Structural and functional models for [NiFe] hydrogenase

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1

General Introduction

Abstract. The main goal of the research presented in this thesis is the synthesis of suitable structural and functional models for the enzyme [NiFe] hydrogenase, which can reduce protons† into dihydrogen. This chapter starts with a brief survey of the roles of all the known nickel-containing enzymes in biological systems with a focus on the [NiFe] hydrogenases. Structure, function, physicochemical and catalytic properties of the [NiFe] hydrogenase itself and of the reported model complexes are presented. This chapter concludes with the goal of the research and modeling strategies, followed by an outline of the thesis.

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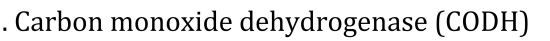
† Although strictly speaking natural isotope ratios require the use of “hydron”, hydride etc., throughout this thesis “proton” is used.

1.1. A Prelude to Nickel Biochemistry

Nickel is a relatively abundant element, constituting approximately 8% of the earth's core and 0.01% of the earth's crust. Organisms in nature have obtained nickel by leaching the most abundant form of nickel, Ni(II), from the earth's crust. It is perhaps puzzling then as to why no protein or enzymatic system containing functionally significant nickel was known until 1975, despite the fact that nickel is readily available.[1-4] Currently there are only nine proteins or enzymatic systems known in nature that encompass functionally significant nickel; the environment around nickel within each protein is different. Several aspects in the bioinorganic chemistry of nickel-containing enzymes are unusual in the context of known coordination chemistry of common nickel salts, as well as the functions in which these enzymes are involved (See Scheme 1.1).[5,6]

1. Urease



$$H_2N–CO–NH_2 + 2H_2O \longrightarrow 2NH_3 + H_2CO_3$$

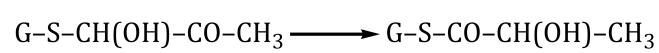
2. Carbon monoxide dehydrogenase (CODH)

$$CO + H_2O \rightleftharpoons 2H^+ + CO_2 + 2e^-$$

3. Acetyl–coenzyme A synthase (ACS)

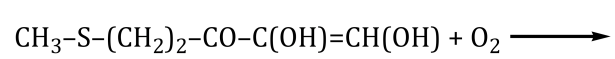
$$CH_3–CFeSP + CoA–SH + CO \rightleftharpoons CH_3–CO–SCoA + CFeSP$$

4. Glyoxalase I (GlxI)



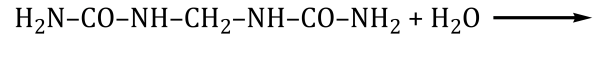
$$G–S–CH(OH)–CO–CH_3 \longrightarrow G–S–CO–CH(OH)–CH_3$$

5. Aci–reductone dioxygenase (ARD)



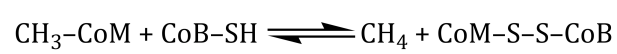
$$CH_3–S–(CH_2)_2–CO–C(OH)=CH(OH) + O_2 \longrightarrow$$
$$CH_3–S–(CH_2)_2COOH + HCOOH + CO$$

6. Methylenediurease (MDU)



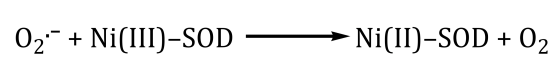
$$H_2N–CO–NH–CH_2–NH–CO–NH_2 + H_2O \longrightarrow$$
$$CO(NH_2)_2 + NH_3 + HCHO + CO_2$$

7. Methyl–coenzyme M reductase (MCR)

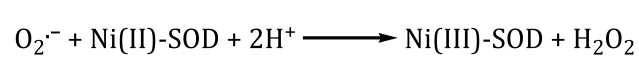


$$CH_3–CoM + CoB–SH \rightleftharpoons CH_4 + CoM–S–S–CoB$$

8. Superoxide dismutase (SOD)

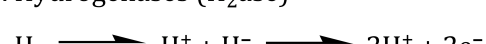


$$O_2^{\cdot -} + Ni(III)–SOD \longrightarrow Ni(II)–SOD + O_2$$



$$O_2^{\cdot -} + Ni(II)\text{-}SOD + 2H^+ \longrightarrow Ni(III)\text{-}SOD + H_2O_2$$

9. Hydrogenases (H_2ase)



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

Scheme 1.1. Nickel-containing enzymes and their roles in biology as known today.

1.2. Nickel-Containing Enzymes and their Role in Biological Systems

1.2.1. Introduction

Nickel-containing enzymes catalyze many critical and distinct biological processes. Most of them are industrially and environmentally significant, such as **(1)** hydrolysis of urea into ammonia and carbamate,[7-10] **(2)** reversible interconversion of carbon monoxide and carbon dioxide, **(3)** decomposition of the acetyl group into separate one-carbon units in some cells or catalyzing acetate synthesis using one-carbon precursors in others,[11-19] **(4)** detoxification of cytotoxic methylglyoxal (MG) via the isomerization of hemithioacetal,[4,20,21] **(5)** oxidation of 1,2-dihydroxy-3-keto-5-methylthiopentane (aci-reductone) into methylthio propionic acid, formic acid and carbon monoxide,[22-26] **(6)** degradation of methylenediurea (slow release fertilizer),[27] **(7)** methane generation,[28] **(8)** dismutation of toxic and cell damaging superoxide radical anions into harmless molecular oxygen[29,30] and on top of all, **(9)** reversible interconversion of dihydrogen into protons and electrons (Scheme 1.1).[31-35] The first eight enzymes are briefly discussed here. The hydrogenase enzymes are described in more detail in Section 1.3.

1.2.2. Urease

Urease (urea amidohydrolase) catalyzes the hydrolysis of urea to form ammonia and carbamate at approximately 10^{14} times the rate of the uncatalyzed reaction.[36] The carbamate formed spontaneously degrades in vivo to form a second molecule of ammonia and hydrogen carbonate.[37] This urease-catalyzed hydrolysis is in contrast with the uncatalyzed reaction, which affords ammonia and cyanic acid.[38] James B. Sumner successfully crystallized the enzyme urease from Jack bean in 1926 after almost nine years of hard work, as the first enzyme to be isolated in crystalline form.[7]

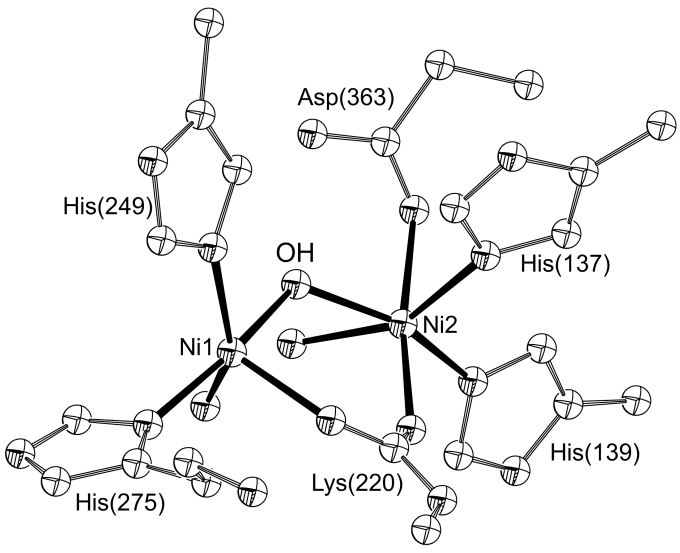


Fig. 1.1. Perspective view of the active site of urease (1FWJ).

The presence of a nickel center in the active site was discovered only fifty years after the isolation of the crystalline urease.[8] It took almost seventy years before the first crystal structure of a urease was reported.[6,9,10] The crystal structure of urease shows the active centre to contain a homodinuclear Ni_2 center. Each nickel ion is coordinated to a water molecule and two histidine nitrogen donors apart from the two bridges between the nickel centers formed by a hydroxido group and a carbamylated lysine (Fig. 1.1). Numerous dinuclear nickel(II) complexes have been reported in recent literature to mimic the structure and function of urease.[39-54]

1.2.3. CO Dehydrogenase/Acetyl-Coenzyme A Synthase (CODH/ACS)

The bifunctional enzyme CODH/ACS has an important role in the global carbon cycle as the C-cluster, an Ni–Fe–S centre, of CODH reduces carbon dioxide to carbon monoxide and the A-cluster, another Ni–Fe–S centre, of ACS assembles acetyl-CoA from a methyl group, coenzyme-A and the CO generated by the C-cluster (Scheme 1.1).[11,14,17,55-59] The A-cluster is a complex metallocofactor, containing an Fe_4S_4 group connected by cysteine bridging to M_p of a dinuclear $[M_pNi_d]$ site. The proximal metal M_p is predominantly Cu in the as-isolated enzyme from native *Moorella thermoacetica*, but [NiNi] and [ZnNi] forms are also known to be isolated and well studied (Fig. 1.2).[55-57,59,60]

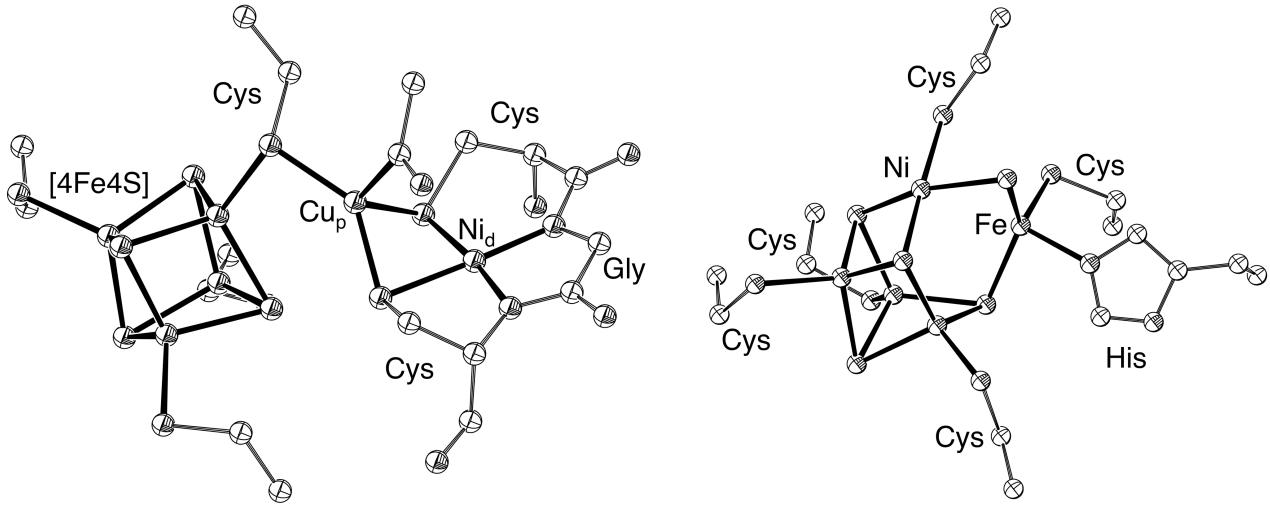


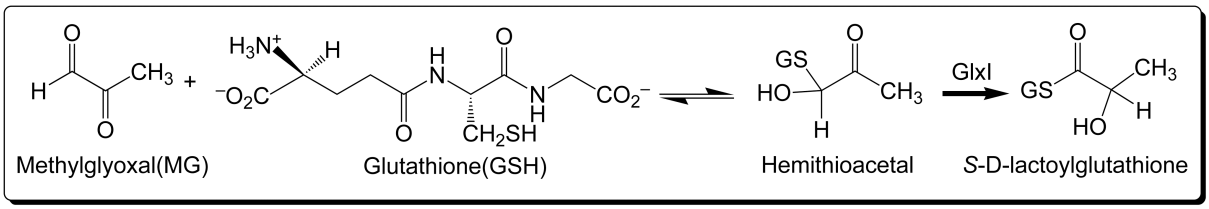
Fig. 1.2. Perspective view of the A-cluster of ACS (left, 1MJG) and the C-cluster of CODH (right, 1JJY).

The distal nickel ion Ni_d is in a square-planar (NiN_2S_2) geometry derived from two backbone carboxamido nitrogens and two Cys-S residues. The Ni_d centre is bridged through the two Cys-S donors to the proximal metal M_p that is in a tetrahedral coordination environment. A fourth nonprotein ligand (CO/acetyl) is bound to M_p to complete its coordination sphere. The C-cluster of CODH is a unique asymmetric $[NiFe_4S_5]$ assembly containing a four-coordinate square-planar nickel center linked to an

iron ion, which is extraneous to the cuboidal-like core, through a bridging sulfide (Fig. 1.2). Numerous model complexes mimicking the structure and functions of CODH/ACS, involving methyl transfer[61,62] and CO insertion[63-68] reactions, have been reported in recent years and have been recently reviewed.[69-73]

1.2.4. Glyoxalase I (GlxI)

Glyoxalase I, a member of the metalloglutathione transferase superfamily, catalyzes the first step in the detoxification of cytotoxic methyglyoxal (MG) via the conversion of nonenzymatically-produced hemithioacetals (HG-GSH) into *S*-D-lactoylglutathione and thereby plays a critical detoxification role in cells (Scheme 1.2). Yet, the mechanism of nickel incorporation into GlxI remains hard to pin down.[4,20,21] The three-dimensional structure of the enzyme is homodimeric with what appears to be two identical active sites (Fig. 1.3).[20] Each active site contains two histidine and two glutamic acid residues that coordinate to the metal ion along with two water molecules so that the catalytic metal ion has a distorted octahedral geometry.



Scheme 1.2. Formation and isomerization of hemithioacetal.

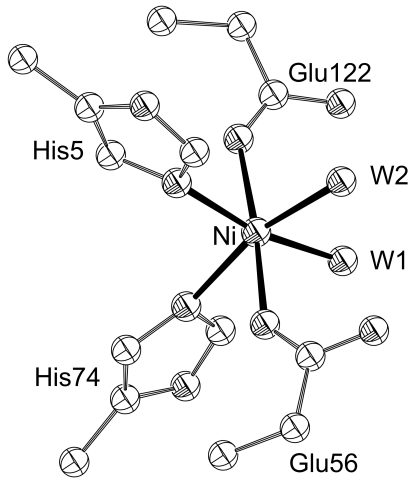
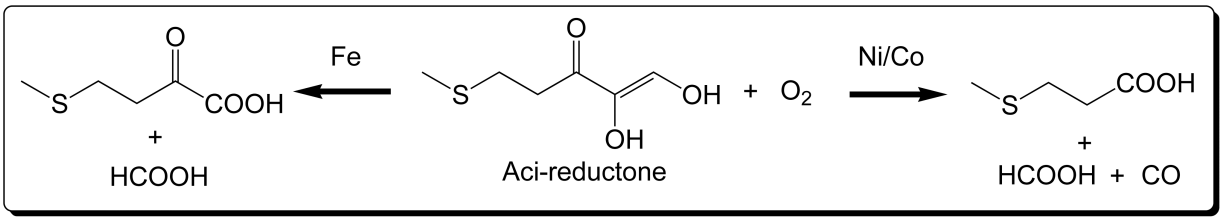


Fig. 1.3. Active site structure of glyoxalase (1F9Z).[20]

1.2.5. Aci-reductone Dioxygenase (ARD)

Aci-reductone dioxygenase was first discovered in 1993 in a study of the methionine salvage pathway in *Klebsiella pneumoniae*.[74] ARD was found to cleave the key intermediate of this pathway namely aci-reductone (1,2-dihydroxy-5-methylthiopent-1-

en-3-one) and its analogues.[75,76] Investigations using *K. pneumoniae* unveiled that aci-reductone is oxidized to two different sets of products. In the productive case, a dioxygenase activity produces formic acid and the α-ketoacid precursor of methionine.[25,75] In addition, a second, non-productive dioxygenase activity converts the aci-reductone into formate, carbon monoxide, and methylthiopropionic acid. Remarkably, these activities belong to the same protein (ARD), but result from the differences in metal content (Scheme 1.3). The reason for the presence of two isoforms of a protein with different metals is a mystery. Further investigations using recombinant protein confirmed that the productive activity is due to the iron-containing ARD and the non-productive activity is from the Ni– or Co–containing ARD.[75]



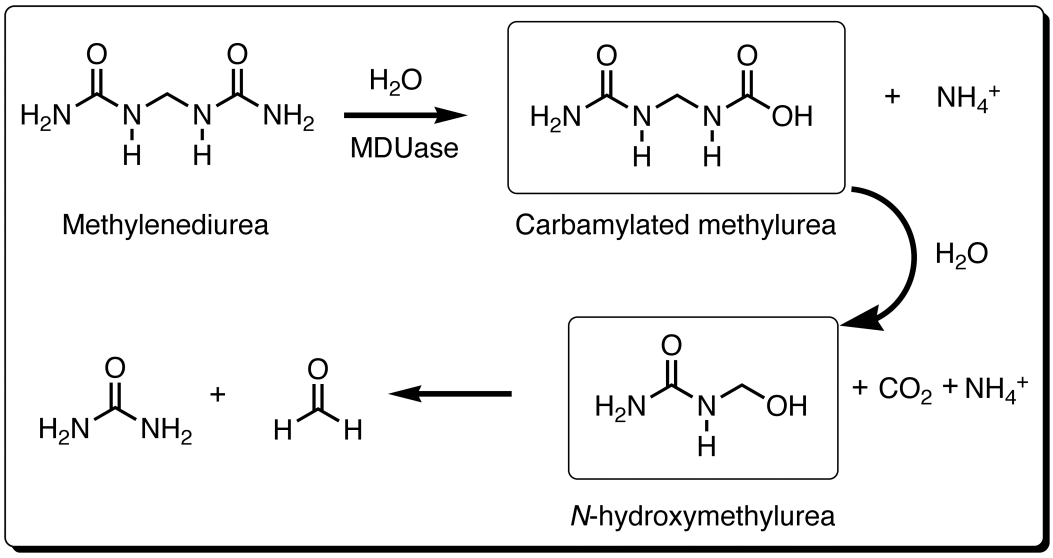
Scheme 1.3. Metal-dependent reactions carried out by ARD.

The global structure of ARD was elucidated employing high-resolution NMR spectroscopy,[24,26] while the active site structure was studied with by X-ray absorption spectroscopy.[23] The active site appears to have an octahedral geometry with three nitrogen donors provided by His96, His98 and His140 together with three oxygen donors provided by Glu102 and two water molecules. Among these six ligands, His96 and Glu102 are trans located at the paramagnetic nickel(II) ion.[23] A limited number of structural[77] and functional[78] models have been reported recently in an effort to understand the catalytic mechanism of ARD.

1.2.6. Methylenediurease (MDUase)

Methylenediurease (MDUase), isolated from *Burkholderia*, was found to be a nickel-dependent enzyme, which is able to degrade methylenediurea into urea and formaldehyde with ammonia and carbon dioxide as byproducts (Scheme 1.4).[27] Methyleneureas or ureaforms are condensation products of urea and aldehyde [(H_2N-(CO–NH–CH_2–NH)$_n$–CO–NH_2); n=1 for methylenediurea] which are potentially applied as slow-release fertilizers in bioremediation processes (more than 300,000 tons per year).[79-81] Significantly, the methylenediurease activity was resolved by anion exchange chromatography from urease activity of the same microorganism, and each enzyme was found to be specific toward its own substrate, such as *Ralstonia paucula* (methyleneureas),[80] *Burkholderia* (methylenediurea and dimethylenetriurea),[79] *Rhodococcus erythropolis* (isobutylidenediurea),[82] *Rhodococcus* (crotonylidenediurea).[79]

Further studies are necessary to characterize the structure and functional mechanism of this enzyme.



Scheme 1.4. Degradation pathway of methylenediurea by MDUase.

1.2.7. Methyl-Coenzyme M Reductase (MCR)

Methyl-coenzyme M reductase (MCR) is the key enzyme in biological methane formation by methanogenic archaea.[28,83,84] In the MCR active site, the nickel ion is present in the tightly, but non-covalently, bound tetrahydrocorphinoid complex called coenzyme F-430 (Fig. 1.4). The upper face of the F-430 cofactor forms the floor of a narrow hydrophobic well leading to the surface of the protein. The nickel ion is coordinated by the four pyrrolic nitrogens in the equatorial plane and by an oxygen of the glutamine side-chain in the lower axial position. The upper axial position contains either the thiolate or the sulfonate group of CoM, depending on the form of MCR isolated.

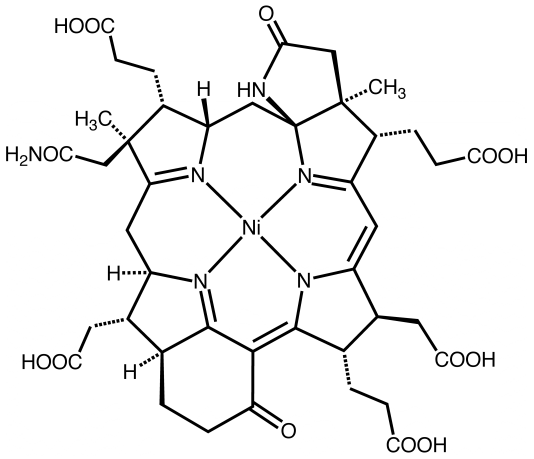
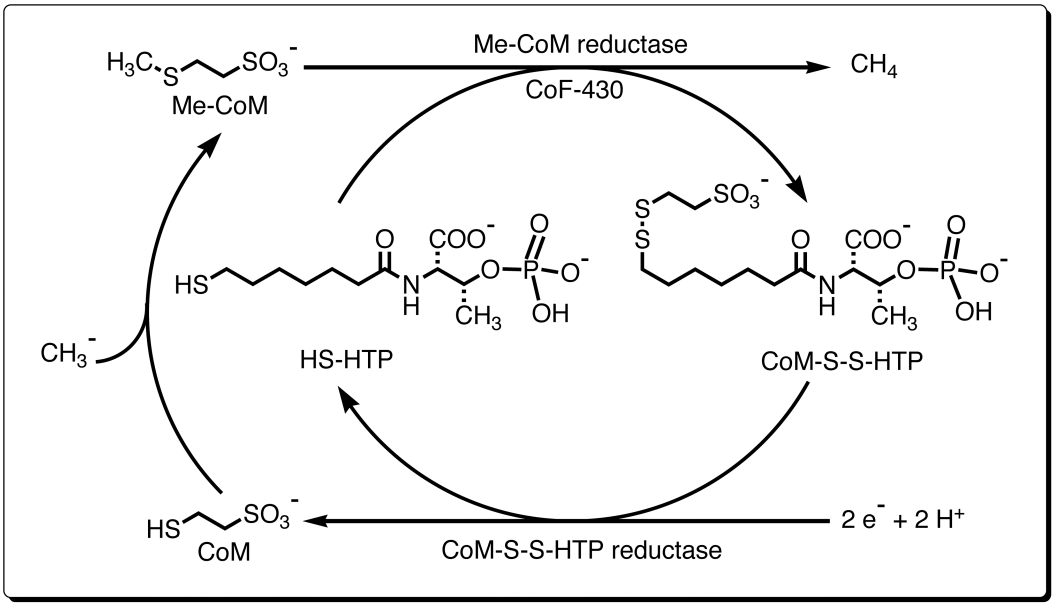
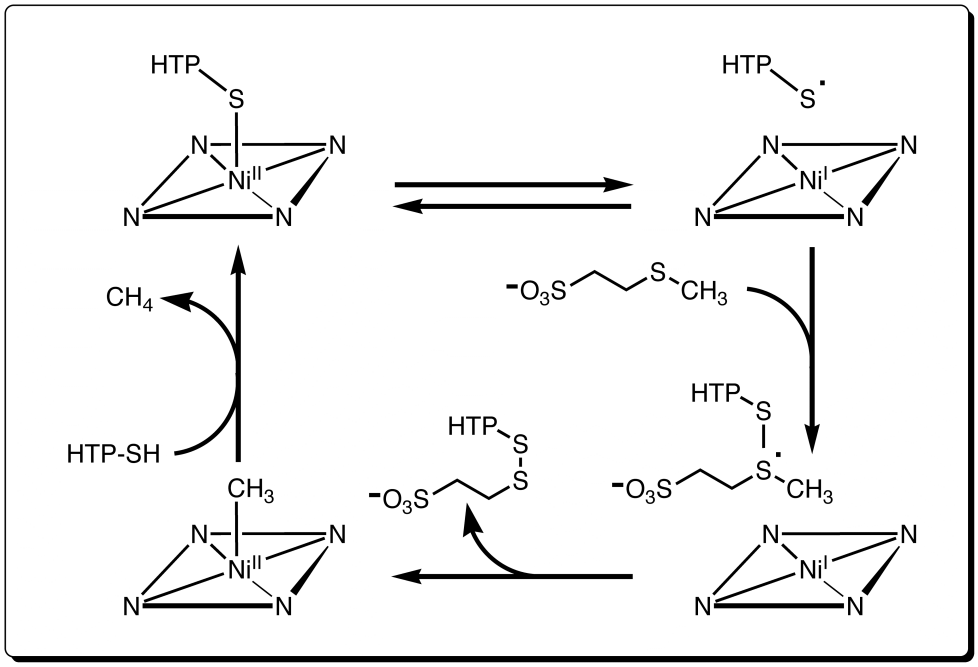


Fig. 1.4. Schematic view of coenzyme F-430 of MCR showing the extensively reduced tetrapyrrole ring in which the π chromophore only extends over three of the four nitrogens. A lactam ring and a six-membered carbocyclic ring enlarge the tetrapyrrole ring.[28,83]



Scheme 1.5. Catalytic cycle involving the coenzyme F-430-assisted methane formation in methanogenic archaea (Adapted from the literature).[85]



Scheme 1.6. Mechanism of F-430-catalyzed methane formation (Adapted from the literature).[85-87]

MCR catalyzes the reaction between the thioether methyl coenzyme M (MeCoM) and the thiol *N*-(7-mercaptoheptanoyl)-*O*-phospho-L-threonine (HS-HPT, coenzyme B) to give methane and the mixed disulfide CoM-S-S-HTP (Scheme 1.5). The nickel center of free coenzyme F-430, as well as its penta-ester or penta-amide derivatives, can be reduced reversibly to the Ni(I) valence state, which exhibits a characteristic quasi-axial

EPR spectrum and UV-visible absorption maxima at 380 and 750 nm. Conventional purification of MCR leads to an inactive enzyme that contains the metal in the Ni(II) valence state. The first isolation of highly active enzyme preparations from reductively preconditioned cells and the reductive reactivation of the so-called MCR_{ox1} state to active enzyme (MCR_{red1}) demonstrated that the enzyme is active only if the metal center of coenzyme F-430 is in the Ni(I) form.[88]

The mechanism shown in Scheme 1.6 postulates the formation of Ni(I) and a thiyl radical. The formed thiyl radical attacks the Me-CoM to form the sulfuranyl radical. The unpaired electron from the Ni(I) $d_{x^2-y^2}$ orbital transfers to the C-S σ^* orbital and induces the homolytic cleavage of the C-S bond to form the Ni(II) methyl-substituted coenzyme F-430 and the unsymmetrical disulfide. This methyl-substituted coenzyme F-430 is further attacked by HSCoB to release methane.

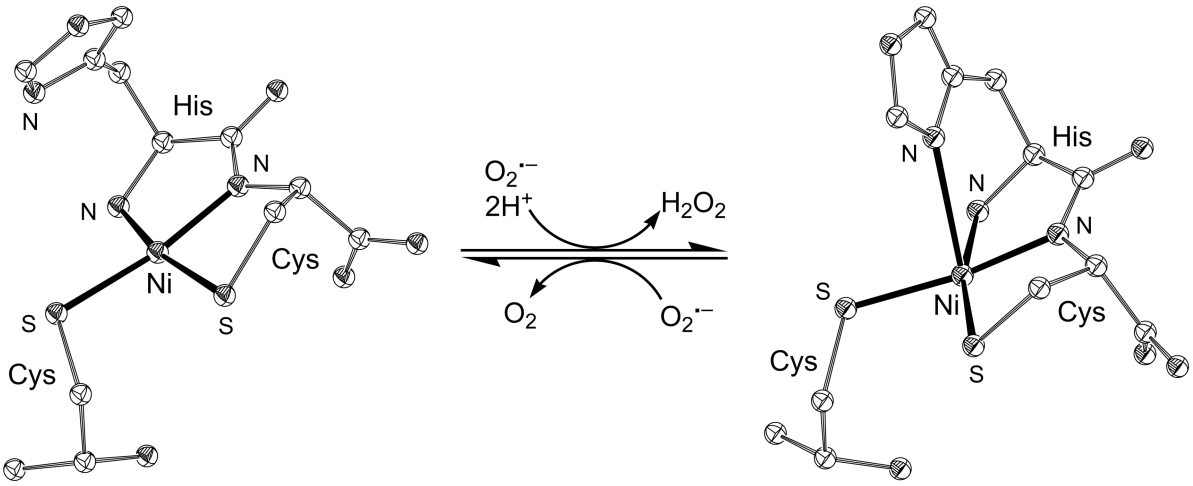
1.2.8. Nickel Superoxide Dismutase (Ni-SOD)

Nickel superoxide dismutase (Ni-SOD) is a recently discovered member of the nickel-containing metalloenzymes and of the SOD class of enzymes that catalyze the disproportionation of highly toxic superoxide ($O_2^{\bullet -}$) into peroxide (O_2^{2-}) and molecular oxygen.[29,30,89] Ni-SOD is the fourth member of this class of enzymes; the other known SODs containing Fe, Mn and Cu/Zn. Reduced Ni-SOD contains nickel(II) in a square-planar N_2S_2 coordination environment derived from the backbone terminal amino group of His1, the amide group, and the thiolate groups of Cys2 and Cys6 (Scheme 1.7).[30,90,91] The Nδ and Nε nitrogens of His1 are not involved in coordination; they are involved in hydrogen-bonding to the main-chain oxygen atom of Val8 (Nδ) and to Glu17 (Nε) of a neighboring subunit.[30] Oxidized Ni-SOD contains a Ni(III) ion in a distorted square-pyramidal N_3S_2 coordination environment derived from same units as reduced Ni-SOD and in addition the Nδ nitrogen of His1 (Scheme 1.7).

The presence of thiolate donors makes the Ni-SOD different from other SODs and the stabilization of these two thiolate ligands against sulfur-based oxidation in the presence of the highly active radical substrate remains elusive. The monomeric unit of this enzyme is a 4-helix bundle accommodating the active site at the N-terminus and six of these bundles make the whole molecule of a Ni-SOD as a homohexamer. The proposed mechanism of dismutation shows that the electron transfer from the nickel(II) ion to the axially bound superoxide must be coupled with a proton transfer to generate the dihydrogen peroxide. Site-specific mutagenesis studies confirm the significance of the histidine ligand, as altering this site tremendously decreases the dismutase activity.

A number of nickel complexes with N_2S_2 and N_3S_2 (bis-amide or bis-amine) coordination environment are available in literature before and after the report of the

crystal structure of a nickel-containing superoxide dismutase (Fig. 1.5).[92-96] NiN_2S_2 complexes can be reactive toward both H_2O_2 and O_2, often yielding S-based oxygenation products.[97] Synthetic studies have demonstrated that NiN_2S_2 complexes in bis-amine ligand environments are more stable toward oxygen than the corresponding bis-amide complexes.[70,92]



Scheme 1.7. Active site structures of reduced Ni-SOD showing the square-planar Ni(II) (1Q0K) and oxidized Ni-SOD showing the square-pyramidal Ni(III) with axially coordinated imidazole of His-1 (1Q0D) along with the detoxification reaction carried out by Ni-SOD.[30]

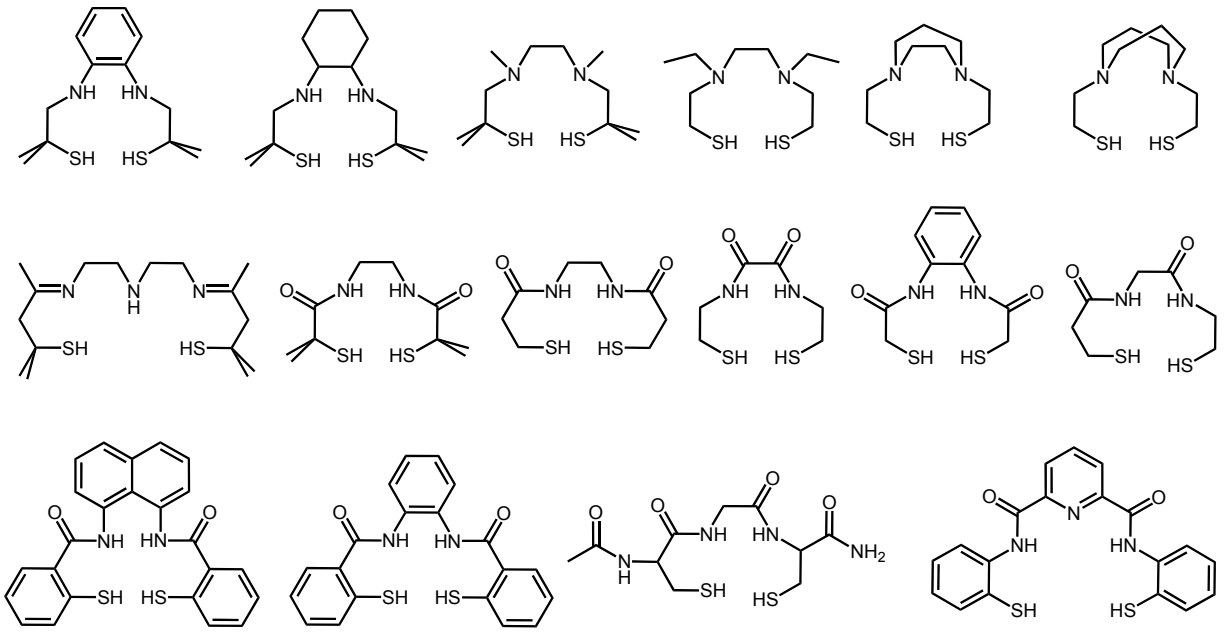
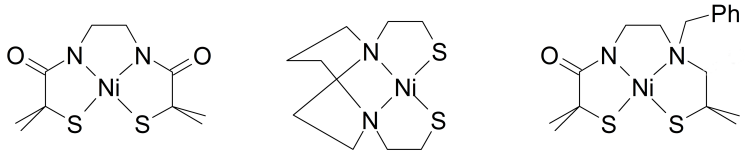


Fig. 1.5. Selection of N_2S_2 and N_3S_2 ligands used in the synthesis of nickel complexes to mimic Ni-SOD.[92-96]

Recently the first NiN_2S_2 complex $[Ni^{II}(beamm)]^-$ [H_2beamm = *N*-{2-[benzyl(2-mercapto-2-methylpropyl)amino]ethyl}-2-mercapto-2-methylpropionamide] (Fig. 1.6) containing amine/amide coordination has been reported with the studies on the difference between amine/amide and bisamide coordination on the models of Ni-SOD.[98] Bis-amine-coordinated NiN_2S_2 complex $[Ni^{II}(bmedach)]$ [H_2bmedach = *N*,*N*'-bis(2-mercaptoethyl)- 1,4-diazacycloheptane] (Fig. 1.6) possess a Ni^{II}/Ni^{III} redox potential far too positive to reduce superoxide ($E_{1/2}$ > 1.2 V *vs* Ag/Ag^+), while bis-amide-coordinated NiN_2S_2 complex $[Ni^{II}(emi)]^{2-}$ [H_2emi = *N*,*N*'-ethylenebis(2-mercaptoisobutyramide)] (Fig. 1.6) is incapable of oxidizing superoxide after accessing the Ni^{III} oxidation state.



$[Ni^{II}(emi)]^{2-}$ $[Ni^{II}(bmedach)]$ $[Ni^{II}(beamm)]^{1-}$

Fig. 1.6. Comparison of NiN_2S_2 complexes with different environments.[98]

A model complex for Ni-SOD should have the Ni^{II}/Ni^{III} redox potential between 0.04 V and 1.09 V *vs* Ag/Ag^+, obviously because the oxidation and reduction potentials of the superoxide radical anion are respectively 0.04 V and 1.09 V *vs* Ag/Ag^+.[99] It has been postulated that the combination of amine and amide in an $Ni^{II}N_2S_2$ coordination environment ensures a Ni-centered one-electron oxidation process, appropriately tunes the Ni^{II}/Ni^{III} redox potential for SOD catalysis, and secures the thiolate donors from oxygenation by O_2.[98] However, $[Ni^{II}(beamm)]^-$ is not reactive towards $O_2^{\bullet-}$, even though it has an amine/amide mixed environment around the nickel ion; this suggests that the fifth axial coordination might be a key component for the SOD activity, as suggested by the site-specific mutagenesis studies.

1.3. Hydrogenases (H_2ases)

1.3.1. Introduction

Hydrogenases are a class of enzymes, which catalyze the interconversion of protons and electrons with molecular hydrogen ($H_2 \leftrightarrows H^+ + H^- \leftrightarrows 2H^+$).[100] The recent surge towards the development of cheap and clean alternatives for fossil fuels has drawn tremendous attention on the research concerning the active site structure of the hydrogenases and the mechanism behind their catalytic function.[101,102] Furthermore, the presence of biologically unusual ligands in the active sites of hydrogenases has drawn particular attention from the coordination and bioinorganic chemists.[103-110] Hydrogenases are classified into three types according to the metal content of the

active site, namely **(1)** [FeFe] hydrogenases, **(2)** [NiFe] hydrogenases and **(3)** [Fe] hydrogenases or iron-sulfur-cluster free hydrogenases. Although the main focus of this thesis is on the modeling of Ni-containing enzymes, all three classes are briefly discussed in the following sections.

1.3.2. [FeFe] Hydrogenases

[FeFe] hydrogenases and their model complexes are the most studied among the three types of hydrogenases.[111] The periplasmic [FeFe] hydrogenase is involved in H_2 uptake while the cytoplasmic [FeFe] hydrogenase is involved in dihydrogen production.[112]

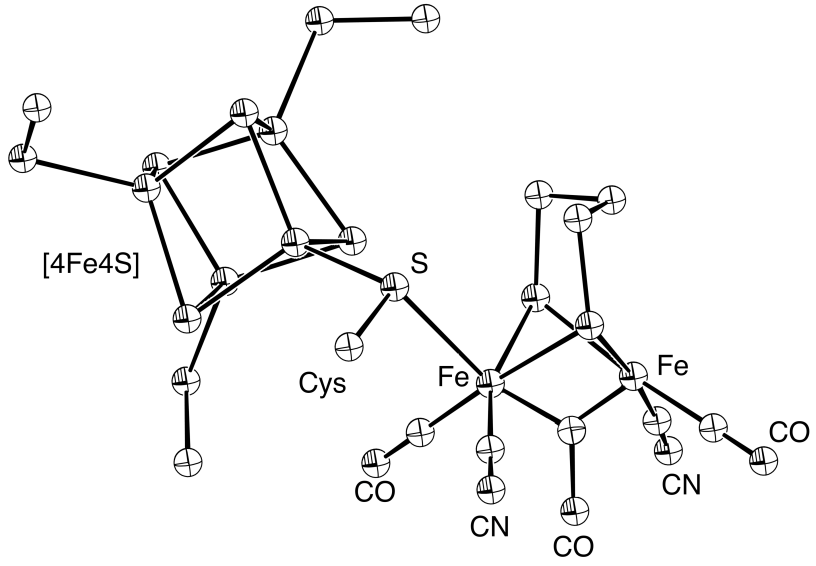


Fig. 1.7. Active site structure of [FeFe] hydrogenase (1FEH).

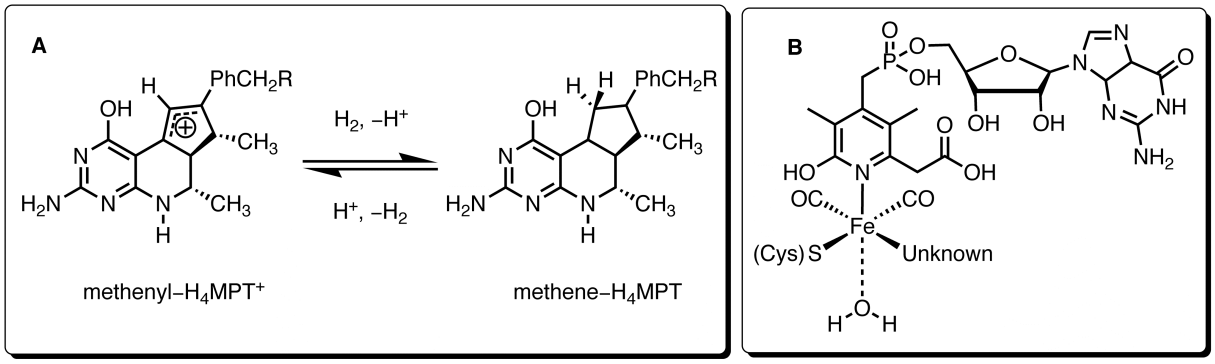
The H-cluster of [FeFe] hydrogenase active site is built up from two parts, namely, an [4Fe4S] cubane and a binuclear [2Fe2S] metal center bridged by a dithiolate ligand, linked to each other by a cysteinyl residue.[113,114] The metal centers in the binuclear site are bridged by the biologically unusual carbonyl ligand and a set of carbonyl and cyanide groups coordinate to each iron center. The coordination environment of the active site of [FeFe] hydrogenase can be simply formulated as $[(H_2O)(CN)(CO)Fe(SCH_2XCH_2S)Fe(CN)(CO)(\mu\text{-}S_{Cys})(Fe_4S_4)]$ (X = CH_2, NH, O). More detailed information on [FeFe] hydrogenase and its model complexes are available in recent reviews.[100,106,112,115-117]

1.3.3. [Fe] Hydrogenases

[Fe] hydrogenase is the relatively new member in the hydrogenase family, and is present in some methanogenic archaea.[118] [Fe] hydrogenase is also called iron-sulfur cluster free hydrogenase, owing to the fact that in contrast with other classes it contains

only a mononuclear iron center in the active site.[119] The [Fe] hydrogenase has been abbreviated as "Hmd" (H_2-forming methylenetetrahydromethanopterin), as it catalyzes the reversible reduction of methenyltetrahydromethanopterin (methenyl-H_4MPT^+) with dihydrogen to methylenetetrahydromethanopterin (methene-H_4MPT), which is an intermediary step in the biological conversion of CO_2 to methane (Scheme 1.8).

The iron ion in the active site of Hmd has a square-pyramidal geometry comprised of a pyridine-type nitrogen of the guanylylpyridone derivative coordinated apically to the iron center. The basal-plane comprises two *cis*-carbonyl ligands, a cysteinyl thiolate and an unknown ligand.[119] A water molecule is located *trans* to the pyridone derivative within a distance of 2.7 Å. The oxidation state of the iron center remains elusive; high-spin Fe(II) has been excluded, as the as-isolated form of the enzyme is not EPR active and Mössbauer experiments suggest low-spin Fe(0) or Fe(II).[120] More detailed information on the enzyme Hmd[118,119,121] and of its model complexes[122-125] are available in recent literature.[111]



Scheme 1.8. (A) Reaction catalyzed by the [Fe] hydrogenase. (B) Schematic representation of the active site of the [Fe] hydrogenase showing the iron guanylylpridone cofactor (FeGP cofactor) from *M. jannaschii* (3DAG).[119]

1.3.4. [NiFe] Hydrogenases

[NiFe] hydrogenases are interesting among the three types of hydrogenases due the presence of the heterodinuclear active site (Fig. 1.8). They are further divided into four subclasses according to the functions in which they are involved namely, **(1)** H_2-uptake, **(2)** H_2-evolution, **(3)** bidirectional H_2-activation and **(4)** H_2-sensing. High-resolution X-ray crystal structures are available for the [NiFe] hydrogenases isolated from *D. gigas*,[31,126] *D. vulgaris*,[32,127-129] *D. fructosovorans*,[33,130,131] *D. sulfuricans*[35] and *Dm. baculatum.*[34]

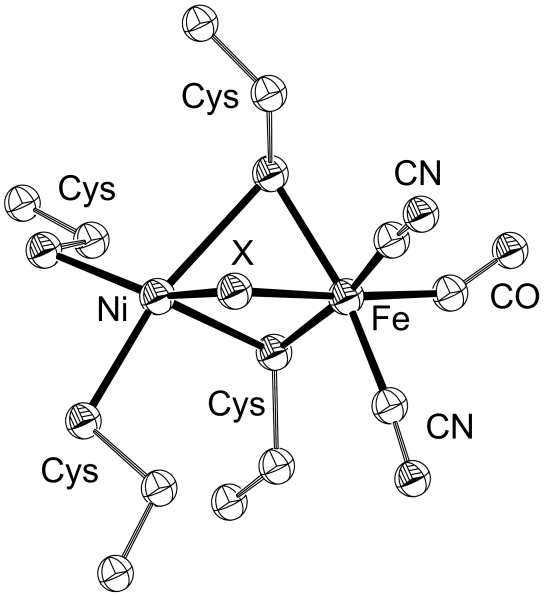
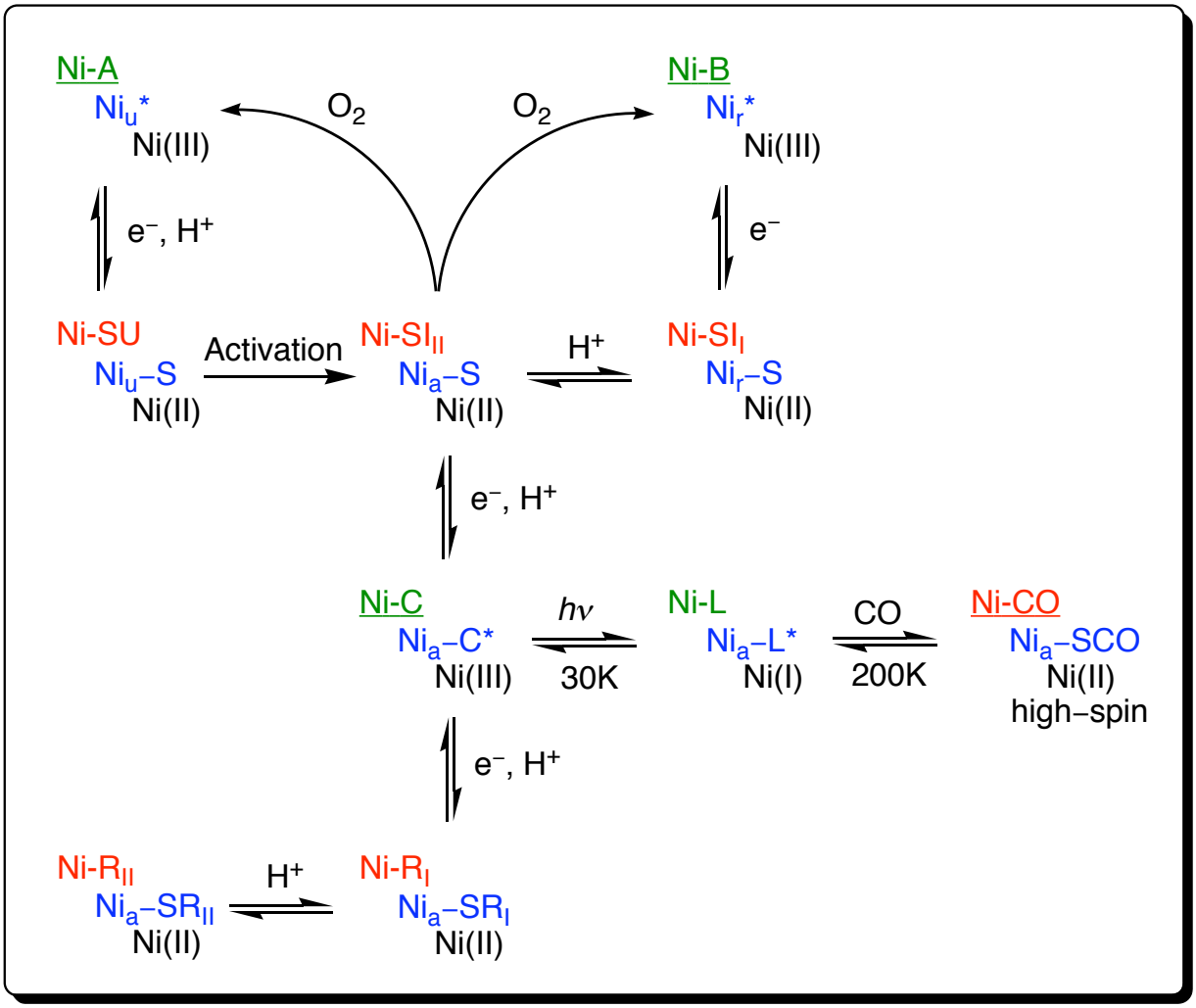


Fig. 1.8. Active site structure of [NiFe] hydrogenase from *D. gigas* (2FRV).

All the known X-ray structures have revealed a heterodinuclear active site which can be formulated as $[(Cys–S)_2Ni(\mu\text{-}S–Cys)_2Fe(CN)_2(CO)]$; it contains a NiS_4 center with four S-donors derived from cysteine residues, two of which bridge the nickel and iron center (Fig. 1.8). Surprisingly, the low-spin iron center is further coordinated by biologically unusual carbonyl and two cyanide groups. Even though the earlier studies speculated that these carbonyl and cyanide ligands are part of the catalytic center,[132] the X-ray structural studies suggest that these groups may just maintain the oxidation state of iron at 2+ in order to preserve its low-spin nature.[31]

As the "gas channel" from the surface of the enzyme ends at nickel[33,133] and the inhibitors CO and HOO^- bind at nickel,[127,128,130] the nickel ion is suggested to be the binding site of dihydrogen; yet DFT studies have shown the possibility of iron being the H_2 binding site.[117,134] Numerous studies suggest that only the nickel center is responsible for the redox state changes in the active site. All the observed redox states of the enzyme together present a highly complicated scheme of the catalytic cycle as shown in Scheme 1.9. The enzyme's different redox states in its active and inactive forms are distinguished by different notations in literature as shown in Scheme 1.9.

For the past ten years the biochemistry and modeling chemistry [NiFe] hydrogenase have grown tremendously and have been the subject of numerous reviews, from which more detailed information can be obtained.[100,104,111,112,116,117,134,135]



Scheme 1.9. Overview of different redox states proposed for [NiFe] hydrogenase showing various redox states of the enzyme (u, unready; r, ready; a, active; S, SI, EPR-silent).[117,134,136] EPR-active species are shown in green.[111] Diamagnetic species are shown in red. Alternative notations are denoted in blue. X-ray crystallographically characterized species are underlined.[34,126-130,137] In some reports Ni-SI$_I$ and Ni-SI$_{II}$ are denoted as Ni-SI$_{(b)}$ and Ni-SI$_{(a)}$, respectively.[138]

1.4. Modeling the Structure of [NiFe] Hydrogenases

1.4.1. Introduction

The report of the first X-ray crystal structure of a [NiFe] hydrogenase enzyme watered the surge towards better structural and functional models.[31] A large number of small molecular models comprising heterodinuclear [NiFe] complexes have been reported since the first structure report in 1996.[139] The field of heterodinuclear complexes modeling [NiFe] hydrogenases has been first reviewed in the year 2001,[110] a later review in the year 2003 focuses on both [NiFe] and [Fe-only] hydrogenases.[138] In the year 2005, Bouwman and coworkers reported a historic overview of the biomimetic models for [NiFe] hydrogenase that have been synthesized since the nickel content of the

enzyme was reported.[103] A large number of reports appeared in special issues of Chemical Reviews,[100,102,111,112,116,117,134,135,140] Coordination Chemistry Reviews[103,104,108,109,141-143] and Chemical Society Reviews,[144] that are helpful for the readers to obtain an overview of the biochemistry and structural properties of the enzyme, the model complexes and the techniques used to assess the functional activity of the model compounds. Some remarkable model systems and the major functional studies of the mimics are discussed in the following sections.

1.4.2. [NiFe] Complexes

A large number of heterodinuclear [NiFe] complexes have been reported as structural models for [NiFe] hydrogenase, since the first report of the X-ray crystal structure of the enzyme.[103,110,138,145] Darensbourg and coworkers reported the first reasonably accurate structural model for the [NiFe] hydrogenase, comprising of a Ni(II) ion in an N_2S_2 environment with one of the two thiolates bridging to an $Fe(CO)_4$ moiety (Fig. 1.9A); the Ni···Fe distance (3.76 Å) is rather large compared to the biological system (2.6-2.9 Å).[139]

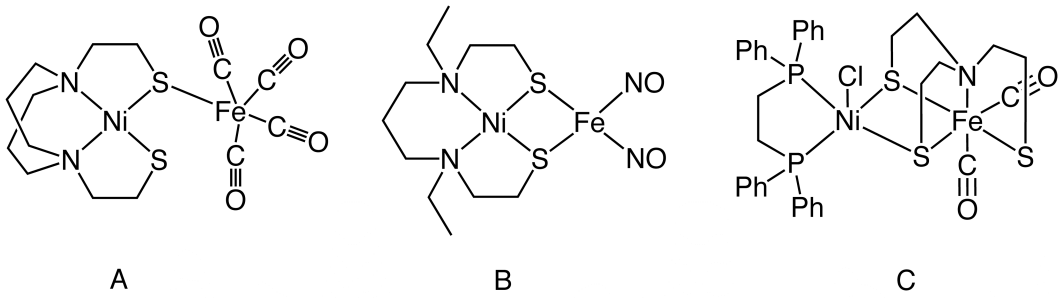


Fig. 1.9. [NiFe] complexes reported as mimics for [NiFe] hydrogenase by Darensbourg et al. (A),[139] Pohl et al. (B)[146] and Evans et al. (C).[147]

Pohl and coworkers reported the first [NiFe] complex in which two thiolates of a NiN_2S_2 metalloligand are bridging to the iron moiety resulting in a Ni to Fe distance of 2.8 Å (Fig. 1.9B).[146] Evans and coworkers reported the first [NiFe] complex containing two thiolates bridging to the iron center containing carbonyl ligands with a Ni···Fe distance of 3.3 Å (Fig. 1.9C).[147] This complex introduced the utilization of soft P-donor ligands instead of N-donor ligands to mimic the S-donor cysteinates of the [NiFe] hydrogenase.

Sellman and coworkers have reported a large series of transition-metal complexes of S-donor ligands. The first [NiFe] complex (Ni···Fe = 3.3 Å) comprising an NiS_4 coordination sphere with two thiolates bridging to the iron moiety with a carbonyl ligand was reported by Sellman and coworkers in 2002 (Fig. 1.10A).[148] In the same year, Bouwman and coworkers reported the S_4 ligand H_2xbsms

(1,2-bis(4-mercapto-3,3-dimethyl-2-thiabutyl)benzene) and its mononuclear low-spin nickel complex[149] which was the basis of a number of structural[150] and functional[145,151,152] models for [NiFe] hydrogenase. The compounds [Ni(xbsms)Fe(NO)$_2$] (Fig. 1.10B), [Ni(xbsms)Fe(CO)$_4$] (Fig. 1.10C) and [Ni(xbsms)Fe(CO)$_2$I$_2$] (Fig. 1.10D) were derived from [Ni(xbsms)] by reaction of the nickel complex with [Fe(CO)$_2$(NO)$_2$], [Fe$_2$(CO)$_9$] and [Fe(CO)$_4$I$_2$], respectively.[150]

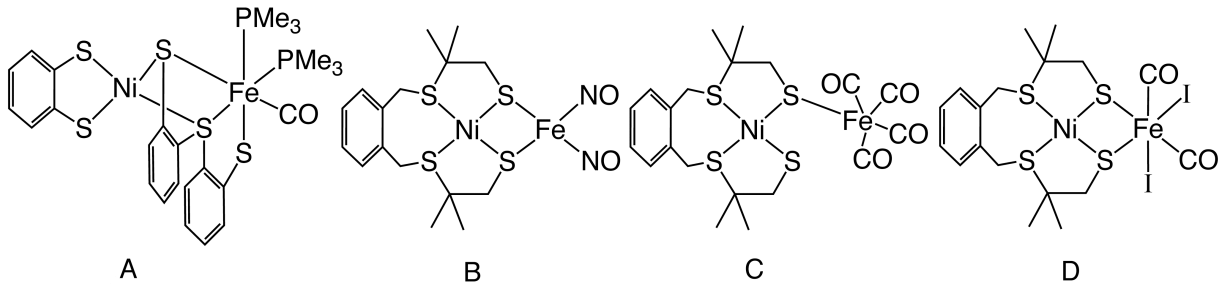


Fig. 1.10. Heterodinuclear [NiFe] complexes reported as mimics for [NiFe] hydrogenase by Sellman et al. (A)[148] and Bouwman et al. (B-D).[149,150]

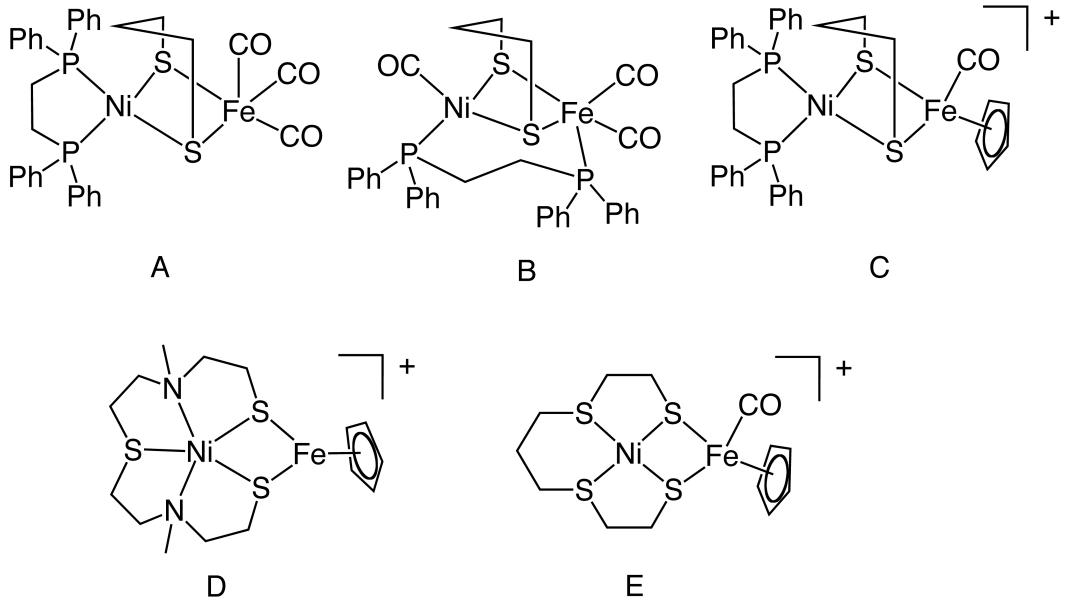


Fig. 1.11. Heterodinuclear [NiFe] complexes reported by Schröder et al.[153]

Breakthrough model complexes were reported by Schröder et al.[153] and Tatsumi et al.[154] in the year 2005. Where all the known [NiFe] complexes contain square-planar or square-based geometry around the nickel center, the complex [(dppe)Ni(μ-pdt)Fe(CO)$_3$] (dppe, 1,2bis(diphenylphosphino)ethane; pdt, propane-1,3-dithiolate) (Fig. 1.11A) interestingly was reported to have a distorted tetrahedral NiS$_2$P$_2$ coordination arrangement (Ni···Fe = 2.46 Å); surprisingly, this complex is diamagnetic.[153] Thus, the

nickel center in the square-planar precursor [Ni(pdt)(dppe)] has undergone a complete tetrahedral twist on binding of the $Fe(CO)_3$ moiety. The complex [(dppe)Ni(μ-pdt)$Fe(CO)_3$] (Fig. 1.11A) is unstable in solution and affords [(CO)Ni(μ-dppe)(μ-pdt)$Fe(CO)_2$] (Fig. 1.11B) upon rearrangement in benzene; this compound also contains a diamagnetic nickel center with the same Ni···Fe distance (2.47 Å). In addition, several other [NiFe] complexes were reported, obtained from [Fe(Cp)$(CO)_2$I] as a precursor. The [NiFe] complexes [(dppe)Ni(μ-pdt)Fe(Cp)(CO)]$^+$ (Fig. 1.11C), [Ni(N_2S_3)Fe(Cp)]$^+$ (Fig. 1.11D) and [Ni(S_4)Fe(Cp)(CO)]$^+$ (Fig. 1.11E) were reported with Ni···Fe distances of 2.78, 2.54 and 3.17 Å, respectively.[153]

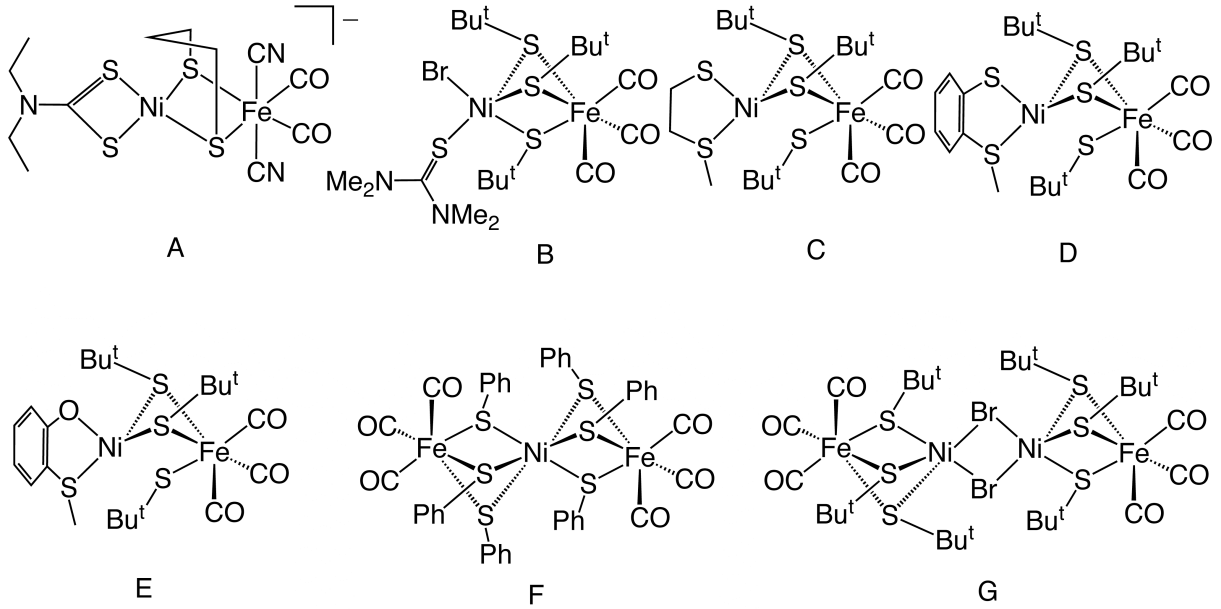


Fig. 1.12. Heterodinuclear and oligonuclear [NiFe] complexes reported by Tatsumi et al.[154,155]

The complex [(dedtc)Ni(μ-pdt)$Fe(CO)_2(CN)_2$]$^-$ (Fig. 1.12A) was reported as the closest yet structural model of that time comprising most of the elements matching the active site of [NiFe] hydrogenase; an S_4 coordination geometry around the nickel center, two thiolates bridging to the iron center (Ni···Fe = 3.05 Å) which coordinates to diatomic ligands (CO and CN).[154] Recently, Tatsumi et al. reported a number of [NiFe] complexes (Fig. 1.12B-E) formed from the reaction between the tetranuclear [Ni_2Fe_2] cluster [$(CO)_3$Fe(μ-S^{t}Bu)$_3$Ni(μ-Br)]$_2$ (Fig. 1.12G) and various S-donor ligands such as $SC(NMe_2)_2$, $NaS(CH_2)_2SMe$, $NaSC_6H_4SMe$ and $NaOSC_6H_4SMe$.[155] The linear clusters [$(CO)_3$Fe(μ-SPh)$_3$Ni(μ-SPh)$_3$$Fe(CO)_3$] (Fig. 1.12F) and [$(CO)_3$Fe($\mu$-S^{t}Bu)$_3$Ni($\mu$-Br)]$_2$ (Fig. 1.12G) were obtained from the reaction between $FeBr_2(CO)_4$ and $NiBr_2(C_2H_5OH)_4$ in the presence of the sodium salts of the corresponding thiols; both the precursors and the

resulting [NiFe] complexes were characterized by X-ray crystallography, despite the fact that these complexes were synthesized and manipulated at –40 °C.

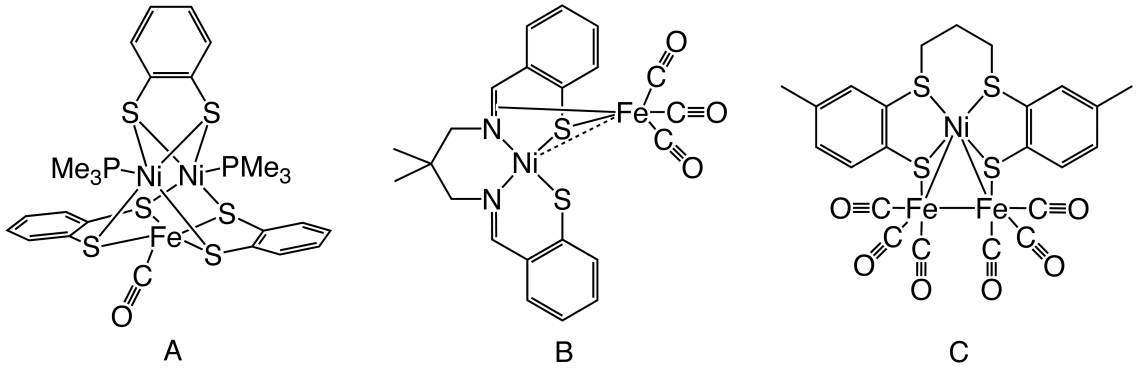


Fig. 1.13. [NiFe] complexes reported by Sellman et al. (A)[156,157] and Schröder et al. (B,C).[158-160]

The trinuclear [Ni_2Fe] complex [(bdt)($NiPMe_3$)$_2$Fe(CO)(bdt)$_2$] [bdt = benzene-1,2-dithiolate] (Fig. 1.13A) was reported as the first *functional* model for [NiFe] hydrogenase; upon reaction with HBF_4 this compound evolved molecular hydrogen and formed the stable one electron oxidized paramagnetic complex [(bdt)($NiPMe_3$)$_2$$Fe_2(CO)_2(bdt)_2$]$^+$.[156] The dinuclear complex [Ni(N_2S_2)Fe(CO)$_3$] (Fig. 1.13B) was reported by Schröder et al. with a diimine-dithiolato ligand coordinated to the nickel(II) ion and with the iron center of the Fe(CO)$_3$ moiety coordinated to the C=N π bond (Ni···Fe = 2.89 Å).[159] The two trinuclear complexes [(bdt)($NiPMe_3$)$_2$Fe(CO)(bdt)$_2$] (Fig. 1.13A) and [Ni(S_4)Fe_2(CO)$_6$] (Fig. 1.13C)[160] are so far the only [NiFe] complexes which show electrocatalytic activity in the reduction of protons into molecular hydrogen at –0.48 V *vs*. NHE[157] and –1.03 V *vs*. Fc/Fc^+,[158] respectively.

More recently, Schröder et al. reported a [$NiFe_2$] cluster (Fig. 1.14A) with interesting structural features formed from the reaction between the mononuclear nickel complex [Ni(S_5)] [H_2S_5 = bis(2-((2-mercaptophenyl)thiol)ethyl)sulfide] and [Fe_3(CO)$_{12}$] as a result of C–S and S–Ni bond cleavages.[161] The origin of and the mechanism by which the bridging sulfide ion is formed are unclear. The [$NiFe_2$] cluster comprises a NiS_3 moiety connected to two Fe(CO)$_3$ moieties by direct Ni-Fe bonds and a sulfide ion capping the [$NiFe_2$] equilateral triangle forming a trigonal pyramid (Fig. 1.14A).

Tatsumi et al.[162] reported the [NiFe] complex [(dedtc)Ni(μ-tpdt)Fe$(CN)_2$(CO)]$^-$ (Fig. 1.14B) formed from the reaction between [$(CN)_2$(CO)Fe($SCH_2CH_2SCH_2CH_2SK$)]$^-$ and [(PPh_3)NiBr(dedtc)] at –40 °C (dedtc = diethyldithiocarbamate; tpdt = 3-thiapentane-1,5-

dithiolate); the CN/CO bands in the IR spectrum of this complex reproduce those of the Ni–A, Ni–B and Ni–SU states of the [NiFe] hydrogenases.

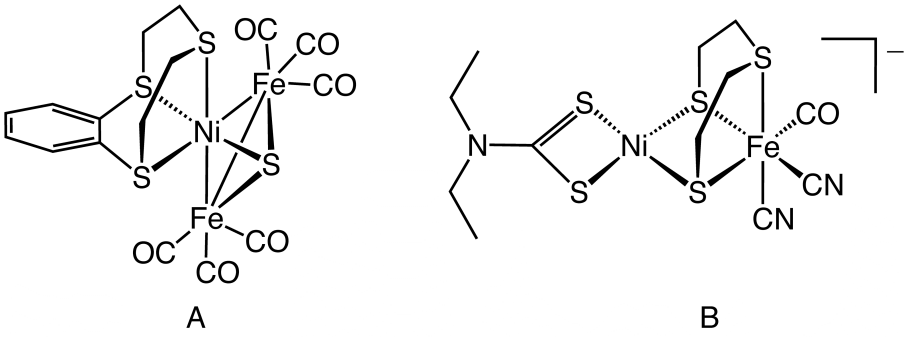


Fig. 1.14. [NiFe] complexes recently reported by Schröder et al. (A)[161] and Tatsumi et al. (B)[162].

A number of nickel-ruthenium complexes[145,151,152,163-167] have also been reported as models for [NiFe] hydrogenases (Fig. 1.15A-D), since the first report of $[Ni(S_2N_2)RuCp^*]_2(OTf)_2$ (Fig. 1.15A) and [Ni(bme*-daco)RuCp*(NCMe)]OTf (Fig. 1.15B) with NiN_2S_2 coordination geometry as models for ACS and [NiFe] hydrogenase.[163] A very recent review[111] from Pickett et al. covers many aspects of [Fe], [FeFe] and [NiFe] hydrogenase enzymes and of their model complexes. The review contains tables of CO stretching frequencies, metal-to-metal distances, redox potentials of selected complexes and other important features that may be useful for the interested readers.[111]

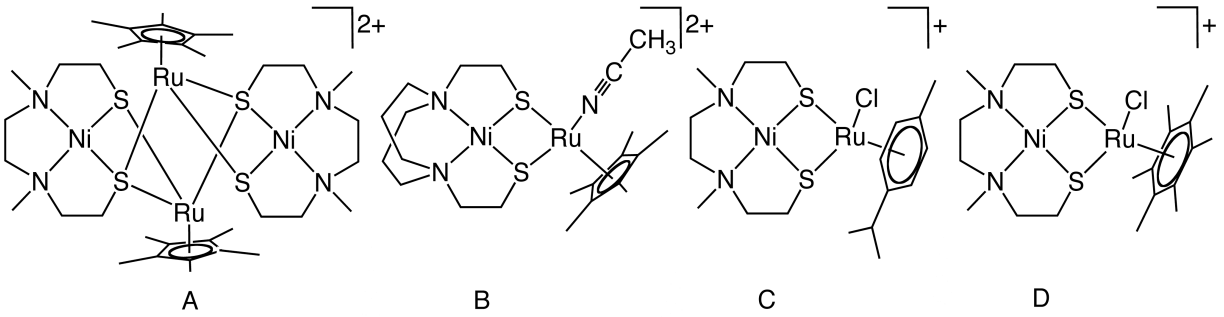


Fig. 1.15. [NiRu] complexes reported by Rauchfuss et al.[163]

1.5. Modeling the Function of Hydrogenases

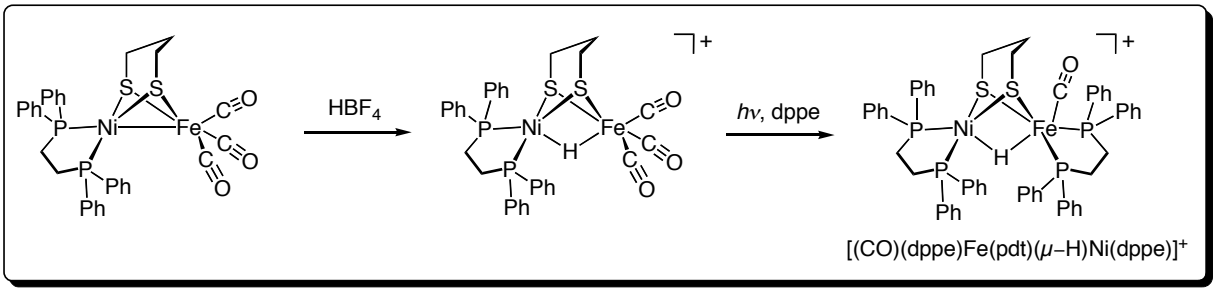
1.5.1. Introduction

Most of the structural mimics of the hydrogenases discussed in the previous section are either not stable or not active towards the reduction of protons or oxidation of dihydrogen; many complexes have not been tested for their activity at all or their

reactivity was not reported. Various groups have synthesized many complexes solely in the aim of mimicking the functions of hydrogenases. Although model complexes have been reported as catalysts for proton reduction, activation of dihydrogen and H/D exchange reactions, only the complexes that are active catalysts for proton reduction are discussed in detail, in the view of the aim of this thesis.

1.5.2. Electrocatalysts for Proton Reduction

Until recently, the complexes $[(bdt)(NiPMe_3)_2Fe(CO)(bdt)_2]$ (Fig. 1.13A)[156] and $[Ni(S_4)Fe_2(CO)_6]$ (Fig. 1.13C)[160] were the only [NiFe] complexes reported to be active as electrocatalyst for proton reduction. The recent report from Rauchfuss et al.[168] presents a new type of synthetic approach towards [NiFe] complexes, with a bridging hydride ion as shown in Scheme 1.10. The hydride complex $[(CO)(dppe)Fe(pdt)(\mu\text{-H})Ni(dppe)]^+$ synthesized by this approach catalyses the reduction of protons at –1.37 V vs Fc/Fc^+ using trifluoroacetic acid as the proton source.



Scheme 1.10. [Ni(μ-H)Fe] complexes reported by Rauchfuss et al.[168]

Due to the pronounced stability of coordination complexes of chelating ligands, there has been considerable interest in stable and efficient electrocatalysts, such as nickel and cobalt complexes of macrocycles and multinuclear metallacrowns, as they can be potentially employed in PEM (Proton Exchange Membrane) water electrolysis cells.[169-172] A handful of transition-metal complexes, away from the interest of modeling the active site of hydrogenases, have also been reported to reduce protons into dihydrogen effectively with various overpotentials ranging between –1.5 and –0.2 V vs. SCE.[151,169,170,173-181]

A series of cobalt difluoroboryl-diglyoximate complexes have been reported recently to catalyze the electrochemical dihydrogen evolution at overpotentials as low as –0.20 V vs. SCE in acetonitrile.[170-172,182] The dinuclear complex $[(CpMo\text{-}\mu\text{-}S)_2S_2CH_2]$ has been reported as an electrocatalyst in the dihydrogen production showing almost 100% current efficiency when *p*-cyanoanilinium tetrafluoroborate was used as a proton

source.[173] The oxothiomolybdenum wheel $Li_2[Mo_8S_8O_8(OH)_8(oxalate)]$ has recently been shown to be a electrocatalyst producing dihydrogen from $HClO_4$, *p*-toluenesulfonic acid, trifluoroacetic acid and acetic acid at –1 V vs. SCE.[169]

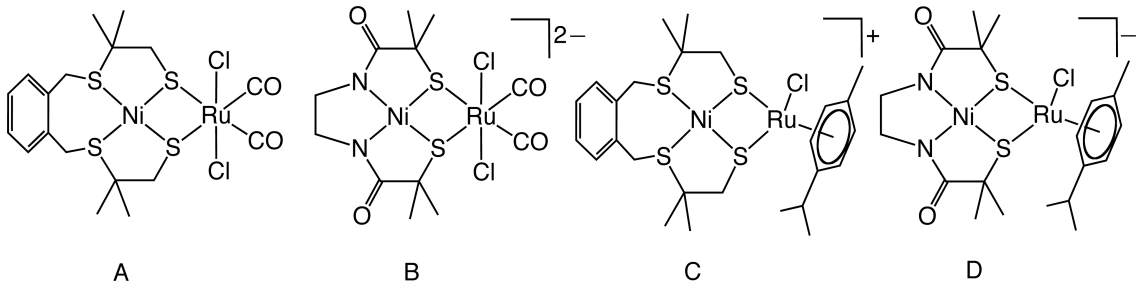


Fig. 1.16. [NiRu] complexes reported by Fontecave et al. as electrocatalysts for H_2 production.[151,152]

$[Ni(xbsms)Ru(CO)_2Cl_2]$ (Fig. 1.16A) was the first [NiRu] complex reported as a functional model for [NiFe] hydrogenase showing electrocatalytic properties to produce H_2 from a DMF solution of TEA·HCl at –1.50 V vs Ag/Ag^+.[152] $(NEt_4)_2[Ni(emi)Ru(CO)_2Cl_2]$ (Fig. 1.16B), $[Ni(xbsms)Ru(p\text{-}cymene)Cl]BF_4$ (Fig. 1.16C) and $(NEt_4)[Ni(emi)Ru(p\text{-}cymene)Cl]$ (Fig. 1.16D) were further reported with similar comparable H_2-evolution properties.[151] However, these complexes are leaving the researchers with the interesting question whether similar [NiFe] complexes can be used as electrocatalysts. A recent review from Artero et al.[141] provides detailed information about electrocatalysts for the proton-reduction reaction along with mechanistic details, while another review from Pickett et al.[111] tabulates the working potentials of a large selection electrocatalysts.

1.5.3. Photocatalysts for Proton Reduction

Due to the fact that dihydrogen production by cheaper and efficient sources is important in the context of the quest for alternative fuels, researchers have successfully made use of light-sensitive materials assisting redox systems in proton reduction. Recent reports from Artero et al.,[183,184] Reek et al.,[185] Song et al.[186,187] and Sun et al.[188-193] have demonstrated the utilization of photoactive complexes in photocatalytic dihydrogen production. These photosystems can be classified into three different types according to their constitution: **(1)** Photosensitizing systems, e.g. $Ru(bpy)_3$ covalently linked to a redox active center, such as a diiron moiety (Fig. 1.21A);[192-195] **(2)** Photosensitizing systems linked to a redox active system through non-covalent linkage such as metalloporphyrins (Fig. 1.21B);[185,186,189] **(3)** Homogeneous solutions containing photoactive materials, which can be reduced in the presence of light and a sacrificial electron donor; the reduced species then reduces the redox active species in order to

undergo the proton reduction.[183-185,190] Recent reviews provide detailed information on photocatalytic proton reduction.[107,111,188]

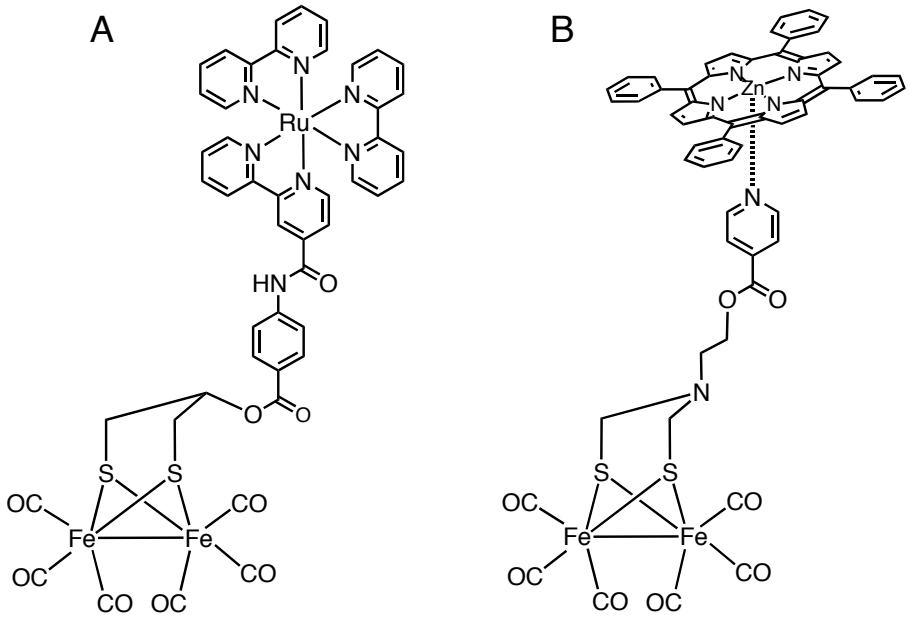


Fig. 1.17. Illustration of photocatalysts in the photoreduction of protons reported by Sun et al.[189,194]

1.5.4. Other Functional Models

Recently, the water soluble [NiRu] complex $[Ni(N_2S_2)Ru(H_2O)(C_6Me_6)](NO_3)_2$ (Fig. 1.18A) has been reported to form the hydride-bridged complex $[Ni(N_2S_2)(H_2O)(\mu\text{-}H)Ru(C_6Me_6)](NO_3)$ (Fig. 1.18B) by the reaction with H_2 in water. The latter complex catalyses the H/D exchange in acidic medium (pH 4-6).[166] Furthermore, $[Ni(N_2S_2)Ru(H_2O)(C_6Me_6)](NO_3)_2$ produces the hydroxido bridged complex $[Ni(N_2S_2)Ru(OH)(C_6Me_6)](NO_3)_2$ in basic medium (pH 7-10), which catalyses the hydrogenation of carbonyl compounds.[167]

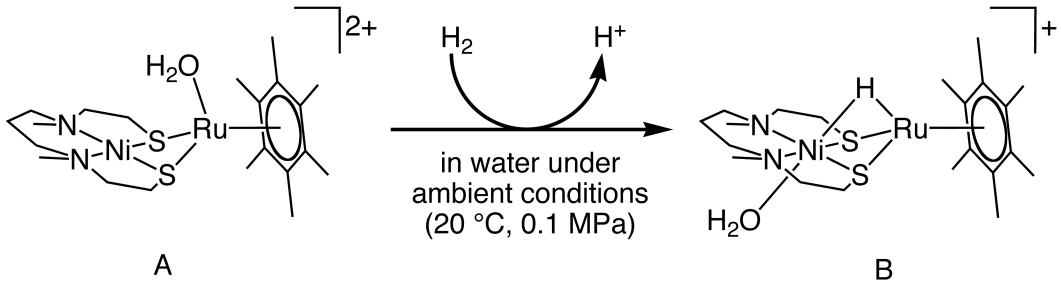


Fig. 1.18. Heterodinuclear [NiRu] complexes reported by Ogo et al.[166]

1.6. Aim and Outline of the Research

1.6.1. Aim

The aim of the research described in this thesis is the synthesis of new structural and functional models for the enzyme [NiFe] hydrogenase. By varying the steric and electronic properties of the ligands, attempts will be undertaken to tune the structural and redox properties of the [Ni] and [NiFe] complexes. Owing to the fact that the research towards the models for [NiFe] hydrogenase has led to a handful of unexpected and exciting findings, this thesis also reports structural and/or functional models of other Ni-containing enzymes such as ACS/CODH and MCR.

1.6.2. Modeling Strategy

The travel along the literature on the models complexes of hydrogenases that appeared after the report of the crystal structure of *D. gigas* provides a clear view of the gradual developments and the interesting facts about this particular discipline of chemistry.

The first phase of the modeling was to make stable mononuclear nickel complexes with NiS_4 coordination spheres, inspired by the complex [Ni(xbsms)][149] introduced by my predecessor, which formed the basis for many stable interesting [NiFe][150,196] and [NiRu][145,151,152] complexes as structural and functional models. A library of tetradentate chelating S_4-donor ligands containing two thioether and two thiolate donors were designed/selected (Fig. 1.19) to be used in the synthesis of stable low-spin nickel(II) complexes. The variation in the bridges (C2, C3 and C4) and the dimethyl substitution were introduced in the view of controlling steric and electronic properties of the complexes.

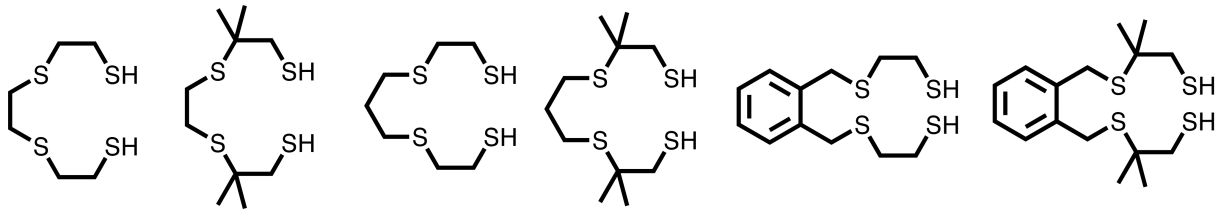


Fig. 1.19. Tetradentate chelating $S_2S'_2$-donor ligands selected for the synthesis of low-spin nickel complexes.

The second phase was to use new bidentate S_2-donor ligands for the synthesis of $Ni(S_2)_2$ complexes thereby providing flexibility around the Ni center (Fig. 1.20). The R groups were varied and the dimethyl groups were introduced in the view of fine-tuning the geometrical and electronic properties of the complexes.

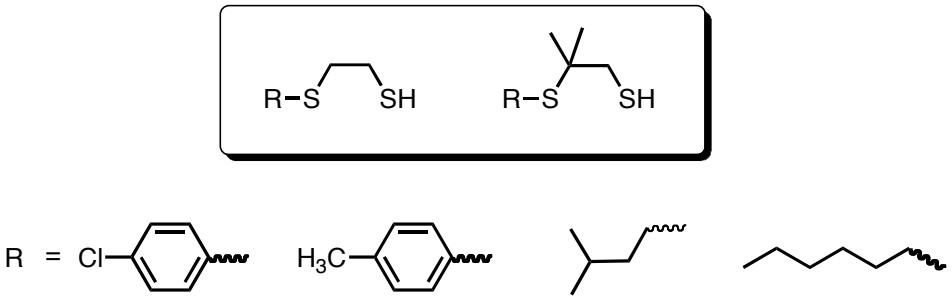


Fig. 1.20. Bidentate chelating SS'-donor ligands selected in the present study.

The third phase was using the low-spin nickel complexes synthesized with the $S_2S'_2$-donor and SS'-donor ligands in the synthesis of heterodinuclear [NiFe] complexes by reacting them with Fe moieties such as $[Fe(Cp)(CO)]^{2+}$ (Cp, cyclopentadienyl).

The final phase was to use Ru-containing moieties such as $[Ru(bpy)_2]^{2+}$ and $[Ru(tpa)]^{2+}$ instead of iron-containing moieties in order to enhance the stability of the model systems. Further to study the effect of attaching photosensitive groups directly to the redox active center (Fig. 1.21) in contrast to the conventional methods (Fig. 1.17).

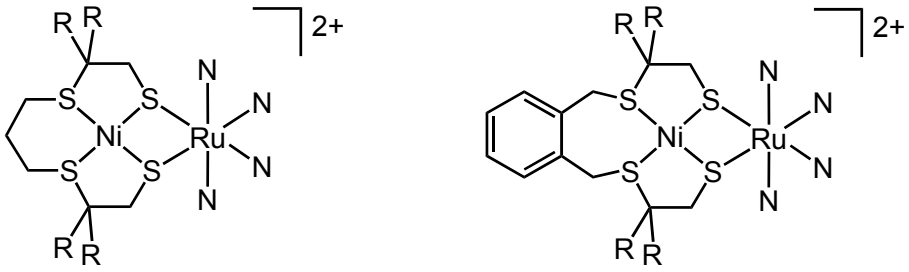


Fig. 1.21. Heterodinuclear [NiRu] complexes planned; R = H or Me; Bipyridine or tripicolylamine are used as N-donor ligands.

1.6.3. Outline of the Thesis

The design, syntheses and characterizations of new tetradentate dithioether-dithiolate ligands and bidentate thioether-thiolate ligands are presented in Chapter **2**; schemes of the syntheses of the ligands and simplified code notations for the ligands and of their precursors, and intermediates have also been provided in this Chapter.

A library of new low-spin nickel complexes of new tetradentate dithioether-dithiolate ligands are reported in Chapter **3**. These low-spin nickel complexes were reacted with $[Fe(C_5H_5)(CO)I]$ to obtain [NiFe] complexes, including one reported complex; their electrocatalytic properties towards proton reduction are also reported in Chapter **3**. Chapter **4** is devoted to analogous [NiFe] complexes based on new $[Ni(S_2)_2]$ complexes. The $[Ni(S_2)_2]$ complexes reported in this chapter were obtained by the reaction of $Ni(acac)_2$ with bidentate thioether-thiolate ligands reported in Chapter **2**.

The reactivity of four $[Ni(S_4)]$ complexes with the $[Ru(bpy)_2(EtOH)_2]$ moiety in order to make [NiRu] complexes and of their proton reducing abilities are presented in Chapter **5**. A serendipitously obtained hexanuclear Ni_6-thiolate metallacrown, its reactivity with iodine, protonation studies and the proton reduction abilities are presented in Chapter **6**. Reactivity of a new $[Ni(S_2)_2]$ complex reported in Chapter **4** towards CuI yielded a heterooctanuclear cage possessing interesting structural features including Ni–H anagostic interactions, which is reported in Chapter **7**. A light-induced C–S bond cleavage in a nickel thiolate complex with relevance to the function of methyl-coenzyme M reductase (MCR) is presented in Chapter **8**.

In Chapter **9**, a summary of all the results reported in the previous chapters, important general conclusions drawn from the studies and future prospects for further research are provided. Parts of this thesis have been published,[197-199] or have been submitted for publication;[200] some manuscripts are under preparation.[201-207]

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