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Metal-Metal Bonds in Biology

Paul A. Lindahl*

Departments of Chemistry and of Biochemistry and Biophysics, Texas A&M University, College Station, TX U.S.A. 77843-3255.

Abstract

Nickel-containing carbon monoxide dehydrogenases, acetyl-CoA synthases, nickel-iron hydrogenases, and diiron hydrogenases are distinct metalloenzymes yet they share a number of important characteristics. All are O₂-sensitive, with active-sites composed of iron and/or nickel ions coordinated primarily by sulfur ligands. In each case, two metals are juxtaposed at the “heart” of the active site, within range of forming metal-metal bonds. These active-site clusters exhibit multielectron redox abilities and must be reductively activated for catalysis. Reduction potentials are milder than expected based on formal oxidation state changes. When reductively activated, each cluster attacks an electrophilic substrate via an oxidative addition reaction. This affords a two-electron-reduced substrate bound to one or both metals of an oxidized cluster. M-M bonds have been established in hydrogenases where they serve to initiate the oxidative addition of protons and perhaps stabilize active sites in multiple redox states. The same may be true of the CODH and ACS active sites – Ni-Fe and Ni-Ni bonds in these sites may play critical roles in catalysis, stabilizing low-valence states and initiating oxidative addition of CO₂ and methyl group cations, respectively. In this article, the structural and functional commonalities of these metalloenzyme active sites are described, and the case is made for the formation and use of metal-metal bonds in each enzyme mentioned. As a post-script, the importance of Fe-Fe bonds in the nitrogenase FeMoco active site is discussed.

Keywords

Bioinorganic chemistry; metalloenzyme reaction mechanisms; multielectron redox processes; small molecule activation; nitrogenase

1.1 Introduction

Nickel-containing carbon monoxide dehydrogenases (CODHs [1]), acetyl-CoA synthases (ACSs [2]), diiron hydrogenases ([FeFe] Hases [3]) and nickel-and-iron hydrogenases ([NiFe] Hases [4]) are distinct, genetically unrelated, metalloenzymes, yet they share a remarkable number of structural and functional properties. When these properties are viewed collectively, a pattern emerges that suggests the formation of metal-metal (M-M) bonds during catalysis and the functional importance of such bonds. The purpose of this article is to highlight these properties and the possible functions of M-M bonds in catalysis.

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*Phone, 979-845-0956; Fax, 979-845-4719; Lindahl@chem.tamu.edu .

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All four metalloenzymes are O₂-sensitive and are found in anaerobic bacteria and archaea. Hases and CODHs catalyze redox reactions involving low reduction potentials; Hases catalyze the reversible reduction of protons to H₂, while CODHs catalyze the reversible reduction of CO₂ to CO. Once reductively activated, ACSs catalyze an overall *non*-redox reaction, namely the synthesis of acetyl-coenzyme A from CO, CoA, and a methyl cation transferred from a corrinoid-iron-sulfur protein.

The active sites of these enzymes are clusters of iron, sulfur and, except for [FeFe] Hases, nickel ions. [FeFe] and [NiFe] Hases contain binuclear clusters (Figure 1) bridged by thiolate groups and coordinated by CO and CN⁻ ligands [5 - 8]. The [NiFe] active site is located near to an [Fe₄S₄] cluster while the active-site *H-cluster* of [FeFe] Hases is coordinated directly to such a cluster. The CODH *C-cluster* has the Fe/S cluster “built-in” so to speak; it consists of a NiFe₄S₄ cluster in which a unique iron (Fe_u, Figure 1) is juxtaposed to the Ni [9 - 13]. Fe_u and Ni form a substrate-binding subsite while the remaining iron and sulfide ions form a [Fe₃S₄] sub-cluster that supports the [Ni Fe_u] subsite structurally and functionally. The ACS *A-cluster* consists of a Ni-Ni binuclear site (Figure 1) coordinated to an [Fe₄S₄] cluster [11, 14].

All of these binuclear metal-sulfur clusters are activated for catalysis when they are reduced relative to an oxidized state. In their activated states, they react with substrates via oxidative addition reactions in which substrates become reduced and bound to a site on the cluster. Hase clusters attack protons to generate metal-bound hydrides [3, 4], the C-cluster reacts with CO₂ to generate a bound carboxylate group [13], and the A-cluster reacts with what is essentially a methyl cation to afford a methyl-bound intermediate [15].

1.2 The Hypothesis

The active-site structures in Figure 1 correspond to inactive *oxidized* states. Current research problems on these enzymes involve characterizing the activated *reduced* state of these sites, and understanding, on the electronic level, the mechanism by which these sites react with substrates. The hypothesis to be considered here is that reduced states of all four enzymes involve metal-metal bonds (either transiently or stably) and that such bonds play critical roles in catalysis. These bonds would stabilize multiple electronic states of these centers, including low-valence states, and they would promote oxidative addition reactions with substrates. These bonds may be covalent, polar covalent, or dative in character. Their strength can vary from weak interactions that can barely be viewed as a bond to strong covalent bonds.

1.3 Characteristics of Metal-Metal Bonds

Polar covalent and dative bonding interactions arise when different metals are used and/or with different ligand environments [16]. Given the different metals involved in the sites considered here, and the asymmetries in ligand coordination spheres, it seems likely that the M-M bonds in these protein-bound clusters will have some polar or dative character. Describing the polarity and dative character of metal-metal bonds in quantitative terms is beyond the scope of this article. However, even minor differences in coordination environment appear sufficient for a M-M bond to have some polar/dative character. For example, the M-M bond in [CH₃N(PF₂)₂]₃M₂ (M = Fe, Co, Ni) is covalent whereas the bond in the same base compound augmented by one CO coordinated to one of the metal atoms is sufficient to be viewed as dative [17].

Another aspect that suggests polar or dative bond character is that in each of the binuclear centers to be considered, one of the metal ions appears to be redox active while the other appears to be redox inactive. In the example of Figure 2, the two neighboring metals in the

oxidized state are assumed to be divalent M_D^{II} (donor) and M_A^{II} (acceptor) and non-interacting [18]. M_A^{II} is redox-inactive and perhaps electron-deficient. M_D^{II} has a softer ligand environment and is redox-active. In the presence of a sufficiently powerful reductant, M_D^{II} accepts two electrons and becomes electron-rich. The same ligand environment would not support such a reduction in an equivalent mononuclear complex, but in the binuclear setting, interactions between the two metal ions stabilize the reduction. The formally M_D^0 species shares its newly acquired electrons with M_A^{II} , affording a $M_D^0 \rightarrow M_D^{II}$ dative bond. A loosely bound ligand (water in this example) dissociates as the bond forms. Dative bond formation is reversible, as required for enzyme-catalyzed reactions. The $M_D^0 \rightarrow M_D^{II}$ bond stabilizes the system, as it relieves the electron richness of the donor and the electron deficiency of the acceptor. Thus, oxidation states that would otherwise be thermodynamically unattainable (or attainable only with non-biological organometallic ligands) become attainable using biological ligands. Relative to covalent M-M bonds, dative bonds are dominated by dispersive (London) and electrostatic forces between the metals; relativistic effects are important [18]. The polarized anisotropic electronic nature of $M_D \rightarrow M_A$ bonds may facilitate small molecule activation and allow multielectron reductions [19, 20]. Systems containing dative bonds react readily with electrophiles, forming oxidative addition products.

$M_D \rightarrow M_A$ bonds have been characterized in numerous inorganic systems. Thomas and coworkers [19] examined the effects of dative bond formation on the redox properties of [Co...Zr] complexes. Formation of a dative bond causes the Co-Zr distance to shorten and allows multielectron reduction of the Co ion at unusually mild potentials. More recently, Thomas demonstrated oxidative addition of CH_3I across Co-Zr bonds in dinuclear complexes with phosphinoamide ligands [21]. Mashima *et al.* [20] prepared a number of $Pt^0 \rightarrow Mo^{II} \equiv Mo^{II} \leftarrow Pt^0$ compounds with trigonal-planar ligand geometry at each Pt. These compounds undergo 1,4-oxidative additions with diaryl disulfides to generate $ArS-Pt^I-Mo^{II} \equiv Mo^{II}-Pt^I-SAr$ (dithiolate Pt^I complexes). E^0 , corresponding to the formation of these bonds is *ca.* -500 mV *vs.* NHE, a shift of about 1 volt toward milder potentials, relative to related systems lacking such bonds. White and Stephan [22] characterized the complex $[Cp_2Ti(SCH_2CH_2CH_2PPh_2)_2Ni]$ in which there is a 2.852 Å $Ni^0 \rightarrow Ti^{IV}$ dative bond. The complex undergoes two reversible $n = 1$ oxidations to afford the Ni^I and Ni^{II} states; the Ti^{IV} ion is redox inactive. Similar mononuclear Ni^0 complexes with P_2S_2 (thioether) coordination showed either irreversible oxidation [23] or a substantially larger spread in reduction potentials corresponding to the Ni^{II}/Ni^I and Ni^I/Ni^0 couples [24].

1.4 Evidence for M-M bonds in Metalloenzymes

In this section, aspects of each active site are highlighted which suggest that these sites, when reductively activated, contain M-M bonds that are involved in catalysis. For each enzyme, the evidence varies and different mechanistic issues are involved. The evidence overall supports the formation and function of such bonds, but “loose ends” certainly remain and further studies are required before the full implications of such bonds can be established. These loose ends are mentioned, but given the broad scope of this article, they cannot be treated in detail.

1.4.1 [NiFe] Hydrogenases

Synthetic models of [NiFe] hydrogenases show clear evidence for the formation of M-M bonds under reducing conditions, and for importance of such bonds in substrate (proton) activation. Schröder synthesized [NiFe] heteronuclear complexes with bridging dithiolates that exhibit metal-metal bonds [25]. Rauchfuss found that protons attack these bonds, affording bridging hydrides [26] (Figure 3, top). Computational studies have established that the interaction between Ni and Fe in this compound is a polar covalent M-M bond, with the

negative end at the Ni and positive end at the Fe [25]. In this state, both metals have a formal oxidation state of 1+. Upon protonation, the Ni-Fe distance lengthens, the hinge angle defined by the intersection of the NiS₂ and FeS₂ planes increases, and the two electrons previously used for the metal-metal bond become associated with the incoming bridging proton [26]. The bond in similar dimetallic systems with a bridging proton has been viewed as a 3c-2e bond involving Ni^{II}, H⁺ and Fe^{II} centers [27]. In any event, the Ni-Fe bond in these compounds is clearly playing a central role in the oxidative addition of the proton.

In the oxidized state of the enzyme, the Ni-Fe distance is ~ 2.9 Å; in the reduced state, it is ~ 2.5 Å, suggesting that a Ni-Fe bond forms upon reduction of the oxidized structure [8]. The oxo/hydroxo ligand that bridges the two metals in the oxidized cluster is absent in the reduced state. The Ni-Fe bond is basic and oxidatively adds a proton to it [28].

The binuclear center can be stabilized in multiple redox states (Ni-B, Ni-SI, Ni-C, Ni-R) as defined from EPR, IR, and electrochemical studies [29, 30, 31, 32]. The Ni-B state (Ni^{III}) can be reduced to the EPR-silent intermediate Ni^{II} state called Ni-SI. Ni-SI can be further reduced to an S = ½ state called Ni-C, and Ni-C can be further reduced to the EPR-silent Ni-R state. Redox potentials for the Ni-B/Ni-SI, Ni-SI/Ni-C and Ni-C/Ni-R couples (*ca.* -140, -350 and -420 mV, respectively at pH ~ 8) are not widely divergent [29, 31]. In all states, the iron is thought to be low-spin Fe^{II} and redox-inactive [33, 34] whereas the Ni is redox-active. This suggests that the Ni-Fe bond may have some dative character. The range of reduction potentials for these redox transitions (three-electron reduction starting from Ni^{III}) is remarkably small and has not been matched in mononuclear Ni complexes.

The short Ni Fe distances of the reduced enzyme suggest that Ni-Fe bonds might be associated with Ni-C and/or Ni-R states. Spectroscopic and computational studies [35, 36] have established that the S = ½ Ni-C state has a bridging hydride, with an electronic state corresponding to [Ni^{III} μ-H⁻ Fe^{II}]. The corresponding reactivity of related model complexes suggests that a Ni^I-Fe^{II} bond forms transiently as the Ni-SI state (Ni^{II}... Fe^{II}) is reduced by one electron. A proton inserts into that bond to form the Ni-C state (Figure 3, top). In this case, the Ni-Fe bond would be present transiently, because the state containing this bond would be generated in aqueous solvent where [H⁺] is ~ 10⁻⁷ M, such that the rate of protonation may be exceedingly fast. Interestingly, the Ni-L state of the enzyme, obtained by exposing frozen solutions in the Ni-C state to light, may contain the Ni^I-Fe^{II} bond after the bridging proton dissociates [37]. Perhaps during catalysis, Ni-L may form transiently, converting rapidly to Ni-C upon protonation (Figure 3, bottom). Thus, a M-M bond might play an important role in catalysis, but isolating such species might not be easy.

The Ni-R state is poorly characterized, largely because it is EPR-silent; however, it is 1e⁻ more reduced than Ni-C and 2e⁻ more reduced than Ni-SI [30]. Ni-R has been proposed to be a dihydride (with one bridging hydride and another hydride coordinated to the Ni or to a ligand of the Ni) and a dihydrogen complex [38]. Although protonated, Ni-R may also contain a Ni-Fe bonding interaction.

The Ni-Fe bonds in the enzyme may have substantial polar or dative bond character. Not only are the metals involved different, the ligands coordinating each metal are different, such that the Ni exhibits substantial redox activity and the Fe is redox inactive. In qualitative terms, this suggests a dative bond, but computational studies will be required to characterize the properties of the bond quantitatively.

1.4.2 The H-cluster of [FeFe] Hydrogenases

The iron ions in this cluster are referred to as Fe_d and Fe_p (distal and proximal, relative to the coordinated [Fe₄S₄] cluster). Fe_d is thought to be the substrate-binding site. Fe_d-Fe_p

distances of ~ 2.6 Å are observed in both fully reduced (H_{red}) and anaerobic-but-reductant-free (H_{ox} [$Fe^I_d Fe^II_p$]) states, suggesting the presence of an Fe-Fe bond [6, 7]. Numerous model compounds in reduced oxidation states have similar Fe-Fe distances [39], and computational studies indicate an Fe^I-Fe^I oxidation state for the H_{red} state [40]. Upon protonation and oxidation to the [$Fe^{II} \dots Fe^{II}$] state, Fe-Fe distances lengthen slightly. Reduction potentials for the $H_{ox(inact)}/H_{ox}$ and H_{ox}/H_{red} redox couples [41] are *ca.* -75 mV vs. NHE (at pH 7) and -400 mV, respectively [42, 43, 44]. The catalytic operating potential for the enzyme, associated with the transition to the active form of the H-cluster, is $E_{mid}' = -421$ mV, indicating no overpotential [45,46].

Numerous model complexes of the H-cluster have been synthesized, most of which are based on an Fe_2 (dithiolate) core that forms an Fe-Fe bond under reducing and non-protonated conditions [1]. The exact site of protonation is uncertain but a terminal site is kinetically favored, followed by rearrangement to the bridging position. In any event, the Fe-Fe bond is thought to be involved and sufficiently electron-rich to react with a proton. In Figure 4, we presume that the M-M bond is present in the fully-reduced formally [Fe^I-Fe^I] H_{red} state, but such bonds might also be present in the $1e^-$ more oxidized H_{ox} state. Although a terminal position is shown, a bridging position for the proton should also be considered. It is interesting to note that the spectroscopically determinable oxidation states of these irons might be more oxidizing than indicated by the formal $1+$ designation, in that some electron density would be delocalized away from the metal centers. This delocalization may stabilize multiple redox states for these binuclear centers. A similar situation was seen with a formally Co^{-I} species with a dative M-M bond; the species behaves spectroscopically and electrochemically as Co^I [19].

1.4.3 The C-cluster of CODH

Evidence for M-M bonds in the CODH C-cluster and evidence for their participation in catalysis is less developed than for the Hases, and no model complexes have been prepared that exhibit M-M bonds. As such we will use the examples of M-M bonding in the hydrogenase sites to support the possibility of such bonds in CODH. Indeed the motivation for this article began with the recent X-ray diffraction structures of the C-cluster, which indicates two positions of Fe_u relative to the other atoms of the cluster [10, 11]. The dominant position is 2.9 Å from the Ni while the minor location is 2.56 Å away. Jeoung and Dobbek [10] recently found that CN^- binding to the C-cluster reverses the relative proportions of these forms, such that the conformation with the shorter Ni- Fe_u distance dominates. This short distance indicates a Ni-Fe bond [10], but whether it plays a role in catalysis is uncertain and, as far as we are aware, has not been considered previously.

Like the Hase clusters, the C-cluster can be stabilized in multiple redox states, including C_{ox} , C_{red1} , C_{int} , and C_{red2} , with each state one-electron more reduced than its predecessor [47]. Redox potentials for the C_{ox}/C_{red1} and C_{red1}/C_{red2} couples (*ca.* -200 and -530 mV, respectively) are not widely separated [29]. CO/CO_2 electrocatalysis is reversible at *ca.* -500 mV indicating no overpotential [48]. The most highly reduced C_{red2} state reacts with CO_2 , reducing it by 2 electrons to CO as the C_{red1} state forms. The presence of more than one C-cluster conformation in X-ray crystal structures is consistent with previous evidence for multiple spectroscopic forms. C_{red1} and C_{red2} are $S = 1/2$ states with EPR signals that quantify to only ~ 0.3 spin/cluster; Mössbauer spectra show the presence of more than one species [49].

Where the two electrons used to reduce C_{red1} to C_{red2} localize on the cluster is unknown. We have previously suggested that both localize on the Ni ion (forming a formally Ni^0 species) [47] but the ligand environment does not seem appropriate to support such a low valance. Here we suggest that the two electrons are stored as a Ni- Fe_u bond and that CO_2

oxidatively adds to that bond during the catalytic cycle. We designate it as a $\text{Ni}^0 \rightarrow \text{Fe}_u^{\text{II}}$ dative bond (Figure 5), but computational studies will be required to reveal how the two electrons are distributed. The proposed electronic state would be equivalent to the Ni-R state of [NiFe] Hases, except that it would not be protonated.

A apparent discrepancy with this possibility is that CN^- reportedly binds to the C_{red1} redox state, rather than to C_{red2} [10]; thus, the reported structure implies that the C_{red1} state should contain the M-M bond. Might the analyzed structure have been a superposition of two types of C-cluster molecules? Some C-clusters could have been in the C_{red1} state with CN^- bound, and others might have been in the C_{red2} state with the Ni-Fe bond. Another concern is that the concentration of CN^- used in the study (70 mM [10]) is two orders-of-magnitude higher than that used in earlier studies that established that CN^- binds to the C_{red1} state [50, 51]. At this high concentration, CN^- may indeed bind to the C_{red2} state.

A $\text{Ni} \rightarrow \text{Fe}_u$ bond in the C_{red2} state would nicely explain a nearly 20-year old conundrum. C_{red1} and C_{red2} are both $S = \frac{1}{2}$ systems that exhibit very similar EPR signals, implying that their electronic configurations and spin-coupling mechanisms are very similar. Mössbauer and ENDOR spectra of C_{red1} indicate that the unpaired electron is primarily associated with the $[\text{Fe}_3\text{S}_4]^{-1}$ subcluster (with 2 formally Fe^{II} and 1 Fe^{III} ions), with little spin density located on the low-spin diamagnetic Ni^{II} ion [49, 52]. The C_{red2} state is two electrons more reduced than C_{red1} but there seemed, until now, to be no site(s) on the C-cluster where these electrons could localize without altering the spin-coupling properties significantly. The Fe_3S_4 subcluster would have all formally Fe^{II} ions after adding a single electron. Given its coordination environment, the Ni^{II} ion could perhaps become reduced by one electron, but a literal Ni^0 state could not be supported by the ligands involved. Conceivable spin-coupling mechanisms associated with a postulated $\text{Ni}^{\text{I}}: [\text{Fe}_3\text{S}_4]^{-2}$ cluster seem dramatically different from those which might generate the $S = \frac{1}{2}$ state of C_{red1} . In contrast, a Ni- Fe_u bond in the C_{red2} state could stabilize a diamagnetic Ni^0 species and permit a spin-coupling arrangement that is similar to that of C_{red1} .

1.4.4 The A-cluster of ACS

The evidence for a M-M bond in this cluster has not been established, but current evidence is consistent with this possibility. The A-cluster is found in each α subunit of an $\alpha_2\beta_2$ ACS/CODH molecule. Two conformations of the α subunit are known, called *Open* and *Closed* [11]. In both conformations, a low-spin square-planar Ni_d^{II} ion is distal to the $[\text{Fe}_4\text{S}_4]$ cluster, with a coordination environment that renders it redox-inactive. In the *Open* conformation, the *proximal* metal-binding site is occupied by a Ni ion. Ni_p is coordinated by three endogenous sulfur ligands (two cys that bridge Ni_d and one cys that bridges the $[\text{Fe}_4\text{S}_4]$ cluster) and a water [14]. The planes defined by the ligands of the two Ni ions intersect on a line that includes the two bridging sulfur cysteinates. In the *Open* conformation, average reported Ni_p - Ni_d distances of *ca.* 3.0 Å [11, 14] are too long for a M-M bond designation.

Besides Ni, the proximal site can also be occupied by Zn^{II} and Cu^{I} [11, 12]. These ions have d^{10} configurations that favor tetrahedral geometries. In both cases, the α subunit is in the *Closed* conformation. Although no structure has been reported with the Ni_p form of the A-cluster in the *Closed* conformation, the α subunit with Ni_p bound is expected to cycle between *Open* and *Closed* conformations during catalysis. The $\text{M}_p \dots \text{Ni}_d$ distance in the *Closed* conformation is slightly shorter than in the *Open* conformation due to a decrease in the dihedral angle between the two planes (See Figure 3 of Ref. 11)). Thus, the two bridging cysteinates may serve as a hinge, functioning along with the conformational changes to form a Ni_p - Ni_d bond in a redox-dependent fashion (Figure 6). An analogous situation may arise

for the [NiFe] Hase structure – namely the two bridging cysteinates may serve as a hinge to modulate Ni-Fe bond formation.

To become activated, the oxidized state of the A-cluster (A_{ox}) must be reduced by two electrons. The redox potential for this process is *ca.* -520 mV, as estimated from the midpoint potential for catalysis [53, 54, 55]. Where these electrons reside on the A-cluster is uncertain; indeed the location of these two electrons has been a decade-old puzzle that has not been resolved. One possibility is that both electrons localize on Ni_p^{II} to afford a $d^{10} Ni_p^0$ species [56 - 59]. Alternatively, one electron may localize on Ni_p^{II} and the other on the $[Fe_4S_4]^{2+}$ cluster to afford a $\{Ni_p^I:[Fe_4S_4]^{1+}\}$ spin-coupled structure. The feasibility of the Ni^0 state has been questioned because two DFT studies favor the $\{Ni_p^I:[Fe_4S_4]^{1+}\}$ configuration [60, 61]. Different ACS preparations exhibit Mössbauer evidence for both electronic configurations [62, 54]. Typically, stable zero-valence nickel complexes require organometallic non-biological ligands such as trialkyl phosphines, cyclooctadiene, or carbonyls for stability. However, in this case, a formal Ni_p^0 state could be stabilized by the simultaneous formation of a $Ni_p^0 \rightarrow Ni_d^{II}$ dative bond.

Tatsumi, Rauchfuss, Riordan and others have synthesized A-cluster model complexes with two Ni ions and bridging dithiolates [59, 63 - 66], reminiscent of the Hase model compounds (Figure 6, top). These $[Ni^0 Ni^{II}]$ compounds show flexible Ni-S₂-Ni hinge angles, ranging from *ca.* 98° to 120° . Those with the smallest angles exhibit the shortest Ni-Ni distances of *ca.* $2.65 \text{ \AA}$, enticingly close to bonding distance. A possible dependence on oxidation state was noted, with $[Ni^{II} Ni^{II}]$ complexes possessing longer Ni-Ni bond distances than the corresponding $[Ni^I Ni^{II}]$ complexes [65]. For the two ACS structures that have been published [11, 14], the average Ni_p - Ni_d distance and hinge angle are *ca.* $3.0 \text{ \AA}$ and 130° , respectively. However, the enzymes appear to have been crystallized in the as-isolated states, in which case both Ni ions would have been in the Ni^{II} oxidation state. In the fully-reduced A_{red} state, the Ni_p - Ni_d distance may be short enough to allow bonding interactions. This state would then be poised to react with a methyl group cation, as shown (Figure 6, bottom).

1.5 The General Pattern

In summary, these four genetically unrelated enzymes share a remarkable number of structural, redox and catalytic properties, including the following.

- They are found in anaerobic organisms and are inactivated by O_2 .
- Their active sites include two “special” iron and/or nickel ions that are most directly involved in catalysis.
- The sites exhibit multielectron redox ability, and are activated by 2-electron reductions.
- The two special metals are juxtaposed within M-M bonding distance.
- One metal is redox-inactive and perhaps electron-deficient; the other can accept 1-2 electrons and becomes electron-rich when reduced.
- Reduction potentials are milder than expected based on formal oxidation state changes.
- When reductively activated, the cluster attacks an electrophilic substrate via an oxidative addition reaction.
- The reduced substrate either binds to the redox-active metal or it binds in bridging fashion to both metals.

1.6 Research Challenges and Potential Rewards

More studies are required to establish the presence of M-M bonds in these enzymes (especially in CODH and ACS). There is no doubt that M-M bonds form when Hase model complexes are reductively activated, and that protons can insert into these bonds. The major problem in identifying and characterizing M-M bonds in the Hase enzymes themselves is that they are bathed in an aqueous environment where protons are in high concentration. The rates at which protons would add across such bonds would likely be fast, such that only the protonated forms would be isolatable under normal conditions. Perhaps unprotonated states could be trapped under cryogenic rapid-freeze conditions using solvent systems that minimize the proton concentration. The possibility that the Ni-L state has a Ni-Fe bond should be testable since this state can be isolated in frozen solution. However, EXAFS analysis of the Ni-Fe bond distances in Ni-C and Ni-L states were both short (*ca.* 2.5 Å in both states) [67], highlighting the challenges of distinguishing what may be a protonated 3c-2e [Ni^{III} H⁻ Fe^{II}] bond in one case and an unprotonated 2c-2e [Ni^I-Fe^{II}] bond in the other.

The situation with the other two enzymes is different because their reduced states can be prepared in the absence of substrates. For CODH, establishing that the short Ni-Fe_u distance is present in the fully-reduced C_{red2} state of the C-cluster and is absent in the C_{red1} state would support the functional role proposed here for this bond. However, care must be taken (e.g. by EPR verification) to ensure that crystals are in the correct redox state. Given the heterogeneity and complexity of these systems, it would not be enough to simply grow crystals in the presence of a reductant, hoping that the C_{red2} state is attained. The issue regarding redox state and CN⁻ binding must be resolved.

Regarding ACS, an X-ray diffraction crystal structure of the reductively-activated state showing a short Ni_p-Ni_d distance would provide unequivocal evidence that a Ni_p-Ni_d bond forms in this state. A structure of the methylated intermediate should show a longer non-interacting Ni_p...Ni_d distance, along with a methyl group bound to Ni_p. The redox inactivity of Ni_d, the $n \geq 1$ redox activity of Ni_p, and the two-electron reductive activation of the A-cluster are generally well established [54, 55].

Although further studies are required to establish such bonds in these enzymes, the general pattern of evidence is strongly consistent with the formation of a M-M bonds in the reductive-activated state of each of the four enzymes. If such bonds can be shown to function in catalysis, such bonds should be rightly regarded as a new motif in mechanistic metalloenzymology. The function of M-M bonds would involve oxidative addition reactions under low-potential anaerobic conditions. Given the difficulty of identifying unifying concepts in biology, this would be very exciting indeed!

1.7 Post-script: Nitrogenase

While researching for this article, I noticed a 2002 article by Dimitri Coucouvanis where he argued that the FeMoco active-site cluster of nitrogenase contained Fe-Fe bonds that functioned in catalysis [68]. This O₂ sensitive enzyme catalyzes the six-electron reduction of N₂ → 2NH₃. Six of the 7 iron ions in the cage-like cluster are located at the corners of a trigonal prism, with Fe-Fe distances substantially shorter than found in standard [Fe₄S₄] clusters (ave = 2.63 Å vs. ~ 2.73 Å). When the article was published, these ions were thought to be coordinated exclusively by three sulfide donors, an unusually low number of ligands. The cluster in its crystallographically-determined state must be reductively activated (by *ca.* 3 electrons) before it can bind and reduce N₂. Coucouvanis *et al.* [68] suggested that the M-M bonds in the cluster stored electrons at early steps of catalysis and delivered them to N₂ in a later step. Soon thereafter, Rees and coworkers reported that an atom of C, N, or

O was located in the center of the prism, coordinating each of the 6 iron ions [69]. These bonding interactions could also rationalize the short Fe-Fe distances and the apparent low coordination numbers, which perhaps defused what would have been a more extensive consideration of the presence and importance of Fe-Fe bonds in the cluster.

After a decade of unsuccessful efforts to identify the central atom, perhaps we should again ask whether the M-M bonds in FeMoco play an important role in catalysis. Electron delocalization and the possibility of multielectron processes should become more extensive as metal centers become more reduced, as the nuclearity of clusters with M-M bonds increases and as the number of M-L bonding interactions decrease. Following these themes, Kim and Han have synthesized related tri-iron clusters with short Fe-Fe bonds and multielectron redox chemistry [70]. This suggests the intriguing possibility that the trigonal prismatic arrangement of six Fe ions in FeMoco, each engaged in M-M bonding with their neighbors, might collectively be sufficient to focus delocalized electrons to a “hot spot” in the center of the structure. Might that electron density be so attractive to various electrophiles (perhaps including N₂) that the cluster is inevitably isolated with a species bound there? Interstitial carbon atoms are common in high nuclearity metal cage clusters [71], perhaps for the same reason. Reductive activation of FeMoco beyond the state crystallized might cause the dissociation of that species, thereby allowing for substrate binding. Thus, the “hot spot” may be more important than the central atom in catalysis. In any event, it is intriguing to consider the implications of M-M bonding in the FeMoco cluster, as this may shed light on our understanding of the catalytic mechanism of this most recalcitrant enzyme.

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1.8 Abbreviations

Hase	hydrogenase
[FeFe] Hases	hydrogenases containing an [Fe Fe] diiron active site called the H-cluster
H_{ox(inact)}, H_{ox}, and H_{red}	fully-oxidized, one-electron-reduced, and fully-reduced states of the H cluster
Fe_p and Fe_d	Fe ions of the H-cluster that are proximal and distal to the associated Fe ₄ S ₄ cluster
[NiFe] Hase	hydrogenases containing a nickel-and-iron active site
Ni-B, Ni-SI, Ni-C and Ni-R	different oxidation/spectroscopy states of the [NiFe] center, ranging from most oxidized to most reduced, in one-electron intervals
ACS	acetyl-coenzyme A synthase
CoA	coenzyme A
Ni_p and Ni_d	Ni ions of the active-site A-cluster that are proximal and distal to the associated Fe ₄ S ₄ cluster, respectively
CODH	nickel-containing carbon monoxide dehydrogenase

C_{ox}, C_{red1}, C_{int}, and C_{red2}	different redox states of the active-site C-cluster, with each subsequent state one-electron more reduced than its predecessor
Fe_u	the unique Fe of the C-cluster
M-M	metal-metal, as in a bond between two metal ions
M→M	a dative M-M bond
M_D and M_A	donor and acceptor metal ions involved in a dative M-M bond
NHE	normal hydrogen electrode
E⁰	thermodynamic reduction potential at pH 7
DFT	density function theory
LS	low spin
EPR	electron paramagnetic resonance

1.9 References

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Figure 1.
Structures of oxidized active sites that are hypothesized to possess M-M bonds when reduced.

Figure 2.

Formation of M→M dative bonds. In this hypothetical example, a divalent donor metal is located near to the acceptor metal. The ligand environment supports redox-activity of the donor but not the acceptor. A two electron reduction is coupled to the formation of a dative bond. The two metals move together slightly and a water/hydroxide that had bridged the two metal ions dissociates. Once formed, the dative bond can initiate oxidative addition chemistry by reacting with an electrophile. After reacting, the reduced electrophile is bound to the donor metal which has become reoxidized, and an hydroxide ion coordinates the other site. In other cases, the electrophile might bridge the metals.

Figure 3. NiFe hydrogenase model complexes (top, from [26]) and catalytic mechanism (bottom), emphasizing the role of Ni-Fe bonds.

Figure 4.
[FeFe] hydrogenase mechanism, emphasizing the role of Fe-Fe bonds.

Figure 5.
CODH mechanism, emphasizing the role of the Ni-Fe bond.

Figure 6. ACS model complexes (top) and catalytic mechanism (bottom). Both complexes are from [59].