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The role of nickel and iron–sulfur centers in the bioproduction of hydrogen

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Abstract - Hydrogenases are generally classified as iron-sulfur-containing proteins with four to twelve iron atoms in different cluster arrangements. Through physiological, chemical and spectroscopic studies,nickel has also been found to be a constitutive metal of several hydrogenases and shown to be involved in a redox linked process. The **[NiFe] hydrogenases** are now the most intensivelly studied nickel enzymes. Here we will focus on the study of [NiFe] hydrogenases isolated from sulfate reducing bacteria (*Desulfovibrio* (*D.*) species). *D.gigas* hydrogenase is used as a model system for the discussion of the spectroscopic features of the metal centers in the native and hydrogen reacted states. A reasonable understanding of the native enzyme metal center constitution has emerged from EPR and Mössbauer (MB) spectroscopic studies,indicating the presence of four non-interacting centers: one **nickel** (assigned to be in the unusual trivalent state),one **[3Fe-4S]** center (EPR active) and two **[4Fe-4S]** clusters (EPR silent). The intermediate redox species generated under H_2 were studied in order to detect the redox processes involved and to determine the associated mid-point potentials. The occuring redox transitions (nickel is assumed to cycle between tri and divalent oxidation states) are related to the redox linked activation step required for the full expression of the enzymatic activity. Special emphasis is given to the interpretation of the EPR and MB results obtained in the enzyme active state and the interplay between the metal centers. The existence of a proton and a hydride acceptor sites are postulated in agreement with a heterolytic cleavage step of the hydrogen molecule. The properties of relevant nickel compounds and functional models are used in order to define the oxidation states involved and the favored coordination of the nickel site. The assemble of the available experimental data is integrated in the enzyme mechanistic framework. **Selenium** is also an essential constituent of some [NiFe] hydrogenases. Its role on the fine tuning of the catalytical properties and as a proximal nickel ligand is discussed.

1. INTRODUCTION

Nickel is now well recognized as a transition metal required for a wide range of biological functions (1-2). The discovery of nickel in purified proteins is relatively recent and many current studies are centered on the characterization of the nickel containing active center as well as the search for structure-function relationships. Nickel-containing hydrogenases constitute a choice enzyme group since the metal, besides being a constitutive element, also plays a diversified role in the redox process involved (H_2 ----> $2H^+ + 2\,e$) and in the regulation of the catalytic activity (3).
Over the past ten years there has been a renewed interest in the physiology and biochemistry of hydrogenases with emphasis on the catalytic properties and the mechanisms involved (3-6), and the applications of the enzyme to bioconversion (chloroplast-hydrogenase interplay in biophotolysis) (7) as well as other biotechnological oriented processes (8). Homogeneous preparations have been purified from strict and facultative aerobic and anaerobic organisms, and in particular sulfate reducing, methanogenic and photosynthetic bacteria. It is generally believed that hydrogenases represent a diverse group of proteins differing not only in respect to their metal content and type of active centers but also in subunits composition, stability and reactivity.

2. NICKEL INVOLVEMENT IN HYDROGENASES

Nickel was considered until recently an uncommon metal in biology. The involvement of nickel in the hydrogenase system was established through accumulated evidences of different origins: physiological studies (nickel requirement for autotrophic bacterial growth (9) and nickel dependent hydrogenase synthesis (10, 11)), chemical determination and mainly EPR spectroscopic studies (see below). The detection of nickel EPR signals in biological systems was first reported by Lancaster (12) in membrane preparations of *Methanobacterium* (*Mb.*) *bryantii*; the assignment of the unusual rhombic signal with g-values at 2.3, 2.2 and 2.0 was based on data available for nickel model compounds and on spectral characteristics of the signal (g-values and relaxation). The signal tentatively

attributed to Ni(III) could be detected up to 120 K. The definitive assignment of the observed rhombic signal was achieved when *Mb.bryantii* was grown in a medium supplemented with ^{61}Ni (I=3/2) (13). Similar signals have been observed for bacterial hydrogenases of different origins. The response and alterations of the EPR features associated with nickel upon hydrogen exposure are also indicative of direct participation of the nickel center in the hydrogenase activity (see below).

3. SPECTROSCOPIC TOOLS – ISOTOPIC SUBSTITUTIONS

EPR is a reasonable sensitive method for detecting paramagnetic nickel species (Ni (III) and Ni(I)) and to give information about the metal environment. The technique does not allow the unambiguous assignment to one of these two redox states and some controversy is still unresolved (3, 15-18). Mid-point redox properties can be readily obtained from EPR measurements (17, 18).

A better understanding of the structural features and functional properties of a metalloenzyme containing this "new biological relevant metal" was achieved by the knowledge of coordination chemistry and redox properties of nickel model compounds. A well of information has been built in this way and proved useful in order to predict reasonable geometries and oxidation states for the nickel site (3, 19).

Mössbauer spectroscopy has also been an indispensable tool for the characterization of the iron-sulfur centers present in the enzyme and its inter-relation with the nickel site. The conjunction of the two techniques is very powerful in order to characterize complex systems and to study spin-coupled structures (20). Also, important information can be extracted about the iron-sulfur oxidation states present in EPR silent states.
The use of isotopic replacements was also a valuable source of information. **^{61}Ni isotope** (1.2 % natural abundance, I=3/2) enhabled unambiguous assignment of EPR active species to the nickel site, due to the appearance of resolved nuclear hyperfine structure. This step of assignment may be crucial when the observed EPR spectrum is the superimposition of signals originated from different nuclei. **^{57}Fe isotope** (2.1 % natural abundance, I=1/2) has an obvious advantage for increasing the experimental sensitivity of the Mössbauer experiments as well as introducing specific broadening in the EPR dectectable iron species. **^{33}S** (0.76% natural abundance, I=3/2) and **^{77}Se** (7.58% natural abundance, I=1/2) **isotopes** have been recently used in order to explore the nickel coordination sphere (21, our unpublished results).
In order to probe the catalytic active site properties, the isotopic exchange D_2-H^+ has been used and the amount of HD plus H_2 formed measured by mass spectrometry (23).
Magnetic Circular Dichroism and EXAFS spectroscopies have also been used in order to probe the nickel site (24, 25).

4. ACTIVE CENTER COMPOSITION – TYPES OF HYDROGENASES

The metabolism of hydrogen in sulfate-reducing bacteria is regulated by reversible hydrogenases and *Desulfovibrio* sp. enzymes are clearly representative of the complexity involved in this process. At least three different types are now recognized within this bacterial group:

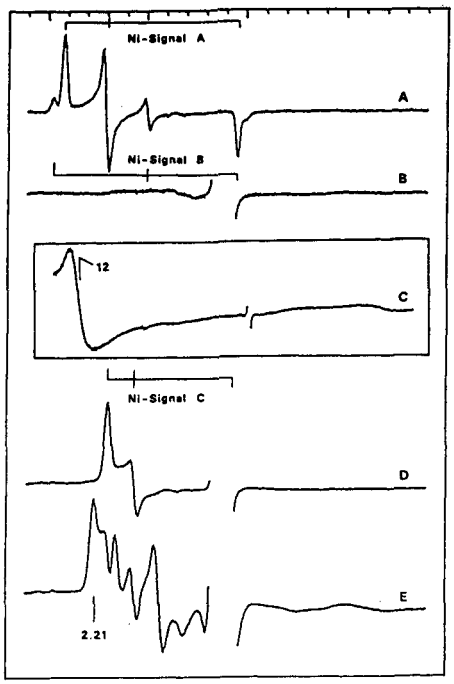
[Fe] hydrogenases - containing only iron-sulfur centers, purified from *Desulfovibrio* (*D.*) *vulgaris* (Hildenborough) (26, 27). The metal cores are arranged as two [4Fe-4S] clusters and a third one considered atypical (26).
[NiFe] hydrogenases - containing one nickel and iron-sulfur centers generally arranged as one [3Fe-4S] and two [4Fe-4S] clusters, purified from *D.gigas* (NCIB 9332) (18, 28-30), *D.desulfuricans* (ATCC 27774) (30) and *D.multispirans* n.sp. (31).
NiFeSe] hydrogenases - containing iron-sulfur centers and equimolecular amounts of nickel and selenium, have been purified from *D.baculatus* (Norway 4) (33, 34), *D.baculatus* (DSM 1743) (35) and *D.salexigens* (British Guiana) (36). A [3Fe-4S] core seems to be absent and two distinct [4Fe-4S] cores have been observed.

Nickel has been associated to hydrogenases by Ni requirement for autotrophic growth, by demonstration of nickel in purified enzymes and by detection of Ni EPR signals. An exaustive list can be consulted in ref. (37).

5. EPR AND MOSSBAUER STUDIES – NICKEL AND [Fe-S] CENTERS

The properties of the purified periplasmic [NiFe] hydrogenase from *D.gigas* (17, 18, 28-30, 38-40) have been extensivelly studied and will be used here as a representative of this enzyme group. It has a molecular mass of 89 KDa, is composed of two non-identical subunits (62 and 26 KDa) and contains **eleven** g-atoms of non-heme iron and **one** g-atom of nickel per enzyme mole. A reasonable understanding of *D.gigas* hydrogenase metal centers constitution has already emerged from EPR and Mössbauer spectroscopic studies, indicating the presence of four non interacting redox centers in the as isolated state: one nickel, one [3Fe-4S] center (EPR active) and two $[4Fe\text{-}4S]^{+2}$ centers (EPR silent).

In the native state, periplasmic *D.gigas* hydrogenase shows a slow relaxing rhombic EPR signal with g-values at g_1=2.31, g_2=2.23 and g_3=2.02 (Ni-signal A) detected from low temperatures up to 100K. Isotopic substitution by ^{61}Ni (I=3/2) induced line broadening in the feature at g_1=2.31 and resolved hyperfine structures in the g_2=2.23 ($^{61}A_2$=1.5 mT) and g_3=2.02 ($^{61}A_3$=2.7 mT) lines, clearly showing that the paramagnetic nickel is the origin of those signals. This species was attributed to a low-spin nickel (III) center with a tetragonally distorted octahedral symmetry in an S=1/2 system with the one unpaired electron in a d_{z^2} orbital. In addition to Ni-signal A another rhombic species with g-values at 2.33, 2.16 and ~2.0 (Ni-signal B) is observed (Figure 1) (29).



Representative EPR spectra of *D.gigas* [NiFe] hydrogenase poised at different redox potentials in the presence of dye mediators, by variyng the partial pressure of hydrogen gas.

A - Native sample (as isolated), poised around +150mV, at 77K. Ni-signals A and B are observed.
B - The so-called "EPR silent state. Sample poised at -207mV, at 20K.
C - The "g= 12" signal. Same as B, but the spectrum is observed in a large field range at 4.2K.
D - Ni-signal C. Sample poised at -357mV, at 20K.
E - "g= 2.21 signal". Same as D, but observed at 4.2K.

The EPR spectrum at low temperature (below 30 K, not shown, see ref. 3) is dominated by an intense signal centered at g=2.02 (8, 9, 19, 23). Hyperfine broadening is observed in the EPR signal of ^{57}Fe enriched samples (3, 18). By conjunction of detailed EPR and Mössbauer spectroscopic studies the presence of an oxidized [3Fe-4S] center with S=1/2 was definitively established. The 4.2 K Mössbauer spectra of native natural abundance and ^{57}Fe enriched samples (18, 40) indicate that besides a paramagnetic component due to the [3Fe-4S] cluster, an intense quadrupole doublet at the center of the spectra accounts to 70-80% of the total iron absorption. The observed spectral parameters (quadrupole splitting and isomer shift) indicate that *D.gigas* hydrogenase contains two oxidized EPR silent (S=0) $[4Fe\text{-}4S]^{+2}$ centers.

The EPR signals observed in this native state are related with inactive species (18, 29, 40), (also see below). The first events observed upon interaction with hydrogen are the disappearance of the g=2.02 signal associated with the [3Fe-4S] center (E_o ~ -70 mV/pH independent) (17, 18), followed by the disappearance of the Ni-signal A and B (for Ni-signal A, E_o=-220 mV, at pH 8.5, 60 mV/pH unit dependent), an "EPR silent" state being attained, as observed in the g=2 spectral region (17, 18, 29, 40). However concomitant with the disappearance of the g=2.02 isotropic signal, a broad feature appears at the very low field region (cross-over point around g=12). This signal is the only EPR feature observed at this oxidation state and is reminiscent of the "g=12" signal recently discussed and assigned to a Δm_s=4 EPR transition within the spin quintet of reduced (S=2) *D.gigas* ferredoxin II which contains a single [3Fe-4S] cluster (41). (Figure 1B and C).

On the basis of EPR and Mössbauer studies of the enzyme at this intermediate reduced states, the redox process at -70 mV was assigned to the reduction of the 3Fe center (as measured by the disappearence of the feature at g=2.02 and concomitant appearance of the "g=12" EPR signal). The "g=12" signal was previously observed (28) but only recently positioned in the redox scheme (our unpublished results).

Following this so-called "EPR silent" state further reduction of *D.gigas* hydrogenase results in the appearance of a new slow relaxing transient rhombic EPR signal with g-values at g_1=2.19, g_2=2.14 and g_3=2.02 (termed Ni-Signal C) (Figure 1D), which subsequently disappears upon longer exposure to hydrogen gas or in the presence of excess of sodium dithionite (29, 39, 40). This EPR signal was also assigned to nickel by isotopic substitution ($^{61}A_3$=2.0 mT) (14).

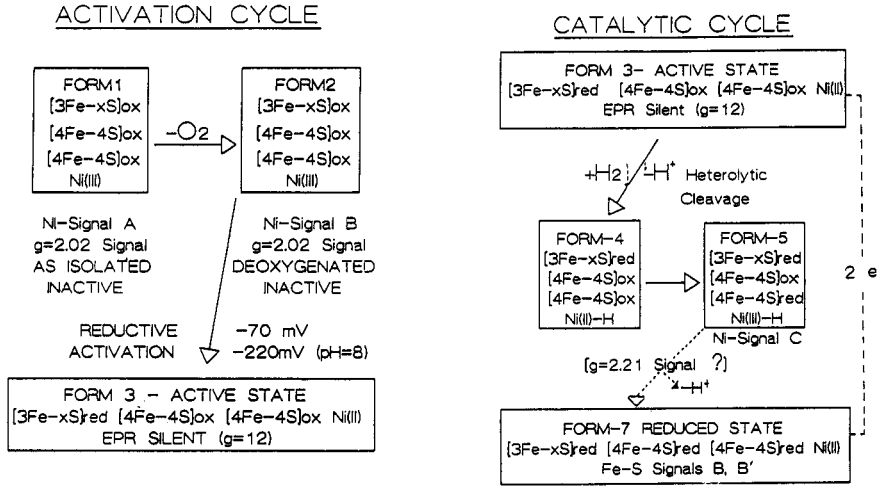
EPR studies conducted at low temperature (generally below 10 K) at redox levels concomitant and below the development of Ni-signal C reveal another complex EPR signal termed "g=2.21" signal (Figure 1E) (16, 29). The observation of this last signal requires a high microwave power (fast relaxing species). An heterolytic cleavage of the hydrogen molecule was proposed to be operating in bacterial hydrogenases (23). Ni-signal C was assigned to an hybride nickel species (29, 39), but the relaxation behaviour of the "g=2.21" signal indicates that it represents a spin-spin interacting species and not a simple S=1/2 paramagnet. The appearance of this "g=2.21" EPR signal was also interpreted as a splitting of the Ni-signal C ("g=2.19") by spin-spin interaction with a $[4Fe\text{-}4S]^{+1}$ cluster (16).

The Ni-signal C and the "g=2.21" signal have similar but not identical redox behaviours. They show a bell-shape redox titration curve appearing around -330 and disappearing below -450 mV, in a process that is pH dependent (29). A detailed analysis of samples poised at redox potentials where both signals develop seem to indicate that the relative intensities of the Ni-signal C and of the "g=2.21" one vary with the redox potential and they may have different origins (our unpublished results).

Mössbauer spectra of fully reduced samples show that the 3Fe center remains reduced (integer spin state, S=2) being not converted to a [4Fe-4S] center (40). This is the first example of a reduced [3Fe-4S] cluster present in a catalitically active state. At this state the [4Fe-4S] center are reduced in the +1 state. The Mössbauer data further reveal that the two [4Fe-4S] centers are spectroscopically distinguishable in their reduced states. One of the [4Fe-4S] centers is found to exhibit atypical magnetic hyperfine parameters. The two reduced [4Fe-4S] centers give origin to broad EPR signals that develop around -320mV and have slightly different redox potentials (our unpublished results).

6. ACTIVATION AND CATALYTIC CYCLES IN [NiFe] HYDROGENASES – INTERMEDIATE SPECIES GENERATED UNDER HYDROGEN

Nickel-containing hydrogenases are reversibly inactivated by oxygen. For example, *D.gigas* hydrogenase is mainly isolated in an inactive (29, 42) or unready (16) state and the catalytic competent form of the enzyme is only attained after a lag phase consisting of two steps: a deoxygenation step demonstrated by the use of oxygen scavengers such as either glucose plus glucose oxidase or *Desulfovibrio* tetrahaem cytochrome c_3, and a reductive step occurring under H_2 or D_2 (42). The complex activation cycle of [NiFe] hydrogenase has been discussed several times based on the activity measurements and spectroscopic data available and was rationalized in terms of an hypothetical activation mechanism (29) (see scheme). Ni-signal A is associated with an inactive or unready form of the oxygenated enzyme (Form 1). Ni-signal B represents a ready state of the enzyme (Form 2), in the sense that the active state of the enzyme can rapidly be attained starting from this form (29).



The [4Fe-4S] clusters are in the 2+ state and are EPR silent both for the unready and ready states . The $[3Fe\text{-}4S]_{oxid}$ cluster is EPR active and exhibits an isotropic g = 2.02 signal observed at temperatures below 30 K. The oxidation state of the EPR active nickel is proposed to be Ni(III). In the oxygenated form (unready) the nickel center exhibits Ni-signal A. In the deoxygenated form (ready) it exhibits Ni-signal B. The amount of ready state can be increased drastically through anaerobic reoxidation (29). EPR and Mössbauer studies in the enzyme "as isolated", indicate that there is no magnetic interaction between these four redox centers (28, 38).

In the active state the *D.gigas* periplasmic enzyme is in the so-called "EPR silent" state (Form 3). It can be attained either from the inactive form through a complex and slow activation process (removal of oxygen followed by a reduction step conversion of Form 1 into Form 2) or it can be reached directly from the ready form (without a lag phase) (cf.Scheme I). During this activation process, both the isotropic g=2.02 and the nickel signals disappear. The loss of the g=2.02 signal is attributed to the reduction of the [3Fe-4S] cluster. Such a mechanism is also supported by optical studies which indicate that the activation process involves the reduction of iron-sulfur clusters (43). However, the oxidation state of the nickel at this state is under discussion.

A recent examination of the intermediates poised at or very near the "EPR-silent" state indicate that no [4Fe-4S] centers are reduced at this redox states (our unpublished results). This suggests the assignment of the redox process at -220 mV to a simple transition Ni(III) ----> Ni(II). Then, Form 3 is defined as the active enzyme state and the [3Fe-4S] reduced cluster (S=2) is the only paramagnet observable by EPR.

Since a hydride intermediate state was anticipated, a proton binding site is required in terms of the proposed mechanistic hypothesis. Plausible candidates are either a [4Fe-4S] cluster or a ligand (sulfur atom) at the nickel site. Preliminar EXAFS data on *D.gigas* hydrogenase indicate that the Ni(III) coordination sphere is dominated by sulfur atoms (25), although N and O atoms were not excluded at this point. However no superhyperfine structure due to nitrogen coordination has been observed by EPR (see below).

Ni-signal C is assumed to represent the hydride-bound nickel center(Forms 4 and 5). Accumulated experimental evidence supports this assignment for the *D.gigas* and *Chromatium* hydrogenases. The development of the "g=2.19" EPR signal under hydrogen is concomitant with the activation of the enzyme (29). This EPR signal is reversibly modified by illumination with visible light in the frozen state originating a new rhombic EPR signal (g-values at 2.28, 2.12 and 2.03) (15, 24). The rate of conversion was found to show a kinetic isotopic effect (slower in D_2O than in H_2O) (48). The light conversion of the EPR signal with g_z ~2 into another signal with g_z >2, led Albracht and co-workers to propose a Ni(I) transient state. EPR is a sensitive spectroscopic tool but does not allow the unambiguous differenciation between Ni(III) and Ni(I) oxidation states and some controversy in this respect is still unresolved. Other possibility involve the proposal of a mechanism where the nickel center operates between the Ni(III) and Ni(II) states during activity avoiding the lower nickel oxidation state, less pausible in terms of nickel chemistry arguments. The presence of a sulfur (and selenium, see below) as nickel ligands as indicated by EXAFS measurements (15, 22, 29) and ^{33}S isotopic replacements (21) and the observation of modulation of the D/H exchange reaction by pH and the presence of selenium, suggests the involvement of a nickel ligand at the proton acceptor site (35).
The midpoint redox potential for the development of Ni-signal C is consistent with a catalytic active species (29, 43).

Most of the features here described for *D.gigas* [NiFe] hydrogenase have been reproduced in the hydrogenases isolated from *D.desulfuricans* (ATCC 27774) (31) and *D.multispirans* n.sp. (32).

7. NICKEL SITE COORDINATION

The nickel EPR active species observed for nickel-containing hydrogenases (Ni-signals A, B and C) differ both in g-values, line-shape (more or less rhombic), and nuclear hyperfine coupling constants (Figures 1). The alterations observed by EPR must reflect variations in the nickel environment (different coordination numbers and/or ligations). Preliminary EXAFS data on *D.gigas* (25) and *Mb.thermoautotrophicum* (H) (45) hydrogenases indicate that the Ni(III) coordination sphere is dominated by sulfur atoms (4 to 6) although N and O atoms were not excluded at this point (25). However, no superhyperfine structure due to nitrogen coordination has been observed by EPR. Also, EPR studies on the hydrogenase isolated from cells of *Wolinella succinogenes* grown on ^{33}S support the existence of sulfur-nickel coordination (21). However most quantitative conclusions must wait a systematic (see also role of selenium, below) study of novel Ni-S and Ni-N model compounds. Additionally, a small narrowing in the g=2.19 (0.2 mT) and g=2.0 (0.3 mT) regions of the Ni-signal C was observed in D_2-reduced *D.gigas* hydrogenase relative to the H_2-reduced enzyme, which may indicate a weak proton coupling (45). Nevertheless, the Ni-site coordination of hydrogenases is different from the one observed for other nickel-containing enzymes. EPR spectroscopic studies on *Cl.thermoaceticum* CO-dehydrogenase, indicate that the enzyme contains a nickel-iron-carbon complex since magnetic hyperfine broadening was induced in the Ni EPR signal by the ^{57}Fe nuclear spin and by ^{13}CO binding (46). The comparison of ^{57}Fe and ^{56}Fe *D.gigas* hydrogenase EPR spectra, clearly shows that there is no observable broadening due to the ^{57}Fe nucleus in Ni-signals A, B, and C, suggesting these EPR active Ni species are magnetically isolated from the iron-sulfur clusters (40) and our unpublished results.

8. THE HETEROLYTIC CLEAVAGE OF THE HYDROGEN MOLECULE – ROLE OF SELENIUM

In addition to nickel, selenium is also found in some bacterial hydrogenases. The [NiFeSe] hydrogenases clearly emerge as a distinct group of enzymes in terms of catalytic and active site composition but the degree of structural homology between [NiFe] and [NiFeSe] hydrogenases is yet to be determined. Selenium may play a role in modulation of the catalytic properties (34). The [NiFe] and [NiFeSe] hydrogenases share identical intermediates after reaction with hydrogen suggesting a common operating mechanism in these enzymes. Ni-signal C and the fast relaxing species (g=2.21) were detected in *D.baculatus* (DSM 1743) and *D.salexigens* (British Guiana) [NiFeSe] hydrogenases, showing a redox behaviour identical to that observed for *D.gigas* [NiFe] hydrogenase (29, 35, 36).

Isotopic exchange between D_2 and H^+ and the ortho/para hydrogen conversion are well suited for the study of the activation of the hydrogen molecule at zero electronic balance. The data obtained with both methods are consistent with the heterolytic cleavage of the hydrogen molecule (23, 47). This last mechanism requires the presence of a metal-hydride complex and of a proton acceptor site as we previously discussed and the stabilization of the proton by a base (external or a metal ligand) is considered to be a necessary requirement:

$$M + H_2 + B \longrightarrow M\text{-}H^- + H^+\text{-}B$$

$$M\text{-}X + H_2 \longrightarrow \underline{M\text{-}H^-}\ \underline{X\text{-}H^+}$$

The first product of the D_2/H^+ exchange reaction is HD, as seen in the presence of whole cells, crude extracts, and purified enzymes. This result has been used in support of the heterolytic cleavage mechanism, assuming that one of the enzyme-bound H or D atoms exchanges more rapidly with the solvent than the other. Thus, HD is the initial product, but D_2 (or H_2) is none the less the final product of the total exchange process, since a secondary exchange step of the HD molecule occurs.

Assuming that the hydride and proton acceptor sites can exchange independently with the solvent, the amount of HD and H_2 produced depends on the relative exchange rates of both sites. According to this assumption the ratio of products should be pH dependent (47, 48). An alteration in the pKa of the proton acceptor or active site can be viewed as responsible for attaining these isotope ratios. The comparison of the experimental data on the exchange reaction measured with different purified enzymes indicate that only the [NiFeSe] hydrogenases from *D.baculatus* (DSM 1743) and *D.salexigens* (British Guiana) have H_2/HD ratios greater than 1 and that the [NiFe] hydrogenases isolated from *D.gigas*, *D.multispirans* n.sp. and *D.desulfuricans* (ATCC 27774) show a ratio of H_2/HD smaller than 1 (0.22-0.40) at pH 7.6 (23). *D.baculatus* (DSM 1743) and *D.gigas* hydrogenases show pH-dependent H_2/HD ratios (35) indicating the protonation of the proton acceptor site.

The different exchange kinetics of the hydrogen binding sites may reflect differences in the active centers. Selenium and nickel are present in equimolecular amounts in the [NiFeSe] hydrogenases, and a suggestion was made that selenium is a ligand to the nickel site (23, 35). Substitution of one of the sulfur ligands to the nickel by the less electronegative one selenium may serve to destabilize the hybride form of this hydrogenase. Experiments using cells ^{77}Se enriched hydrogenase isolated from *D.baculatus* (DSM 1743) and selenium EXAFS experiments were recently performed in order to determine the involvement of selenium in the nickel-binding site (22).

The EPR spectrum of H_2 reduced *D.baculatus* [NiFeSe] hydrogenase purified from cells grown in ^{77}Se enriched medium clear indicates a broadening of the spectral feature associated with Ni-signal C. Also Ni edge and Ni EXAFS data shows that the Ni site in *D.baculatus* [NiFeSe] hydrogenase is structurally distinct from that of the *D.gigas* [NiFe] hydrogenase (22). Inclusion of Ni-Se interaction at 2.46 A significantly improves the simulation of the Ni EXAFS of *D.baculatus* hydrogenase. Also the Se EXAFS requires the presence of a heavy scatterer (Ni or Fe) at ~2.4 A. This preliminar results are indicative of the presence of selenium in the Ni-site coordination sphere.

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