

On the function of nitrogenase FeMo cofactors and competitive catalysts: chemical principles, structural blue-prints, and the relevance of iron sulfur complexes for N_2 fixation

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Abstract

This article tries to rationalize why low molecular weight complexes have not yet been able to copy nitrogenase catalyzed reactions or to act as competitive catalysts with nitrogenase-like activity. An answer is sought in that such complexes must rather fulfil the principles governing FeMoco function than duplicate its structure. Such principles, e.g. metal sulfur bonds, reversible M–S bond dissociation, Brønsted basicity, vacant sites, redox activity, are illustrated with metal complexes of multidentate thioether thiolate ligands. A structure–func-

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tion relationship of metal sulfur [MS] centers is described, revealing that [MS] centers can stay structurally invariable in spite of considerable electronic changes. Complexes with [MS] centers further accomplish strong coupling of H^+/e^- fluxes, the heterolytic activation of H_2 , and the stabilization of the N_2 reduction key intermediate diazene N_2H_2 . Low-spin states of Fe(II) centers can be enforced by sterical constraints. These coordination chemistry results, combined with X-ray structural and biochemistry findings, form the basis of a model for the FeMoco function. It proposes the breakage of one Fe–S–Fe bridge of FeMoco and the formation of two unique five-coordinate low-spin Fe(II) centers when the enzyme passes from the resting into the turnover state. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Nitrogen fixation; Iron sulfur complexes; Structure–function

1. Introduction

The chemical inertness of the N_2 molecule, the drastic conditions of the Haber–Bosch process (500°C, 200 bar), and the contrastingly mild biological N_2 fixation (20°C, 1 bar, redox potential ~ -500 mV) represent a persistent challenge for chemists.

Antagonisms, such as principles versus copies, invariability versus change, closed versus open, iron versus molybdenum, determine the controversial discussion of the mechanism(s) of nitrogenase and its cofactors, indicating that biological N_2 fixation is an still unsolved puzzle.

In 1965, Allen and Senoff reported $[Ru(N_2)(NH_3)_5]^{2+}$, the first transition metal dinitrogen complex [1]. It had formed from $RuCl_3 \cdot xH_2O$ and hydrazine. Chemists tended to believe the solution of the N_2 fixation problem had come within reach, and the dream of a competitive catalyst for nitrogenase, a low-molecular weight compound with nitrogenase-like activity, could become true. Indeed, reactions of the N_2 molecule at standard pressure and temperature were discovered. They led to numerous transition metal N_2 complexes. In a few cases, the N_2 ligand of these complexes even could be reduced to give nitrogen hydrogen species down to ammonia [2]. The reduction of N_2 ligands induced by electrophilic (H^+) [3], radical ($CH_3^\bullet$) [4], or nucleophilic ($C_6H_5^-$, CH_3^-) [5] attack are noted, because they are mechanistically well understood. Aqueous transition metal systems were shown to reduce N_2 to NH_3 or N_2H_4 [6,7]. Recently, seemingly simple compounds such as Mo(III) trisamido complexes were found to cleave molecular N_2 to give trisamido nitrido molybdenum(VI) complexes [8]. Coordinated N_2 entities were demonstrated to react, directly [9] or indirectly [10], with dihydrogen to yield N_2H_x products.

Nevertheless, the dream of finding a catalyst which can compete with nitrogenase has remained a dream as yet. None of the synthetic chemical systems truly works catalytic. Their redox potentials are either unknown or definitely very negative and biologically incompatible. Frequently, alkaline metals are involved, if not in the actual N_2 reduction reaction then in the synthesis of the starting materials, e.g. the N_2 complexes [11].

In such a situation, inevitably many hopes were put on the X-ray structure analysis of nitrogenase. The structures of the nitrogenase cofactors, in particular the

FeMo cofactors, were expected to give hints on how these cofactors function and a potentially competitive catalyst should look like.

The X-ray structure analysis of FeMo nitrogenases was solved in 1992 [12]. In the 6 years since then, however, it has become increasingly clear that a molecular structure does neither necessarily tell how it functions nor yield blue-prints for constructing competitive catalysts of nitrogenase-like activity.

With regard to FeMoco function, the pivotal question from the chemical point of view is how one N_2 , 8H^+ and 8e^- are combined by FeMoco to give ammonia and dihydrogen at standard conditions and biological redox potentials (Fig. 1).

Several models have been proposed and calculated for the interaction between N_2 and FeMoco [13–17]. Practically all of these models assume the FeMoco to retain the ‘closed’ structure which has been determined for the resting state of the enzyme. The activation of N_2 is suggested to be achieved, for example, by binding N_2 inside the cage-like FeMoco structure to six Fe centers (**A**), outside to four Fe centers (**B**) or to the Mo center after dissociation of the endogeneous homocitrate ligand (**C**).

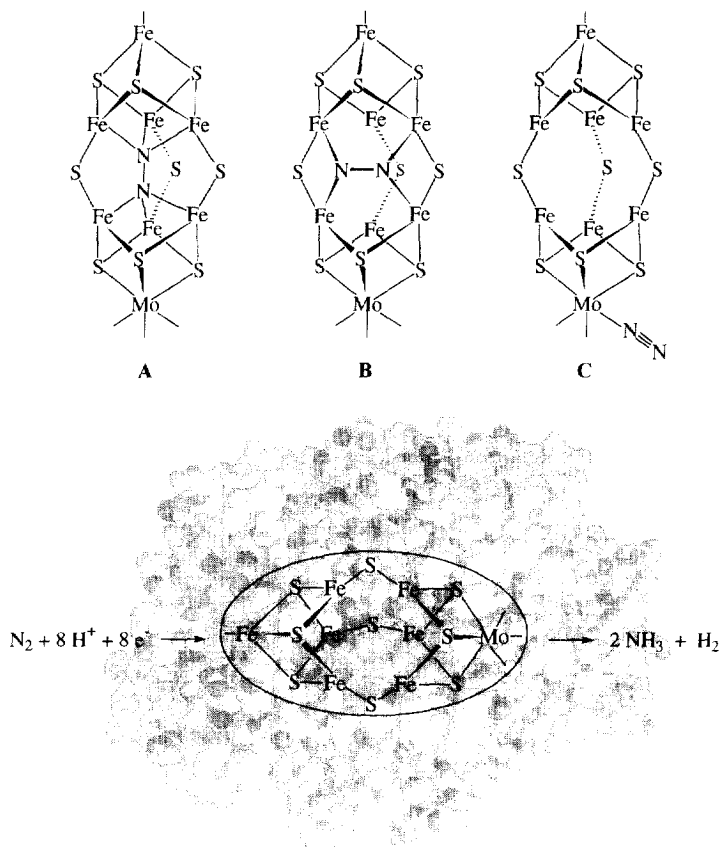


Fig. 1. Schematic drawing of the molecular structure of FeMoco, and the pivotal question from the chemical point of view.

The problems of these models have been discussed in detail elsewhere [18]. Here it may suffice to say that the interior size of the FeMoco is just large enough to accommodate N_2 , but not its reduction intermediates such as N_2H_4 or two NH_3 .

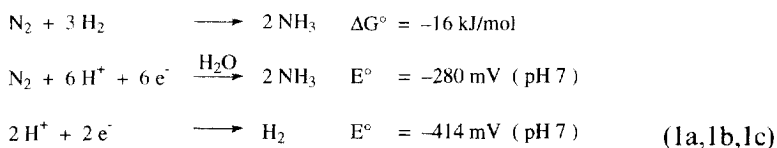
Dinitrogen reduction at the Mo center has chemical precedents in so far as molybdenum phosphine complexes are known which allow the reduction of N_2 . But these complexes require strong reductants and cannot answer the question how the cofactors of 'Fe-only' nitrogenases function, which are assumed to have FeMoco analogous structures.

Summing up, the question arises why all chemical model reactions, despite the undeniably great chemistry which they represent, could not yet meet the nitrogenase challenge and hit the two major chemical targets: provide an understanding of the FeMoco function on the molecular level and the synthesis of a competitive catalyst [19].

2. Chemical principles of FeMoco and potentially competitive catalysts

An answer to this question and a working hypothesis is that all known chemical systems lack too much the principles governing the FeMoco function. A logical consequence thereof, which at first glance sounds paradoxical, is that a competitive catalyst fulfilling these principles cannot be a structural FeMoco copy. But what are the principles? In order to find out, summarizing the following facts and conclusions can help.

Facts: (1) FeMoco has got transition metals. Sulfur donors and iron are dominant constituents. The 3-coordinate Fe centers are clearly undercoordinated. Isolated native FeMoco is labile and does not catalyze N_2 reduction [20]. (2) Thermodynamics state that reaction (1a) is exothermic. The electrocatalytic N_2 reduction (Eq. (1b)) thermodynamically requires less negative reduction potential than the reduction of protons (Eq. (1c)). (3) In addition to N_2 , nitrogenase catalyzes the reduction of a large number of other substrates (H^+ , C_2H_2 , N_3^- , HCN, MeCN) which all are reduced by multiples of $2H^+/2e^-$ reactions [21].

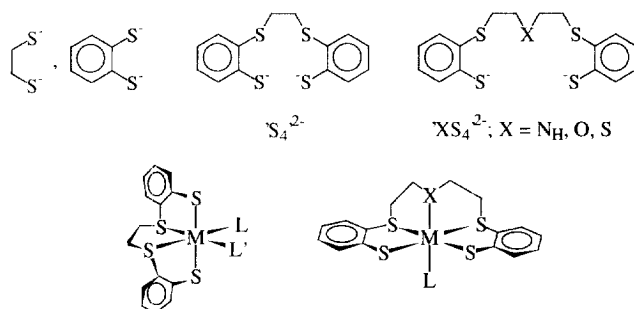


From these facts, it can be concluded for the FeMoco: S donors and M–S bonds are likely and the nitrogenase protein is imperatively essential for the FeMoco function. For the structure and function of competitive catalysts, it follows: competitive (low molecular weight) catalysts must not structurally copy FeMoco. Unlike FeMoco, they must be robust in the absence of proteins. However, like FeMoco, they may need M–S bonds, should allow $2H^+/2e^-$ reactions, and must not require strong reductants. In any case, they (1) must have vacant sites for the

coordination of N_2 (and other nitrogenase substrates); (2) must exhibit Brønsted acid–base behaviour for proton transfer and (3) must be redoxactive to allow electron transfer. The last three points are minimum conditions for the catalysis of reaction (1b) which requires the activation of N_2 and the transfer of protons and electrons.

These conditions we tried to meet with multidentate organosulfur ligands and complexes of the types shown in Chart 1 which shows selected multidentate organosulfur ligands, metal complex structures and small molecules acting as coligands L [19].

Chart 1. Selected multi-dentate organosulfur ligands, metal complex structures and small molecules acting as coligands L.



M = Fe, Mo, Ru, Os; Cr, W, Ni, Pd, Pt; Co Rh

L or L' = N_2H_2 , N_2H_3 , N_2H_4 , NH, NH_2 , NH_3 ; CO, NO, N_3^- ; $\{H^+\}$, H_2 , H

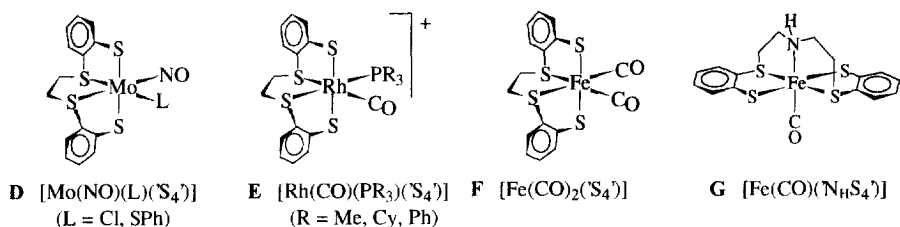
The ligands coordinate many transition metals, and the resulting metal sulfur ligand [MS] complex fragments bind numerous biologically relevant small coligands. Chart 1 shows those molecules which are relevant to nitrogenase. Our interest focussed on Fe and Mo complexes, but with Fe complexes we got further than with any other metal. For this reason, and because iron is dominant in FeMoco, the following discussion will concentrate on Fe complexes.

3. Structurally invariable [MS] centers and the coupling of H^+ /e flux: a structure–function relationship of [MS] complexes

A fundamental question for [MS] mediated catalytic reductions according to the general reductase equation (Eq. (2)) is the order of proton and electron transfer steps, and how protonation influences the metal sulfur cores, the [MS] bound substrate A and the electron transfer (or redox potentials).



Investigation of complexes **D–G**, which contain ligands (NO, CO) suitable as IR probes, yielded in all cases practically identical results [22–25]. They are well illustrated for $[Fe(CO)(N_HS_4)]$ [25].



The thiolate donors of $[\text{Fe}(\text{CO})(\text{NHS}_4')]$ can be stepwise and reversibly protonated, proving the Brønsted basicity of the $[\text{Fe}(\text{NHS}_4')]$ fragment. Protonation blue-shifts the $\nu(\text{CO})$ frequencies 35–40 cm^{-1} per step, indicating that the electron density at the iron center strongly decreases. Exactly the same effects occur upon alkylation of the thiolate donors. These effects are summarized in Fig. 2.

The alkyl derivatives could be characterized by X-ray structure analyses. They revealed an unexpected phenomenon. Electronic changes also usually cause structural changes. Here, however, the $[\text{FeNS}_4]$ cores do not change. The FeN and, in particular, the FeS distances in the parent complex, in the monoalkylated and in the dialkylated derivative stay invariant, although the $\nu(\text{CO})$ frequencies demonstrate a significant electronic variation at the metal centers. It approaches that which is found in other metal carbonyl complexes only upon uptake or release of one electron, for example, in the $\text{V}(\text{CO})_6$

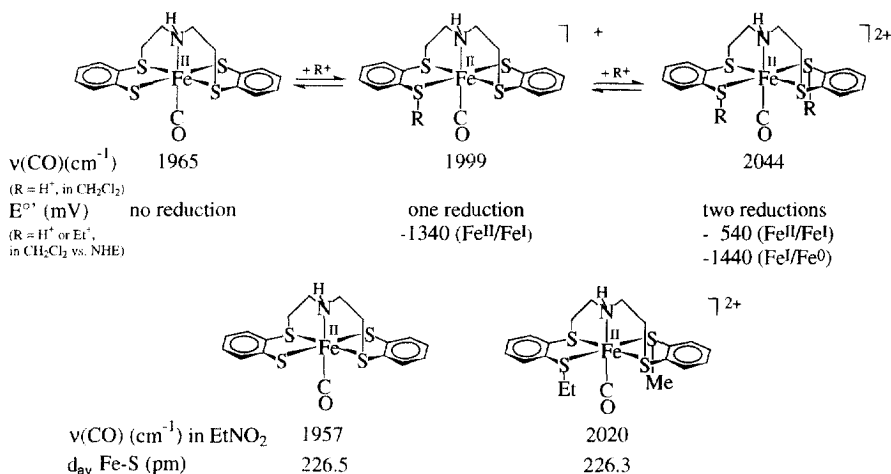
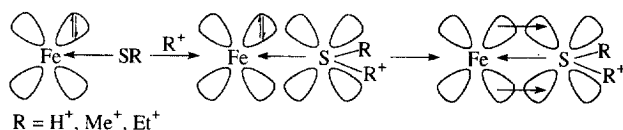


Fig. 2. Electronic, structural, and redox effects in $[\text{Fe}(\text{CO})(\text{NHS}_4')]$ upon reversible protonation ($\text{R} = \text{H}^+$) or irreversible alkylation ($\text{R} = \text{Me}^+$, Et^+).

(1976 cm^{-1})/ $\nu(\text{CO})_6^-$ (1860 cm^{-1}) couple. Comparison of $[\text{Fe}(\text{CO})(\text{N}_\text{H}\text{S}_4)]$ and the dialkylated derivative $[\text{Fe}(\text{CO})(\text{N}_\text{H}\text{S}_4\text{-Me,Et})](\text{BF}_4)_2$ illustrates that both complexes have Fe–S distances of ~ 226 pm while their $\nu(\text{CO})$ frequencies differ by 63 cm^{-1} .

The following bonding scheme yields an explanation for the invariance of Fe–S distances [24].



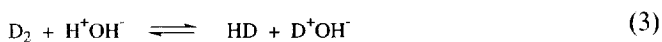
The Fe–S(thiolate) bonds are assumed to have predominantly σ donor bond character. Protonation and, likewise, alkylation of the thiolate donors leads to a weakening of the respective $\text{S} \rightarrow \text{Fe}$ σ -bonds and an inductive withdrawal of electron density from the Fe centers. The thiolate donors that turned into thiol or thioether donors, however, gain π -acceptor properties such that partial $\text{Fe} \rightarrow \text{S}$ π -back bonds form. The π -back bonds lead to a further decrease of electron density at the Fe centers. The Fe–S distances, on the other hand, remain invariant because weakening of the $\text{S} \rightarrow \text{Fe}$ σ -donor bond and formation of the $\text{Fe} \rightarrow \text{S}$ π -back bond compensate each other. In figurative terms, electron rich and electron poor metal sulfur centers are like sponges which show identical appearance in wet and dry state.

The electronic differences indicated by the $\nu(\text{CO})$ differences are even more clearly reflected by the redox potentials. The redox potentials anodically shift by up to 700 mV per step of protonation or alkylation. These large redox potential shifts have important consequences. The parent complex can be oxidized showing a $\text{Fe}^{\text{II/III}}$ redox wave at 0.35 V (vs. NHE) in the cyclic voltammogram (CV), but it is not reducible down to -1.8 V. The monoalkylated derivative can be reduced once at very negative potential ($\text{Fe}^{\text{II/I}}$: -1240 mV). The two-fold alkylated derivative, however, shows two CV redox waves ($\text{Fe}^{\text{II/I}}$: -540 mV, $\text{Fe}^{\text{I/0}}$: -1440 mV) and the $\text{Fe}^{\text{II/I}}$ couple appears anodically shifted by 700 mV in a redox range which is biologically compatible.

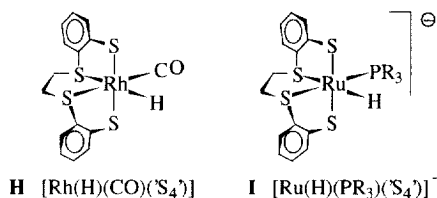
These results demonstrate that primary protonation (or alkylation) can make complexes reducible which are otherwise non reducible. In other words, protonation becomes essential for reduction. The invariable $[\text{FeNS}_4]$ cores in conjunction with the variable electron density at the Fe centers and the redox potential shifts can be considered a structure–function relationship of [MS] centers. This relationship is anticipated to facilitate redox reactions for kinetic and thermodynamic reasons. When it holds for mononuclear [MS] centers, there is no reason why it should not also hold for multinuclear [MS] centers of enzymes. Last but not least, the order of successive proton and electron transfer steps in the course of N_2 fixation, which is generally assumed to involve first reduction and then protonation of N_2 , has to be reconsidered.

4. Heterolytic H₂ activation at [MS] centers

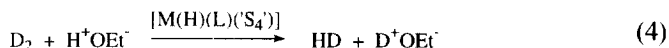
Inseparably connected with enzymatic N₂ reduction is hydrogen evolution and the N₂ dependent exchange of molecular D₂ with protons (of water) to give HD [21b]. In other words, nitrogenases exhibit hydrogenase activity. One of the (two) key reactions catalyzed by hydrogenases is the D₂/H⁺ exchange according to Eq. (3) [26].



Eq. (3) requires the heterolytic cleavage of D₂ or H₂, respectively. Therefore, when the principles are sought which govern the function of the nitrogenase FeMoco, the mechanism of H₂ activation at [MS] centers can be anticipated to yield insights into these principles. In our search for [MS] complexes catalyzing the heterolytic cleavage of H₂, we found the rhodium and ruthenium hydride complexes **H** and **I** [27,28].

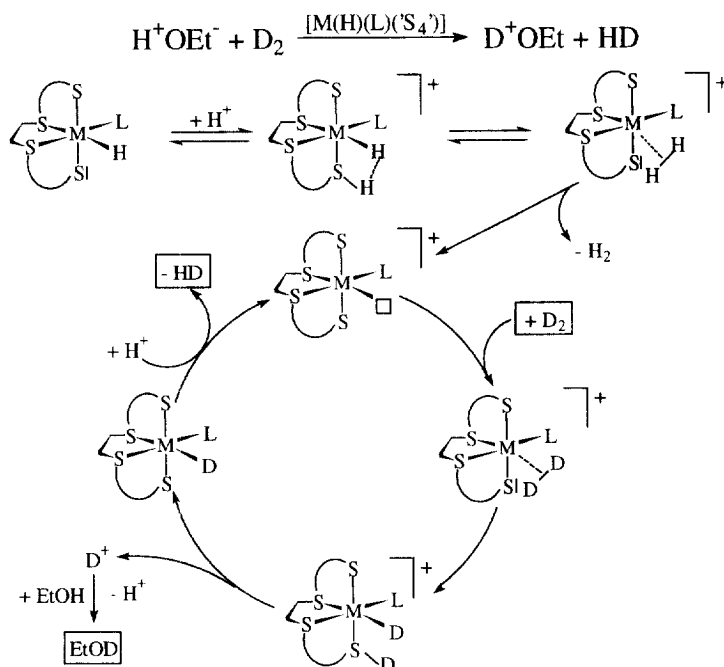


None contain a biological metal, but ruthenium is a homologue of the nitrogenase dominating iron. Complexes **H** and **I** both catalyze the exchange reaction between D₂ and H⁺. For solubility reasons, EtOH was used as proton source according to Eq. (4).



In both cases, the hydrides are not the actual catalysts but intermediates which give the catalytically active species upon protonation and release of H₂ (Scheme 1).

Scheme 1 indicates that the reaction sequence starts by forming a thiol hydride. Via a [H⁺ + H⁻] reaction, the thiol proton and the hydride ligand give a non-classical η²-H₂ ligand and a Kubas type complex, respectively [29]. H₂ is released to generate the catalytically active species [M(L)(S₄')] having a vacant site. D₂ adds to this vacant site and, in the reversal of the starting sequence, the D₂ ligand is cleaved into D⁺ (binding to one thiolate donor) and a deuteride D⁻ (binding to the metal center). This heterolytic cleavage is achieved by the concerted attack of the Lewis acidic metal center and the Brønsted basic thiolate donor upon the η²-D₂ ligand. The resulting thiol deuterion is acidic and can exchange with EtOH protons to give EtOD and a free proton, which reacts with the deuteride complex in order to form HD and to regenerate the coordinatively unsaturated catalyst.



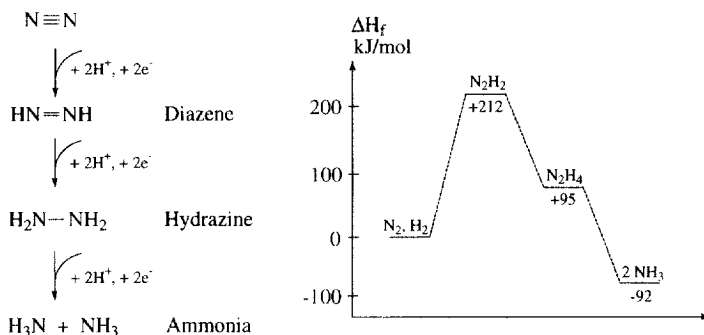
Scheme 1. Mechanism of heterolytic H_2 activation catalyzed by $[\text{M}(\text{H})(\text{L})(\text{'S}_4\text{'})]$ complexes ($\text{M} = \text{Rh}, \text{Ru}$; $\text{L} = \text{CO}, \text{PCy}_3$).

All the details and proofs of this mechanism are reported elsewhere [27b,28]. The important point to be made here is that these $[\text{M}(\text{'S}_4\text{'})]$ complexes catalyze a key reaction of the metal sulfur enzymes nitrogenase and hydrogenase although their structures significantly differ from those of the active enzyme centers [12,30]. However, the complexes show features and fulfil principles which hold or must also hold for the FeMoco and hydrogenase centers, i.e. metals, sulfur donors, Brønsted basicity, vacant sites, etc.

5. Diazene complexes as key intermediates of biological N_2 fixation

Numerous results indicate that biological N_2 fixation proceeds via $2\text{H}^+ / 2\text{e}^-$ reduction steps, the intermediates diazene N_2H_2 , and hydrazine N_2H_4 , and that the first step is the most difficult step, the cleavage of the first bond in the N_2 triple bond [21b] (Scheme 2).

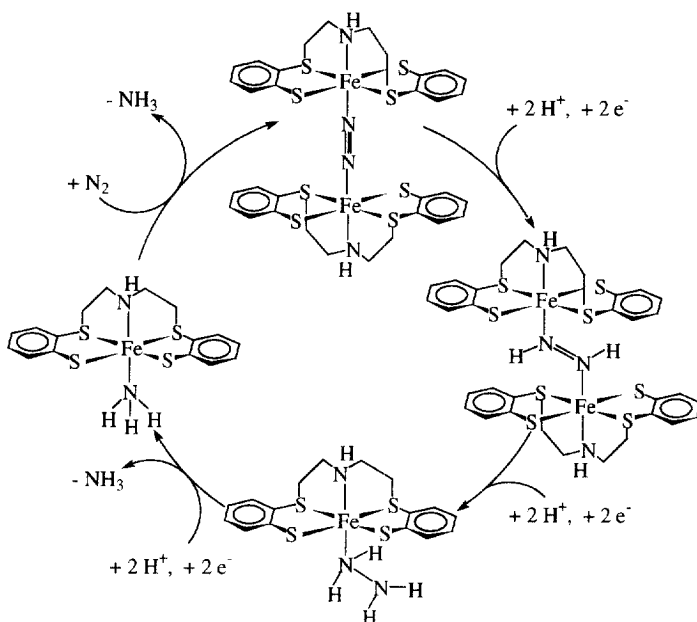
In free state, diazene is an endoergic and extremely unstable molecule which decomposes at temperatures above -180°C [31]. This raises the question of how such a molecule can be stabilized in the metal sulfur coordination sphere of FeMoco or, more general, $[\text{MS}]$ complexes in order to avoid insurmountably high barriers on the reaction coordinate.



Scheme 2. Intermediates of N_2 fixation, and a reaction coordinate stressing the high-barrier if diazene occurred in free state.

This question was answered by the diazene complex $[\mu-N_2H_2\{\text{Fe}(\text{N}_4\text{S}_4)\}_2]$ which is part of our hypothetical 'dream' cycle of N_2 fixation shown in Scheme 3.

In this cycle, the $[\text{Fe}(\text{N}_4\text{S}_4)]$ fragment binds N_2 , and the resulting N_2 complex is subsequently reduced via the N_2H_2 and N_2H_4 complexes down to the NH_3 complex which exchanges NH_3 for N_2 . The starting N_2 complex could not be established, however, the NH_3 , N_2H_4 and N_2H_2 complexes were synthesized and completely characterized [32], in particular, the diazene complex [33]. In a kind of 'retro N_2 fixation' it was oxidatively obtained from the hydrazine complex. Its structure is shown in Fig. 3.



Scheme 3. Hypothetical model cycle of N_2 fixation by $[\text{Fe}(\text{N}_4\text{S}_4)]$ fragments.

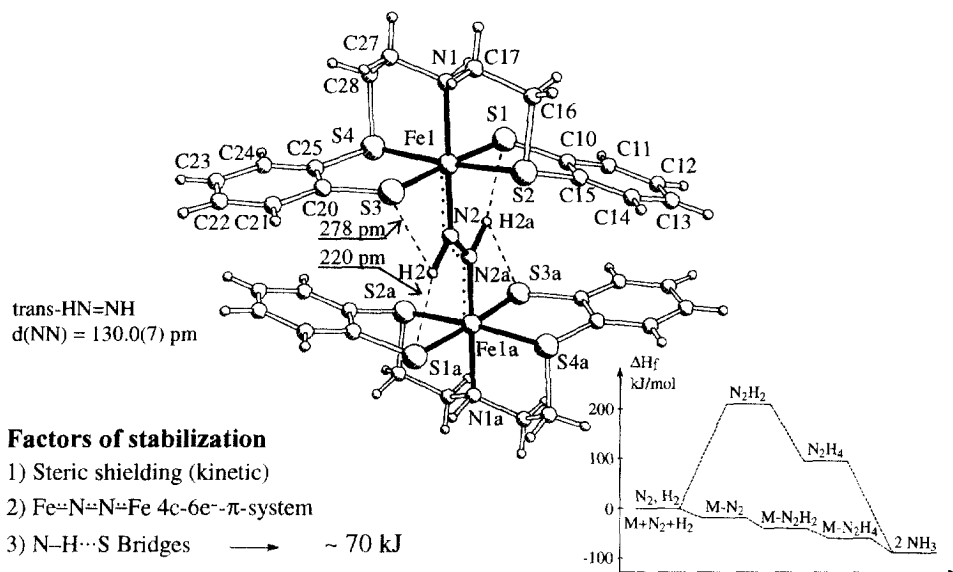
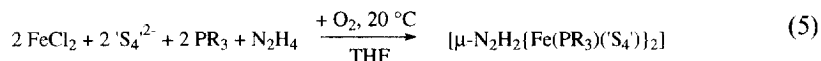


Fig. 3. Molecular structure of $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{N}_4\text{S}_4)\}_2]$ (redrawn after Ref. [33]), factors stabilizing complex-bound N_2H_2 , and reaction coordinates for the $\text{N}_2 \rightarrow \text{NH}_3$ transformation in the absence or presence of suitable metal catalysts.

The N_2H_2 ligand bridges two $[\text{Fe}(\text{N}_4\text{S}_4)]$ fragments and the resulting $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{N}_4\text{S}_4)\}_2]$ is thermally stable far above room temperature. Thus, the diazene experiences an enormous stabilization by complexation. This stabilization can be traced back to three major factors: (1) steric shielding of the N_2H_2 bridge by the bulky $[\text{Fe}(\text{N}_4\text{S}_4)]$ fragments. (2) The formation of $4c\text{-}6e^-\pi$ -molecular orbitals formed from iron d and diazene p orbitals. This π bonding gives rise to the very intense dark blue color of $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{N}_4\text{S}_4)\}_2]$ and probably represents the largest stabilization factor. (3) Strong intramolecular bifurcated $\text{N-H}\cdots(\text{S})_2$ bridges, which are indicated by the distances between thiolate S donors and diazene NH protons. A very cautious estimate, based on the extremes of weak hydrogen bridges in H_2S ($\Delta H = 7.7 \text{ kJ mol}^{-1}$) [34] and calculated very strong $\text{N-H}\cdots\text{S}$ bridges in ferredoxines ($\Delta H = 80 \text{ kJ mol}^{-1}$) [35], leads to about 70 kJ of additional stabilization energy for N_2H_2 that results from the two bifurcated $\text{NH}\cdots(\text{S})_2$ bridges. These hydrogen bridges are not expected in the corresponding hypothetical N_2 complex, and they alone would 'neutralize' one third of the positive ΔH_f of free diazene. All three effects (steric shielding, $4c\text{-}6e^-\pi$ bonds, H bridges) taken together signify that even the first reduction step from N_2 to N_2H_2 could become exergonic such that all steps of N_2 fixation would proceed downhill in small steps. This is the way biological processes usually proceed and the secret of many catalyses.

Through its structure and spectroscopic properties $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{N}_4\text{S}_4)\}_2]$ had become a landmark for our research. The other properties, however, represented severe handicaps. $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{N}_4\text{S}_4)\}_2]$ formed in very low yields only (15–30 mg)

and proved so sparingly soluble that its chemistry in homogeneous phase could not be investigated. Other diazene complexes were needed which exhibited identical features as far as possible but were better accessible and soluble. In the search for such complexes, we found the $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{PR}_3)(\text{'S}_4')\}_2]$ complexes ($\text{R} = \text{'Prop, 'Bu}$). They form in high yields (10 g) in one-pot syntheses according to Eq. (5).



The molecular structure of the P''Pr_3 derivative (Fig. 4) showed that the bridging N_2H_2 ligand is stabilized by the identical three effects as in $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{N}_\text{H}\text{S}_4')\}_2]$.

Analogous, and also structurally characterized, complexes of ruthenium such as $[\mu\text{-N}_2\text{H}_2\{\text{Ru}(\text{PR}_3)(\text{'S}_4')\}_2]$ [37] support that these bonding features of diazene in metal sulfur complexes are a general phenomenon. These N_2H_2 complexes proved well soluble. They contain stable ($\text{M}-\text{NH}=\text{NH}-\text{M}$) chromophores that are retained in PR_3/PR'_3 phosphine substitution reactions. The diazene protons undergo base catalyzed H^+/D^+ exchange reactions [38]. Last but not least, these complexes are redox-active and yielded cyclic voltammograms (CV) that allowed key conclusions (Fig. 5) [36,38].

Fig. 5 depicts the cyclic voltammogram of $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{P''Pr}_3)(\text{'S}_4')\}_2]$ at -20°C showing three quasi-reversible anodic redox waves. At -78°C , even a fourth (irreversible) wave at 1.23 V can be observed. The unequal intensities of the waves can be traced back to concomitant very rapid deprotonation/protonation equilibria [38]. In general, the CV's of these diazene complexes sensitively depend upon the addition of bases or acids. They also depend upon the scan range demonstrated by the CV's of $[\mu\text{-N}_2\text{H}_2\{\text{Ru}(\text{PCy}_3)(\text{'S}_4')\}_2]$ (Fig. 3(b)), in which the first two anodic redox waves become reversible only when the scan is reversed at $+0.8$ V [37].

The important point is that these diazene complexes can reversibly be oxidized in at least two steps. These steps are assigned to the formation of the corresponding

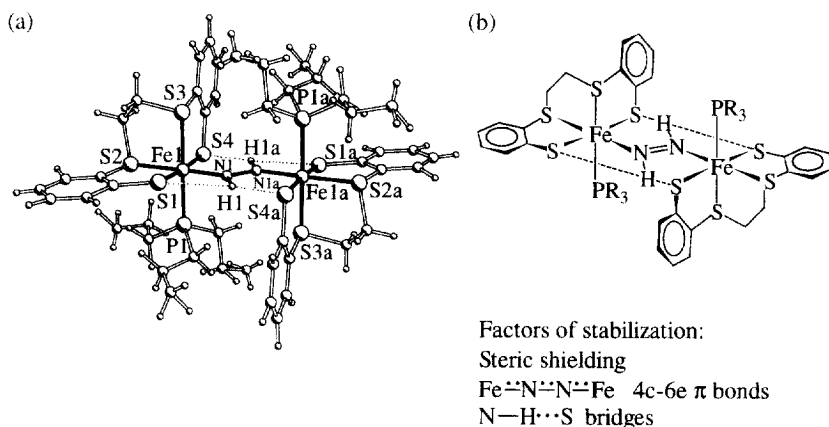


Fig. 4. (a) Molecular structure of $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{P''Pr}_3)(\text{'S}_4')\}_2]$; (b) schematical drawing stressing the planar arrangement of Fe centers, thiolate donors and N_2H_2 atoms.

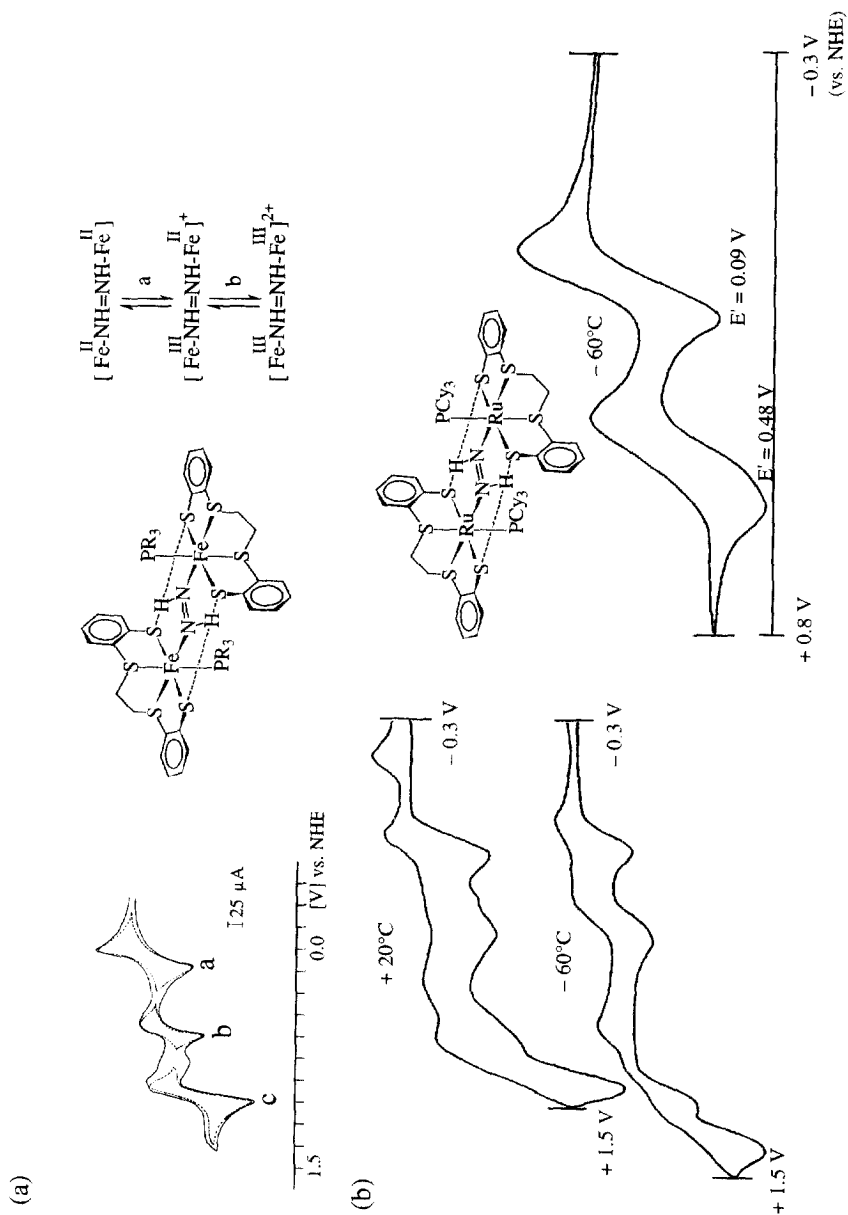


Fig. 5. (a) Cyclic voltammogram of $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{PPTr})(\text{S}_4)\}_2]$ at -20°C (CH_2Cl_2 , $v = 20 \text{ mV s}^{-1}$) and assignment of (formal) Fe oxidation states. (b) Cyclic voltammograms of $[\mu\text{-N}_2\text{H}_2\{\text{Ru}(\text{PCy}_3)(\text{S}_4)\}_2]$ at $+20$ and -60°C (CH_2Cl_2 , $v = 50 \text{ mV s}^{-1}$) within different scan ranges.

monocation and dication. Formal assignment of metal oxidation states then results in diazene complex dications which in the iron case contain two Fe(III) centers. The reversibility of these two steps forms the basis of our working hypothesis to achieve the first and most difficult step of N_2 reduction under mild conditions.

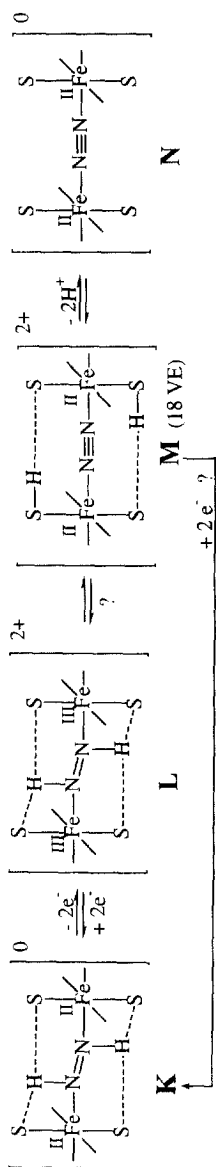
Redrawing the relevant core atoms of the dication (Fe , N_2H_2 , S (thiolate) donors) (Scheme 4) shows that the dicationic diazene complex **L** is a redox isomer (or valence tautomer) of the doubly protonated N_2 complex **M**.

Transfer of two electrons from the diazene ligand to the two iron centers, cleavage of the NH bonds and formation of the SH bonds transforms species **L** into species **M**. Loss of two protons yields the neutral (and hypothetical) $Fe(II)-N_2$ complex **N**. In accordance with the reversible protonations of the carbonyl complex $[Fe(CO)(N_{11}S_4)]$ and related species, this last step can be expected to take place reversibly and in a stereocontrolled way favored by formation of $S-H\cdots S$ bridges.

Conversion of the neutral $Fe(II)-N_2$ complex **N** into the neutral N_2H_2 complex **K** would be equivalent to the first reduction step of N_2 to N_2H_2 in Schemes 2 and 3. This conversion would be easy if, after two-fold protonation of **N**, the resulting **M** and species **L** were in real equilibrium, because the CV of Fig. 5(a) shows that the dication **L** can be reduced to give the neutral diazene complex. However, more probably, two electrons will have to be transferred directly to species **N** or **M**. Species **N** is an 18 valence electron complex which, if accessible, can be expected to have a very negative reduction potential like the isoelectronic CO complex $[Fe(CO)(N_{11}S_4)]$ (see above). But exactly as has been observed for $[Fe(CO)(N_{11}S_4)]$, two-fold protonation of **N** can be anticipated to anodically shift the redox potential by 1.0–1.4 V. Therefore, as soon as the N_2 complex **N** has formed, it should be easy to protonate it to give **M** and then to reduce **M** to **K** at reduction potentials around -500 mV, i.e. biologically compatible mild reduction potentials.

The remaining, and probably toughest, problem is the formation of the N_2 complex **N**. In this context it is to be noted that N_2 complexes with sulfur rich coordination spheres are conspicuously rare and such complexes which form at redox potentials above -500 mV (needing no strong reductants for synthesis) are unknown. It is therefore worth noting that evidence could be obtained for the existence of **M** and **N**, respectively [38]. Oxidation of the diazene complex $[\mu-N_2H_2\{(P''Pr_3)Fe(S_4')\}_2]$ with two equivalents of Cp_2FePF_6 at $-78^\circ C$ yielded stoichiometric amounts of Cp_2Fe and a compound analyzed as $[N_2\{Fe(P''Pr_3)(S_4')\}_2] \cdot 2HPF_6$. This species is so thermally labile that it decomposes above $-40^\circ C$ releasing one N_2 that was volumetrically determined and identified by isotopic labeling. The additionally resulting $[Fe(P''Pr_3)(S_4')]$ fragment was identified by trapping experiments with CO or PMe_3 yielding $[Fe(CO)(P''Pr_3)(S_4')]$ or $[Fe(PMe_3)(P''Pr_3)(S_4')]$. Similar results were obtained when $[\mu-N_2H_2\{Fe(N_{11}S_4')\}_2]$ was oxidized with Cp_2FePF_6 . Again, N_2 was released and the coordinatively unsaturated $[Fe(N_{11}S_4')]$ fragment formed which could be characterized even by X-ray structure analyses [39].

The question arises why the N_2 complexes presumably formed as intermediates are so unstable or why the five-coordinate $[Fe(P''Pr_3)(S_4')]$ and $[Fe(N_{11}S_4')]$ frag-



Scheme 4. Redoxisomerism of the $[\mu\text{-N}_2\text{H}_2\{\text{Fe}(\text{P}^n\text{Pr}_3)(\text{S}_4')\}_2]^2+$ dication.

ments bind N_2 only so labile. One possible reason could be that these fragments are highly fluxional and exhibit high-spin Fe(II) centers. Their spin-state and stereochemical non-rigidity can be expected to disfavor the coordination of N_2 and, in turn, to cause instability of the N_2 derivatives. The $[Fe('N_HS_4')]$ fragment, for example, can exist in the two diastereomeric forms **O** and **P** indicated in Fig. 6 [32,39].

Diastereomer **O** has *cis*-thiolate donors and binds σ ligands such as N_2H_4 or NH_3 to give high-spin adducts which are labile because they have two electrons in antibonding e_g^* orbitals. Diastereomer **P** has *trans*-thiolate donors and binds σ - π ligands such as CO, N_2H_2 or phosphines to give the diamagnetic and stable low-spin adducts. Only isomer **P**, however, would be suited to bind N_2 and to form the above mentioned thiolate-diazene $NH...(S)_2$ bridges in subsequent protonation and reduction reactions of the N_2 complex.

For this reason, we tried to enforce the *trans*-thiolate configuration in complexes with $[FeNS_4]$ cores by replacing the flexible NH (dialkyl) bridge of the ' N_HS_4 '²⁻ ligand through a sterically rigid dialkyl pyridine bridge in the new ' pyS_4 '²⁻ ligand [40]. The dialkyl pyridine bridge indeed enforces the *trans* coordination of the thiolate donors in the resulting $[FeNS_4]$ cores (Fig. 7).

In a significant way, now also σ ligand adducts such as the structurally characterized hydrazine complex $[Fe(N_2H_4)('pyS_4')]$ have low-spin Fe centers and are diamagnetic [41]. The target N_2 complex with the $[Fe('pyS_4')]$ fragment remains to be established. However, THF or DMF solutions of $[Fe('pyS_4')]$, when treated with N_2 , yield mass spectra showing a peak at $m/z = 910$ which corresponds to the binuclear N_2 complex $[\mu-N_2\{Fe('pyS_4')\}_2]$ [41]. Thus, the wanted competitive catalyst for N_2 reduction still awaits its isolation, but signs of hope exist.

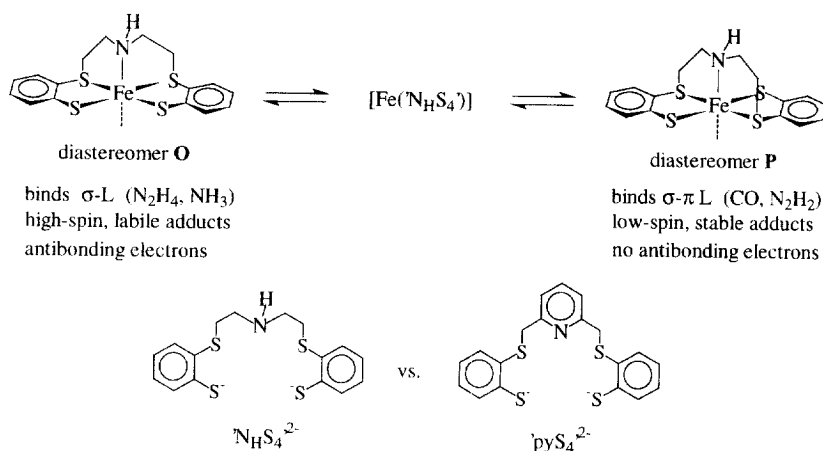


Fig. 6. Diastereoisomerism and low-spin versus high-spin states of $[Fe('N_HS_4')]$, and schematical drawings of ' N_HS_4 '²⁻ and the new ' pyS_4 '²⁻ ligand.

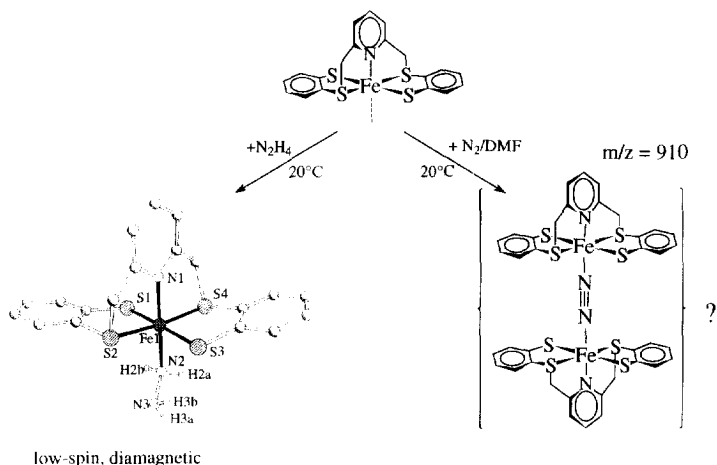


Fig. 7. Sterical enforcement of *trans* thiolate coordination and Fe(II) low-spin states in $[\text{Fe}(\text{'pyS}_4\text{'})]$ complexes.

Another question is whether all these experiments and results can yield an idea of how FeMoco functions. This is attempted in the last section.

6. A model for FeMoco function

The model rests on the combination of all results described above. Enforcement of low-spin Fe(II) states by sterical constraints, for example, could be one reason why the nitrogenase protein is essential for the FeMoco function. The kinetic lability of MS bonds in FeMoco, which is also found for many synthetic metal sulfur ligand complexes with $[\text{M}(\text{'S}_4\text{'})]$, $[\text{M}(\text{'N}_4\text{'S}_4\text{'})]$ and related fragments [32,39,42], suggests reversible M–S bond dissociation reactions.

The Brønsted basicity of sulfur donors and the structure–function relationship of [MS] complexes outlined above for $[\text{Fe}(\text{'N}_4\text{'S}_4\text{'})]$ complexes allows the coupling of proton and electron flux. Further points are: in native nitrogenase, the FeMoco is surrounded by water molecules and two essential amino acids (Gln α 191, His α 195) whose site directed mutagenesis causes complete or nearly complete inactivity of the enzyme [43,44]. In the resting state, these amino acids do not bind to the FeMoco, but they are close to it and possess O or N donors with which they could coordinate to metal centers. An increasing number of enzymes are shown to have different structures in the resting and in the turn-over state. Examples are carboxypeptidase and aconitase. Kinetic data on the FeMo protein redox cycle (the Thorneley–Lowe cycle) indicate that the FeMo protein must take up at least two electrons before it can bind N_2 [45]. All of these observations taken into account allow us to envisage that, in the turn-over state, the FeMoco opens one Fe–S–Fe bridge and coordinates the amino acid donors and nearby water molecules to the respective Fe centers. In this way, two unique five-coordinate Fe(II) centers could result, which are also low-spin via sterical constraints from the protein, as indicated in Fig. 8.

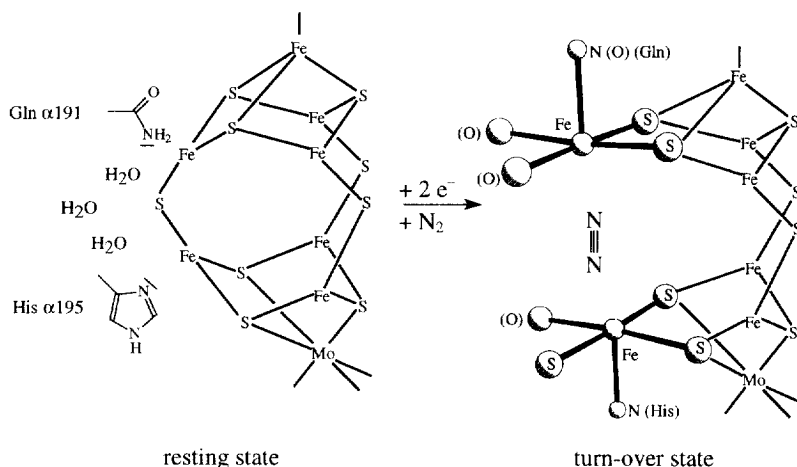


Fig. 8. Opening the FeMoco after take-up of two electrons, and generation of two unique five-coordinate low-spin Fe(II) centers.

These two unique five-coordinate Fe(II) centers can be anticipated to favor the binding of N_2 that is subsequently reduced by coupled proton/electron flux in the same way as suggested for the diazene–dinitrogen complexes in Scheme 4 above.

A further pillar of this model is the structure of $[\mu\text{-}N_2H_2\{\text{Fe}(\text{'N}_{11}\text{S}_4\text{'})\}_2]$. It contains only biologically compatible donor atoms around the metals and allows us to illustrate the first N_2 -reduction stage (Fig. 9).

Fictitious removal of all carbon atoms from the diazene complex and merging the remaining core with the two unique Fe centers of the 'open' FeMoco demonstrates that the Fe centers and their donors are in the correct position to favor the binding of N_2 or N_2H_2 and, in addition, the formation of essential hydrogen bridges.

The 'rest' of the FeMoco can act as a flexible spacer for the two unique Fe centers which must be able to move apart in order to accommodate not only N_2 but also the increasingly larger reduction intermediates N_2H_2 and N_2H_4 . Also, the variable distance between the two unique Fe centers plausibly explains the reduction of other nitrogenase substrates. In conjunction with the heterolytic activation of H_2 at $[M(\text{'S}_4\text{'})]$ centers, last but not least, such a model also explains the nitrogenase catalyzed H_2 reactions. They require a vacant site which can form by dissociation of one Fe-donor bond in the presence, as well as in the absence, of bound N_2 or N_2H_x reduction products.

Substitution of the molybdenum atom by vanadium or iron finally would yield models for the alternative 'FeV' or 'all-Fe' nitrogenase cofactors. In the end, the heterometals may act as 'fine-tuners' only for the electron transfer steps.

This model, first published in 1996 [46], 'destroys' the fascinating beauty of FeMoco in its resting state and certainly needs further experimental evidence. However, it is interesting to note that Hales, Hoffman et al. late last year concluded from spectroscopic investigations that CO, the strongest nitrogenase inhibitor,

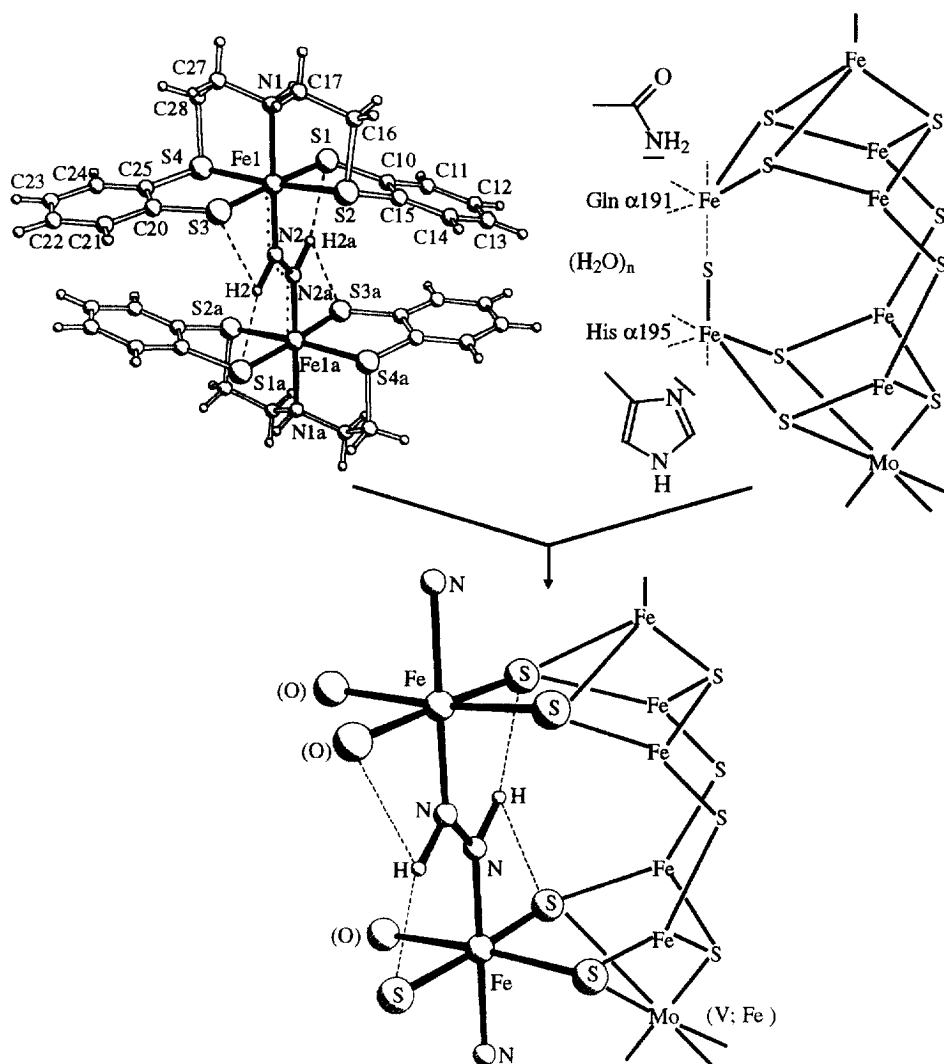


Fig. 9. The 'ultimate' nitrogenase model.

binds to exactly the same two iron centers in the FeMoco 'waist-region' [47], which this model utilizes to bind N_2 .

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