7.5: Multisite Redox Enzymes

In making the transition from the relatively simple electron-transfer proteins to the far more complex Fe-S-containing enzymes, we must recognize that the difference in our degree of understanding is enormous. Not only are the catalytic proteins ten to twenty times larger than the redox proteins, but they often also have several subunits and multiple copies of prosthetic groups. Moreover, very few crystal structures are known for the redox enzymes, and none is known at high resolution. In the absence of three-dimensional protein structural information, we do not know the arrangement, relative separation, or orientation of the prosthetic groups. Finally, studies on model systems have not yet approached the sophisticated state that they have for the structurally known centers.

In general, multicomponent redox enzyme systems appear to be organized in two distinct ways to effect their substrate reactions. In the first mode, the enzyme is designed to bring the oxidant and reductant together so that they may directly interact. For example, oxygenases bring O_2 and an organic molecule together, and activate one or both of these reactants to cause them to react directly with each other. This mode can be called *proximation*, as the reactants are brought near each other by the enzyme catalyst.

In contrast to proximation, many redox enzymes keep the oxidant and reductant well-separated, and use rapid (usually long-distance) internal electron transfer to bring electrons from the reductant to the oxidant. We can term their mode of action *electrochemical*. The oxidant and reductant are separated spatially. The enzyme provides the "anode" site to interact with the reductant, the "cathode" site to interact with the oxidant, and the wire to allow electronic flow between the "anode" and "cathode" sites. Hydrogenases and nitrogenases adopt the electrochemical mode of redox activation. In hydrogenases, the electron acceptor, even if it must formally take up hydrogen (e.g., $NAD^+ \rightarrow$ NADH), does not interact at the same site as the H_2. There is no direct transfer of H^- from H_2 to NAD^+. Rather, H_2 reduces the enzyme at one site, and NAD^+ or other acceptors, such as methylene blue, retrieve the electrons at other sites following internal electron transfer. For nitrogenase, the redox partners are even more removed, as a separate protein, the Fe protein, delivers electrons to the FeMo protein, that eventually end up at the FeMoco site ready to reduce N_2 to NH_3. These enzymes work much like electrochemical cells.

Hydrogenase and Nitrogenase

Hydrogenase is the enzyme responsible for the uptake or evolution of H_2. Nitrogenase is the enzyme that catalyzes the ATP-dependent reduction of N_2 to NH_3, with concomitant evolution of H_2.

The relationship between H_2 and N_2 in biology is intricate.[184] Metabolically, H_2 use and N_2 use are tightly coupled in many nitrogen-fixing organisms, with H_2 serving indirectly as the reductant for N_2. Moreover, H_2 and N_2 react in related ways with various transition-metal complexes, which are at present the closest (albeit quite imperfect) models of the enzyme active sites. The biological fixation of molecular nitrogen is dependent on iron-sulfide proteins that also contain molybdenum or vanadium. The biological production or uptake of H_2 depends on the presence of iron-sulfide proteins, which often also contain nickel and sometimes selenium. Spectroscopic and model-system studies, which have played such a key role in advancing the understanding of simple Fe-S sites, are now helping to foster an understanding of these more complex enzyme sites, although we have much yet to learn about structure and mechanism in these enzymes. The remainder of this chapter seeks to convey the state of our rapidly evolving knowledge.

Hydrogenases

1. Physiological Significance

Molecular hydrogen, H_2, is evolved by certain organisms and taken up by others. For either process, the enzyme responsible is called hydrogenase. The raison *d' être* for hydrogenases in particular organisms depends on the metabolic needs of the organism. Properties of some representative hydrogenases are given in Table 7.5.[184,184a-g]

Hydrogenases are found in a wide variety of anaerobic bacteria, such as the eubacterial *C. pasteurianum* and *Acetobacterium woodii* and the archaebacterial *Methanosarcina barkerii*. Interestingly, *C. pasteurianum* sometimes evolves H_2 during its growth on sugars. This H_2 evolution is required for continued metabolism, since it allows the organism to recycle (reoxidize) cofactors that are reduced in the oxidation of sugars (or their metabolic descendants, lactate or ethanol). In effect, H^+ is acting as the terminal oxidant in clostridial metabolism, and H_2 is the product of its reduction. In contrast, methanogens such as methanosarcina take up H_2 and in effect use it to reduce CO_2 to CH_4 and other carbon products. Clearly, either H_2 uptake or H_2 evolution may be important in particular anaerobic metabolic contexts. The hydrogenases of the anaerobic sulfate-reducing bacteria of the genus *Desulfovibrio* have been particularly well-studied (see Table 7.5).

Table 7.5 - Properties of some representative hydrogenases.

Organism (designation)	MW (subunits)	Approximate Composition	Reference
Clostridium pasteurianum (Hydrogenase I)	60,000 (1)	12Fe 22Fe	191 194
Clostridium pasteurianum (Hydrogenase II)	53,000 (1)	8Fe 17Fe	191 194
Acetobacterium woodii	15,000	Fe	192
Megasphaera elsdenii	50,000 (1)	12Fe	188
Desulfovibrio vulgaris (periplasmic)	56,000 (2)	12Fe	171
Desulfovibrio gigas	89,000 (2)	11Fe, 1Ni	363
Desulfovibrio africanus	92,000	11Fe, 1Ni	364
Methanobacterium thermoautotrophicum	200,000	Fe, 1Ni	145
Methanosarcina barkeri	60,000	8-10Fe, 1Ni	77
Methanococcus vanielli	3400,000	Fe, Ni, 1Se, FAD	365
Desulfovibrio baculatus	85,000 (2)	12Fe, 1Ni, 1Se	365

In nitrogen-fixing organisms, H_2 is evolved during the nitrogen-fixation process, and hydrogenase is present to recapture the reducing equivalents, which can then be recycled to fix more nitrogen. In N_2-fixing organisms, such "uptake" hydrogenases can make an important contribution to the overall efficiency of the nitrogen-fixation process. In fact, certain species of rhizobia lacking the hydrogen-uptake system (*hup*$^-$ strains) can be made more efficient by genetically engineering the hup activity into them.

Aerobic bacteria such as *Azotobacter vinelandii*, *Alcaligenes eutrophus*, and *Nocardia opaca*, and facultative anaerobes, such as *Escherichia coli* and various species of *Rhizobium* and *Bradyrhizobium* (the symbionts of leguminous plants), also contain hydrogenase, as do photosynthetic bacteria such as *Chromatium vinosum*, *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*), and *Anabaena variabilis* (a filamentous cyanobacterium). The thermophilic hydrogen oxidizer *Hydrogenobacter thermophilus*, which grows in alkaline hot springs above 70 °C, obviously has a critical requirement for hydrogenase.

In certain aerobic organisms, such as hydrogenomonas, H_2 and O_2 are caused to react (but not directly) according to the Knallgas reaction[185]

$$2H_2 + O_2 \rightarrow 2H_2O \qquad \Delta G^o = -54.6 kcal/mol \tag{7.7}$$

These organisms break up this thermodynamically highly favorable redox process by using intermediate carriers, thereby allowing the large negative free-energy change to be captured in biosynthetic capacity.

Hydrogenases seem to be especially prevalent in anaerobic, nitrogen-fixing, and photosynthetic organisms. However, although hydrogenases are obviously found widely among prokaryotes, unlike nitrogenase, their domain is not restricted to prokaryotes. Eukaryotic green algae such as *Chlorella fusca* and *Chlamydomonas reinhardtii* possess hydrogenase. The anaerobic protozoan *Trichomonas vaginalis*, which lacks typical aerobic organelles, such as mitochondria and peroxisomes, has an organelle called a hydrogenosome, whose function is to oxidize pyruvate to acetate, producing H_2, via hydrogenase, in the process.

The various hydrogenase enzymes are all transition-metal sulfide proteins. However, before we discuss these enzymes, we turn briefly to the dihydrogen molecule and its physical and chemical properties.

2. Dihydrogen: The Molecule

Diatomic H_2 has a single H—H bond formed by overlap of the two 1s orbitals of the two hydrogen atoms. In molecular orbital terms, this overlap forms bonding σ and antibonding σ^* orbitals, shown in the energy-level diagram in Figure 7.21A and displayed spatially in Figure 7.21B. The H-H distance is 0.74 Å, and the bond dissociation energy is 103.7 kcal/mole. The isotopes of

hydrogen $^1H_1$, $^2H_1$ = $^2D_1$, $^3H_1$ = $^3T_1$ are called protium (a designation seldom used), deuterium, and tritium, respectively. Deuterium, at natural abundance of 0.015 percent, is a stable isotope with nuclear spin I = 1, whereas both ^1H and ^3T have nuclear spin I = $\frac{1}{2}$. NMR has been fruitfully applied to all the hydrogen isotopes, including tritium. Tritium is radioactive, decaying to $^3He_2$ by β^- emission with a half-life of 12 years. The nuclear properties of deuterium and tritium make them useful as labels to probe structure and mechanism in hydrogen-containing compounds. "Exchange" reactions involving the formation of HD or HT have played a significant role in mechanistic studies of both hydrogenase and nitrogenase.

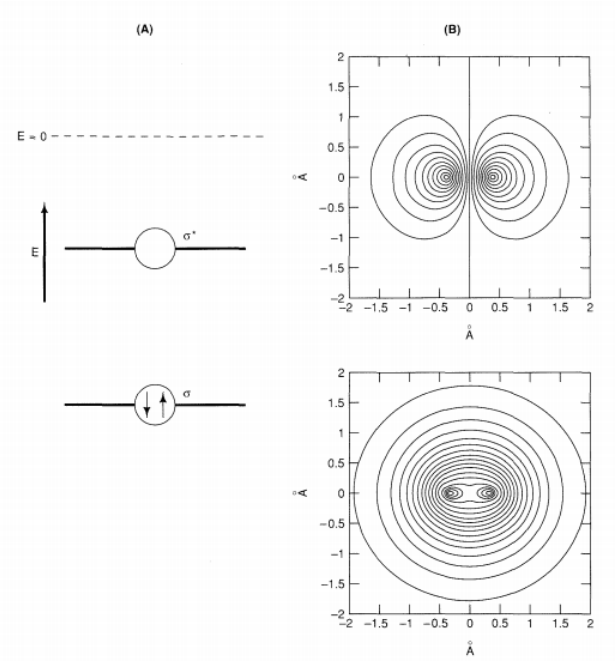

Figure 7.21 - Molecular orbital scheme for binding in H_2: (A) energy-level diagram; (B) bonding and antibonding orbitals.

In molecular hydrogen, the existence of nuclear-spin energy levels is responsible for the distinction between *ortho* and *para* hydrogen, which correspond to the triplet and singlet (i.e., parallel and antiparallel) orientations, respectively, of the two nuclei in H_2. Because of the coupling of the rotational and spin levels, *ortho* and *para* hydrogen differ in specific heat and certain other properties. The correlated orientation of the nuclear spins in *para* H_2 has recently been shown to constitute a powerful mechanistic probe, wherein NMR may be used to trace the relative fate of the two H nuclei in the original molecule.[186,187] Although this technique has not yet been applied to any enzyme systems, hydrogenase is known to catalyze the interconversion of the *ortho* and *para* forms of H_2 (as does the hydrogenase analogue Pd).

Dihydrogen is a reducing agent. The H_2/H^+ couple at $[H^+]$ = 1 M defines the zero of the potential scale. At pH = 7 the hydrogen half-reaction

$$H_2 \rightarrow 2H^+ + 2e^- \tag{7.8}$$

has E_o' = -420 mV. Dihydrogen is therefore one of the strongest biological reductants.

Although many hydrogenases are reversible, some "specialize" in the uptake of H_2. One hydrogenase has been reported[188] to specialize in the evolution of H_2. This "specialization" seems curious, since it appears to contradict the notion of microscopic reversibility, and seems to violate the rule that catalysts increase the speed of both forward and backward reactions without changing the course (direction) of a reaction. In fact, there is no contradiction or violation, since the overall reactions catalyzed by the various types of hydrogenases are fundamentally different. The electron acceptor in uptake hydrogenases *differs* from the electron donor/acceptor in the reversible hydrogenases. The difference involves structure and, more importantly, redox potential. The reaction catalyzed by the uptake hydrogenase involves an acceptor of such high positive redox potential that its reaction with H_2 is essentially irreversible. The enzyme appears to be designed so that it can transfer electrons only to the high potential acceptor.

A selection of hydrogenases from various organisms is given in Table 7.5. All hydrogenases contain Fe-S centers. The hydrogenases from more than 20 organisms[189] have been found to contain Ni by analysis and/or spectroscopy. Many more Ni hydrogenases are likely to be found, given the nutritional requirements[189] for hydrogenase synthesis or growth on H_2. Hydrogenases may be cytoplasmic (as in *C. pasteurianum*), membrane-bound (as in *E. coli*), or located in the periplasmic space (as

in *Desulfovibrio v*ulgaris). The isolation of hydrogenases is sometimes complicated by their air sensitivity or membranebound nature. Many hydrogenases have now been isolated and studied in detail; they can be divided into two categories, the iron hydrogenases and the nickeliron hydrogenases.
