contained nickel in the divalent oxidation state (Ni^{2+}), whose electronic configuration (d^8) favours square-planar coordinated complexes; these have no unpaired electrons and are therefore diamagnetic (‘EPR silent’). Moreover the square-planar NiS_4 units were usually found to form polynuclear complexes via common edges, either resulting in zigzag chains or closed ring structures (for examples, see e.g. Krebs and Henkel 1991). However, none of the compounds synthesized was found to possess any of the chemical or spectroscopic characteristics of the biological metal centre. The same is true for mononuclear complexes with tetrahedral symmetry, which were formed with *thio*-phenolate or ring-substituted ligands – Fig. 8.5(4) (e.g. Müller and Henkel 1995). Although mononuclear complexes with square-planar symmetry, which are formed with difunctional ligands of the 1,2-dithiolate type in Fig 8.5(5), could be reversibly oxidized in cyclic voltammetric experiments (Fox *et al.* 1991, Krüger *et al.* 1991), none of the oxidation products could be isolated (Köckerling and Henkel 2000).

Stable mononuclear complexes of nickel in formal oxidation states of +1 or +3 were found with – at least partially – a coordination of ligands, that are classified as ‘harder’ (i.e. more ionic in bonding character) than the rather ‘soft’ (i.e. covalent in bonding character) thiolate sulfur function, such as nitrogen or oxygen donor atoms. These complexes usually possess anisotropic EPR signals, and can have coordination numbers in the range of four to six – Fig. 8.5(6, 7, 8) (Krüger and Holm 1989, 1990; Krüger *et al.* 1991; Köckerling and Henkel 1993). Penta-coordinate nickel complexes with a mixed nitrogen (oxygen) and sulfur coordination have attracted much interest, because of the results of XAS measurements on the [NiFe] hydrogenase from *Thiocapsa roseopersicina* and other bacterial sources (see Section 13 in Chapter 7; Gu *et al.* 1996; Müller *et al.* 1997a,b). In order to obtain penta-coordinate complexes, however, one has to employ multifunctional chelating ligands,

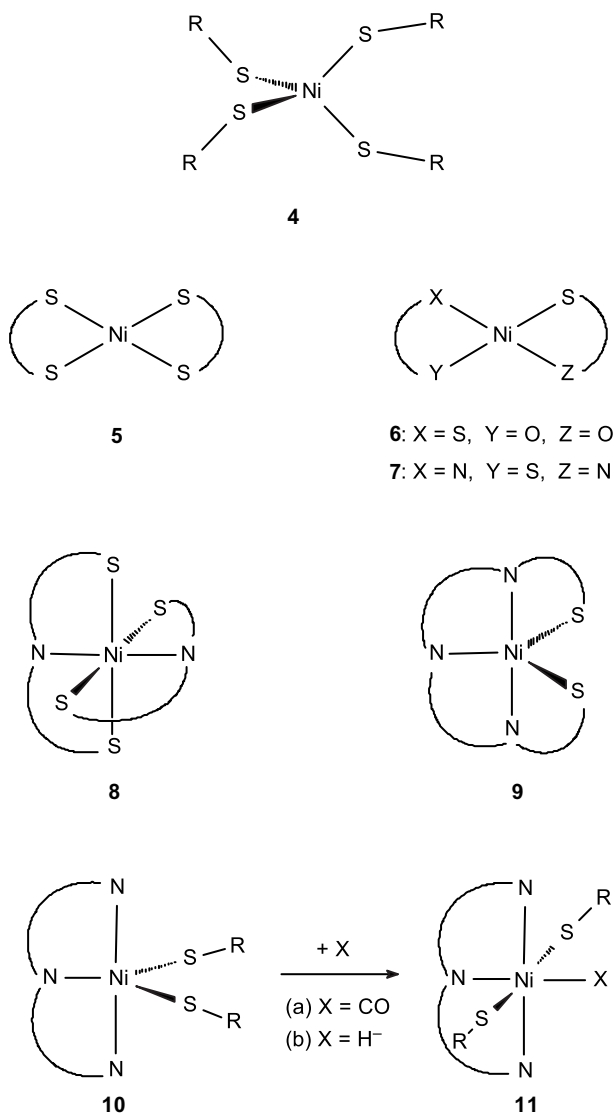


Figure 8.5 (4–11) Structures of chemical models for the active sites of hydrogenases. For references, see text.

that provide a set of donor functions in a suitable arrangement and thus determine the structure of the formed complex in a similar way to the protein. An example is the specially designed pentadentate ligand in complex 9 (Shoner *et al.* 1994). Some remarkable knowledge on the reactivity of such nickel compounds was gained from the penta-coordinate complexes with trigonal bipyramidal geometry, which are formed with a trifunctional nitrogen donor ligand and two monofunctional thiolates – Fig. 8.5(10) (Baidya *et al.* 1992; Marganian *et al.* 1995). These compounds could not

only be reversibly transferred through nickel oxidation states of +1, +2 and +3, but were also shown to bind a carbon monoxide molecule or a hydride ion, respectively, in the Ni^{1+} state. The penta- and hexa-coordinate compounds in Fig. 8.5(10, 11) showed EPR spectra that closely resembled the characteristic hydrogenase Ni-EPR signals. This series of model compounds was the first not restricted to modelling only a single aspect (structural, spectroscopic, functional) of the biological nickel centre, as they modelled most of the properties known at this date reasonably well.

However, despite the great progress made in the field of nickel-thiolate chemistry, the surprising discovery of the dinuclear nature of the active nickel site in the [NiFe] hydrogenase by protein crystallography (Chapter 6) found model chemistry rather ill prepared. Although at that time numerous dinuclear nickel complexes with two thiolate bridges were known, only two contained nickel ions coordinated solely by thiolate ligands, in a geometry other than square planar (Halcrow and Christou 1994; Halcrow 1995; Fontecilla-Camps 1996). The homodinuclear complexes $[\text{Ni}_2(\text{S}'\text{C}_4\text{H}_9)_6]^{2-}$ in Fig. 8.5(12) and $[\text{Ni}_2(\text{S}-2,4,5\text{-}i\text{Pr}_3\text{C}_6\text{H}_2)_5]^-$ in Fig. 8.5(13) each contain two nickel ions with a distorted tetrahedral thiolate coordination, linked by two or three bridging thiolate ligands, respectively (Müller and Henkel 1995; Silver and Millar 1992). Heterodimetallic compounds containing thiolato-bridged nickel-iron units were only known with nickel ions chelated by ligands of the N_2S_2 -type and additional chloride ligands on the iron ions (Mills *et al.* 1991; Colpas *et al.* 1992), but S-metalation reactions using similar nickel chelates as precursors readily produced dinuclear nickel-iron complexes such as $[\text{Ni}(\text{N},\text{N}'\text{-bis-2-mercaptoethyl-1,$

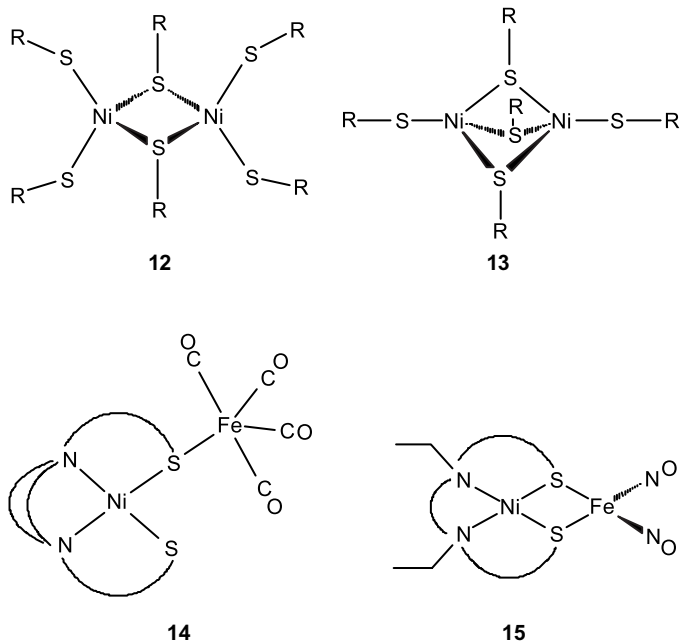


Figure 8.5 (12–15) Structures of chemical models for the active sites of hydrogenases. For references, see text.

5-diazacyclooctan)Fe(CO)₄] in Fig. 8.5(14) or [Ni(N,N'-diethyl-3,7-diazanonane-1,9-dithiolate)Fe(NO)₂] in Fig. 8.5(15) (Lai *et al.* 1996; Osterloh *et al.* 1997). These contain iron-carbonyl or iron-nitrosyl fragments that are bridged to a nickel moiety by one or two thiolate functions, respectively. An impressive model for the iron part of the heterodimetallic centre was found with the organometallic compound [(η⁵-C₅H₅)Fe(CN)₂(CO)]⁻ in Figs 7.4 and 8.5(16), in which the pyramidal Fe(CN)₂(CO) fragment is bound to a cyclopentadienyl ligand, that mimics the six-electron donating ability of the Ni(μ-SCys)₂(μ-O) donor face of the enzymatic centre (Darensbourg *et al.* 1997). Subsequently, a series of related compounds [(η⁵-C₅R₅)Fe(CO)_{3-x}(CN)_x]ⁿ⁻ (R = H, Me; x = 0–2; n = 0–1) (Lai *et al.* 1998) was used to probe the effects of electronic pressure and hydrogen bonding, respectively, on the vibrational modes of the triply bonded diatomic molecules, that are used to distinguish between the different enzymatic states in FTIR spectroscopy (Sections 2.1 and 8 in Chapter 7).

Although hitherto no structural analogue of the complete heterodimetallic nickel–iron centre of [NiFe] hydrogenase has been reported, some compounds with similarities to the biological centre were recently synthesized using specially designed

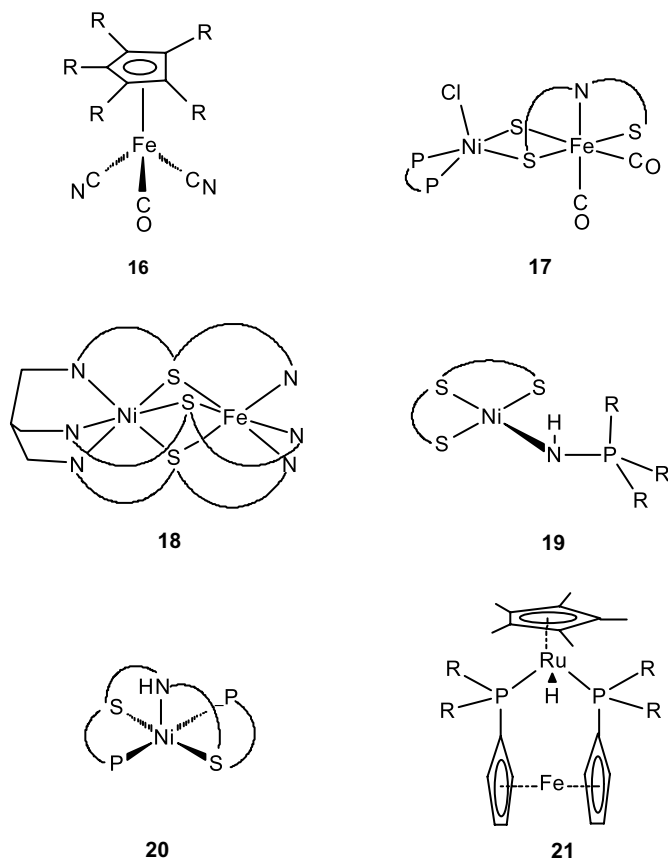


Figure 8.5 (16–21) Structures of chemical models for the active sites of hydrogenases. For references, see text.

chelate ligands in Fig. 8.5(17, 18) (Davies *et al.* 1999; Steinfeld and Kersting 2000). The necessity for the restriction induced by these tailored ligands is not surprising, as all the evidence points to the extraordinary character of the enzymatic centre, for whose construction even Nature has to resort to complicated procedures (Chapter 4). However, studies towards functional models for the hydrogenase suggest that the complete heterodimetalllic centre might not be necessary. Mononuclear nickel complexes such as $[\text{Ni}(\text{NHP}(\text{C}_3\text{H}_7)_3)(\text{bis}(2\text{-sulfanylphenyl)sulfide})]$ in Fig. 8.5(19) catalyse the hydrogenase-specific H_2/D_2 exchange reaction (Sellmann *et al.* 2000), and compounds such as $[\text{Ni}(\text{bis}(5\text{-(diphenylphosphino)-3-dithiapentanyl)amine})]^{1+}$ in Fig. 8.5(20) were found to produce H_2 gas from protons without even having thiolate ligands (James *et al.* 1996). The catalytic activity of the hydrogenase in hydrogen consumption coupled to methyl viologen reduction was modelled by the organometallic ruthenium–iron compound in Fig. 8.5(21) (Hembre *et al.* 1996), which might indicate an active function of the iron part in the reaction, other than the mere fine-tuning of reactivity properties.

In conclusion, model compound studies have contributed valuable information for our understanding of the structure and reactivity of the enzymatic centre by probing the chemical possibilities. But apart from the help in the complete understanding of the catalytic principle, they also point to potential alternatives for functional catalysts, which are needed for the cheap production of hydrogen on a large scale to meet the increased demand expected after its introduction as a fuel in the future (Chapters 9 and 10).

8.7. A dinuclear iron(II) compound mimicking the active site of [Fe] hydrogenases

Elisabeth Bouwman, Richard K. Henderson and Jan Reedijk

We have reported the first dinuclear iron(II) compound which may be regarded as a promising structural model for [Fe]-only hydrogenases (Fig. 8.6A). The synthesis and crystallographic characterization of this mixed-spin dinuclear iron(II) complex, containing sulfur bridges and terminal carbon monoxide ligands, have been described (Kaaajager *et al.* 1998). The compound has been synthesized by refluxing a mixture of $[\text{Fe}(\text{II})(\text{dsdm})]_2$ ($\text{H}_2\text{dsdm} = \text{N,N}'\text{-dimethyl-N,N}'\text{-bis}(2\text{-mercapto-ethyl})\text{ethylenediamine}$) with $\text{K}[\text{HFe}(\text{CO})_4]$ in the presence of 2-bis(mercaptoethyl)sulfide (H_2bmes). The structure shows two octahedrally coordinated iron(II) atoms, each having different ligand environments. Fe1 is coordinated to the ligand dsdm, and the two thiolate sulfurs from bmes, resulting in an FeN_2S_4 chromophore. Fe2 is bound to the ligand bmes, to two terminally coordinating carbon monoxide groups and one of the thiolate sulfurs from dsdm, resulting in an FeC_2S_4 chromophore. The two octahedrons are face sharing through three asymmetric μ_2 -thiolato bridges. The dinuclear molecule is electrically neutral, and charge considerations lead to the conclusion that both irons are divalent.

The IR spectrum of the compound in the solid state reveals strong absorptions for the carbonyl ligands at $2,011\text{ cm}^{-1}$ and $1,957\text{ cm}^{-1}$, well within the range as is observed for the [Fe]-only hydrogenases.

The spin states of the iron(II) atoms have been verified with Mössbauer spectroscopy. A Mössbauer spectrum recorded at room temperature, shows two doublets

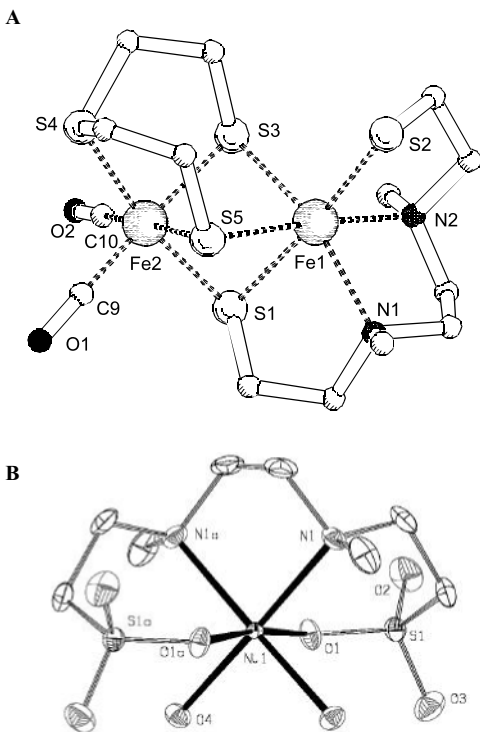


Figure 8.6 (A, B) Dinuclear models for the active sites of hydrogenases. For references, see text.

of equal intensity but with different isomer shifts and quadrupole splittings, confirming the presence of two distinct Fe(II) atoms.

In conclusion, the presented dinuclear iron structure is the first example of a bio-mimetic iron compound, which can be regarded as a first generation model for the class of [Fe]-only hydrogenases. The complex incorporates both relevant carbon monoxide ligands, as well as three bridging thiolato ligands, which could be possibly present in the active site of these enzymes.

8.7.1. A nickel disulfonato complex obtained by oxidation of a mononuclear nickel dithiolate complex

The oxidation with H_2O_2 of the mononuclear nickel dithiolate complex $[\text{Ni}(\text{dsdm})]$ ($\text{H}_2\text{dsdm} = \text{N,N}'\text{-dimethyl-N,N}'\text{-bis(2-mercaptoethyl)ethylenediamine}$) led to the high-yield formation of a disulfonato nickel complex $[\text{Ni}(\text{dsodm})(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ ($\text{dsodm} = \text{N,N}'\text{-dimethyl-N,N}'\text{-bis(2-sulfonatoethyl)ethylenediamine}$) (Fig. 8.6B). This is the first example of oxidation of a nickel thiolate to a nickel sulfonato compound (Henderson *et al.* 1997). The coordination around the nickel is octahedral, with the nickel sitting on a two-fold axis. The tetradentate ligand occupies four coordination sites with the two sulfonate oxygens in trans-positions. Two water molecules cis

to each other complete the octahedral coordination set. The nickel to amine nitrogen distances are 2.143(2) Å. The nickel to oxygen bonds in $[\text{Ni}(\text{dsodm})(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ are 2.044(1) and 2.084(1) Å for the sulfoxides and the water molecules, respectively.

The use of six equivalents of dihydrogen peroxide leads to a clean conversion of the dithiolate complex to the disulfonate compound. Earlier studies on oxidation of nickel thiolates showed that oxidations with dioxygen stop at monosulfonates. Our observation and the characterization of the first chelating bis-sulfonato nickel complex formed from the direct oxidation of a mononuclear nickel dithiolate, may also provide new insight into the chemistry of sulfur-rich nickel-containing enzymes in the presence of oxygen.