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Modeling of the molybdenum center in the nitrogenase FeMo-cofactor

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Contents

Abstract

The functional, structural and theoretical chemical approaches to specifically model the molybdenum center of the nitrogenase enzyme are reviewed. We show how dinitrogen can be reduced at monometallic centers and highlight attempts to develop a nitrogenase-relevant dinitrogen reduction chemistry at molybdenum–sulfur complexes and clusters. The theoretical work addressing the molybdenum issue is also reported together with models for nitrogenase function, some of them directly involving the molybdenum atom.

Keywords: Nitrogenase; FeMo-cofactor; Molybdenum; Sulfur; Nitrogen fixation; Modeling

1. Introduction

Biological dinitrogen fixation is the crucial life process by which the nitrogenase enzymes, present only in restricted classes of micro-organisms, catalyze the reduction of atmospheric nitrogen into ammonia under

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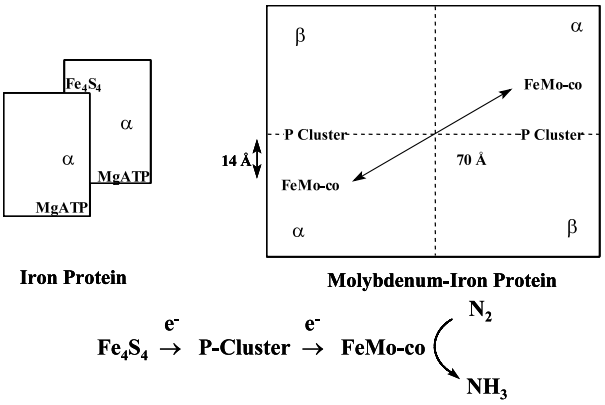


Fig. 1. Schematic representation of the Fe-protein (60 kDa, α_2 dimer) and the MoFe-protein (250 kDa, $\alpha_2\beta_2$ tetramer) of the Mo-nitrogenase, together with the outline of the electron transfer path through the metallo clusters.

ambient temperature and pressure. In the form of ammonium, 'fixed dinitrogen' enters the plants' anabolism as the primary source of nitrogen for the biosynthesis of N-containing molecules as diverse and essential as, for example, nucleotides and amino-acids. Among the different types of nitrogenase enzymes known, the Mo–nitrogenase is the most extensively studied[1,2] and the only one for which detailed structural information is available [3,4]. The enzyme consists of two metalloproteins schematically drawn in Fig. 1. The iron protein is a specific, nucleotide-dependent, reductant of the molybdenum–iron protein. The electrons are shuttled from the Fe_4S_4 cluster of the iron protein to the active site of the enzyme via the Fe_8S_7 cluster (P-cluster) of the iron–molybdenum protein, in a MgATP hydrolysis dependent process. The active site of the enzyme is called the iron–molybdenum cofactor or FeMo-co, at which dinitrogen is bound and reduced to ammonia along with the overall limiting approximate stoichiometry outlined in Fig. 2 [5].

The cofactor structure [3,4], (Fig. 2), consists of a $MoFe_7S_9$ cluster that can be viewed as the assembly of two incomplete $Fe_4(\mu_3\text{-}S)_3$ and $MoFe_3(\mu_3\text{-}S)_3$ sub-cubes, linked together by three central μ_2-S bridging sulfide ligands. Six of the seven iron atoms in the cluster core possess the rare distorted trigonal geometry. The pseudo-octahedral coordination of molybdenum is completed by the exogenous bidentate (*R*)-homocitrate ligand and by imidazole from a neighboring histidine residue of the polypeptide backbone. A cysteine residue, covalently bound to the tetrahedral apical iron atom, provides the cofactor with its other protein ligation.

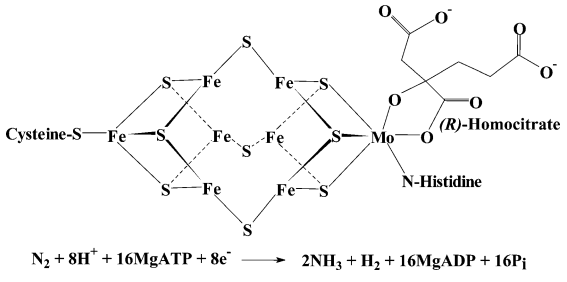


Fig. 2. Structure of the FeMo-co and function of the Mo–nitrogenase.

Although there is still no experimental evidence as to whether the N_2-binding and activation site on FeMo-co is located at molybdenum or at the approximate trigonal prism formed by the six central iron atoms array, the latter is often referred to as the likely site for dinitrogen activation. This is largely due to the unprecedented structure of the cluster core and to the apparent undercoordination of the trigonal iron centers that might be highly reactive and well suited for N_2 binding. Moreover, the fact that the molybdenum atom is coordinatively saturated seems, at first sight, to proscribe substrate activation at this center. However, the molybdenum atom could still be involved in catalysis either by an increase of its coordination number[6] or by the opening of a coordination site after the loss of a ligand, possibly induced by electron transfer. It is known from stopped-flow kinetics experiments that dinitrogen binds to the active site only after three electrons have been transferred to the cofactor [7]. In addition, on the basis of recent molybdenum-iron protein Mössbauer spectra analyses, it has been suggested that the first one-electron reduction of FeMo-co could be centered principally on the molybdenum atom [8]. The decrease of the molybdenum oxidation state might lead to changes in its coordination sphere. It should also be stressed that the available protein structures are derived from the enzyme in the resting state and that no information is available on the structure of FeMo-co during catalysis. Structural changes are likely to occur and this has been demonstrated for two oxidation states of the P-cluster [4,9,10]. In the reduced state, (Fig. 3 (top)), the Fe_8S_7 cluster can be described as two Fe_4S_4 cubes sharing one common sulfur atom, the iron atoms being linked to the protein by cysteinate ligands, two of them bridging the sub-

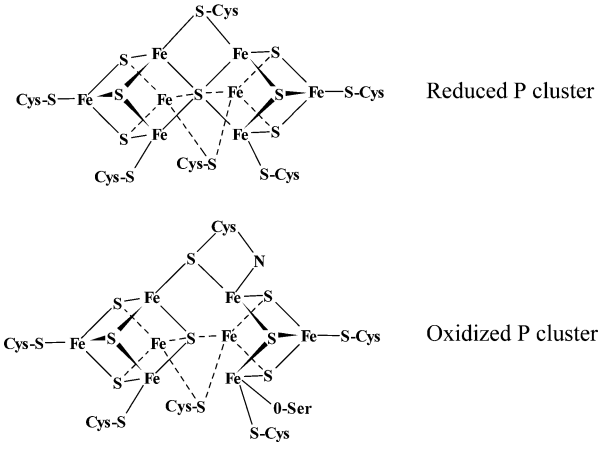


Fig. 3. Structure of the P-cluster in two oxidation states.

cubes. In the oxidized state, (Fig. 3 (bottom)), the central sulfur atom looses two bonds with two iron atoms in one of the sub-cubes, thus becoming more open. The tetrahedral coordination of these two iron atoms is then completed by extra ligations from neighboring protein residues.

With this in mind, and the question of the precise location of the N_2-activation site at FeMo-co remaining open, it appears appropriate to review the model chemistry that assesses the idea of a direct role for molybdenum in biological nitrogen fixation.

Approaches available to the inorganic chemist are: (i) functional model chemistry that consists of establishing the mechanism of N_2-reduction at synthetic metal centers (ii) structural model chemistry that consists of the synthesis and study of clusters containing some structural features akin to those of the cofactor, or better, its total synthesis, which is far from straightforward and has not been achieved to date, and (iii) theoretical calculations on the cofactor itself to investigate the feasibility of speculative reaction pathways.

Ideally, these three different approaches should merge to give a clear picture on how molecular nitrogen could be activated and reduced at the bio-metallo cluster.

In this account, results from studies in the three approaches, specifically relevant to the modeling of the molybdenum center of the FeMo-co are reviewed.

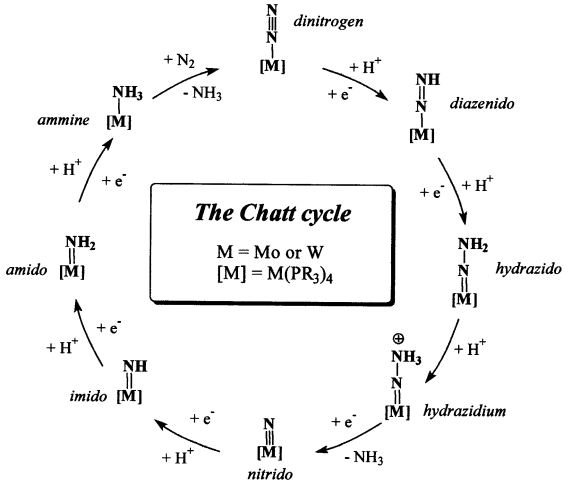
First we discuss the well-established dinitrogen reduction chemistry involving mononuclear molybdenum or tungsten functional models with low or high metal oxidation state (Sections 2 and 3, respectively). Then we consider more relevant structural models that better take into account the actual environment of molybdenum in the FeMo-co. These models are mononuclear Mo-thiolate centers (Section 4), dinuclear Mo-thiolate centers (Section 5) and $MoFe_3S_4$ cubane-like clusters (Section 6). Despite the lack of interaction between these structural models and molecular nitrogen, we also highlight their partial functional chemistry involving more reduced diazo derivatives. In Section 7 are reviewed several speculative biological mechanisms inspired by different synthetic models and involving different sites at FeMo-co. The theoretical work undertaken to assess the possibility of molybdenum as the active site in FeMo-co is then addressed in Section 8. Finally, the different proposed models for biological nitrogen fixation are summarized and critically assessed and the conclusion offers some perspectives on the future of chemical and theoretical modeling of nitrogenase structure and function (Section 9).

2. N_2-reduction at the MP_4 center (M = Mo, W)

For a metal center to deserve the title of true functional model of nitrogenase requires at the very least that it interacts with N_2. A more accurate functional model would also allow the activation of dinitrogen up to ammonia formation, and isolation or synthesis of possible intermediates on the path to NH_3 synthesis. An even more refined functional model would also account for other aspects of nitrogenase function such as the reduction of other substrates or the peculiarities of H_2 evolution which is coupled to N_2 reduction in the enzyme [1,2]. An excellent functional model would also have to be catalytic.

Meeting all the above requirements, as it might be anticipated, has proven to be rather challenging. Nevertheless, the MP_4 center chemistry (where M = Mo or W and P_4 is a combination of mono or diphosphine ligands) has provided so far the most complete functional model with respect to the criteria defined above. This chemistry, developed largely in the groups of Hidai [11,12], Chatt [13–15] and George [16], has shown that the metal–phosphine center is able to bind N_2 in an end-on fashion leading to its activation towards protonation, in some cases up to ammonia formation.

A stoichiometric yield of ammonia has been obtained by protolysis of *cis*-$[M(N_2)_2(PMe_2Ph)_4]$ or *trans*-$[M(N_2)_2(PMePh_2)_4]$ with sulfuric acid in methanol, M = Mo or W [17,18]. An electrocatalytic cycle of N_2-reduction to ammonia is possible with *trans*-$[W(N_2)_2(dppe)_2]$: NH_3 is produced by protonation and electrochemical reduction of the complex at low potential (−2.7 V vs. the ferrocene/ferrocenium couple in tetrahydrofuran–$[NBu_4][BF_4]$ 0.2 M electrolyte). The $W(dppe)_2$ motif is retained which allows the recovery of the bis-N_2 complex under dinitrogen and the repetition of the cycle upon addition of *para*-toluenesulfonic acid (TsOH) [19,20]. Not only is ammonia synthesis from molecular nitrogen possible at the MP_4 center but also



Scheme 1. The Chatt cycle for dinitrogen reduction at a molybdenum or tungsten metal phosphine center. '$P(R_3)_4$' is a combination of mono- or di-tertiary phosphine ligands: for example dppe (diphenylphosphino ethane) or PMe_2Ph.

numerous likely intermediates of N_2-reduction to NH_3 have been isolated or indirectly synthesized and characterized by X-ray diffraction: these are the $MNNH_2$ hydrazido(2-) [21–28], $MNNH_3$ hydrazidium [28,29], MN nitrido [30], MNH imido [31,32] and MNH_2 amido [33] derivatives. The MNNH diazenido complex which is most likely the first intermediate of N_2-reduction can be prepared by treatment of the $MNNH_2$ hydrazido(2-) precursor with a weak base but its X-ray crystal structure is not yet available [34]. On these bases, the Chatt cycle (Scheme 1), involving successive N_2-reduction and protonation steps, has been proposed to be of possible relevance to the biological process involving the Mo center of nitrogenase [15,35,36].

More recent electrochemical studies [35,37] on $[MH_2(\eta^2\text{-}O_2CR)(dppe)_2]^+$ complexes (M = Mo or W) have shown that a η^1-carboxylate ligand can function as a leaving group at a reduced molybdenum dihydride center, generating a coordination site where dinitrogen can bind in an end-on fashion, which is followed by dihydrogen loss. Protic attack of the coordination site affords a trihydride intermediate, which can partake in a dihydrogen evolution cycle. The features of H_2 evolution at this MP_4 site are consistent with the Lowe–Thorneley scheme [7] which describes the first steps of Mo–nitrogenase turnover in the presence and in the absence of the substrate. In the biological cluster, the carboxylate of the exogenous (*R*)-homocitrate bidentate ligand on molybdenum could act as a tethered leaving group, producing or protecting the coordination site for N_2 at this metal. Subsequent reduction and protonation of the end-on Mo-coordinated N_2 could then proceed as described in the Chatt cycle (Scheme 1) [35,36].

The 'carboxylate as a tethered leaving group' proposal has been found useful to rationalize recent kinetic data [38] on the coordination of thiophenolate to extracted cofactors possessing either (*R*)-homocitrate (in a cofactor extracted from a wild type bacterial strain), or citrate (in a cofactor extracted from a mutated bacterial strain) as the exogenous ligand at Mo. In the extracted cofactors, the protein ligations are replaced by coordinating N-methyl formamide (NMF) solvent molecules. NMF can be displaced at the apical iron atom by PhS^- or CN^- and at molybdenum by N_3^- or imidazole ligands, H^+ is believed to bind to bridging sulfur. Reactivity of both extracted cofactors towards PhS^--binding are similar when CN^-, N_3^- or H^+ bind to the cluster core, but different when imidazole binds to molybdenum. These results have been interpreted in terms of hydrogen bonding between one carboxylate arm of (*R*)-homocitrate and the NH group of the imidazole ligand; the shorter polycarboxylate citrate ligand being unable to form this type of weak bonding interaction. Using molecular mechanics modeling, the authors have shown that for the hydrogen bond to occur in the protein, the (*R*)-homocitrate ligand must go monodentate, thus opening a coordination site at molybdenum to which N_2 could bind (Fig. 4). This model provides an elegant explanation for the high-specificity of (*R*)-homocitrate for enzyme activity, and can be seen as a refinement step of the MP_4 center as a functional model.

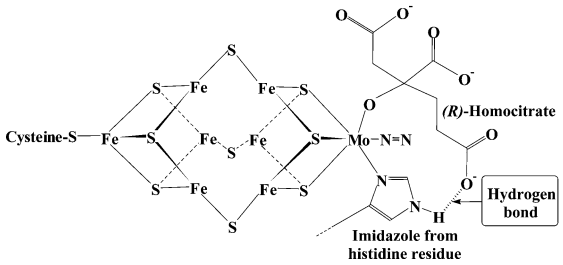


Fig. 4. A model for the specific requirement of the (*R*)-homocitrate ligand for nitrogenase function. It includes (i) a monodentate homocitrate ligand, (ii) hydrogen bonding between the long carboxylate arm of homocitrate and imidazole, (iii) dinitrogen bound at molybdenum.

Hidai and co-workers have provided recent interesting developments to the protonation of coordinated N_2 at the metal-phosphine center [39]. They have shown that hydrosulfido-bridged dinuclear complexes such as $[Cp^*Ir(\mu\text{-}SH)_3IrCp^*]^+$ or $[P_3Fe(\mu\text{-}SH)_3FeP_3]^+$ (P_3 is bis(2-diphenylphosphinoethyl)phenylphosphine) are able to protonate coordinated dinitrogen to produce either moderate yields of ammonia when the N_2 complex is *cis*-$[W(N_2)_2(PMe_2Ph)_4]$ or high yield of the hydrazido derivative when the N_2 complex is *trans*-$[W(N_2)_2(dppe)_2]$ [40,41]. This might be of relevance to nitrogen fixation since the sulfides of the biological metal–sulfur cluster are likely to be basic sites and may mediate protonation of coordinated N_2 and/or hydrogen evolution. In the context of dihydrogen production by the enzyme (which happens in the absence of substrate as a general H_2 evolution process, and that is also intimately coupled to N_2 reduction as an obligatory H_2 evolution process) it is worth mentioning the work of Hidai and co-workers on the use of dihydrogen to protonate coordinated N_2 via sulfido-bridged di-molybdenum complexes [42] or acidic metal hydrides [43–46].

The dinitrogen chemistry of the molybdenum (or tungsten) phosphine center described here provides a detailed picture on the stepwise reduction of N_2, and in some cases, includes other nitrogenase relevant aspects such as H_2 evolution or protonation of coordinated N_2 mediated via hydrosulfido complexes.

However, the relevance of the metal–phosphine model to nitrogenase function can be questioned by the following criticisms: (i) the model relies on low oxidation states complexes, typically Mo or W(0), incompatible with the biological system where the molybdenum atom is in the +III or +IV oxidation state when the enzyme is in the resting state [8]; (ii) the complexes possess abiological phosphine co-ligands,

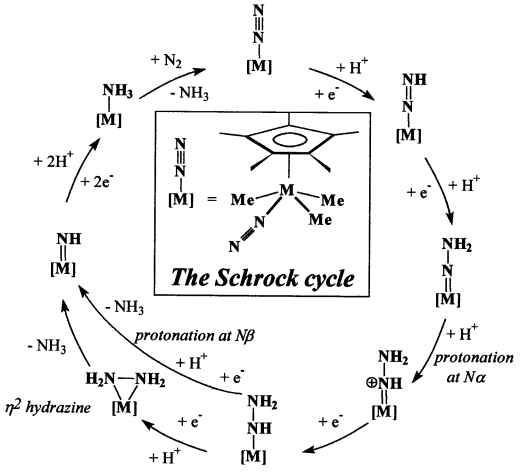
and (iii); the complexes are mononuclear whereas the molybdenum atom belongs to an heteropolymetallic cluster in the enzyme.

We highlight in the next section, N_2-reduction chemistry at Mo or W metal centers bearing a higher oxidation state, that may respond to the first criticism to the MP_4 model.

3. N_2-reduction at high oxidation state metal centers

Schrock and co-workers have shown that dinitrogen activation by molybdenum or tungsten complexes with high oxidation state is possible at the MCp^*Me_3 core, M = Mo or W, Cp* = pentamethylcyclopentadienyl [47].

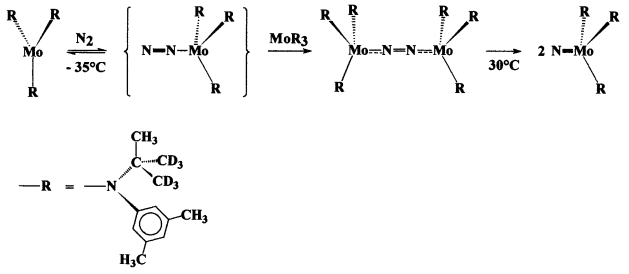
For instance, the following reactions have been carried out at the conserved WCp^*Me_3 site. Reduction by sodium of $WCp^*Me_3(OTf)$ (OTf = triflate) under dinitrogen leads to the formation of the N_2-bridged complex $[Cp^*Me_3W]_2(\mu\text{-}N_2)$ [48]. Ammonia production is nearly quantitative by reduction of the N_2-bridged complex in the presence of protons [49], but weak (< 3%) by reduction in the same conditions of $WCp^*Me_3(OTf)$ under N_2. The difference in reactivity between the dimer and the monomer could be accounted for by the protonation of the metal site that would prevent interaction with N_2 [50]. However $[WCp^*Me_3(\eta^2\text{-}N_2H_4)]^+$ and $WCp^*Me_3(\eta^1\text{-}NNH_2)$ allow stoichiometric ammonia formation and support the catalytic reduction of hydrazine to ammonia in the presence of a proton source [50,51]. One-electron reduction of $[WCp^*Me_3(\eta^2\text{-}N_2H_4)]^+$ yields the imido derivative and NH_3 almost quantitatively [51]. Moreover the complexes $WCp^*Me_3(NH)$, $WCp^*Me_3(NH_2)$ and $[WCp^*Me_3(NH_3)_x]^+$ (x = 1 or 2) have been isolated and characterised [52], and represent possible N_2-reduction intermediates. These observations led to suggest that the initial activation of N_2 is probably monometallic and to the proposal of an alternative N_2-reduction cycle at a mononuclear metal center (Scheme 2) [47,51]. The main mechanistic difference with the Chatt cycle is the presence of an hydrazine-bound intermediate prior to the splitting of the N–N bond. The often very stable metal nitride intermediate, that implies an increase of the metal center oxidation state by three units and could represent a dead end for ammonia synthesis, is thus avoided. One of the striking features of the MCp^*Me_3 center is to possess two coordination sites in a *cis* position that allow the N_2H_x intermediates of N_2-reduction to rearrange in an η^1-η^2 fashion. This is not possible in Chatt-type complexes with the $M(dppe)_2$ core where the coordination sites are *trans*.[1]



Scheme 2. The Schrock cycle for dinitrogen reduction at a molybdenum or tungsten 'high oxidation state' metal center.

At a different metal site (Mo or W), coordinated by aryl-substituted triamidoamine tripodal ligands of the type $[N(CH_2CH_2NR)_3]^{3-}$, Schrock and co-workers have developed another somehow parallel 'high oxidation' metal-dinitrogen chemistry where N_2 is activated towards electrophiles [47,54,55a]. Very recently, Yandulov and Schrock reported the reduction of dinitrogen to ammonia at a well-protected Mo–triamidoamine site [55b].

The Cummins group has reported the six-electron reductive cleavage of molecular nitrogen mediated by the three-coordinate Mo(III) anilide complex $Mo(NRAr)_3$ ($R = C(CD_3)_2CH_3$, $Ar = 3,5\text{-}C_6H_3Me_2$) to yield the corresponding $N{\equiv}Mo(NRAr)_3$ nitride (Scheme 3) [56–59]. However, ammonia synthesis from the nitride was not reported, presumably because of the great stability and low reactivity of the Mo≡N bond. Recently, the N_2-splitting reaction has been found to be redox-catalyzed and the reduced $[Na(12\text{-crown-}4)_2][N_2Mo(NRAr)_3]$ intermediate complex has been isolated and structurally characterised [60]. In this system also, the initial activation of dinitrogen is therefore monometallic.



Scheme 3. Splitting of the N≡N bond by a three coordinate Mo(III) complexes.

[1] N_2 itself can switch from an end-on to a side-on bonding mode at a single coordination site as recently showed crystallographically. However, the side-on bonding mode is, only at best, as efficient as corresponding end-on N_2-metal complexes for dinitrogen activation. F. Barrière, unpublished EHMO calculations [53].

These examples show that molybdenum centers with a formal oxidation state as high as +III or +IV are capable of activating N_2, some of them allowing NH_3 synthesis. However, from the functional chemistry point of view, these models are still subject to criticism (ii) and (iii) defined at the end of Section 2: i.e. they have abiological co-ligands (Cp* and Me or $[N(CH_2CH_2NR)_3]^{3-}$ or NRAr) and they are mononuclear. The strongly donating Me- or R_2N- ligands in the Schrock and Cummins systems, significantly increase the electron density at the formally +III metal center. This feature, together with the geometry of the metal coordination site, certainly allows N_2 activation since efficient backbonding interaction from the metal to the dinitrogen antibonding orbitals is crucial to weaken and polarize the N≡N bond.

The next section reviews the efforts aimed at introducing more relevant thiolate ligands to mononuclear functional models.

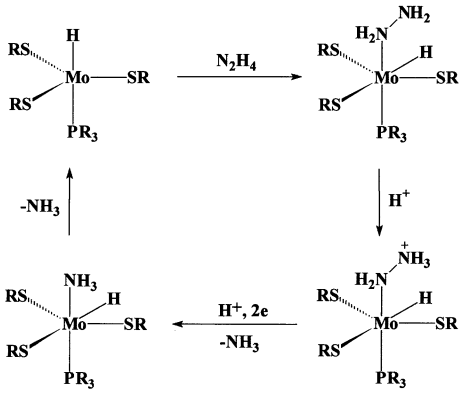
4. Diazo chemistry at mononuclear molybdenum-thiolate centers

Several groups have sought the synthesis of functional models where the metal center possess thiolate co-ligands in order to better take into account the $(\mu_3\text{-}S)_3$ sulfide environment of molybdenum in FeMo-co (Fig. 1). The synthesis of these complexes can be viewed as a first step towards a structural modeling. However, it is striking to note that, while modeling more closely the structural and electronic environment of Mo in FeMo-co, they also seem to have lost a key functional feature: so far, none of them has been shown to interact with N_2!

The Mo(0) bis-N_2 complex *trans*-$[Mo(N_2)_2(Me_8[16]\text{-ane-}S_4)]$ synthesized by Yoshida and co-workers and from which low yields of ammonia can be obtained is an exception in this context and relies on a low metal oxidation state and less relevant thioether ligations [61].

Nevertheless, dinitrogen interaction with metal–thiolate centers might be achieved when the right set of co-ligands with the adequate electronic properties will be found. In the meantime, they have been shown to coordinate and activate more reduced intermediates of possible relevance to biological N_2-fixation.

Catalytic hydrazine reduction has been achieved with the complex $[MoH(SC_6H_2{}^{i}Pr_3\text{-}2,4,6)_3(PMePh_2)]$ [62]. The phenylhydrazine $[MoH(SC_6H_2{}^{i}Pr_3\text{-}2,4,6)_3(PMePh_2)(PhNHNH_2)]$ and ammine $[MoH(SC_6H_2{}^{i}Pr_3\text{-}2,4,6)_3(PMePh_2)(NH_3)]$ derivatives have been obtained at low temperature by reaction of $[MoH(SC_6H_2{}^{i}Pr_3\text{-}2,4,6)_3(PMePh_2)]$ with $PhNHNH_2$ and N_2H_4, respectively. This allowed the proposition of an N_2H_4-reduction cycle at a mononuclear Mo–thiolate center (Scheme 4). In the context of the quest for Mo–dinitrogen interaction by this core, it is worth mentioning the existence of the analogue rhenium N_2-complex $[Re(SC_6H_2^{i}Pr_3\text{-}2,4,6)_3(PPh_3)(N_2)]$ [63].



Scheme 4. Catalytic hydrazine reduction cycle at $[MoH(SC_6H_2{}^{i}Pr_3\text{-}2,4,6)_3(PMePh_2)]$.

More recently, the use of the tripodal $N(RS)_3$ ligand $N(CH_2CH_2S^-)_3$ has allowed the synthesis and structural characterization of the mononuclear Mo(III) diazenido derivatives $[Mo(N(RS)_3)(N_2R')]$ with R′ = Me or Ph [64]. The diazenido group in both compounds can be protonated by HBF_4 or HCl to yield the corresponding hydrazido derivative, that in turn can deprotonate in more basic solvents to regenerate the parent complexes. $[Mo(N(RS)_3)(N_2Me)]$ also reacts with $[Me_3O][BF_4]$ to yield the hydrazido $[Mo(N(RS)_3)(N_2Me_2)][BF_4]$ complex.

Cummins and co-workers have reported the synthesis and structure of the molybdenum nitrido–thiolate complex $N{\equiv}Mo(SAd)_3$ (Ad = 1-adamantyl) by a ligand exchange reaction with the nitrido-butoxide $N{\equiv}Mo(O^tBu)_3$ complex and $Ti(SAd)(O^{i}Pr)_3$ as the source of thiolate [65]. N-atom abstraction chemistry from $N{\equiv}Mo(SAd)_3$ with the azophilic $Mo(N[^tBu]\text{-}Ph)_3$ complex yielded the unsymmetrical (μ-nitrido)dimolybdenum complex $(SAd)_3Mo(\mu\text{-}N)Mo(N[^tBu]\text{-}Ph)_3$ whose thermal decomposition generated $N{\equiv}Mo(N[^tBu]\text{-}Ph)_3$ and the transient $Mo(SAd)_3$ fragment. Although this $Mo(SR)_3$ fragment is analogous to the $Mo(NR_2)_3$ fragment described at the end of Section 3, it does not support the same efficient N_2-binding and activation chemistry. The lack of N_2-interaction by this MoS_3 site has been attributed to its substantially less π-basic character [65].

The molybdenum–thiolate centers described in this section exhibit very interesting diazo chemistry but lack the key interaction with dinitrogen. In addition, despite modeling more closely the molybdenum–sulfide environment found in FeMo-co, these complexes are mononuclear and therefore remain subject to criticism (iii) defined at the end of Section 2.

In the next section we describe no less interesting diazo chemistry occurring at the conserved bimetallic $Mo_2(\mu\text{-}SR)_3$ motif.

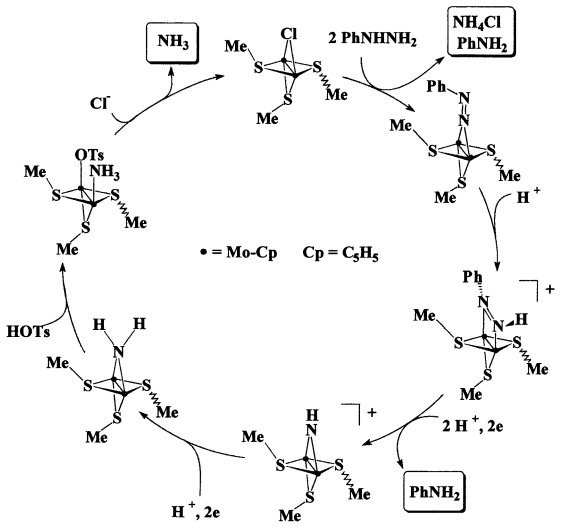
5. Diazo chemistry at dinuclear molybdenum–thiolate centers

The structural modeling of the Mo-center of the active site of MoFe-nitrogenase must take into account the polymetallic nature of the active site that comprises an extensive array of metal–metal bonding interactions. The binuclear complexes with the $\{Mo_2Cp_2(\mu\text{-}SR)_3\}$ motif (Cp = cyclopentadienyl) can be seen as a starting point to respond to this concern. These compounds have proven to support a very rich functional model chemistry involving N_xH_y or N_xR_y ligand binding and transformation requiring the two adjacent metal centers [66,67]. These complexes still belong to the 'high oxidation state' family and the Mo-environment still contains three thiolate ligands.

Reaction of $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}Cl)]$ [68] with N_2H_4 yields the amido-bridged derivative, $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}NH_2)]$ [69,70] whereas reaction with two equivalent of phenylhydrazine allows the isolation of two η^1-η^2 structural isomers containing the phenyldiazenido ligand coordinated to both metal centers: $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}\eta^1\text{-}NNPh)]$ and $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}\eta^2\text{-}NNPh)]$, with the concomitant formation of aniline and ammonium chloride [71].

$[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}\eta^1\text{-}NNPh)]$ can be protonated to the $\mu\text{-}\eta^1$-phenylhydrazido derivative that rearranges to the $\mu\text{-}\eta^2$-phenyldiazene $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}\eta^2\text{-}HNNPh)]^+$ [71]. Electrolysis of the latter [72] in the presence of 3 equivalent of HX (X = TsO or CF_3CO_2) produces in good yield the known amido-bridged complex $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}NH_2)]$ [69,70] after consumption of nearly 4 Faraday per mole of starting material. In the presence of larger quantity of acid (> 3 eq.) the ammine complex $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(NH_3)(X)]$ and aniline $PhNH_2$ are produced, demonstrating the cleavage of the N=N bond. A likely intermediate of this reaction is the imido complex $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}NH)]^+$ [70]. Indeed electrolysis of the imido derivative in the presence of one equivalent of acid yields the amido complex whereas in the presence of two equivalents of acid the ammine complex was obtained [67]. These observations provide a self-consistent picture [72], Scheme 5, of the stepwise conversion at the conserved $\{Mo_2(Cp)_2(\mu\text{-}SMe)_3^+\}$ dinuclear metal–sulfur site, of the $\mu\text{-}\eta^1$-NNPh diazenido species by successive proton and electron transfer events into an ammine complex from which ammonia can be released [70].

All the intermediates and products in Scheme 5 have been characterized and represent dinuclear analogues of intermediates from the Chatt and Schrock cycles



Scheme 5. Cycle for the reduction of phenylhydrazine to aniline and ammonia at a conserved dinuclear molybdenum sulfur site.

(Schemes 1 and 2). In addition, the observed $\mu\text{-}\eta^1 \Leftrightarrow \mu\text{-}\eta^2$ rearrangement of the substrate parallels, to some extent, the different $\eta^1 \Leftrightarrow \eta^2$ possible coordination modes of the diazo ligands in Schrock's monometallic system (cf. Section 3). The model described here argues in favor of a polymetallic active site in the enzyme: it could be either heterodimetallic involving an Mo–Fe edge of the cuboidal $\{MoFe_3S_3\}$ fragment of FeMo-co, or homopolymetallic involving at least two iron atoms from the cofactor core. An Mo_2 bimetallic active site is obviously not possible since a second molybdenum atom is not present in the biological cluster: what has been modeled here is the reactivity and transformation of diazo substrates at a dinuclear site where the two bridged-metals are strongly interacting. The emphasis has been laid on the dinuclear nature of the site and its geometry: notably the Mo–Mo distance of ca. 2.6 –2.7 Å is of the same order of the metal–metal distances found in FeMo-co. So far, no interaction with N_2 have been obtained with these complexes. Also these models contain the abiological Cp co-ligand.

In many respects, this model is comparable to the thiolate-bridged diruthenium system of Hidai and co-workers that catalyses the dismutation of hydrazine to ammonia and dinitrogen [12,73]. Indeed, the Ru–Ru distance of ca. 2.8–3.0 Å in the complexes crystallographically characterized correspond to a significant metal–metal interaction [74a], and the two close unsaturated metal centers can accommodate diazo substrates in the $(\mu\text{-}\eta^1\text{:}\eta^1)$ bonding mode. Fryzuk's complex [74b] $\{[(PhNSiMe_2CH_2)_2PPh]_2TaH\}_2N_2$ does not rely of any FeMo-co relevant metal or ligand. It displays however the unprecedented coordination of dinitrogen to two interacting metals (the Ta–Ta distance is 2.83 Å). Moreover, N_2 coordination occurs by displacement of H_2 from the bringing hydride precursor, reminiscent of

the obligatory hydrogen evolution coupled to N_2 reduction by nitrogenase [1], and the unsymmetrical (μ-η^2:η^1) dinitrogen bonding mode in this complex is also unprecedented.

These systems can be seen as modeling a reactive edge of a larger polymetallic cluster and are fundamentally different from those bridged bimetallic complexes where N_2 itself, or more reduced diazo species, solely hold the dimetallic structure together, and where no direct metal-metal interaction occur. For example, in the complex [μ-*trans*-N_2H_2{Fe('NS_4')}$_2$], formed by oxidation of the hydrazine analogue, the otherwise very unstable diazene molecule is stabilized by bridging two iron sulfur fragments [75] (Fig. 5). The two metals do not interact directly, but the sulfur atoms are however directly involved in the stabilization of diazene by specific NH–S hydrogen bonds, underlining a possible role for sulfur in the biological system [76–78].

The next section focuses on model clusters of higher nuclearity, the $MoFe_3S_4$ cubanes, where the fine structural modeling of molybdenum in FeMo-co has been achieved.

6. Diazo chemistry at the Mo-center of $MoFe_3S_4$ clusters

The accurate structural modeling of the Mo center of nitrogenase has been achieved by the Holm group at a time when only analytical and spectroscopic data on FeMo-co were available and while its precise molecular structure was not known [79–81]. Among the synthetic MoFeS clusters proposed as models for the nitrogenase active site, the compounds with the {$MoFe_3S_4$} monocubane-type core have later proven to closely model the Mo environment in the biological system. Indeed, the first and second coordination spheres of Mo in FeMo-co are well reproduced in the model systems by three μ_3-S sulfide ligands, each of them also bridging two of the three iron atoms in the synthetic cubes as found in the bio-metallo cluster. The iron atoms in the model compounds however, possess a tetrahedral geometry rather than the trigonal arrangement found in FeMo-co.

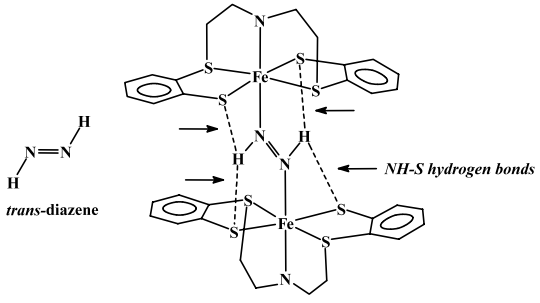
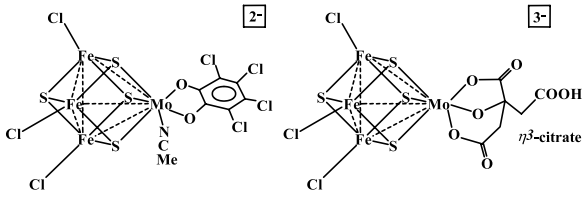


Fig. 5. Stabilisation of *trans*-diazene by two iron-sulfur fragments and specific NH–S hydrogen bonds.



$[MoFe_3S_4Cl_3(C_6Cl_4O_2)(MeCN)]^{2-}$ ref. 81 $[MoFe_3S_4Cl_3(\eta^3\text{-citrate})]^{3-}$ ref. 87

Fig. 6. $\{MoFe_3S_4\}^{3+}$ clusters efficient for the catalytic reduction of hydrazine at the molybdenum center.

The octahedral coordination of Mo may be completed by O or N-containing donor ligands as in nitrogenase.

For example, in the synthetic cluster $[MoFe_3S_4(Cl)_3(C_6Cl_4O_2)(MeCN)]^{2-}$ (Fig. 6) (left), the molybdenum atom is chelated by the oxygen atoms of the tetrachlorocatecholate ligand and solvated by one acetonitrile ligand [81]. The Mo-environment in this compound was found to be similar to the one in nitrogenase by comparison to the interatomic distances in FeMo-co, available at that time only from extented X-ray absorbtion fine structure studies [82].

The labile acetonitrile ligand at Mo has been shown by NMR, and in some cases by X-ray diffraction structure determinations, to be displaced by several other ligands including CN^-, N_3^-, N_2H_4, $PhNHNH_2$ and piperidine. It is of much interest in the context of the enzyme function that such nitrogenase-related substrates bind at the Mo site of the model cluster [81].

With the knowledge of the FeMo-co structure in hand [1], this class of compounds has been extended to $MoFe_3S_4$ cubes containing polycarboxylate ligands bound at molybdenum in order to model the homocitrate ligation of Mo in FeMo-co [83–89] (Fig. 6 (right) for example [87]).

Interestingly enough, the structural models depicted in Fig. 6 have also been found to catalyze efficiently the reduction of hydrazine to ammonia [86–91], or acetylene to ethylene or ethane [91,92], thus supporting some functional model chemistry features [83,84,89]. Evidence of the direct involvement of the Mo atom in the catalytic process was provided. However, it is rather surprising that the $[MoFe_3S_4Cl_3(C_6Cl_4O_2)MeCN]^{2-}$ cluster, (Fig. 6 (left)), with a labile acetonitrile ligand at Mo, was found less efficient for catalysis than $[MoFe_3S_4Cl_3(\eta^3$-citrate$)]^{3-}$, (Fig. 6 (right)), where the Mo octahedral coordination is saturated by the η^3-citrate ligand. These observations suggest that the polycarboxylate citrate ligand can open up a coordination site at Mo in the presence of acid, and that it could further play the role of an efficient local proton source for the reduced substrate [83,84,89].

The $[MoFe_3S_4Cl_3(C_6Cl_4O_2)MeCN]^{2-}$ cluster (Fig. 6) (left), has also been found to catalyze the splitting of the N=N bond of *cis*-methyldiazene yielding exclusively

methyl amine, whereas *cis*-methyldiazene reduction by nitrogenase yields also ammonia and methane [93]. Phosphine inhibition experiments strongly suggest the involvement of molybdenum in the substrate activation process in the synthetic model. Analogue VFe_3S_4 clusters were also found to be efficient for hydrazine reduction catalysis at the vanadium heterometal site [94].

The catalytic reactions borne out by the structural model clusters described in this section suggest that molybdenum might be directly involved in nitrogenase function, at least in the latter stages of dinitrogen reduction, since no interaction of $MoFe_3S_4$ model clusters with N_2 has been obtained so far. From a structural point of view, the weakness of these models is the presence of tetrahedral rather than trigonal iron atoms. This is the most challenging aspect of the synthetic modeling of FeMo-co: only high-spin mononuclear iron II in the coordination number two [95] or three [96–98] with a sulfur environment have been obtained with encumbered thiolate ligands. Crystallographic and ^{57}Fe Mössbauer spectroscopy analyses [99] showed that these complexes have in fact a higher coordination number due to agostic interactions with their ligands. It remains one example of a quasi-planar homoleptic tricoordinate mononuclear iron–thiolate complex: $[Fe(SC_6H_2^tBu_3\text{-}2,4,6)_3]^-$ [98].

A strategy has been proposed by Holm for the synthesis of a symmetric topological analogue of the FeMo-co, in principle easier to achieve than the dissymmetric cofactor itself: a forced structural rearrangement of a sulfide bridged di-cubane μ-S$\{MoFe_3S_4\}_2$ could yield a symmetric cluster with the $\{Mo_2Fe_6S_9\}$ stoichiometry [100].

Exploring the reactivity of the edge-bridged double cube core $\{Mo_2Fe_6S_8\}^{4+}$, Holm and co-workers have obtained clusters of high nuclearity containing fragments of structural relevance to the P-cluster of nitrogenase [101,102], whereas Coucouvanis and co-workers obtained sulfur-voided cubanes of structural relevance to the $MoFe_3S_3$ subunit of the FeMo-co [103]. Despite these latest synthetic developments, a faithful structural model of FeMo-co is still currently missing.

7. Speculative biological mechanisms from different synthetic models

7.1. Introduction

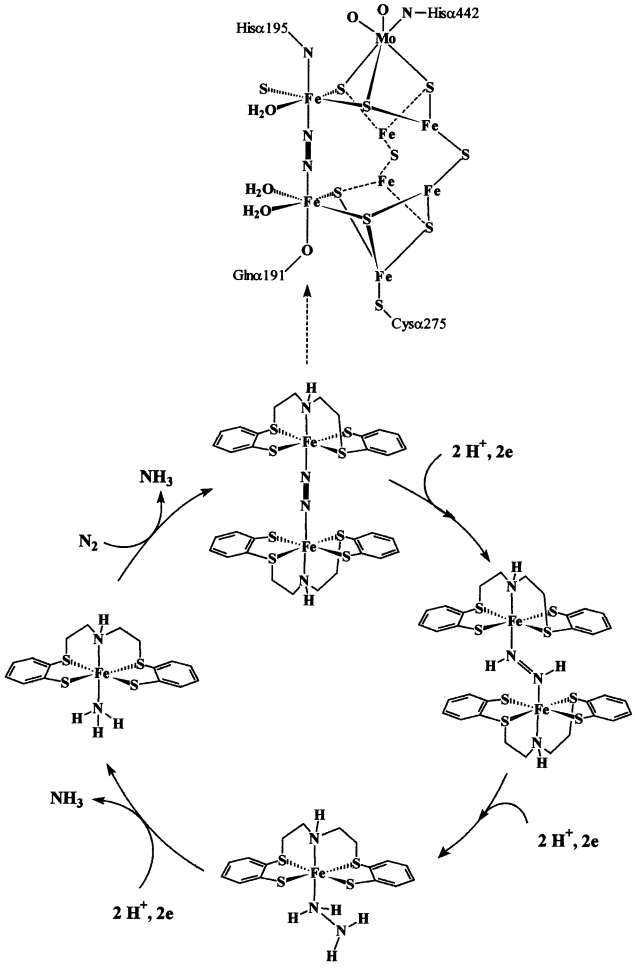
The current failure of mono- or polymetallic model systems with molybdenum in a sulfur environment to even interact with dinitrogen, (cf. Sections 4–6) can lead to conflicting speculations concerning the precise location of N_2 binding and activation at FeMo-co. The results obtained by different model complexes can be interpreted in different ways and it has been proposed that iron, molybdenum, or even sulfur could be involved in N_2 reduction.

These different speculative biological mechanisms, extrapolated from experimental model chemistry, are reviewed here.

7.2. Iron-based model

First, one may be tempted to reject a molybdenum-based dinitrogen activation by nitrogenase. The obvious alternative, having in mind the unprecedented structure of FeMo-co, would be a multi-iron activation site. This has little support to date in model chemistry precisely because of the current chemists' inability to synthesize iron–sulfur clusters with the three-coordinate iron atom array and also because of the somehow less developed iron-based dinitrogen chemistry.

An example of ammonia synthesis with an iron–tetraphosphine complex has been reported and a cyclic system in alcoholic solution has been proposed. The different reactions composing the cycle, however, were studied separately and the mechanism of ammonia formation is not clear [104–107]. The reductive splitting of the N–N bond in 1,2-diphenylhydrazine by the iron amide aryl–thiolate complex of putative formula $Fe[N(SiMe_3)_2]_2(SAr)$, yields the all ferric cluster $[Fe_4(\mu_3\text{-}NPh)_4(SAr)_4]$ with iron–iron distances $\sim$2.6–2.7 Å [108]. This demonstrates the reactivity of a presumably tricoordinate iron complex towards the N–N reductive cleavage of a reduced diazo species (the hydrazine derivative) but also provides precedence for the resulting reduced (phenyl) imido fragment to be part of an iron cluster. Smith et al. [109] have used different bulky bidendate diketoimidate ligands (L) to stabilize and control low coordinate iron complexes: reduction of the three-coordinate iron (II) complex LFeCl under N_2 yields the dinitrogen-bridged complex LFeNNFeL (d(NN) = 1.18 Å). Further reduction of the dimer by two electrons with alkali metals (Na or K) resulted in the lengthening of the NN bond to 1.23–1.24 Å, and to the coordination of the alkali metal cations to the bridging N_2 and to the aryl rings of the ligand [110]. This work shows that a low coordinate iron complex can bind dinitrogen and allow its reduction in a stepwise fashion. As for other dinuclear bridging N_2 complexes, the two iron atoms in this compound do not share any direct metal–metal interaction: the bridging dinitrogen ligand is solely responsible for holding the complex together. In line with the well-documented bridging-diazene chemistry, involving two independent iron thiolates fragments (Fig. 5 and Scheme 6 (bottom)), Sellmann has proposed a model for the activation of dinitrogen at FeMo-co involving two of the central iron atoms. This mechanism implies the opening of a FeMo-co side to yield two reactive iron centers, capable of

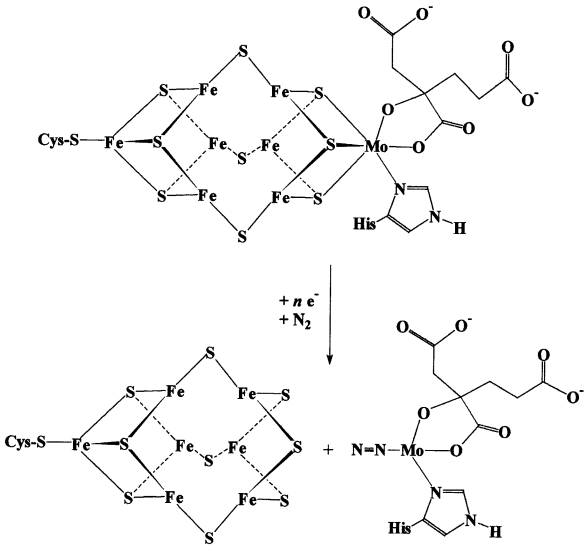


Scheme 6. The iron-based open-side FeMo-co model for nitrogenase function (top) proposed from the bridging diazene chemistry (bottom).

bridging dinitrogen, and whose coordination is completed by neighboring amino acid residues (Scheme 6) (top) [75–78]. This mechanism would require a large structural rearrangement of the cluster core and of its protein environment, the breaking of many metal–metal interactions, and therefore demand a high energy cost.

7.3. Molybdenum-based model

Secondly, one could analyze the results of the molybdenum-based model chemistry (cf. Sections 2–6) in the following way: mononuclear Mo complexes can be suitable for strong dinitrogen activation provided they are not coordinated by sulfur containing ligands. Thus, a mechanism for dinitrogen activation by FeMo-co could imply the redox-linked decoordination of the active Mo(histidine)(homocitrate) end of FeMo-co from the rest of the iron–sulfur cluster. The redox linked dissociation model, depicted in Scheme 7, was first proposed by Schrauzer on the basis of efficient specific catalysis of acetylene reduction to ethylene (also a nitrogenase-catalyzed reaction) by an equimolar mixture



Scheme 7. The molybdenum-based redox-linked dissociation model for nitrogenase function.

of molybdate, *N*-methylimidazole and homocitrate in aqueous buffered solution in the presence of a reductant [111]. Recently, low yields of N_2 reduction have also been claimed [112].

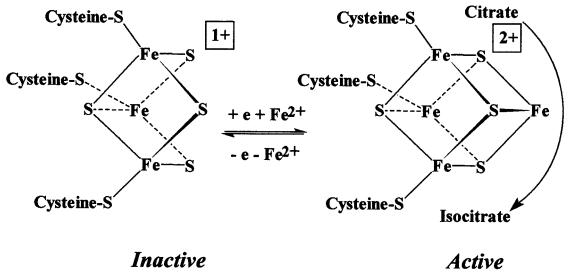
The mononuclear molybdenum active site model, implying decoordination of the heterometal from the cofactor iron–sulfur core, seems a sensible conclusion that one can draw from the analysis of the Mo-based model chemistry reviewed in Sections 2–6. We also have been led to consider this model as a possibility in our theoretical work [113] covered in Section 8.

While it is known that mononuclear centers with different sets of ligands and metal oxidation states are able to support N_2 reduction (cf. Sections 2 and 3), introduction of thiolate or sulfide ligands at mono and polynuclear molybdenum centers seems to have prevented any dinitrogen binding so far (cf. Sections 4–6).

Despite the speculative nature of the model, there is a biological and chemical context that makes it worth considering for nitrogenase function.

There is precedent in biology for the reversible redox-linked decoordination of a metal corner of a Fe_4S_4 cube: the prosthetic group of the aconitase enzyme in the oxidized 'open cube' $\{Fe_3S_4\}^{1+}$ structure is inactive. After one electron reduction it binds a fourth iron metal ion at the free corner, acquiring the cubic $\{Fe_4S_4\}^{2+}$ structure and the ability to catalyze the isomerisation of citrate to isocitrate at the fourth iron site (Scheme 8) [80,114].

The reversible redox-linked decoordination of a heterometal fragment from a cubane-like cluster has not yet been shown in synthetic models. However, the structural elongation of the $\{MoFe_3S_4\}^{0}$ core with respect to the $\{MoFe_3S_4\}^{3+}$ core [115,116] (Fig. 7), could represent an intermediate step towards a decoor-



Scheme 8. Redox-linked cluster interconversion switching 'on' and 'off' the function of the aconitase enzyme.

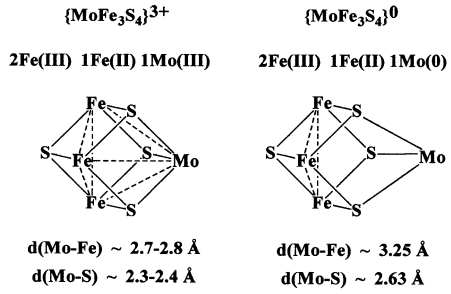


Fig. 7. Comparison of the structure and metal oxidation state in $\{MoFe_3S_4\}^{3+}$ and $\{MoFe_3S_4\}^{0}$ clusters.

dination of the Mo-fragment upon reduction of the cluster, in spite of the very different nature of the molybdenum center in both cores (three CO ligands in the $\{MoFe_3S_4\}^{0}$ cluster). Electrochemical studies on synthetic $\{MoFe_3S_4\}^{3+}$ cubes [79–81,117] have shown that reduction to the $\{MoFe_3S_4\}^{1+}$ level occurs at ca. -2.5 ± 0.3 V vs. ferrocene in common organic solvent electrolytes media. The following (and not observed) electron transfer to the $\{MoFe_3S_4\}^{0}$ state would be expected to occur at ca. one Volt more negative (-3.5 ± 0.3 V). These extremely low redox potentials, unrealistic for biological systems, could be dramatically raised by the combination of three stabilizing factors: (a) structural changes in the cluster core. Indeed, it is known that the primary reduction of synthetic $\{MoFe_3S_4\}^{3+}$ and $\{MoFe_3S_4\}^{0}$ clusters [79–81,115–117] takes place in the same potential range (ca. $-1.5\ \pm0.3$ V vs. ferrocene). (b) Electrostatic protein interactions stabilizing the reduced species. These effects have been probe in synthetic Fe_4S_4 clusters bearing functionalized thiolate ligands in aprotic solvent [118]. (c) Protonation at basic bridging sulfide sites [119], compensating for the additional negative charge upon one electron reduction.

The reduced cuboidal $\{Fe_3S_4\}^{0}$ cluster, found in ferrodoxin proteins, has been shown to coordinate a variety of homo- or heterometal ions at the open site such as divalent Fe, Co, Ni, Cu, Zn, Cd or monovalent Tl to yield the corresponding $\{MFe_3S_4\}^{1+/2+}$ cubic structures [80].

The range of synthetic $\{MFe_3S_4\}^{n+}$ is also quite wide in terms of the nature of the heterometal (M = Mo, W, Nb, V, Re, Fe, Co, Ni) and the different core-oxidation states attainable ($n = 0$–5) [80].

These chemical and biochemical observations have led to define the 'open-cube' Fe_3S_4 cluster as a conceptual versatile tridentate ligand, capable of accommodating various additional monometallic fragment at the free site [80]. This concept might be extended to the Fe_7S_9 core of FeMo-co, since in the biological system, nitrogen-fixing organisms living in a Mo-deficient environment have developed alternative nitrogenases where the cofactor contains either vanadium or iron in place of molybdenum [120,121].

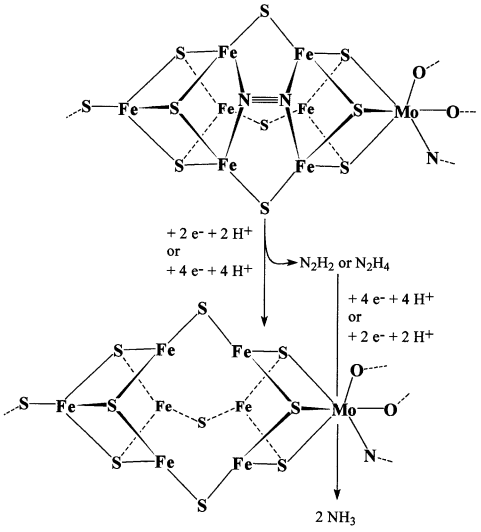
It is also interesting to note in this context that the final stage of FeMo-co biosynthesis has been proposed to involve the capping of the incomplete Fe_7S_9 fragment by the Mo-end [122]. The protective coordination process of the Mo-end would occur in the oxidized form of the cluster, whereas its decoordination, required for activity, would be triggered, in the presence of the substrate, by the first reduction and protonation steps in the Lowe–Thorneley scheme [7]. After completion of catalysis, the protective device would be switched back on.

This model implies that the sulfur environment of Mo in FeMo-co plays a protective role of a very reactive mononuclear Mo-active site, and does not take part directly in the activation of N_2. This would be consistent with the current lack of molybdenum–thiolate or sulfide dinitrogen chemistry. The efficiency of Mo(III) tricoordinate complexes for N_2 activation (cf. Cummins' results [56–60]) and the results of our calculations [113] support the idea that a putative mononuclear Mo(homocitrate)(imidazole) site could be a strong N_2-activator. Finally, the analysis of the MoFe-protein crystal structure shows that the iron cage of FeMo-co is wrapped by the polypeptide backbone whereas the Mo-end sits in a water pocket of ca. thirty water molecules, perhaps more conducive to chemistry involving rearrangements at this latter site [38].

This model however, as for Sellmann's proposal, implies a high energy cost by the breaking and making of many chemical bonds in the reversible large structural change of FeMo-co.

7.4. Iron-(molybdenum)-based model

The catalysis of diazo species reduction (and other nitrogenase-related substrates) borne out by synthetic $MoFe_3S_4$ clusters and directly involving molybdenum (cf. discussion in Section 6 and Refs. [83–94,103]), coupled to the absence of any interaction with N_2, led Coucouvanis to propose the sequential involvement of first iron for dinitrogen reduction, then molybdenum for diazene (or hydrazine) reduction (Scheme 9) [83,89,103]. The central iron sites could be suitable for activation and reduction of dinitrogen, bound end-on or side-on

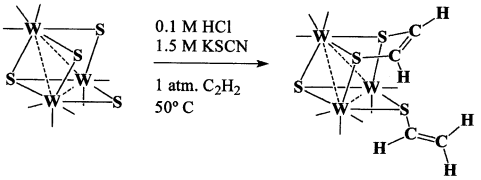


Scheme 9. Iron-based model for dinitrogen reduction up to the diazene or hydrazine level whose reduction would be completed at the molybdenum center.

on an Fe_4-face of the central trigonal iron atoms prism. However, after the transfer of two electrons and two protons, the diazene N_2H_2 molecule (or hydrazine after 4e, $4H^+$) would have to migrate to the heterometal site for the reduction to ammonia to be completed.

7.5. Sulfur-based model

A fourth model would not require any interaction with a metal site but the possible direct involvement of the sulfide ligands bridged to metal centers into a 'metal-free' activation process. Although no model complexes have been shown to activate dinitrogen in that fashion, dinuclear complexes bridged by sulfide [123,124], hydrosulfide or thiolate, were found for example to cleave the triple bond of cyanide or isocyanide groups (by insertion into the S–H bond of a bridging hydrosulfide) or allow the cyclic reduction (hydrogenation) of azobenzene to 1,2-diphenylhydrazine and of alkynes to alkenes [125–127]. An interesting recent development, illustrating this kind of chemistry, is the reaction with acetylene gas in 0.1 M HCl of a sulfur-bridged isothiocyanate tungsten cluster of the incomplete cubane-type, $[W_3(\mu_3\text{-}S)(\mu\text{-}S)_3(NCS)_9]^{5-}$, to yield a new cluster with three



Scheme 10. Reactivity at the bridging sulfides centers of a W_3S_4 cluster with the acetylene molecule (isoelectronic with dinitrogen).

carbon–sulfur bonds by coordination of acetylene (or a derivative) in two coordination modes (Scheme 10) [128]. That the isoelectronic dinitrogen molecule could undergo similar chemistry has never been shown, but an a-metallic activation process cannot be ruled out for sure.

7.6. Conclusion

The different synthetic models trying to account for the presence of sulfur at the active site of nitrogenase have thus lead to very different conclusions or suggestions. The shortcomings of all the different models discussed above is obviously their partial structural as well as functional relevance to the biological process.

In order to take into account the whole structure of FeMo-co, and when possible its protein environment, in proposing and assessing N_2 reduction mechanisms, the methods of theoretical chemistry can assist valuably, as discussed next.

8. Theoretical calculations assessing the molybdenum center as the possible active site in the FeMo-cofactor

8.1. Introduction

The first publication of the FeMo-co structural model, deduced from protein crystallography [129], has offered few clues as for the precise mechanism of biological dinitrogen fixation, and therefore paved the way for renewed speculations on the matter. Given the unprecedented structure of the active site in chemistry or biology (that remains true to date), and the importance of the reaction catalyzed at FeMo-co, the availability of the structural information attracted many chemists to apply a theoretical approach in an attempt to enlighten the unsettled issue [113,130–149]. However, the size of the cluster and the complexity of the nitrogenase enzyme lead to many shortcomings due to the simplifications in the computed structural models, the nature and limitations of the calculation methods used, and the difficulty in taking into account the supramolecular protein interactions required for activation. In spite of these drawbacks, the breadth of biochemical and model chemistry data available has helped to simplify the models and interpret the results in a rationale fashion. It appears that the approximate trigonal prism at the center of the cluster core (Fig. 1) has been the a priori site of choice for numerous theoretical investigations of speculative N_2-binding and activation at FeMo-co. Indeed, nearly all of the theoretical papers that have been published on this issue so far [113,130–149], have focused on a possible active site involving all or some of the six trigonal iron atoms of the central cage. As a result, the molybdenum center in FeMo-co has been less

studied theoretically as a possible site for N_2 activation and reduction. However, the very rich functional and/or structural model chemistry that has been developed over the years (cf. Sections 1–6), and that may suggest an intimate role for molybdenum in the biological process, should draw more care in evaluating theoretically the question of Mo as the possible active site of the enzyme. Those studies based on different calculation methods, and that investigated the molybdenum site, are now reviewed.

First, we examine the calculations using the extended Hückel theory (EHT). This method has the advantage to require very little computing time so that a large number of known or imaginary complexes can be calculated, and the results confronted and discussed with respect to known chemistry. The activation of coordinated N_2 is a problem of choice for the application of an EHT methodology. However, since the calculated energies are often unreliable, it is not always possible to predict the stability of an imaginary complex from a thermodynamic point of view. Secondly, the studies relying on the density functional theory are discussed. This sophisticated calculation method is demanding in computing time so that it is often necessary to simplify to models on which calculations are performed. This also implies that fewer models are generally calculated. The results of the calculations however, and especially the thermodynamic parameters can be treated with confidence, so that detailed mechanistic pathways can be suggested or proscribed on energetic grounds.

Thirdly, the availability of protein crystal structures and the methods of molecular mechanics can valuably complete the former two quantum chemical approaches by considering the protein surrounding of the active site, and suggest acceptable simplifications of the models or biologically realistic speculations on the mechanism.

8.2. Calculations based on extended Hückel theory (EHT)

In the first theoretical study involving a model of the FeMo-co, Deng and Hoffmann have used the extended Hückel calculation method to screen possible efficient activation sites for dinitrogen, including mono iron or molybdenum sites as well as poly-iron sites [130]. It is striking to note that for the models where N_2 is bound to a mononuclear metal site, the activation was calculated equally inefficient for N_2 activation whether the metal was a trigonal iron site or the octahedral molybdenum center (respectively, model nos. 11 and 12 in Ref. [130]).

The involvement of two or four three-coordinate iron atoms improves the N_2 activation parameter and the authors concluded that the site most suited for N_2 activation is a Fe_4 face at which N_2 would bind in an end-on fashion.[2]

Having in mind the breadth of molybdenum–dinitrogen chemistry and the proposal that this metal could be the site of molecular nitrogen activation at FeMo-co, we sought to deduce from calculations on known N_2 complexes, a theoretical scale for N_2 activation in order to evaluate how strong would dinitrogen be activated in an elusive FeMo-co with N_2 bound at Mo.

Our approach [113] was as follows: we used extended Hückel calculations on a wide range of structurally characterized monometallic N_2-complexes to define the parameters required for N_2-activation. We then looked how could these requirements be met at the molybdenum center of $MoFe_3S_4$ model clusters, finally we investigated the FeMo-co itself with a putative Mo–N_2 bond.

We can summarize our findings in the following fashion: We have obtained a good correlation between experimental dinitrogen activation by known N_2-complexes and the calculated NN overlap population in the corresponding calculated models. We used this result as an experimental scale to define whether N_2 could be activated at Mo in the model clusters.

N_2-activation was found theoretically not possible at Mo in $MoFe_3S_4$ cubes unless the cluster core is distorted and reduced to the $\{MoFe_3S_4\}^0$ level. The $\{MoFe_3S_4\}^0$ cluster core consists of a $MoFe_3S_4$ cube showing a trigonal elongation along the μ_3-S(Fe_3)-Mo axis with significantly longer Mo–S and Mo–Fe bonds, and containing three extra electrons with respect to the $\{MoFe_3S_4\}^{3+}$ core (Fig. 7) [80,115,116]. The iron atoms have the same mean oxidation state of +2.67 in both cores and Mo(III) in $\{MoFe_3S_4\}^{3+}$ bears the formal three-electron reduction of the cluster core to Mo(0) in $\{MoFe_3S_4\}^0$. We found that in calculated $MoFe_3S_4$ cubes with the 'zero' core and geometry, and N_2 bound at Mo, the occupied frontier molecular orbitals acquire or reinforce a substantial Mo–N≡N π–π^* character that is sufficient to significantly weaken the N≡N bond and theoretically activate dinitrogen. In that contextit is interesting to note that a $Mo_2Ir_2S_4$ cubane cluster has been shown to undergo the condensation of 2,2-methylphenylhydrazine at molybdenum yielding the organohydrazido derivative. Interestingly enough, the reacting molybdenum site is the one not sharing any metal–metal bonds with the two iridium atoms [150]. This may support the idea that a monuclear-like diazo chemistry at a single metal center in a cluster could be allowed if metal–metal interactions are diminished.

[2] Other models reported in Ref. [130] actually yielded better activation parameter but were not favored for steric reasons (N_2 in the center of FeMo-co or too close N–S contacts) or based on the (now lifted) uncertainty on the nature of the third brigind sulfide ligand between the two sub-cubes [129].

Calculations on a FeMo-co model also showed that the apparent under-coordination of the trigonal iron atoms is largely compensated by stronger inter and intra-cube metal–metal bonds. In addition, the calculated overlap population of the Mo-carboxylate bond suggested that it could easily decoordinate. In light of these results we found legitimate to investigate a FeMo-co model with N_2 bound at Mo.

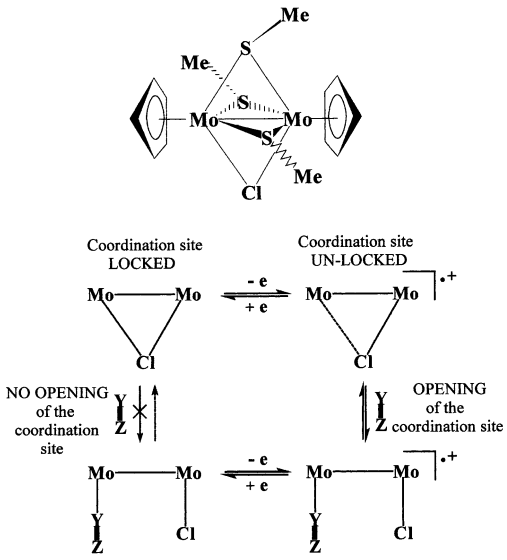
Although N_2 was found unactivated at the Mo center of FeMo-co, the results obtained with $MoFe_3S_4$ cubes led us to investigate the effect of structural deformations of the cluster core. Several distortions were examined including the deformation of the $MoFe_3S_3$ sub-cube of FeMo-co from a +III geometry to a 'zero' geometry. Surprisingly, none of the distortions screened, neither in combination with reduction and/or protonation of the cluster, did lead to a significantly activated dinitrogen. This was attributed to the central iron-atom cage that acts as an electron sink at the expense of molybdenum, thus preventing any dπ-back-bonding from Mo into the π* molecular orbitals of N_2.

We concluded that classical mononuclear Mo–N_2 activation (i.e. the Chatt and Schrock chemistry described in Sections 2 and 3) was not transposable to the Mo center of FeMo-co unless it decoordinates from the cluster core (Scheme 7), thus yielding an efficient mononuclear active site. Although this decoordination process was calculated by the extended Hückel method to be energetically unfavored, it is important to address this mechanism, and the possibility of molybdenum as the active site in general, with higher-level calculation methods, yielding more reliable calculated energies.

8.3. Calculations based on the density functional theory (DFT)

Recent theoretical investigations using the density functional theory (DFT) and considering the molybdenum site have been published [146,147].

Szilagyi et al. have modeled the molybdenum center as a pseudo-octahedral mononuclear complex with three HS^- ligands, a simple bidentate alkoxy-carboxylato lactate ligand and methylimidazolate. They found that decoordination of the carboxylate ligand from molybdenum can only occur upon one-electron reduction of the complex, protonation of the carboxylate and coordination of the substrate to Mo. The major drawback of the model is its mononuclear nature. Indeed, the effect on molybdenum of the poly-iron network in the cluster seems to be important as we suggested in our EHT calculations [113]. Even when higher level calculation methods were applied to $\{MoFe_3S_4\}^{3+}$ clusters [151,152], a discrepancy was noted between the formal +III molybdenum oxidation state in the real cluster and its essentially fully oxidized state according to the theoretically deduced electronic structure, which is in



Scheme 11. Example of electrochemical control of a metal coordination site 'unlocking'. After electron transfer (unlocking step) the actual opening of the site occurs only in the presence of the unsaturated substrate Y≡Z (Y≡Z = CO, isocyanides or nitriles).

agreement with the results of EHT calculations as well [113]. There is however an experimental precedent for the type of mechanism suggested by Szilagyi et al.: a coordination site at a metal–sulfur center is unlocked by an electron transfer step in $[Mo_2(Cp)_2(\mu\text{-}SMe)_3(\mu\text{-}Cl)]$ but it only opens up in the presence of the unsaturated substrate (Scheme 11) [68]. The kinetics of the coordination to the site, and the stability of the reduced substituted complex depend on the nature of the unsaturated substrate. Szilagyi et al. concluded that the imidazole N(ε) from the histidine bound to Mo is not protonated. The imidazolate ligation would therefore be strongly bond to molybdenum via N(δ). From other DFT calculations [145] on the homocitrate ligand of FeMo-co and its water molecule solvation shell, and comparison with the protein crystal structure, it was deduced that the alcoxy arm of homocitrate should be protonated.

Durrant also investigated by DFT truncated mononuclear models of subsections of the FeMo-co [147]. It is the first attempt, at this level of theory, to consider and compare both the trigonal iron site and the molybdenum site with respect to dinitrogen coordination, and to relate the results of the calculations on imaginary systems to benchmark calculations on known N_2 complexes. In agreement with other authors that performed DFT calculations on larger FeMo-co models, end-on N_2 binding at the trigonal iron site was found to be weak: binding energy (BE) = −9 kcal mol^{-1}. The N_2 binding energy to the Mo site was found significantly stronger (BE = −16 kcal mol^{-1}), which suggests a higher affinity of this site for dinitrogen. An octahedral vanadium complex gave a similar result (BE = −19 kcal mol^{-1}),

but the N_2 binding energy in the analogue octahedral iron complex (BE = −7 kcal mol^{-1}) was found close to that of trigonal iron site (−9 kcal mol^{-1}). The study was extended to the investigation of a dinuclear MoFeS complex. The splitting of a Mo(IV)-bound hydrazido ligand into a Mo(V) nitride and a Fe(III) amide was found energetically favorable. That a reactive MoFe edge of the cofactor is required for the biological process may find some experimental support in the diazo chemistry of bimetallic sulfur sites discussed in Section 5.

8.4. Structural analyses of protein crystal structures and molecular mechanics studies

Szilagyi et al. [145] have identified two channels in a graphical analysis of the *Azotobacter vinelandii* MoFe protein crystal structure. The protein surface is connected to the Mo-site by one channel and to the central iron prism by another channel. It is proposed that the products leave the enzyme by the Mo-site channel and that the reactant enter by the iron-prism channel, although it is not clear why the reverse path could not be allowed.

Durrant studied the available crystal structures of the MoFe protein of nitrogenase from *Clostridium pasteurianum*, *A. vinelandii* and *Klebsiella pneumoniae* [148]. A possible candidate for a water-filled channel was identified and is apparently conserved in all three bacterial strains. It connects the water filled-pocket, where the homocitrate ligand stands, to the protein surface. The interstitial channel seems large and flexible enough to accommodate the diffusion of substrate or products such as N_2, H_3O^+, H_2 or NH_4^+, and therefore was proposed to be the path for substrate/product diffusion to/from FeMo-co, as well as the channel for proton delivery to the coordinated substrate.[3] The direct protonation of FeMo-co is then addressed. It is accepted from DFT calculations on FeMo-co that the three central μ_2-S bridging sulfide are the most basic sites [144]. These three likely protonation sites are equivalent when considering the idealized $MoFe_7S_9$ core symmetrized to C_{3v} point symmetry. The protein surrounding of FeMo-co however, gives each sulfur site its own specificity. The three different μ_2-S bridging sulfide are labeled S2B, S5 and S3A according to the crystallographic nomenclature. A hydrogen bond network between S2B and the protein surface is identified: it consists of a S2B–histidine–water–tyrosine sequence that seems conserved in the different MoFe protein crystal structures. S5 is also connected to the protein surface by a hydrogen bond network involving water molecules and amino acid residues, however more complicated than in the case of S2B. The proton channel to S5 starts with a water molecule in the vicinity of FeMo-co. H_2O seems to compete however, with a conserved neighboring arginine residue for the interaction with S5. Durrant proposes that the arginine residue could tune the basicity of S5 with respect to that of S2B. Thus, because of its reduced basicity and more convoluted proton channel to the protein surface, it is proposed that S5 receives the second H^+/e^- couple and S2B the first. The third sulfide site (S3A) is hydrogen-bonded only to the backbone of the protein to four NH groups from consecutive residues and is therefore unlikely to protonate. Proton migration from S2B and S5 to trigonal iron sites would drive hydrogen evolution as an unwanted side-reaction, which would return FeMo-co in its unreduced non-catalytic resting state.

[3] As pointed out by one referee, this proposed interstitial channel corresponds remarkably well with that of the proposed cofactor insertion channel as deduced from the recently published crystal structure of a cofactor deficient nitrogenase MoFe protein [153].

9. Conclusion and perspectives

In this account, we have reviewed the functional, structural and theoretical model chemistry of the Mo center of the nitrogenase FeMo-co. The possibility of reducing dinitrogen at mononuclear molybdenum complexes with different geometry and oxidation state has been clearly shown in Sections 2 and 3. Taking into account the sulfide environment of Mo in FeMo-co in mono- di- or polynuclear structural model complexes has allowed interesting functional chemistry involving more reduced diazo ligands (cf. Sections 4–6). The key interaction with N_2, however, has been lost in most of these cases. It is interesting to compare those related complexes having or lacking a sulfur environment to illustrate this point. The low oxidation state Chatt type metal–phosphine dinitrogen complexes (Section 2) can be compared with Yoshida's low oxidation state thioether N_2 compounds [61]: although both centers allow the coordination and characterization of dinitrogen coordination, the activation of the ligand towards protonation is less pronounced in the latter case and only low yield of ammonia synthesis is possible from the metal–sulfur N_2 complex. The high oxidation state Schrock center supports N_2 reduction chemistry (Section 3) whereas the high oxidation state metal thiolate center studied by Richards is only active for hydrazine reduction (Section 4). The thiolate equivalent of Cummins three-coordinate Mo(III) anilide complex that splits the N≡N bond does not interact with dinitrogen [59,65]. Clearly, the sulfur ligations in those complexes yield a less electron rich metal center, less prone to coordinate and activate dinitrogen by efficient π-back-bonding. Another factor that seems to prevent strong metal–N_2 interaction is the dilution of the metal

electronic density into a metal–metal bond network. To date, apart from Fryzuk's complex [74b], there is no precedent for a molecular bi- or polymetallic cluster with metal–metal interaction that coordinates dinitrogen. Even in the first metal cluster-hydrazido derivative obtained by Hidai [150], the molybdenum–hydrazido site is found elongated from the other metal centers.

Yet, the biological FeMo-co combines both of these features: an extended network of sulfur and interacting metals. Still, it reduces dinitrogen to ammonia. Theoretical calculations confirm that, the coordination of N_2 to FeMo-co, be it at Fe or Mo, is weak, and, when coordinated, π-backbonding from the metal, crucial for activation and protonation, is poor.

From an inorganic chemistry point of view, it therefore seems that FeMo-co is not the most well suited site for dinitrogen reduction. A way to overcome these unfavorable conditions is to accept a drastic structural change of the FeMo-co. In the Sellmann model [76], the opening of a FeMo-co side yields two reactive iron centers, essentially free of interactions from other metals, and able to coordinate N_2 by a chelate effect. On the molybdenum front, the Schrauzer model proposes a redox-linked decoordination of the Mo-end of FeMo-co yielding a reactive, sulfur-free, mononuclear molybdenum center [112]. It might be argued that the structural changes involved in the Sellmann or Schrauzer models are too energy costly and not biologically realistic. However the consumption of 16 MgATP for each N_2 molecule reduction might provide the energy required for large alteration of the FeMo-co structure. The mechanism of the aconitase enzyme and the structural changes of the P-cluster discussed previously, also provide some biological support to consider these proposals. On the other hand, it might be that any structural change experienced by FeMo-co during enzymatic catalysis remains only marginal. Then, mechanism such as those proposed by Coucouvanis on iron [103], or by Durrant on molybdenum [147], might occur. The driving force to overcome the poor quality of the metal site must then come from the protein supramolecular surrounding of the biological cluster, known to be crucial for N_2 reduction catalysis to happen. This poses a real challenge for a more realistic nitrogenase-relevant functional model chemistry: future synthetic models will have to take into account to some extent both the sulfur and metal–metal network together with a minimum supramolecular requirement, forcing the protonation and reduction of a nitrogen molecule, only loosely bound to a mediocre coordination site. As for structural model chemistry, the challenge of the total synthesis of FeMo-co remains intact.

It is ironic that the inorganic site that is likely to have provided living organisms with a fixable nitrogen source for millions of years be dubbed 'inefficient', although this is true from a thermodynamic point of view: even the energy cost of artificial ammonia synthesis compares favorably [154]. The nitrogenase enzyme, however, can be considered very efficient, within the constraints, common to all biological reactions, imposed on its modus opperandum: an aqueous medium at physiological pH, extracellular atmospheric pressure and temperature, a protein scaffold depending only on twenty different amino acids. This is further limited by the chemical necessity for the enzyme to be compatible with the complexity of the interdependent biological reactions making up living organisms. In this respect, the making of the FeMo-co cluster does not differ from other iron-sulfur clusters widely found in metalloproteins as electron transfer relays or as catalytic sites [155–158]. Thus, the nature of its constituents, mainly sulfur and iron, may be rationalized partially on evolutionary grounds. Its particular structure, however, may be justified in part by the difficult task of reducing the stable dinitrogen molecule: the metal site where catalysis occurs is bound to the ultimate electron transfer site, the metal–metal bond network, whose potentials is tuned by interaction between the sulfide scaffold and the protein residues. The natural system also shows some flexibility since alternative 'conventional' nitrogenase enzymes exist with vanadium or iron in place of molybdenum [120,121]. It is also possible that biological nitrogen fixation exhibits some diversity since a 'non-conventional' nitrogenase, also containing iron and molybdenum but depending on oxygen and superoxyde, has been reported [159,160].

The complexity of the nitrogenase system, implied by the difficulty of the reaction catalyzed and the constraints imposed by its biological nature and the simplicity of the available building blocks (amino acids, sulfur, iron and molybdenum, homocitrate), is fascinating [161]. However, the search for an efficient synthetic homogeneous catalyst for dinitrogen reduction should take advantage of the diversity of elements and experimental conditions available to the chemists [161] rather than trying to mimic a complex and energy costly natural system. Such a diversity may be illustrated by the numerous approaches and complexes that chemists have used to attempt to coordinate and reduce N_2 [159,162–164]. The heterogeneous process for ammonia synthesis is now well understood and is being refined, especially by a theoretical approach [165–170]. Such a level of understanding might be at reach for the biological reaction by the continuing research and interplay between the inorganic, theoretical and biological chemistry fields, from which the answer of the

[4] Reduction of C_2D_2 by the wild-type nitrogenase is highly stereospecific (ca. 96% of the ethylene formed is in the *cis*-1,2-$C_2H_2D_2$ stereoisomer). It is possible that dinitrogen gets reduced in an analogous manner [171].

precise location of N_2-binding and activation at FeMo-co will ultimately come [171,172].[4]

For historical reasons, the molybdenum site has originally been attractive and has been studied a lot; after the publication of the Kim and Rees crystallographic model of the active site in 1992, it became almost ignored; it now seems to regain some attention. This article was intended to review the experimental and theoretical chemistry that help to better understand the molybdenum site in FeMo-co and contribute to assess its debated role in the nitrogenase enzyme. Whether molybdenum is indeed intimately involved in biological nitrogen fixation remains to be proved, or disproved.

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