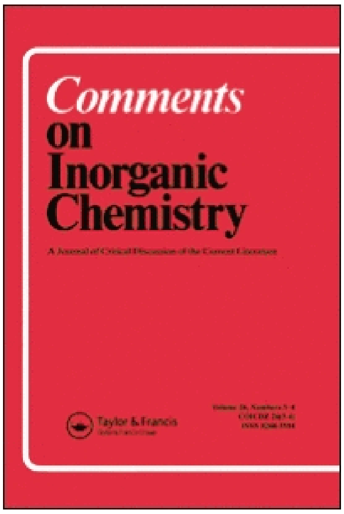

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Reactions of H_2 with the Nickel Site(s) of the [FeNi] and [FeNiSe] Hydrogenases: What Do the Model Complexes Suggest?

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In recent years, the structure(s) of the active site(s) of the nickel-containing hydrogenases (H_2ases) and the mode of H_2 activation at the nickel site(s) of these enzymes have been explored in detail. In such pursuit, spectroscopic and kinetic measurements have been performed on several H_2ases. Despite all these data, the mechanism(s) of H_2 assimilation and H_2 evolution by the microorganisms remain rather elusive at the present time. Several groups, including ours, have therefore adopted the modeling approach to elucidate the structure and function of the biological nickel site. This review contains a concise account of the spectroscopic experiments on the enzymes as a background for the subsequent modeling studies. It also provides a comprehensive summary of the modeling work by our group and others. Specific attempts have been made to correlate the spectral and reactivity parameters of good model complexes with those of the H_2ases and derive conclusions regarding structural and functional characteristics of the biological nickel site.

Key Words: *nickel-containing hydrogenases, model complexes, hydride intermediates, reactions with dihydrogen*

INTRODUCTION

A large number of bacteria and algae are known to contain hydrogenases (H_2ases), enzymes that catalyze the reversible oxidation of dihydrogen:

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

Methanogenic,[1] sulfate-reducing,[2] phototrophic[3] and N_2-fixing bacteria,[4] to name a few, all contain H_2ases but employ them in a variety of metabolic roles that are specific to the needs of the respective organism.[5] In fact, a particular microorganism may contain more than one type of H_2ase in order to maintain its cellular requirements. Many factors govern the cellular function of a H_2ase in an organism, including the reaction direction (uptake or production of H_2), the cellular location (membrane-bound, periplasmic, or cytoplasmic) and the identity and location of outside electron/proton transfer group(s).

CLASSIFICATION OF HYDROGENASES

H_2ases are considered a heterogeneous class of enzymes, differing in immunological, catalytic, and structural (metal content, types of clusters, amino acid sequences) properties, in addition to their sensitivity to inhibitors.[6] Nevertheless, these enzymes can easily be grouped into two main classes based on their metal content:

(1) Fe-only H_2ases ([Fe] H_2ases).
(2) Ni-containing H_2ases ([FeNi] H_2ases), some of which also contain selenium and therefore constitute a subdivision of this class, the [FeNiSe] H_2ases.

The [Fe] H_2ases perform their enzymatic function using only Fe/S clusters at their active sites. The [Fe] H_2ase from the sulfate-reducing bacterium *Desulfovibrio (D.) vulgaris*[7] is one such enzyme that contains two [4Fe-4S] clusters and one [3Fe-xS] cluster.

The [FeNi] H_2ases contain Fe/S clusters as well as one nickel atom. The Fe/S clusters in *D. gigas*[8] are arranged as two [4Fe-4S] and one [3Fe-4S] clusters, while one [4Fe-4S] and one [3Fe-xS] core exist in the enzyme from the purple photosynthetic bacterium *Thiocapsa roseopersicina.*[9–11] The [FeNiSe] H_2ases which constitute a subset of the [FeNi] H_2ases, contain equimolar amounts of nickel and selenium in addition to the Fe/S clusters. The H_2ases from *D. baculatus*[12] and *D. desulfuricans*[13] each contain two [4Fe-4S] clusters, one nickel and one selenium, and are examples of [FeNiSe] H_2ases.

STRUCTURAL STUDIES ON HYDROGENASES

The medium-resolution three-dimensional structure of the [FeNi] H_2ase from *T. roseopersicina* has been determined with the aid of electron microscopy and crystallographic image processing on microcrystals of the enzyme.[14] The enzyme is cylindrical in shape (outer diameter, 11 nm; height, 8 nm) and comprises six $\alpha\beta$ dimeric units. The wall of the cylinder appears to be 3 nm thick; this results in a 5 nm wide channel along the long axis of the cylinder. The total volume of the $\alpha_6\beta_6$ hexamer consisting of 62 and 26 kDa subunits was determined to be 6.3×10^5 Å^3. The structure of this enzyme can also be described as consisting of two rings, one stretched on top of the other in a slightly staggered manner.[14]

Very recently, the 2.85 Å structure of the [FeNi] H_2ase from *D. gigas* has been reported.[15] The results of this study reveal that the three Fe/S clusters are sequentially arranged in the small subunit (28 kDa), while the nickel site, located in the large subunit (60 kDa), remains in close proximity to only one [4Fe-4S] cluster of the small subunit. The Fe/S clusters appear to be neatly aligned so as to provide a pathway for electrons to flow to and from the nickel site during enzymatic turnover.[15] However, they are arranged such that the [3Fe-4S] cluster, with a comparatively more positive midpoint potential (E_m) of −35 mV, lies between the two [4Fe-4S] clusters (E_m = −290 and −340 mV, respectively). This [3Fe-4S] cluster would therefore remain reduced during catalytic activity and thus be responsible for limiting the rate of electron flow. The absence of this cluster in other nickel-containing H_2ases, specifically [FeNiSe] H_2ases,[16] raises questions as to the nature of its role during electron-transfer in the *D. gigas* enzyme. It is likely that alternative electron pathways exist in different classes of nickel-containing H_2ases.

The structural data for the *D. gigas* enzyme also identify a possible pathway for protons to travel to and from the nickel site.[15] Four histidines and one glutamate residue have been located between the active site and the molecular surface. These amino acids (the glutamate and three of the four histidines) are highly conserved among the [FeNi] and [FeNiSe] H_2ases[17,18] and are capable of transferring protons (pK_a (His) ~6.0–7.0, pK_a (Glu) ~4.0–4.5).

The same crystallographic study depicts the active site as containing two metal ions, one nickel and the other of unknown identity (X).[15] The nickel atom is coordinated to four thiolates from cysteine residues, two of which are bridging and serving as ligands to the other metal atom. Previous XAS studies[19] indicated that four sulfur atoms at 2.20 Å were ligated to nickel in this enzyme; no other scatterer(s) around nickel was positively identified in such measurements. Nevertheless, the structural parameters and the iron content of the [FeNi] H_2ase from *D. gigas* (12 + 1 iron atoms per molecule) have prompted the authors of the crystallographic study to tentatively assign the X site as a fully occupied iron atom.

The assignment of X as an iron atom, however, faces several immediate problems. For example, the structural results assign three putative water molecules, in addition to the two bridging thiolates, as ligands for the iron site.[15] This represents a highly unlikely coordination environment for an iron center. In addition, results of the EPR studies performed on unenriched as well as isotopically (^{57}Fe) labeled *D. gigas* enzyme suggest an active site composed of mononuclear nickel,[20] with no other EPR-active metal center in its vicinity. It is also quite difficult to explain all the EPR spectra of the nickel site in different states (vide infra) in terms of a bimolecular Fe-Ni composition.

Clearly, the structural data on the *D. gigas* enzyme have raised more questions regarding the coordination structure of the nickel site. At this time, a high-resolution structure of another [FeNi] H_2ase is essential to establish the identity of X and the coordination structure of the nickel site in general.

SPECTROSCOPIC STUDIES

Various spectroscopic methods have been used successfully to probe the architecture of the active site(s) of the nickel-containing H_2ases. These techniques include Electron-Nuclear Double Resonance (ENDOR) and Electron Paramagnetic Resonance (EPR) spectroscopy, and X-ray Absorption Spectroscopy (XAS). The measurements have provided in addition to structural information concerning the active site(s), insights into the mechanism of H_2 activation that appears to be common to all nickel-containing H_2ases (Fig. 1).

The [FeNi] H_2ases, in the "as-isolated" form (derived from crude extracts or purified enzymes), exhibit EPR signals at low temperatures (~20 K) that most likely arise from the oxidized [3Fe-xS] cluster. These enzymes also display EPR signals at comparatively higher temperatures (~120 K) that are associated with the Ni center. These EPR signals are termed "Ni-A" with g-values of 2.31, 2.23, and 2.02, and "Ni-B" with g-values 2.32, 2.16, and 2.02.[21,22] The two rhombic signals Ni-A and Ni-B have been assigned to Ni(III) centers in the enzymes on the basis of the hyperfine splitting values observed with $^{61}Ni(I = 3/2)$ enriched enzymes.[23-25]

Incubation of the enzyme with reductants like dithionite and $H_2(g)$ leads to the disappearance of both Ni-A and Ni-B signals. It is believed that Ni remains in the +2 oxidation state in this EPR-silent form. Prolonged reduction with $H_2(g)$ eventually produces a new EPR signal, "Ni-C", with g-values 2.19, 2.16, and 2.02.[24,25] The Ni-C signal is also observable at ~120 K. If the EPR spectrum is recorded at 20 K, a more complicated signal that arises from interac-

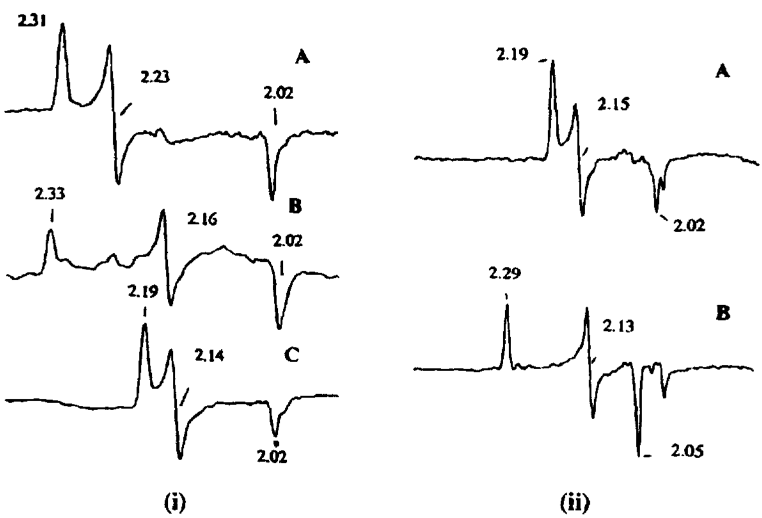


FIGURE 1 (i) EPR spectra of *D. gigas* H_2ase (A) Ni-A Signal, (B) Ni-B signal, (C) Ni-C signal, and (ii) T. roseopersicina (A) Ni-C signal, and (B) Ni-L signal.

tion of the nickel site in the C form with a reduced $[4Fe\text{-}4S]^{+}$ cluster is obtained. Upon further reduction with $H_2(g)$, the Ni-C signal disappears (Ni-R state). This entire sequence of spectral changes can be achieved in the reverse order; chemical oxidation of the most reduced EPR-silent species (Ni-R) with oxidants like $[Fe(CN)_6]^{3-}$ affords each intermediate species (EPR-active or silent) in a reverse sequence.

Although some [FeNiSe] H_2ases are EPR-silent with respect to Ni in the as-isolated state, several do exhibit weak signals (g = 2.33, 2.20, 2.02; g = 2.34, 2.16, 2.02) that are reminiscent of the Ni-A and Ni-B signals of the [FeNi] H_2ases.[12,13,26,27] Like the [FeNi] H_2ases, reduction of [FeNiSe] H_2ases with sodium dithionite or $H_2(g)$ produces an EPR-silent state. However, unlike the [FeNi] H_2ases, only a short incubation period with $H_2(g)$ is required for the corresponding Ni-C signal (g = 2.22, 2.17, 2.02) to appear.

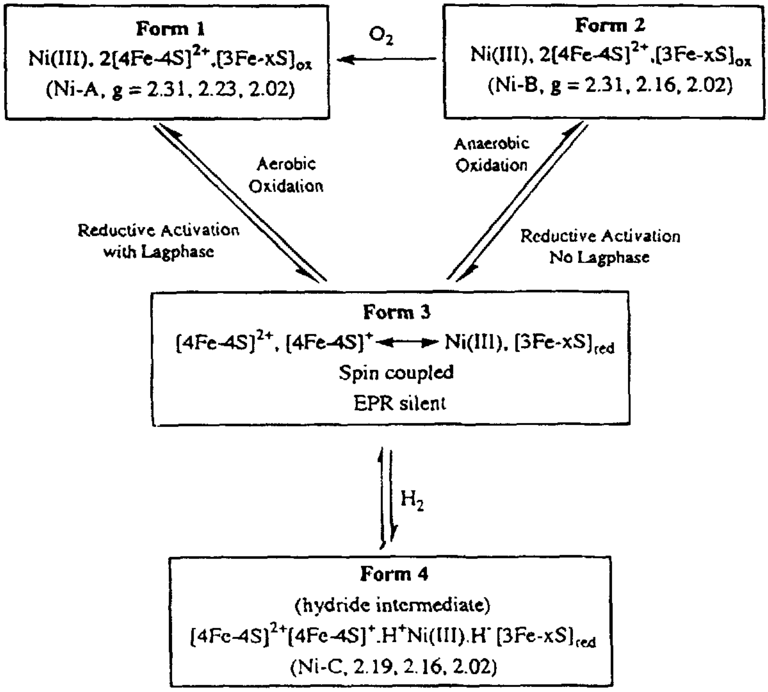
The appearance of the Ni-C signal under reductive conditions with $H_2(g)$ strongly suggests that the nickel site and the H_2 molecule are two key players in the catalytic cycle of these enzymes. Indeed, ^1H ENDOR data on the Ni-C form of the [FeNi] H_2ase from *T. roseopersicina* reveal the presence of a solvent-exchangeable proton that originates from dihydrogen and is directly ligated to the Ni center.[28] Several other observations also support the presence of a hydrogenic ligand at the biological nickel site. For example, the nickel species responsible for the Ni-C signal is sensitive to light.[22] When the enzymes in the C form are illuminated at very low temperatures (22 K), the Ni-C signal is replaced with a new "Ni-L" EPR signal (g = 2.29, 2.13, 2.05 for [FeNi] H_2ase).[28] Although this transformation is an irreversible one at very low temperatures, one can recover the Ni-C signal by "thawing" the sample at 200 K. This has been explained in terms of photodissociation of the hydrogenic ligand from the nickel site and its religation to the nickel center in the semifluid medium at comparatively higher temperatures. The ENDOR data are consistent with this explanation; the exchangeable proton is no longer observed upon formation of the Ni-L signal, but its presence is reestablished once the sample is annealed back to Ni-C. Further support in favor of the hydrogenic ligand comes from the fact that when the identical illumination experiment is performed in D_2O, the rate of disappearance of the Ni-C signal is found to be six times slower than that observed in H_2O.

Almost all [FeNi] and [FeNiSe] H_2ases are reversibly inhibited by carbon monoxide.[29-31] While the Ni-A and Ni-B EPR signals of the as-isolated enzymes remain unperturbed in the presence of CO for as long as five hours at room temperature, the Ni-C signal is sensitive to the presence of CO. CO supresses the hydrogenase activity and transforms the Ni-C signal into another which is often referred to as the "Ni-CO" signal. This species, like Ni-C, is also light-sensitive.[29,32] Since illumination of samples exhibiting the "Ni-CO" signal also produces the "Ni-L" species, it is evident that the hydrogenic ligand and CO occupy the same binding site on nickel.[31] The [FeNiSe] H_2ases appear to be more sensitive to CO-inhibition.[33]

ROLE OF NICKEL IN THE [FeNi] AND [FeNiSe] H_2ases: PROPOSED MECHANISMS

Since the [FeNi] and [FeNiSe] H_2ases consistently exhibit the Ni-C signal under reductive conditions with $H_2(g)$, it is most likely that this EPR signal represents one key intermediate species in the catalytic cycle of the enzymes. The assignment of this and all other EPR signals associated with Ni and the Fe/S clusters in these enzymes has led to various *proposed* mechanisms for the activation of H_2 by nickel-containing H_2ases. These mechanisms differ mainly with respect to the sequence of changes in the oxidation state of the nickel center of the enzyme.

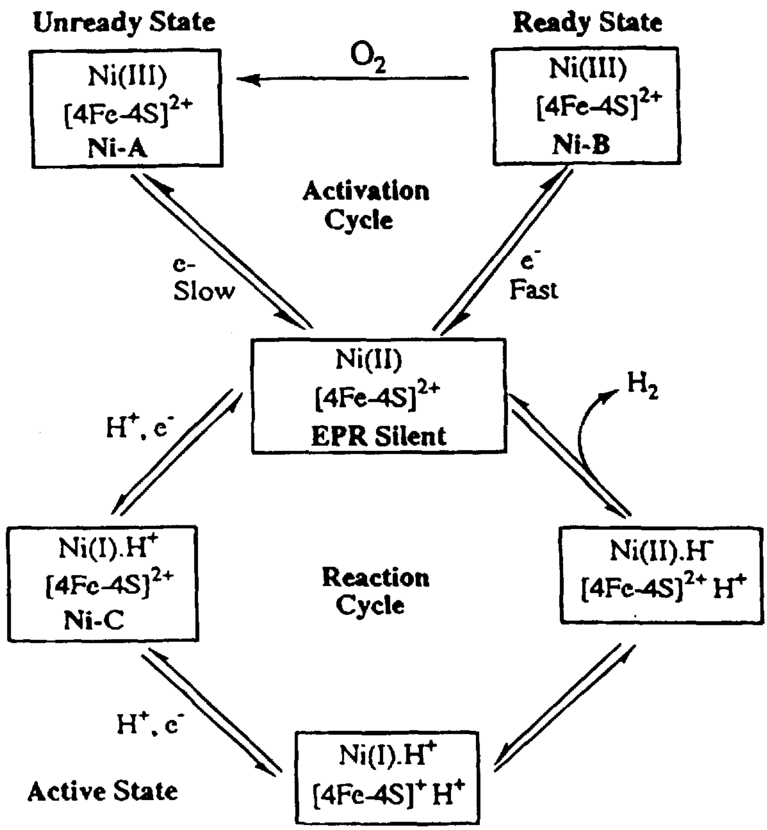
In one proposed (Class I) mechanism,[20] the nickel site is believed to shuttle between Ni(III) and Ni(II). As shown in Scheme 1, the Ni-A and Ni-B signals are both assigned to Ni(III) in the oxidized forms of the enzymes; they belong to the oxygenated and deoxygenated forms, respectively. The [4Fe-4S] clusters are EPR silent (+2 oxidation state) and the [3Fe-xS] cluster is EPR active with g = 2.02. Upon incubation under $H_2(g)$, an EPR-silent form is achieved in which not only is the [3Fe-xS] cluster reduced to an EPR-silent state, but one of the $[4Fe\text{-}4S]^{2+}$ clusters is also reduced to a $[4Fe\text{-}4S]^{+}$ cluster and remains magnetically coupled with the Ni(III) center, thus giving rise to an EPR-silent species. This spin coupling between the Fe/S cluster and Ni(III) is broken when Ni(III)-H^- and $[4Fe\text{-}4S]^{+}H^{+}$ are formed following heterolysis of H_2 at the active site. Ni(III)-H^- is therefore the species responsible for the Ni-C signal



SCHEME 1 Class I mechanism for the catalytic activation cycle of [FeNi] hydrogenases (proposed by Moura and co-workers).

in the Class I mechanism. Further reduction under H_2 converts Ni-C into an EPR-silent Ni(II) state.

The Class II mechanism,[34,35] on the other hand, invokes the use of Ni(III), Ni(II) and Ni(I) throughout the cycle (Scheme 2). The Ni-A and Ni-B signals are associated with the "unready" and "ready" forms of the enzymes and, like the Class I mechanism, they arise from Ni(III) centers. The EPR-silent form generated under H_2 corresponds to Ni(II), while further reduction under H_2 gives rise to a Ni(I) species that is responsible for the Ni-C signal. Cammack and his group have suggested a Ni(I)-H^+ (isoelectronic with Ni(III)-H^-) formalism for this state, whereas Albracht and co-workers describe it as a hydride intermediate, Ni(I)-H^-.



SCHEME 2 Class II mechanism for the catalytic cycle of [FeNi] hydrogenases (proposed by Cammack *et al*).

In addition to the Ni(III)-H$^-$ and Ni(I)-H$^-$ species, a Ni-dihydrogen (Ni-η^2-H_2) moiety has also been suggested as a possiblity for the key intermediate Ni-C.[31,32] This is an interesting suggestion since both Ni(III)-η^2-H_2 and Ni(I)-η^2-H_2 species represent unprecedented examples in biological systems. Chemically, a stable dihydrogen complex (stable enough to allow EPR studies) in aqueous solution at ambient temperature is also unlikely. The brief discussion of dihydrogen complexes in the following section supports this statement.

TRANSITION METAL–DIHYDROGEN COMPLEXES

The transition metal–dihydrogen chemistry is quite extensive.[36,37] Results to date indicate that, in addition to being reaction intermediates, metal–dihydrogen species (M-η^2H_2) are also stable enough to be detected by spectroscopic techniques and even isolable in several cases.[38] The nature of the bonding that exists between the H_2 ligand and the metal center in a dihydrogen complex (M-η^2-H_2) consists of $H_2(\sigma) \rightarrow M(\sigma)$ donation as well as $M(\pi) \rightarrow H_2(\sigma^*)$ back-donation. Both the σ donation and the $\pi \rightarrow \sigma^*$ back-donation contribute to weakening of the H–H bond.[36–38] The stability of this metal–dihydrogen unit in a complex is therefore greatly influenced by the electron density at the metal center as well as the nature of the ancillary ligands in the complex. In general, strong back-donation from an electron-rich metal to the σ^* orbital of H_2 results in the formation of the classical metal dihydrides (with concomitant oxidation of the metal center), while limited back-donation from the metal gives rise to a weakly bound H_2 ligand. Weak binding of H_2 often leads to facile H_2 elimination from the metal center.[36,37]

In between these two extremes lie the activated H_2 complexes. The presence of a metal center is always required to promote any kind of activation of the H_2 molecule (H–H bond energy = 103 kcal/mol). Although the H_2 molecule itself is a very weak acid (pK_a = 35), coordination of H_2 to a transition metal ion substantially activates the molecule toward heterolysis.[39] For example, the pK_a of coordinated H_2 in the ruthenium complex $[(\eta\text{-}C_5H_5)Ru(CO)(PCy_3)(\eta^2\text{-}H_2)]^+$ is 17.6.[39] Comparison of the kinetic selectivity for deprotonation of a metal hydride versus a metal–dihydrogen species indicates that dihydrogen has greater kinetic acidity. This is due to polarization of the bonding electrons toward the metal center which in turn creates partial positive charges on the H atoms.[36] Along the same line, in metal hydrides, protonation occurs at the hydride and not at the metal atom.

Reaction of H_2 with metal centers under basic conditions often leads to heterolysis of the H–H bond. In the so-called *base-assisted hydride donation by H_2*, the combination of a base and free H_2 serves as an effective reagent for the syntheses of metal hydrides.[36] Indeed, base-assisted heterolytic cleavage of dihydrogen has proven to be a more effective means of obtaining some metal–hydride complexes than the use of the standard hydride reagents $LiAlH_4$ and $NaBH_4$. For example, the complexes $[M(H^-)_2(PR_2CH_2CH_2PR_2)_2]$ (R = Et, M = Fe, Os; R = Ph, M = Ru, Os) are prepared from the reaction of the Fe group dichlorides with two equiv of a relatively weak base and $H_2(g)$.[40]

In many cases, the reverse reaction takes place upon protonation of the metal–hydride complexes. An example of protonation of a metal–hydride complex that generates an (η^2-H_2) moiety is the formation of $[(\eta\text{-}C_5H_5)Ru(CO)(PCy_3)(\eta^2\text{-}H_2)]BF_4$ from $[(\eta\text{-}C_5H_5)Ru(CO)(PCy_3)(H^-)]$.[39] The presence of the (η^2-H_2) ligand in this complex has been verified by H-D coupling in its ^1H NMR spectrum (protonation of deuteride provides the HD complex). Another example of a metal–hydride complex that gives rise to an (η^2-H_2) intermediate is the reaction of H^+ with $[Rh(H^-)(CO)(BuS_4)]$ (BuS_4 = 1,2-bis((2-mercapto-3,5-di-tert-butylphenyl)thio)ethanato(2^-)).[41] In this reaction, $H_2(g)$ is released after formation of the intermediate (Rh-η^2-H_2) species.

The two reactions, namely, base-assisted heterolytic cleavage of H_2 at the metal site and formation of (metal-η^2-H_2) species (and subsequent release of H_2) upon protonation of the metal–hydride complexes, are relevant to the enzymatic activity of the [FeNi] and [FeNiSe] H_2ases. We and others[42,43] believe that a base-assisted hydride donation is taking place during the catalytic cycles of the [FeNi] and [FeNiSe] H_2ases. Catalytic studies with the nickel-containing H_2ases indicate that the consumption of $H_2(g)$ occurs with the heterolytic cleavage of the dihydrogen molecule to form a nickel–hydride species (detected by EPR spectroscopy) and a proton. A nearby basic amino acid residue is believed to act as the proton-acceptor sites in such reactions.[43] Along the same lines, the production of H_2 by the nickel-containing H_2ases could also proceed via protonation of the nickel–hydride intermediate(s). This is supported by the fact that while consumption of H_2 by the enzymes takes place at higher pH values, production is maximal in more acidic media.[44]

To date, very few metal–dihydrogen complexes have been isolated. The most notable one is the crystalline complex $[W(H_2)(CO)_3(Pcy_3)_2]$ (cy = cyclohexyl), the structure of which has been confirmed by NMR spectroscopy and neutron diffraction methods.[45] It is quite evident that the preparation and characterization of most metal–dihydrogen complexes require stringent reaction conditions like low temperature and high H_2 pressure.[41,45,46] Such conditions are very different from the ones for the enzymatic processes.

Various groups have suggested that the Ni-C signal in the [FeNi] H_2ases arises from a (Ni-η^2-H_2) species.[31,32,47] This conclusion is derived from the lack of any observable hyperfine coupling in the EPR spectra that would confirm the presence of hydride ($I = 1/2$) as a ligand to nickel. Given what is now known about the reactivity of the metal–dihydrogen species, it is very reasonable to suggest that a (Ni-η^2-H_2) moiety could exist as a short-lived reaction intermediate during the catalytic cycles of the [FeNi] and [FeNiSe] H_2ases, but *not* as the relevant species that gives rise to the strong and persistent Ni-C signal. Moreover, the exact oxidation state of Ni during catalytic activity of the enzyme(s) must be established before the possibility of such a (Ni-η^2-H_2) moiety is considered seriously.

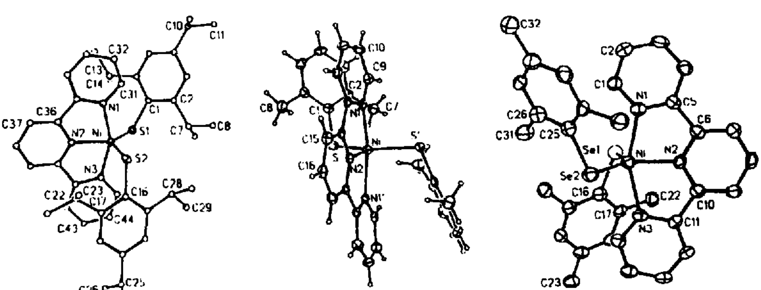
MODEL COMPLEXES THAT MIMIC THE BIOLOGICAL NICKEL SITE(S)

Since the detection of nickel as an integral component of many H_2ases,[48] various groups have undertaken the task of determining its functional role in these enzymes.[49–52] Most of these groups have adopted the synthetic analogue approach[53] to elucidate the structure(s) and function of the biological nickel site. In this approach, relatively low molecular weight complexes are synthesized and their spectral parameters are compared with those of the enzyme. Promising models, or synthetic analogues, are then further studied to establish functional resemblance. The goal of this approach is to obtain a good mimic of the active site and establish the structure and function of the biological unit by comparing its properties with those of the synthetic species.

In our analogue approach to the nickel-containing H_2ases which we initiated in 1986,[42] we focussed our attention on the nickel site of the [FeNi] H_2ase from the purple photosynthetic bacterium *T. roseopersicina*. To date, the nickel site of this enzyme is assumed to be mononuclear and hence our modeling attempts so far have been restricted to the design of mononuclear nickel complexes.

In this first phase of our analogue approach to the nickel site(s) in [FeNi] and [FeNiSe] H_2ases, the research efforts were directed toward syntheses of discrete mononuclear nickel thiolates and selenolates. Several nickel complexes of S- and Se-donor ligands were prepared and structurally characterized.[54,55] In such pursuit, a synthetic strategy that led to the successful isolation of the desired monomeric complexes and avoided the prevalent formation of thiolato-bridged dimeric/trimeric and insoluble polymeric species was developed.

The XAS data for *T. roseopersicina* H_2ase indicate a trigonal bipyramidal (tbp) geometry around nickel at the active site. The coordination sphere consists of 3–4 N/O and ~2 S donor atoms.[56] This structural data prompted us to advance the synthetic analogue approach into its second phase, in which model complexes with $[NiN_3S_2]$ chromophores were synthesized and characterized by crystallographic techniques. The mononuclear nickel complexes $[Ni(terpy)(C_6F_5S)_2]$ (**1**), $[Ni(terpy)(2,4,6\text{-}(i\text{-}Pr)_3C_6H_2S)_2]$ (**2**)[57] and $[Ni(terpy)(2,6,\text{-}(Me)_2C_6H_3S)_2]$ (**3**)[58] were synthesized by using terpy as the the N-donor ligand and aromatic thiolates as the S-donors (Fig. 2). The three N-donor atoms from terpy, along with two S-donor atoms from the bulky and less basic aryl thiolates, provide the desired pentacoordination around nickel in all three complexes. In the crystalline state, **2** and **3** remain pentacoordinated (distorted tbp geometry), while **1** exists as a distorted octahedral complex with a labile acetonitrile (solvent) molecule at the sixth site. The steric crowding created by two bulky thiolates in **2** and **3** allows isolation of the pentacoordinated species but leaves enough space for small



(**2**) (**3**) (**4**)

FIGURE 2 Structures of $[Ni(terpy)(2,4,6\text{-}(i\text{-}Pr)_3C_6H_2S)_2]$ (**2**), $[Ni(terpy)(2,6\text{-}(Me)_2C_6H_3S)_2]$ (**3**) and $[Ni(terpy)(2,4,6\text{-}(Me)_3C_6H_2Se)_2]$ (**4**).

molecules like CO to bind to nickel at the sixth site. The XAS data of these three complexes are very similar to one another and also match very well the XAS data for the nickel site in *T. roseopersicina* H_2ase.[57,58] These three pentacoordinated complexes containing the [NiN_3S_2] chromophore (**1–3**) are therefore examples of good structural models of the nickel site in [FeNi] H_2ases. A fourth tbp complex with Se ligation, namely [Ni(terpy)(2,4,6-$(Me)_3C_6H_2Se)_2$] (**4**), was also synthesized. This complex with the [NiN_3Se_2] chromophore allowed us, for the first time, to examine the features of the Ni-Se coordination in the active site of the [FeNiSe] H_2ases.[59] The existence of these analogues now provides a means by which the reactivity of the sixth site in coordinatively unsaturated nickel complexes can be explored.

HYDRIDE (H^-) ADDUCTS OF THE Ni(I) MODEL COMPLEXES EXHIBIT EPR SPECTRA THAT RESEMBLE THE Ni-C SIGNALS OF THE [FeNi] AND [FeNiSe] H_2ases

Low temperature EPR measurements performed in DMF solutions establish that the model complexes **1–4** are all easily reduced to the corresponding Ni(I) species with a reductant like dithionite.[57–59] These Ni(I) complexes of the type [Ni^I(terpy)$(SAr)_2$]$^-$ bind CO reversibly, much like the nickel-containing H_2ases.[57–59] More interestingly, they also bind hydride (H^-) to provide [Ni^I(terpy)(2,4,6-(*i*-Pr)$_3C_6H_2S)_2(H^-)]^{2-}$ (**5**), [Ni^I(terpy)(2,6-$(Me)_2C_6H_3S)_2(H^-)]^{2-}$ (**6**) and [Ni^I(terpy)(2,4,6-$(Me_3)C_6H_2Se)_2(H^-)]^{2-}$ (**7**).[57,59,60] The EPR spectra of these Ni(I)-H^- adducts closely resemble the Ni-C signals of the [FeNi] and [FeNise] H_2ases. Clearly, this is good evidence in favor of a Ni(I)-H^- formalism for the catalytically active Ni-C species.

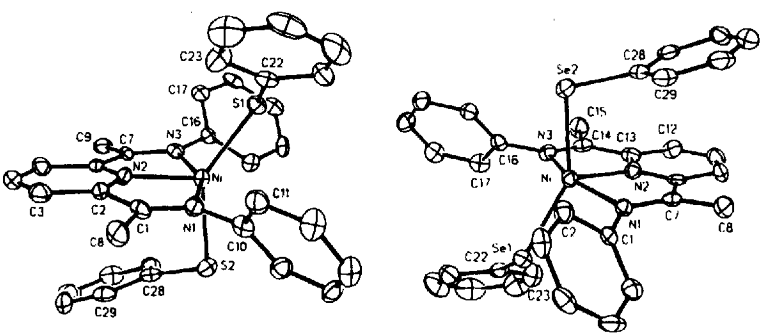
Although the Ni(II) complexes **1–4** can be readily reduced to the corresponding Ni(I) species, oxidation of **1–4** in DMF or DMSO solutions proceeds only marginally at low temperature. The complexes **1–4** therefore fall short of being qualified as functional models since the Ni(I) ↔ Ni(II) ↔ Ni(III) transformation is not readily achieved in this type of complex. Also, these model complexes exhibit minimal reactivity toward H_2(g) at ambient temperature and pressure.

TOWARD FUNCTIONAL MODELS OF THE BIOLOGICAL NICKEL SITE

In the next phase of our analogue approach, we sought model complexes that would readily provide the corresponding Ni(I) and Ni(III) species. In such a pursuit, the terpy ligand was replaced with the 3N donor ligand 2,6-bis[1-(phenylimino)ethyl]pyridine (DAPA). DAPA contains "harder" N-donor atoms and a less extensive π system and is accordingly expected to be less effective than the terpy ligand in removing electron density from the metal center in $[NiN_3E_2]$ (E = S, Se) complexes. This in turn ensures the integrity of the Ni(III) center in the oxidized species.

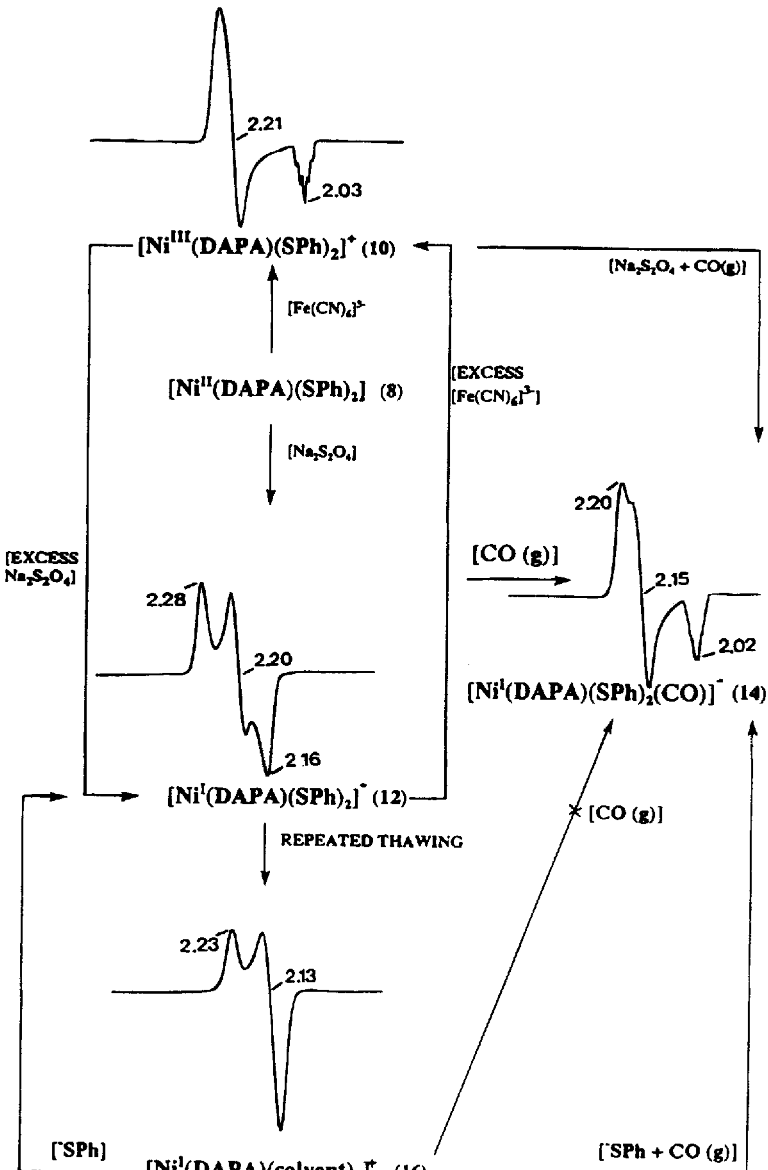
Quite in line with these expectations, the two complexes $[Ni(DAPA)(SPh)_2]$ (**8**) and $[Ni(DAPA)SePh)_2]$ (**9**) with $[NiN_3E_2]$ (E = S,Se) chromophores (Fig. 3) do exhibit superior stability upon both oxidation and reduction of the Ni(II) center(s).[60,61] In **8** and **9**, the DAPA ligand provides enough steric constraint for isolation of the desired pentacoordinated complexes with simple PhS^- and $PhSe^-$ ligands; with the more compact terpy ligand, only bulky thiolates and selenolates provide the pentacoordinated species.

The two DAPA complexes **8** and **9** are readily oxidized and reduced by biologically relevant oxidants and reductants, and the transformation Ni(III) ← Ni(II) → Ni(I) is reversible. The reactions of the Ni(I) and Ni(III) species are included in Schemes 3 and 4. Interestingly, the

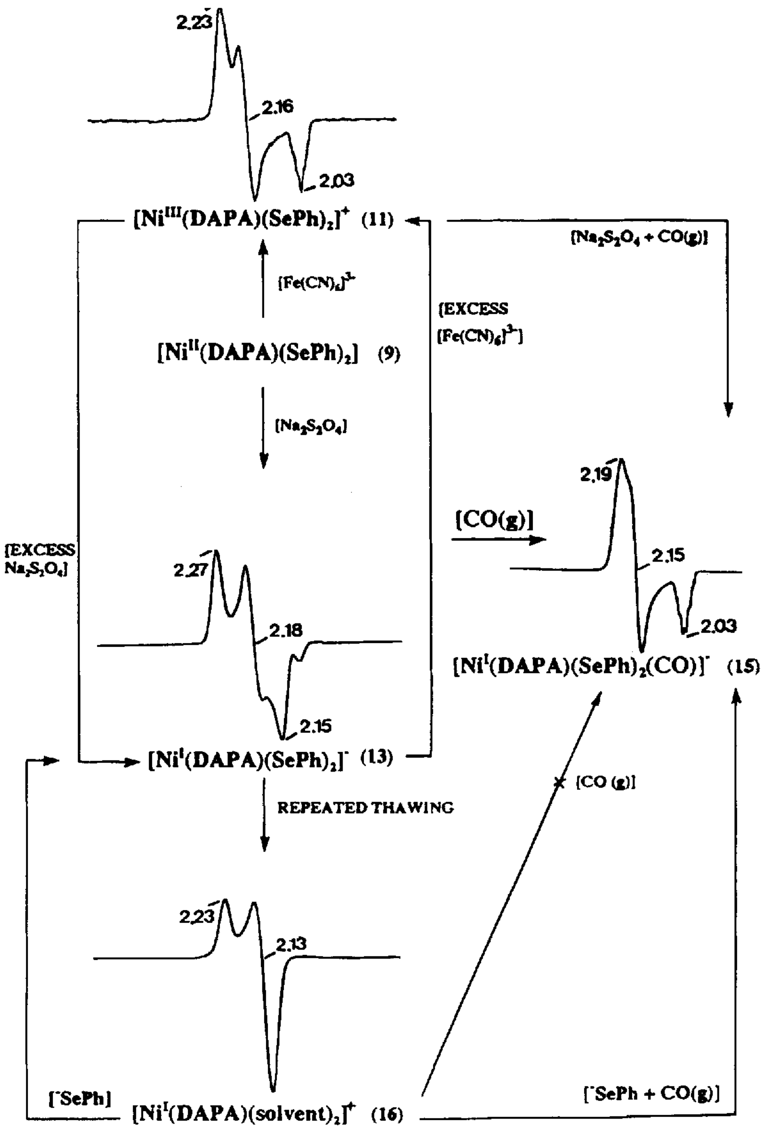


(**8**) (**9**)

FIGURE 3 Structures of $[Ni(DAPA)(SPh)_2]$ (**8**) and $[Ni(DAPA)(SePh)_2]$ (**9**).


2.21
2.03
[Ni^{III}(DAPA)(SPh)$_2$]$^+$ (10)
[Fe(CN)$_6$]$^{3-}$
[Ni^{II}(DAPA)(SPh)$_2$] (8)
[Na$_2$S$_2$O$_4$]
[EXCESS [Fe(CN)$_6$]$^{3-}$]
[Na$_2$S$_2$O$_4$ + CO(g)]
[EXCESS Na$_2$S$_2$O$_4$]
2.28
2.20
2.16
[Ni^{I}(DAPA)(SPh)$_2$]$^-$ (12)
[CO (g)]
2.20
2.15
2.02
[Ni^{I}(DAPA)(SPh)$_2$(CO)]$^-$ (14)
REPEATED THAWING
[CO (g)]
2.23
2.13
[$^-$SPh]
[Ni^{I}(DAPA)(solvent)$_2$]$^+$ (16)
[$^-$SPh + CO (g)]

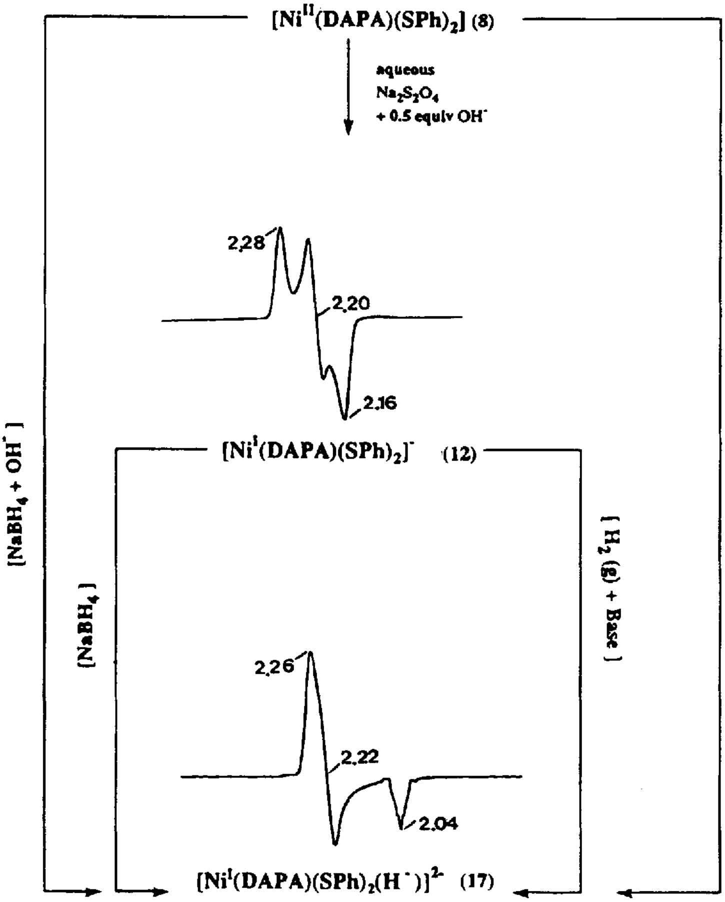
SCHEME 3


2.23
2.16
2.03
[Ni^{III}(DAPA)(SePh)$_2$]$^+$ (11)
[Na$_2$S$_2$O$_4$ + CO(g)]
[Fe(CN)$_6$]$^{3-}$
[EXCESS [Fe(CN)$_6$]$^{3-}$]
[Ni^{II}(DAPA)(SePh)$_2$] (9)
[Na$_2$S$_2$O$_4$]
2.19
2.15
2.03
[CO(g)]
[EXCESS Na$_2$S$_2$O$_4$]
2.27
2.18
2.15
[Ni^I(DAPA)(SePh)$_2$(CO)]$^-$ (15)
[Ni^I(DAPA)(SePh)$_2$]$^-$ (13)
[CO (g)]
REPEATED THAWING
2.23
2.13
[$^-$SePh]
[Ni^I(DAPA)(solvent)$_2$]$^+$ (16)
[$^-$SePh + CO(g)]

SCHEME 4

Ni(III) species $[Ni^{III}(DAPA)(SPh)_2]^+$ (**10**) and $[Ni^{III}(DAPA)(SePh)_2]^+$ (**11**), both stable at low temperatures, do not bind CO; in much the same way, CO does not bind to the Ni-A and Ni-B forms of [FeNi] and [FeNiSe] H_2ases. The single electron in these Ni(III) d^7 species resides in the d_{z^2} orbital. The Ni(I) species $[Ni^I(DAPA)(SPh)_2]^-$ (**12**) and $[Ni^I(DAPA)(SePh)_2]^-$ (**13**) are readily obtained from **8** and **9**, respectively, upon the addition of aqueous dithionite. The single electron in these Ni(I) d^9 species resides in the $d_{x^2-y^2}$ orbital.[60] The Ni(I) complexes **12** and **13**, like the nickel-containing H_2ases in the reduced states, bind CO reversibly. The CO stretching frequencies of the CO adducts of the Ni(I) model complexes are good indicators of the electron density at the Ni(I) centers. Since $[Ni^I(DAPA)(SPh)_2(CO)]^-$ (**14**) exhibits a slightly higher ν_{CO} (2040 cm^{-1}) than $[Ni^I(DAPA)(SePh)_2(CO)]^-$ (**15**, ν_{CO} = 2024 cm^{-1}), it is evident that between PhS^- and $PhSe^-$, the thiolate ligand donates less electron density to the nickel(I) center. This is expected on the basis of the comparatively "harder" nature of the RS^- ligand. Although the Ni(I) complexes **12** and **13** are quite stable at low temperatures, they slowly lose thiolates/selenolates at room temperature to give $[Ni^I(DAPA)(solv)_2]^+$ (**16**). The much faster rate of loss of thiolate ligands from **12** again supports the notion that a comparatively hard–soft interaction exists between RS^- and the Ni(I) center in this species. It is important to note that $[Ni^I(DAPA)(solv)_2]^+$ by itself does not bind CO, but does so in the presence of two equiv of thiolate/selenolate ligand. Clearly, binding of the strong π-acceptor CO at the Ni(I) centers in **12** and **13** is possible only in the presence of good electron donors like thiolates and selenolates. These results suggest that thiolate/selenolate coordination to the active site nickel is necessary for CO binding and formation of the EPR observable Ni-CO species.[60] The soft/soft interaction between RSe^- and Ni(I) present in **15** could also exist in the reduced state of the [FeNiSe] enzyme and thus be responsible for the increased sensitivity of these H_2ases to CO inhibition.[33]

Like the terpy analogues, the two DAPA complexes $[Ni(DAPA)(SPh)_2]$ (**8**) and $[Ni(DAPA)(SePh)_2]$ (**9**) readily react with $NaBH_4$ at low temperature to produce the hydride adducts $[Ni^I(DAPA)(SPh)_2(H^-)]^{2-}$ (**17**) and $[Ni^I(DAPA)(SePh)_2(H^-)]^{2-}$ (**18**).[60] As shown in Scheme 5, the five-line hyperfine splitting pattern around $g \approx 2$ (A = 14.5 G) in the EPR spectra of **17** and **18** indicates that



SCHEME 5

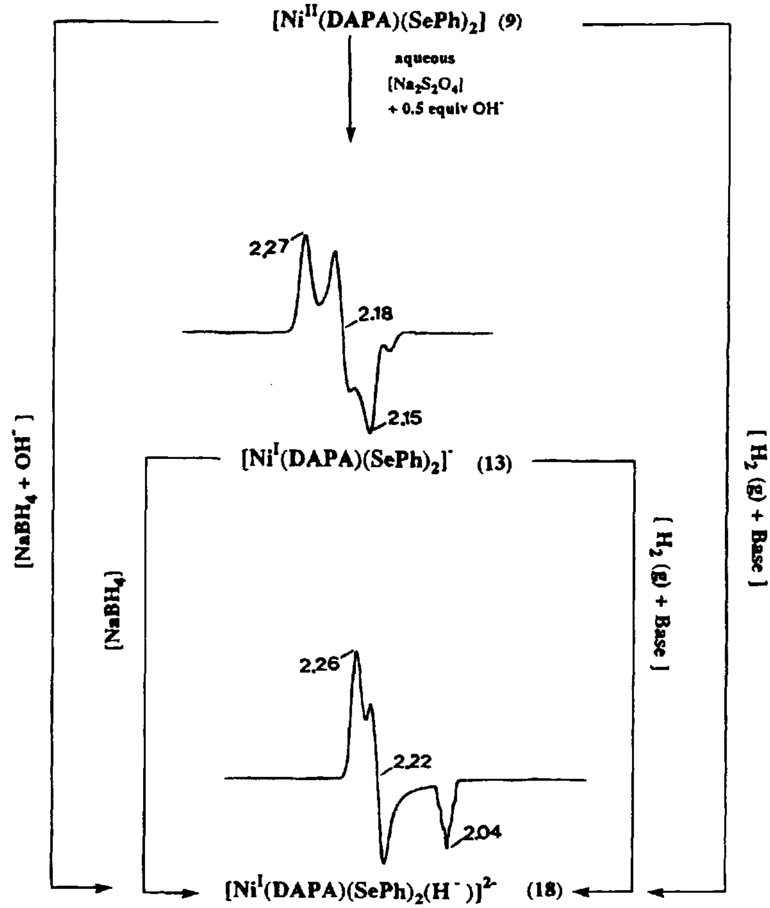
in both hydride adducts, the unpaired electron occupies the d_{z^2} orbital and interacts with two N(I = 1) nuclei at the axial positions. Binding of hydride at the sixth site of $[Ni^I(DAPA)(SePh)_2]^-$ (**12**) and $[Ni^I(DAPA)(SePh)_2]^-$ (**13**) therefore converts the $d_{x^2-y^2}$ ground state to d_{z^2}, presumably by changing the relative positions of the d orbitals following conversion of the tbp species into the octahedral ones. In the presence of a base like NaOH, the yields of the hydride adducts increase to a considerable extent. If the reduced species **12** and **13** are generated with aqueous $Na_2S_2O_4$, subsequent formation of the hydride adducts **17** and **18** with additional $NaBH_4$ is only possible in the presence of a base. Clearly, the hydride adducts are more stable in basic solution. Among all the model compounds with the $[NiN_3E_2]$ (E = S, Se) chromophore, the DAPA complexes exhibit superior hydride-binding capabilities.[60]

REACTIONS OF 8 AND 9 WITH DIHYDROGEN PRODUCE Ni(I)-H^- ADDUCTS

The most remarkable reaction of the model complexes with the $[NiN_3E_2]$ (E = S, Se) chromphores is the formation of the Ni(I)-H^- species (with Ni-C-like EPR spectra) in their reaction with dihydrogen at ambient temperature and pressure.[60,62] At 298 K, passage of H_2 (g) through solutions of $[Ni(DAPA)(SPh)_2]$ (**8**) and [Ni(DAPA) $(SePh)_2]$ (**9**) in DMF affords samples that exhibit weak EPR signals of the Ni(I)-H^- adducts **17** and **18** (Schemes 5 and 6). Interestingly, the presence of a base like NaOH in the reaction mixtures enhances the intensities of the EPR signals to a considerable extent. This enhancement of hydride formation in the presence of NaOH is reminiscent of the aforementioned *base-assisted hydride formation* in reaction with H_2(g).[36] When H_2(g) is passed through solutions of the reduced (Ni(I)) species **12** and **13** at 298 K, the hydride adducts **17** and **18** are formed in much higher yields. The presence of a small amount of base in the reaction mixtures also enhances the formation of the hydride adducts in these cases. The presence of thiolate/selenolate in the coordination sphere of Ni(I) is required for the successful formation of the Ni(I)-hydride adducts. This is indicated by the failure of $[Ni^I(DAPA)Cl_2]^-$ to produce a Ni(I)-hydride adduct upon incubation with H_2(g) under basic conditions.[60] Mention must be made here of the fact that the reactions of the terpy complexes **2–4** with H_2(g) in the presence of a base also provide the Ni(I)-hydride adducts in *low* yields.[60,62]

A Ni(I)-H^- FORMALISM COULD ACCOUNT FOR THE Ni-C SIGNAL

It is important to note that although the EPR spectra of all the hydride adducts (**5–7**, **17**, and **18**) resemble the Ni-C signal to a reasonable



SCHEME 6

extent, no hyperfine coupling due to the presence of H^- is observed in any case.[58,60,62] Instead, the well-resolved five-line hyperfine splitting pattern in the g = 2 region indicates that the two axial sites in each of these pseudooctahedral complexes are occupied by two N (I = 1) donor atoms from the DAPA or the terpy ligand. Clearly, the H^- ligand in each of these Ni(I)-H^- species occupies an equatorial position, and such placement of the H^- ligand does not give rise to any observable hyperfine splitting. No hyperfine splitting due to any coordinated hydrogenic ligand is observed in the Ni-C signals of the [FeNi] and [FeNiSe] H_2ases either. Our results now indicate that it is still possible that a hydride ligand is coordinated to the reduced (incubated under H_2) nickel site in the C forms of these enzymes and it exists in the equatorial plane of a pseudooctahedral Ni(I) center.

CONCLUSIONS

The following conclusions could be drawn from the results of our analogue studies. The biological nickel site appears to exist in a trigonal bipyramidal or pseudooctahedral coordination sphere with ~3 N/O and ~2 S donor atoms ligated to the metal center. Such a site can be oxidized and reduced by biologically relevant oxidants and reductants, and the transformation Ni(III) $\leftarrow$ Ni(II) $\rightarrow$ Ni(I) is reversible. CO binds only to the reduced Ni(I) site and shows no affinity toward the oxidized (Ni(III)) metal center. The presence of S/Se in the first coordination sphere allows the nickel site to readily bind CO and H^- in the reduced states. Binding of the sixth ligand, in each case, converts the $d_{x^2-y^2}$ ground state of the Ni(I) center into d_{z^2}. Incubation under $H_2(g)$ produces the Ni(I)-H^- adducts (with H^- in the basal plane) that are most likely responsible for the Ni-C signals. The hydride adducts arise from heterolytic cleavage of H_2, either coordinated to or situated in the vicinity of the active site. A neighboring basic residue could assist the process of heterolytic H–H bond scission. Ligation of Se to nickel enhances formation of the hydride adduct, a fact that could account for the enhanced H_2/HD ratios of the [FeNiSe] H_2ases.[63,64]

Acknowledgments

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