ELSEVIER

Coordination Chemistry Reviews
200–202 (2000) 545–561

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

The nitrogenase catalyzed N_2 dependent HD formation: a model reaction and its significance for the FeMoco function

Dieter Sellmann *, Anja Fürsattel, Jörg Sutter

Institut für Anorganische Chemie der Universität Erlangen–Nürnberg, Egerlandstraße 1, D-91058 Erlangen, Germany

Received 27 July 1999; received in revised form 20 October 1999; accepted 4 November 1999

Contents

Abstract 545
1. Introduction 546
2. FeMo, FeV, FeFe nitrogenases and the obligatory H_2 evolution 546
3. The N_2 dependent HD formation 547
4. HD formation catalyzed by $[\mu\text{-}N_2H_2\{Ru(PCy_3)(\text{'}S_4\text{'})\}_2]$ 549
5. The N_2 dependent HD formation in the 'open-side' FeMoco model 553
6. The constraints imposed on the N_2 dependent HD formation 556
7. The noncompetitive inhibition of N_2 fixation by CO 559
8. Conclusions 559
Acknowledgements 560
References 560

Abstract

The first completely characterized model for the nitrogenase catalyzed 'N_2 dependent HD formation' from D_2 and protons is described. This key reaction of nitrogenases is most plausibly rationalized by the 'open-side' FeMoco model, which enables us to explain the severe constraints imposed on the N_2 dependent HD formation as well as the noncompetitive inhibition of N_2 fixation by CO.

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

* Corresponding author. Tel.: +49-9131-8527360; fax: +49-9131-8527367.
E-mail address: sellmann@anorganik.chemie.uni-erlangen.de (D. Sellmann).

0010-8545/00/$ - see front matter
PII: S0010-8545(99)00240-4

1. Introduction

Photosynthesis and biological N_2 fixation are two of those fundamental syntheses that form the basis of life. Both processes have remained poorly understood on the molecular level. No low-molecular weight compounds have ever been found that truly enable us to mimic biological photosynthesis or N_2 fixation. Such 'competitive catalysts', also anticipated to yield a deeper insight into the biological processes, have been ranked among the 'Holy Grails of Chemistry' [1]. Their discovery has to wait until the next century.

This article will focus on the problem of N_2 fixation [2], and a reaction which is intimately coupled to N_2 reduction: The nitrogenase catalyzed N_2 dependent HD formation.

2. FeMo, FeV, FeFe nitrogenases and the obligatory H_2 evolution

A giant step forward in solving the N_2 fixation problem has been the X-ray structural characterization of FeMo nitrogenases and its FeMo cofactors by D.C. Rees and his co-workers in 1992 [3]. The FeMo cofactors are the site of N_2 activation and reduction. Their structure is schematically depicted in Fig. 1.

The X-ray structure determination of FeMo nitrogenase provided the structural basis for all future mechanistic discussions of N_2 fixation, but could not afford a direct answer to the pivotal question from the chemical point of view: How are inert N_2 molecules, protons derived from water, and electrons combined to give NH_3 under standard conditions at mild biological reduction potentials.

A characteristic feature of all nitrogenases is the evolution of H_2 that always occurs with N_2 reduction. This H_2 evolution, which cannot be suppressed even under high pressures of N_2, is termed 'obligatory H_2 evolution'. (When no other reducible substrates are present, only protons are reduced to give H_2.) Eq. (1) indicates the (probably) limiting stoichiometry for FeMo nitrogenases. The N_2:H_2 ratio of 1:1 in Eq. (1) has been emphasized in many discussions [4]. It is therefore to be noted that there are also 'alternative' FeV [5] and FeFe nitrogenases [6], which contain vanadium instead of molybdenum or no heterometal at all. They catalyze the reactions according to Eqs. (2) and (3).

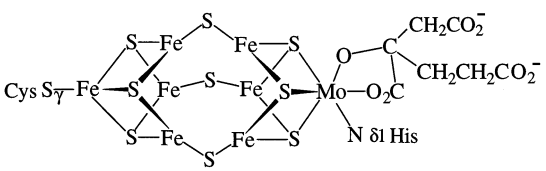


Fig. 1. Schematic representation of the FeMoco structure in FeMo nitrogenase.

$$\text{FeMo} \qquad N_2 + 8\ H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2 \tag{1}$$

$$\text{FeV} \qquad N_2 + 12\ H^+ + 12\ e^- \rightarrow 2\ NH_3 + 3\ H_2 \tag{2}$$

$$\text{FeFe} \qquad N_2 + 21\ H^+ + 21\ e^- \rightarrow 2\ NH_3 + 7.5\ H_2 \tag{3}$$

The reduction of N_2 and protons further requires ATP. The 'classical' FeMo nitrogenase needs 16 ATP for Eq. (1) [4]. Another FeMo nitrogenase, most recently discovered and also catalyzing the stoichiometry of Eq. (1), requires only 4 ATP [7]. These few points are to indicate that mechanistic conclusions drawn from the N_2:H_2 ratios and number of ATP molecules must be taken with caution. However, the phenomenon of the obligatory H_2 evolution is taken for granted [4–7]. It has most thoroughly been investigated with FeMo nitrogenases and led to the discovery of the N_2 dependent HD formation that has remained a mystery for 40 years.

3. The N_2 dependent HD formation

In 1960, Burris et al. observed for the first time that the molecular hydrogen evolved in the course of the 'obligatory H_2 evolution' also contains HD if D_2 is added to the N_2 gas phase (Eq. (4)) [8].

$$N_2 + 8\ H^+ + 8\ e^- \xrightarrow{+D_2} 2\ NH_3 + (H_2,\ HD) \tag{4}$$

This HD must result from protons of water and gaseous D_2. Biochemical electron balance studies later proved that one electron is required per each HD molecule formed [9]. Thus, the HD formation is not a hydrogenase-like D_2/H^+ exchange that requires no electrons [9]. Rather it is to be described by the seemingly simple redox Eq. (5).

$$2\ H^+ + 2\ e^- + D_2 \rightarrow 2\ HD \tag{5}$$

Severe constraints are imposed on this equation.

1. The HD formation is observed only and exclusively when N_2 is the reduction substrate. HD does not form when any other nitrogenase substrate, for example, C_2H_2, N_3^- or N_2O is reduced in the presence of D_2. For this reason, this HD formation was termed 'N_2-dependent HD formation' [3,9].
2. When nitrogenase turns over in the presence of HD, no D_2 can be detected [10].
3. Under the same conditions, but in the presence of T_2, less than 2.4% of T^+ is incorporated into the aqueous phase [9].

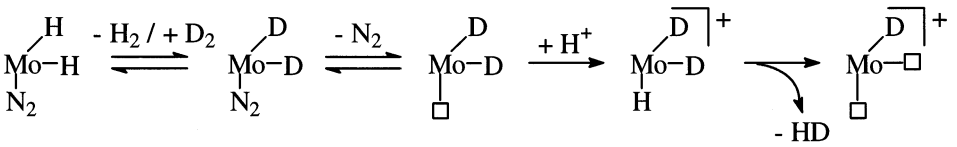
Every mechanism or model trying to explain molecularly the N_2 dependent HD formation must take into account these constraints. Before knowing the intimate mechanism of Eq. (5), however, the constraints enable to draw a couple of compelling conclusions.

1. N_2 reduction and HD formation are integral parts of one and the same reaction. They are inseparably coupled.
2. The formation of HD must depend on a reduction intermediate which is specific for N_2.

3. HD formation must occur via a predominantly intramolecular mechanism in the coordination sphere of the FeMoco, because so little T^+ is released into solution.
4. Elucidation of the HD formation mechanism thus can be anticipated to shed light also on the N_2 reduction mechanism and even on the FeMoco function.

Essentially two mechanisms have been postulated in order to explain the HD formation. Both, the 'trihydride' and the 'diazene' mechanism, suggest that nitrogenase (as isolated in the dithionite reduced state) must first be reduced before HD formation can occur, explaining that NH_3, H_2 as well as HD formation are electron-requiring processes.

The 'trihydride' mechanism is based on the kinetic Thorneley–Lowe scheme of nitrogenase [11] and model reactions of molybdenum phosphine dinitrogen complexes such as $[Mo(N_2)_2(PR_3)_4]$ [4b–d]. Successive electronations and protonations transfer nitrogenase into a stage in which the Mo center of the FeMoco binds N_2 and hydride ligands (Scheme 1). Exchange of the hydride ligands with D_2, loss of N_2, and protonation of the resulting vacant site (□) gives a $[Mo(H)(D)_2]$ species which liberates HD.



Scheme 1. Trihydride mechanism of the N_2 dependent HD formation.

A direct interaction between the N_2 and hydrogen/deuterium ligands occurs at no stage. In the decisive step of HD formation, N_2 does not bind to the Mo center, and N_2 essentially acts as 'stand-in' ligand only. This raises the question why N_2 is indispensable for HD formation and cannot be replaced by other nitrogenase substrates known to function likewise as 2-electron ligands, for example, N_3^- or N_2O.

This is the basic problem of the trihydride mechanism, which also could not be resolved by various modifications of the mechanism, proposing, for example, that bound N_2 enables the enzyme to assume a more reduced stage in which HD formation takes place [12].

The diazene mechanism avoids this problem by proposing diazene, N_2H_2, as pivotal N_2 reduction intermediate. Diazene as mediator of the HD formation had already been suggested by Burris et al. [8]. It received support by the electron balance studies (2 e^-/2 HD) and was proposed to form as enzyme bound N_2H_2 according to Eq. (6) [9]. D_2 attack upon the N_2H_2 intermediate was postulated to result in formation of HD and enzyme bound N_2 according to Eq. (7). Eq. (7) sums up to a 'D_2-catalyzed N_2H_2 decomposition' [9].

$$\text{Enzyme}(N_2) + 2\ H^+ + 2\ e^- \rightarrow \text{Enzyme}(N_2H_2) \quad (6)$$

$$\text{Enzyme}(N_2H_2) + D_2 \rightarrow \text{Enzyme}(N_2) + 2\ HD \quad (7)$$

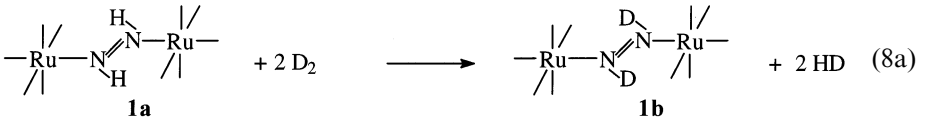
The 'diazene' mechanism raises the question why N_2 should first get reduced in order to be subsequently reoxidized by D_2(!). D_2 would be an unusual oxidation reagent. The intimate molecular mechanism of the 'D_2 catalyzed N_2H_2 decomposition' had to remain open. Other authors, in particular Bazhenova and Shilov, entirely rejected a diazene mechanism for thermodynamic reasons. They stated that free diazene is an extremely unstable compound ($\Delta H_f = +212$ kJ mol^{-1}), and forbiddingly high-energy barriers would exist to reach the N_2H_2 stage by reducing N_2 in a [2 H^+/2 e^-] reduction step [12]. This argument disregards that free and complex bound N_2H_2 behave differently. For example, N_2H_2 is considerably stabilized by metal coordination, the essence of most catalytic reactions is to circumvent energetically disfavored steps by stabilizing unstable intermediates, and, last but not least, the redox couples [enzyme(N_2)/enzyme(N_2H_2)] and [N_2/N_2H_2] can have as different redox potentials as the textbook examples [Au/Au^+] versus [Au/{$Au(CN)_2^-$}].

In summary: both, the trihydride mechanism or its modifications and the diazene mechanism, left unanswered important questions. The major problem of both mechanisms, however, was that they had to remain speculative as long as no compound was known that catalyzed a HD formation from D_2 and protons and stringently depended on N_2 or a reduction product specific for N_2.

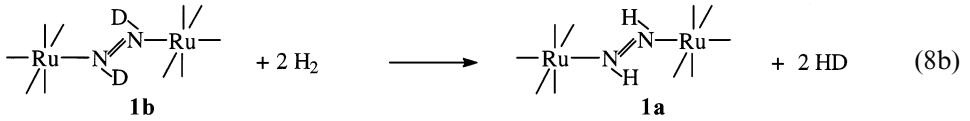
4. HD formation catalyzed by [μ-N_2H_2{Ru(PCy_3)('S_4')}$_2$]

This situation has changed on account of the ruthenium complex [μ-N_2H_2{Ru(PCy_3)('S_4')}$_2$] (**1**). Complex **1** is obtained by oxidation of [Ru(N_2H_4)(PCy_3)('S_4')] or by trapping N_2H_2 (generated in situ from $K_2N_2(CO_2)_2$ and acetic acid) with [Ru(DMSO)(PCy_3)('S_4')] [13]. The molecular structure of **1** has been determined by X-ray crystallography [14] and is depicted in Fig. 2. The schematic drawing illustrates the atom connectivity of **1**. In complex **1**, a *trans*-HN=NH ligand bridges two homochiral [Ru(PCy_3)('S_4')] fragments. The diazene ligand is stabilized by steric shielding, a 4c–6e π-bond system in the [Ru···NH···NH···Ru] entity, and N–H···(S-thiolate)$_2$ bridges. These three factors of stabilization are a characteristic feature of μ-N_2H_2 transition metal sulfur complexes [15]. The PCy_3 ligands evidently cause considerable steric crowding which probably is one reason for the observed reactivity of **1**.

When treated with molecular D_2 at standard conditions (1 bar, 25°C), the N_2H_2 complex **1a** gives the N_2D_2 complex **1b** and HD (Eq. (8a)). This reaction is reversible, proved by the reaction of the N_2D_2 complex **1b** with molecular H_2 (Eq. (8b)). Isolation and identification of *both* the products **1b** (or **1a**) and HD enabled the unambiguous proof of these reactions [13].

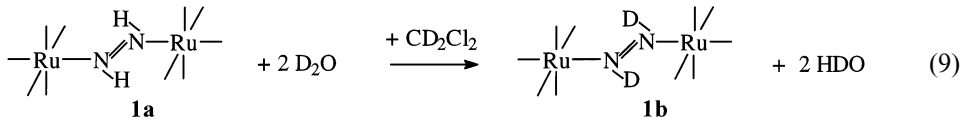


1a **1b** (8a)



(8b)

The Eqs. (4) and (5) and the Eqs. (6) and (7) of the 'diazene' mechanism imply that the protons of the resulting HD (or the reductively formed N_2H_2) must derive from H_2O. For this reason, also the H^+/D^+ exchange of complex **1a** was probed and found to occur according to Eq. (9).



(9)

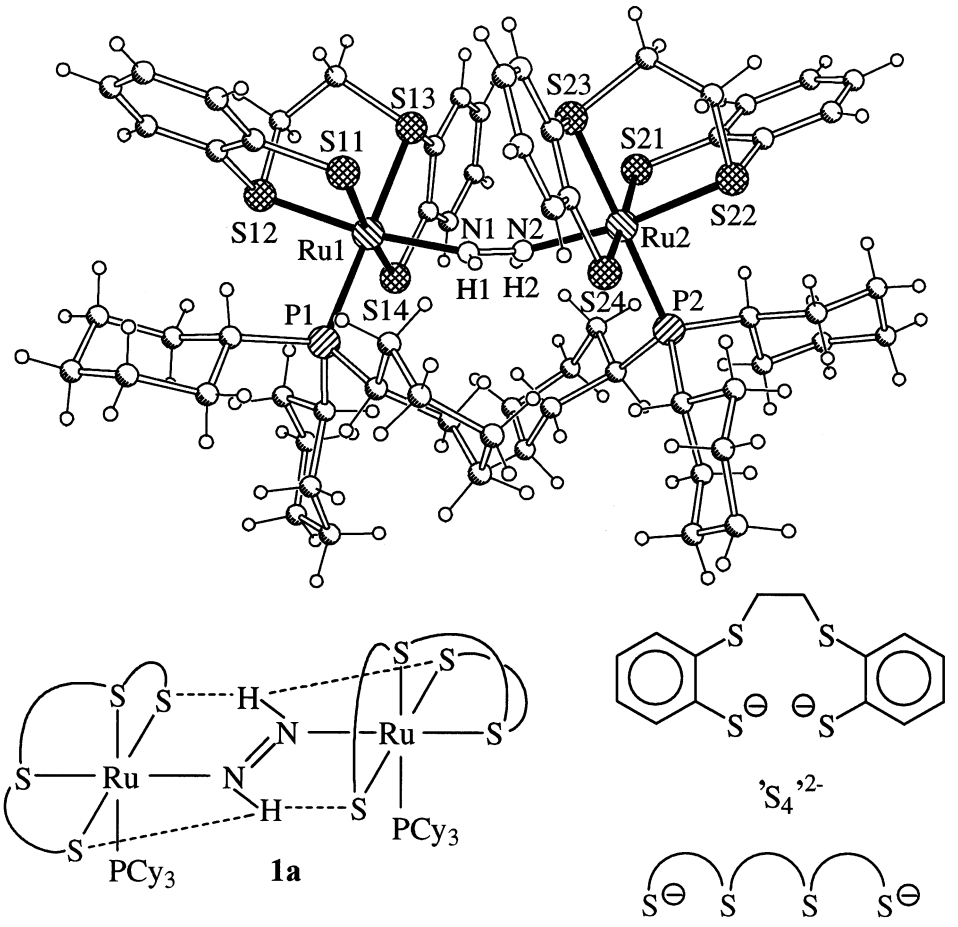


Fig. 2. Molecular structure and schematical drawing of [μ-N_2H_2\{Ru(PCy_3)('S_4')\}$_2$] (**1**).

It is significant that this H^+/D^+ exchange takes place about seven times slower than the D_2/H^+ exchange according to Eqs. (8a,b) [13].

Combining reactions (8) and (9) thus proves that HD formation results from D_2 exchange with ligating N_2H_2 whose protons can derive from water. This represents the first chemical example modeling the N_2 dependent HD formation catalyzed by nitrogenase.

Insight into the D_2/H^+ exchange mechanism of Eqs. (8a,b) results from a combination of previous findings.

The complex [Ru(DMSO)(PCy_3)('S_4')] (**2a**) has a labile DMSO ligand and forms the neutral η^2-H_2 derivative **2b** (Fig. 3). The H_2 ligand of **2b** can be heterolytically cleaved to give the hydride-thiol species **2c** which can be deprotonated to afford the anionic hydride complex **2d** [16].

Because all reaction steps are reversible, the complexes **2** catalyze reaction (10), which requires the heterolytic cleavage of D_2 into D^+ and D^- (Eq. (11)). In the catalytic cycle of Eq. (11), the species **2b**, **2c** and **2d** represent key intermediates [16].

$$D_2 + H^+OH^- \rightleftharpoons HD + D^+OH^- \tag{10}$$

$$D_2 \rightleftharpoons D^+ + D^- \tag{11}$$

The diazene complex **1a** exchanges its PCy_3 for P^iPr_3 ligands under retention of the [μ-N_2H_2{Ru}$_2$] core according to Eq. (12). This indicates that, through dissociation of PCy_3, **1a** can provide vacant Ru sites for the addition and heterolytic cleavage of H_2.

$$[\mu\text{-}N_2H_2\{Ru(PCy_3)(\text{'}S_4\text{'})\}_2] + 2\ P^iPr_3 \rightarrow [\mu\text{-}N_2H_2\{Ru(P^iPr_3)(\text{'}S_4\text{'})\}_2] + 2\ PCy_3 \tag{12}$$

Combination of these results and Eqs. (8a,b) suggests the mechanism of Scheme 2 for the NH/D_2 or ND/H_2 exchange of **1**.

Essential core atoms of **1** are the Ru centers, thiolate donors and diazene atoms. Dissociation of PCy_3 (step a) yields vacant sites (□) to which D_2 adds (step b) that is cleaved heterolytically into D^- and D^+ by the concerted action of the Lewis acidic Ru centers and Brønsted basic thiolate donors (step c). Intramolecular scrambling of the acidic diazene protons and thiol deuterons (steps d and e) gives the N_2D_2 species, which releases HD (step f). Re-addition of PCy_3 yields **1b** (step g).

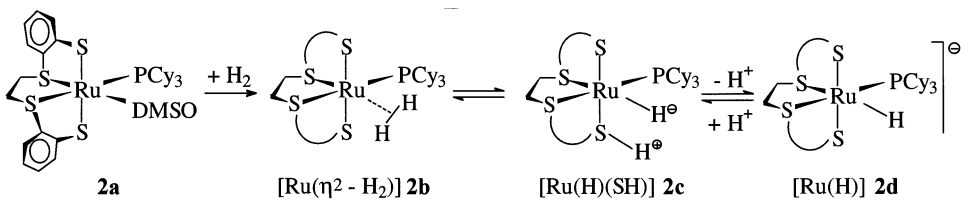
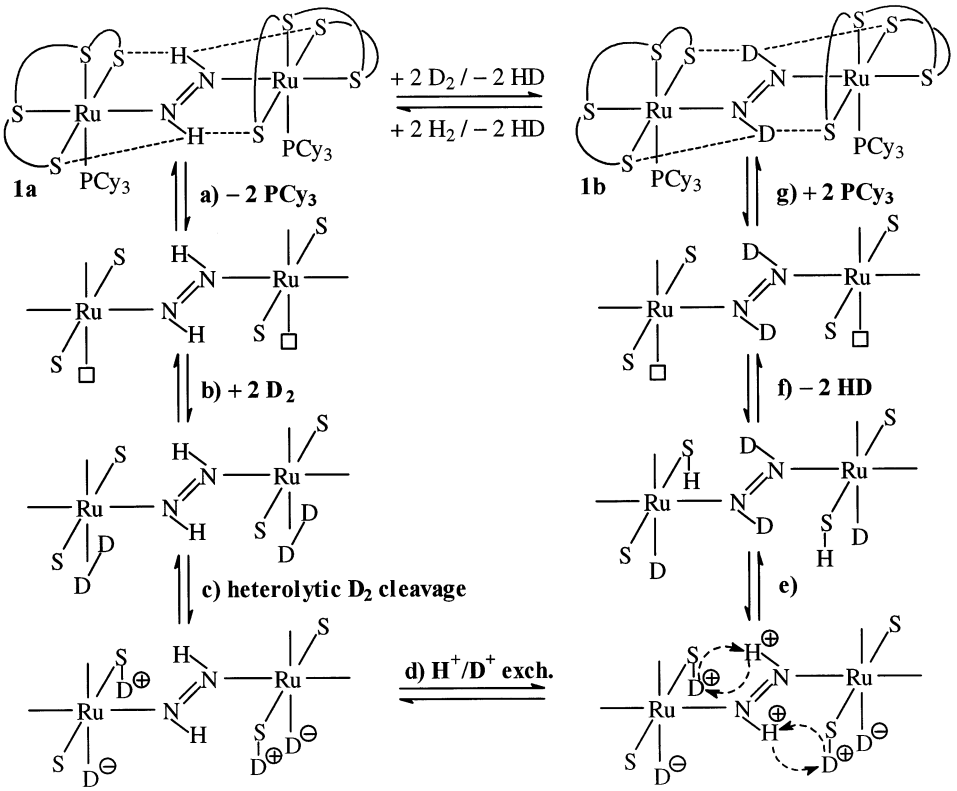


Fig. 3. Dihydrogen, hydride thiol, and hydride complexes of the [Ru(PCy_3)('S_4')] fragment.



Scheme 2. D_2/NH exchange of [μ-N_2H_2{Ru(PCy_3)('S_4')}$_2$].

These results support the diazene mechanism for the nitrogenase catalyzed N_2 dependent HD formation. They need further discussion, because Eqs. (8a,b) evidently contrast with Eq. (7). Eqs. (8a,b) show that attack of H_2 or D_2 upon bound diazene *does not* decompose N_2H_2 to give N_2. They rather demonstrate that the N_2 dependent HD formation takes place on the diazene level. In other words: the diazene reduction stage stays preserved in the course of HD formation and does not switch back to the N_2 level. This necessitates an important conclusion with regard to the overall mechanism of the N_2 dependent HD formation.

Reaction (8a), rewritten as Eq. (13), utilizes only one half of the D_2 for HD formation, binds the other half in the diazene, and does not require electrons.

$$N_2H_2[M]_2 + 2\ D_2 \rightarrow N_2D_2[M]_2 + 2\ HD \tag{13}$$

In order to utilize the diazene bound deuterium, to make the HD formation catalytic, and to fulfill the electron balance of HD formation the reaction according to Eq. (14) must take place.

$$N_2D_2[M]_2 + 4\ H^+ + 4\ e^- \rightarrow N_2H_2[M]_2 + 2\ HD \tag{14}$$

Adding Eq. (13) to Eq. (14) gives Eq. (15). Eq. (15) fulfills the experimentally established 1 e^-/HD stoichiometry of the nitrogenase catalyzed N_2 dependent HD formation.

$$4\ H^+ + 4\ e^- + 2\ D_2 \xrightarrow{N_2H_2[M]_2} 4\ HD \quad (15)$$

These considerations show that even on the diazene level two distinguishable pathways of HD formation have to be taken into account, the non-reductive one of Eq. (13) and the reductive one of Eq. (14). Both pathways can be assumed also for nitrogenase, because in nitrogenase the H^+/e^- flow will continue when the diazene stage has been reached. In order to achieve reaction (14), a scheme can be suggested which is quite similar to Scheme 2. As additional elementary reaction, it contains a H^+ reduction at iron sulfur centers for which a chemical precedent at iron sulfur centers is given by the reaction according to Eq. (16).

$$2\ H^+ + 2\ [Fe^{II}(S_2C_6H_4)_2]^{2-} \rightarrow H_2 + [Fe^{III}(S_2C_6H_4)_2]_2^{2-} \quad (16)$$

Protonation of the planar Fe(II) complex $[Fe(S_2C_6H_4)_2]^{2-}$ leads to evolution of H_2 and formation of the binuclear $[Fe^{III}(S_2C_6H_4)_2]_2^{2-}$ [17]. It is significant that this reaction involves two Fe^{II}/Fe^{III} redox centers.

5. The N_2 dependent HD formation in the 'open-side' FeMoco model

The discovery of the HD formation from D_2/H^+ exchange catalyzed by [μ-N_2H_2{$Ru(PCy_3)$('S_4')}$_2$] (**1**) and mediated via its N_2H_2 ligand not only supports diazene as the key reduction intermediate in biological N_2 fixation, it also sheds light on the functioning of the FeMoco, because it is most readily explained through the open-side FeMoco model. (It is noted that this model is compatible with the kinetic Thorneley–Lowe model [11] demonstrating that nitrogenase can assume several kinetically distinguishable stages of reduction.)

The open-side FeMoco model has been described in detail previously [18]. It takes into account a large number of biological, biochemical and chemical results and tries to combine them. The results considered most important are:

Not only in 'Fe-only', but in all three nitrogenase types, iron is the dominant metal. The Fe centers in the FeMoco (and probably also in the FeV and FeFe cofactors) are clearly 'undercoordinated' (cf. Fig. 1). Sulfur donors prevail. Transition metal thiolate or sulfide bonds frequently are labile. So is isolated FeMoco, which readily decomposes in aqueous media ($\tau_{1/2} \sim 2$ h). Isolated FeMoco does not catalyze N_2 reduction, indicating that the enzyme protein is essential [19]. Thermodynamically, the electrochemical N_2 reduction to NH_3 requires only -280 mV reduction potential. All nitrogenase substrates, e.g. N_2, N_2O, HCN, C_2H_4, etc., are reduced in multiples of [2 H^+/2 e^-] reductions [4]. Diazene, HN=NH, is efficiently stabilized through coordination to transition metal thiolate centers as in the ruthenium complex **1** and related iron complexes [15]. These complexes can be electrochemically oxidized in several reversible steps. The diazene complex dications resulting from 2 e^- oxidations are valence isomers of the corresponding twofold

protonated dinitrogen complexes [15]. Primary protonation of non-reducible (18 valence electron) transition metal sulfur ligand complexes makes them reducible in biologically compatible redox ranges of ca. −500 mV [15,20]. The FeMo protein of FeMo nitrogenase contains two essential amino acids, Gln α191 and His α195. These amino acids do not bind to FeMoco in the resting state, however, they are close to the FeMoco (5 Å), they have N and O donors suited for metal coordination, and their replacement by site-directed mutagenesis causes inactivity of the enzyme [4]. H_2O molecules, which also can act as ligands, surround the FeMoco in native nitrogenase. Nitrogenase as isolated in the dithionite reduced state must take up at least two electrons before it can bind N_2. Crystallographic studies have shown that oxidoreductases frequently have different structures in the resting and in the active turnover state.

Putting together these pieces (and some more outlined elsewhere [15]) suggests that the FeMoco does not have identical structures in the resting and in the turnover state. In contrast to all calculations on FeMoco/N_2 interactions that assume essentially identical FeMoco structures for the resting and turnover states of FeMoco [21], the 'open-side' FeMoco model proposes that the two electron reduction preceding the binding of N_2 leads to an 'opening' of the FeMoco. Dissociation of one labile Fe–S–Fe bridge, coordination of the nearby N or O donors from Gln α191 and His α195, and coordination of three H_2O molecules results in two unique five-coordinate Fe(II) centers which are in low-spin states through steric constraints of the surrounding protein (Fig. 4) [18b]. These two Fe centers bind N_2 which gives NH_3 in the course of subsequent [2 H^+/2 e^-] reduction steps.

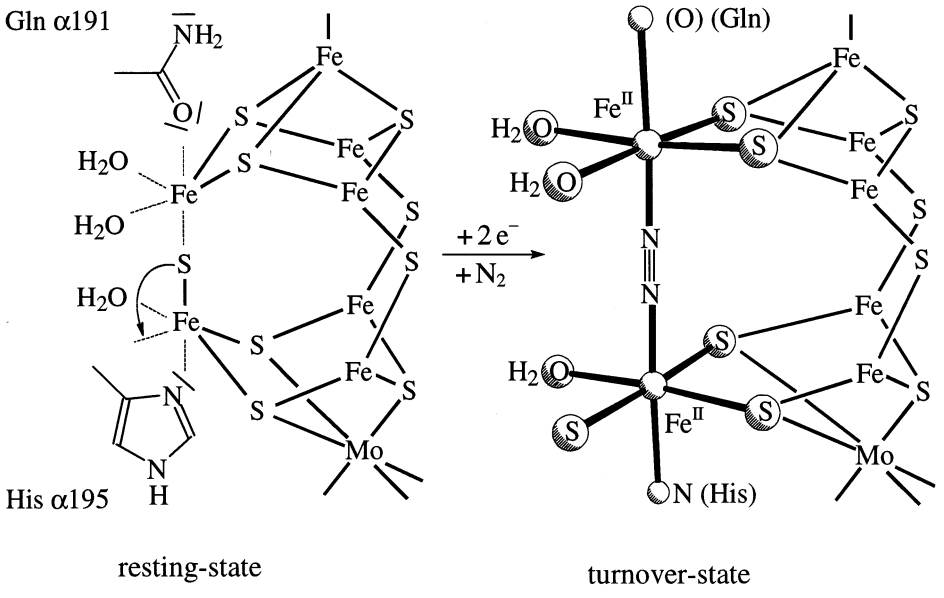


Fig. 4. Opening the FeMoco and binding N_2 to two five-coordinate low-spin Fe(II) centers.

The structure of the iron diazene complex [μ-N_2H_2{Fe('N_HS_4')}$_2$] (**2**), which only contains biologically compatible donor atoms [22], enabled us to illustrate the first reduction stage (Fig. 5).

Fictitious removal of all 'N_HS_4' atoms from **2** and merging the remaining [N_2H_2{Fe}$_2$] 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. In addition this arrangement enables the formation of the essential hydrogen bridges for alleviating the reduction barrier between the N_2 and N_2H_2 stage. It is pointed out that the formation of the open-side FeMoco structure does not require the loss of any FeMoco sulfide donor but only the coordination of additional ligands to under-coordinated Fe centers. It is also noted that the core structures of the hexa-coordinate Fe centers in **2** and the hypothetical FeMoco–N_2H_2 complex differ only through the replacement of three sulfur donors by H_2O ligands.

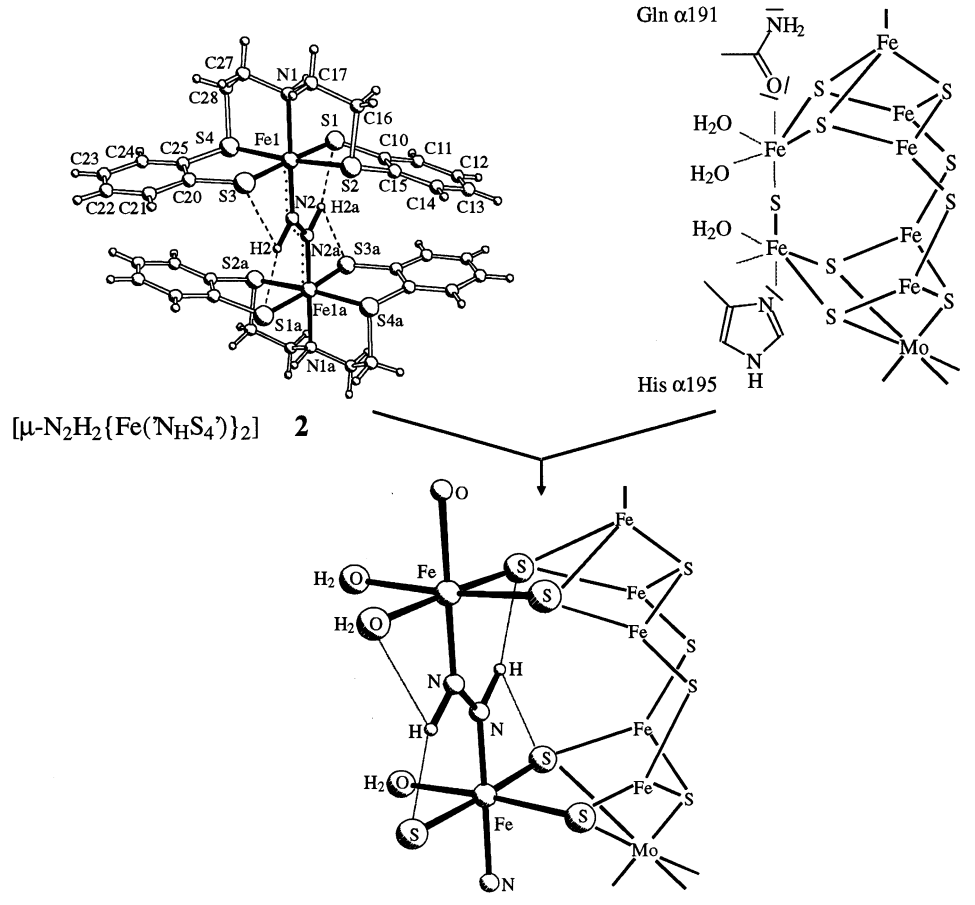


Fig. 5. Illustration of the diazene stage of the open-side FeMoco model.

The 'rest' of the FeMoco can be envisaged as flexible spacer and electron relays for the two unique Fe centers whose oxidation states in the course of N_2 reduction shuttle between Fe(II) and Fe(III) like the Fe centers in the reversible oxidation of, e.g. [μ-N_2H_2{Fe(PPr_3)('S_4')}$_2$] [23], which is a homologue of complex **1**.

The diazene stage depicted in Fig. 6 now yields a plausible explanation for the 'N_2 dependent HD formation' mediated by a diazene intermediate.

'Hard' aqua ligands binding to 'soft' sulfur coordinated low-spin Fe(II) centers are anticipated to be labile. They can exchange for D_2, and as soon as D_2 binds to the Fe centers it gets cleaved by exactly the same mechanism as shown for the Ru complex **1** in Scheme 2. It is only to be kept in mind that low-spin Ru(II) centers are homologous to low-spin Fe(II) centers.

6. The constraints imposed on the N_2 dependent HD formation

The open-side [FeMoco-diazene] model thus yields an explanation for the stringent dependence of HD formation on N_2 as reduction substrate. The question arises, whether it can pass also the crucial test of explaining the other severe constraints imposed on the HD formation. These constraints are that no D_2 forms when nitrogenase operates under HD, and that only extremely little T^+ is released into solution when operating under T_2. A detailed analysis of the various possibil-

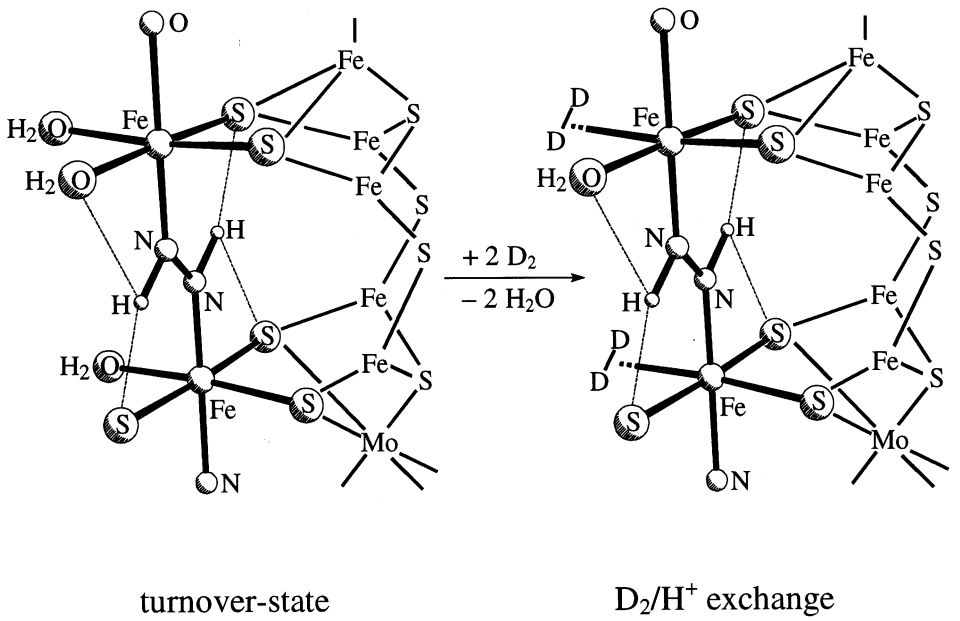


Fig. 6. D_2/H_2O exchange at the two unique Fe(II) centers of the open-side FeMoco–N_2H_2 stage as primary step of the subsequent D_2 heterolysis.

ities to activate H_2, HD, D_2 or T_2 at the [μ-N_2H_2{Fe}$_2$] site of Fig. 6 affords significant answers.

The characteristic feature of the ruthenium complex **1** and the [FeMoco–N_2H_2] model of Fig. 6 is the arrangement of two Lewis-acidic metal centers, a bridging HN=NH ligand and Brønsted basic sulfur donors. Fig. 7 depicts this arrangement (**I**) and illustrates that HD can be cleaved heterolytically, into either H^+ and D^- (a) or into H^- and D^+ (b) resulting in the species **III** or **IV**. Intramolecular H^+/H^+ (**III**) or H^+/D^+ (**IV**) exchange, and reversal of the heterolysis to reform molecular hydrogen can give only HD (a) or H_2 (b) but no D_2, if one N_2H_2 mediated turnover of HD is considered.

(In these cases, identical HD heterolyses are assumed for both M centers in either **III** or **IV**. The net result does not change, when one M center carries a hydride and the other one a deuteride ligand).

When, after H^+/D^+ scrambling, H_2 is released from species **IV**, the N_2D_2 species **V** remains. This species **V** has two options. One is that the diazene bound deuterium is released as HD via the reductive pathway (c) according to Eq. (14) (see above) to give the initial starting species **I**. The second option is that species **V** reacts another time with HD resulting in either the species **VI** (d) or the species **VII** (e). After D^+/D^+ scrambling, species **VI**, too, can release only HD. Solely species **VII** could yield D_2 (e). However, these considerations show that the pathway e) is only one out of five principal possibilities. Moreover, the probability of formation is much lower for **VII** (and likewise for **VI**) than for **I** and **II**, as the formation of **VII** requires a second turnover of the initially formed diazene intermediate.

The same considerations hold for the exchange with T_2 and explain why HT forms as major product. A special consideration is needed for the release of T^+ into solution. Most review articles on the problem of HD formation state that no T^+ is released. However, though small, a certain percentage of 2.4% T^+ was found in solution [9]. This may or may not be significant. If it is significant, it needs an explanation. Here, the observation becomes important that the H^+/D^+ exchange of complex **1a** with D_2O is seven times slower than the D_2/NH exchange. When applied to a [FeMoco–N_2T_2] intermediate, this observation implies that the intramolecular HT formation is much more rapid than the H^+/T^+ exchange with protons of surrounding water. However, the latter exchange is not entirely impossible. Vice versa, it can be concluded that the lifetime of a diazene intermediate is long enough to enable a slow and thus small exchange with protons of water, but too short in order to enable several N_2H_2 mediated turnovers of H_2, HD, D_2 or T_2.

In summary: The mediation of a D_2/H^+ (or T_2/H^+) exchange by a diazene intermediate of limited life-time explains the stringent dependence of HD (or HT) formation on the presence of N_2 as reduction substrate. The NH bonds of the diazene intermediate represent additional reactive centers, which strongly diminish the possibility of HD molecules to react with themselves to give D_2 and H_2. The D_2/NH exchange takes place intramolecularly and more rapidly than the D^+/NH exchange with water, explaining that only an extremely small quantity of labeled hydrogen is incorporated into solution.

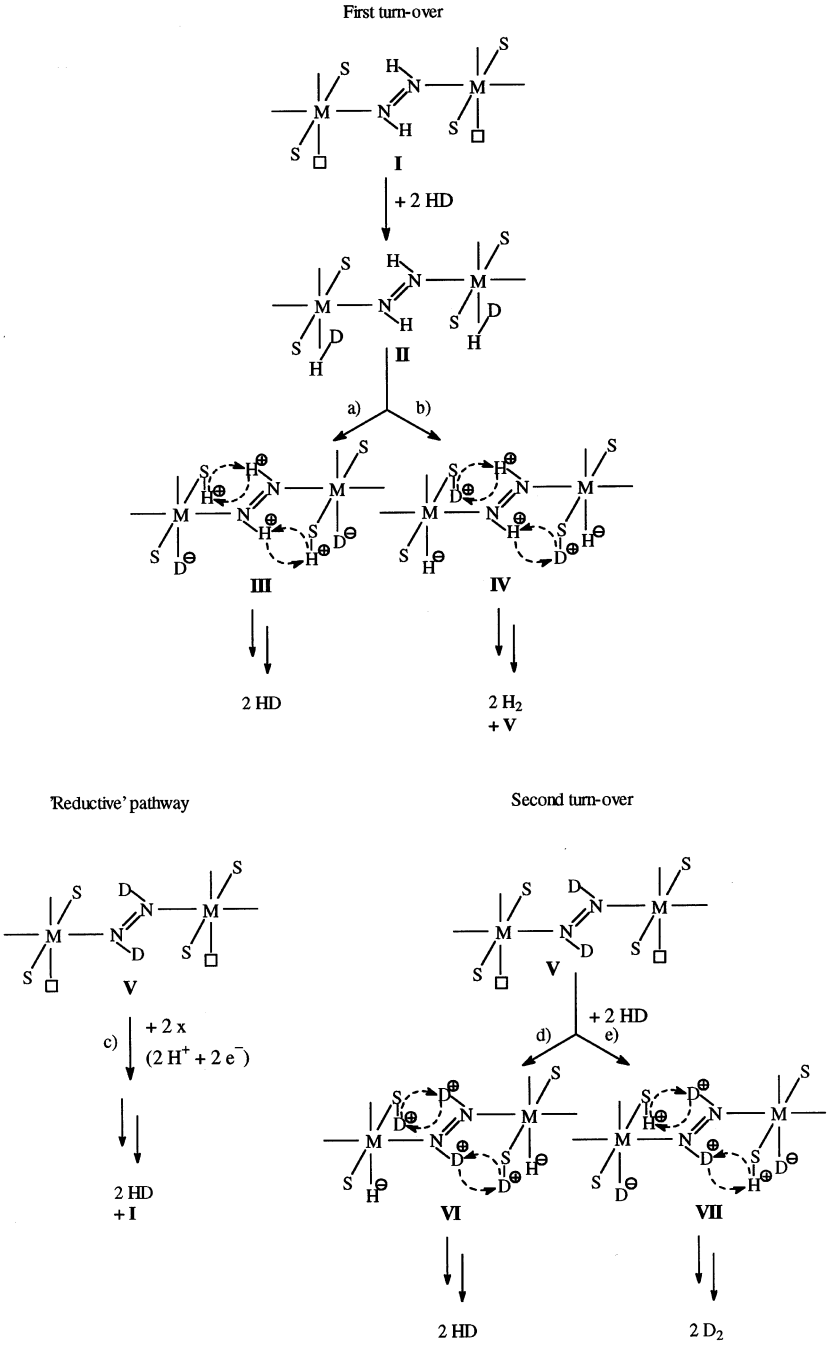


Fig. 7. The various possibilities of HD heterolysis and H/D exchange reactions at a $[(S)_2M–NH=NH–M(S)_2]$ site.

7. The noncompetitive inhibition of N_2 fixation by CO

The last question posed here is, whether the open-side FeMoco model can also explain the mysterious noncompetitive inhibition of N_2 fixation by CO. In catalysis, it can be distinguished between competitive and noncompetitive inhibition. In brief terms and with regard to nitrogenase: competitive inhibitors, e.g. N_2O or CN^-, block the N_2 binding site and prevent N_2 fixation. Noncompetitive inhibitors do not block the N_2 binding site and N_2 can bind to nitrogenase. Noncompetitive inhibitors rather decrease the N_2 reduction rate and 'redirect' incoming electrons toward reduction of protons to give H_2. CO is the most potent of all noncompetitive inhibitors. All incoming electrons are used for H_2 evolution.

The open-side FeMoco model yields a simple explanation for these phenomena, realizing that different complexes have different redox potentials and, quite general, different reactivity. Fig. 8 illustrates this for the open-side FeMoco stage binding N_2 that is not yet reduced to diazene. If CO is present, it can substitute one of the labile H_2O ligands to give a CO/N_2 species. The redox potential (and the entire electronic structure) of this species must differ from that of the N_2/H_2O precursor such that now incoming electrons are directed no longer to the N_2 ligand, but rather to sulfur bound protons or directly to protons at the remaining H_2O ligands to give H_2.

8. Conclusions

The nitrogenase catalyzed 'N_2 dependent HD formation' has been discussed in the light of recent results obtained with the diazene complex [μ-N_2H_2-{$Ru(PCy_3)('S_4')$}$_2$] (**1**). This complex catalyzes the N_2H_2 mediated exchange between molecular D_2 and protons derived from water. Because N_2H_2 is the most plausible intermediate of a [2 H^+/2 e^-] reduction of N_2, the results enable to

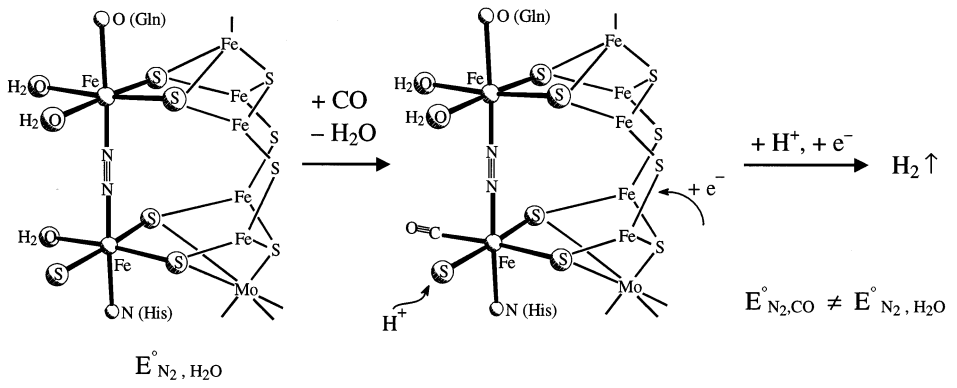


Fig. 8. Explanation of the noncompetitive N_2 fixation inhibition by CO through the different redox potentials of [FeMoco–N_2/H_2O] and [FeMoco–N_2/CO] intermediates.

rationalize the stringent N_2 dependence as well as the electron balance of the HD formation. Vice versa, they support diazene as the key intermediate of N_2 reduction. Heterolysis of D_2 as one of the pivotal steps is achieved by the concerted attack of Lewis-acidic ruthenium centers and Brønsted basic thiolate donors upon η^2-D_2 ligands.

The results also enable to explain plausibly the severe constraints imposed on the 'N_2 dependent HD formation', and thus support the open-side model of the FeMoco in the turnover state. Labile H_2O ligands at the two unique low-spin five-coordinate Fe(II) centers of this model which can be replaced by CO account for the noncompetitive inhibition of N_2 fixation by CO.

In the end, a key reaction of nitrogenase could be mimicked by a low-molecular weight complex which does not structurally copy the FeMoco, but rather meets principles governing the function of the FeMoco. Meeting these principles, which comprise, inter alia, Lewis acidic transition metal centers in sulfur-rich coordination spheres, holds promise to find also compounds that enable the catalytic reduction of N_2 under truly mild conditions. Such conditions must include biologically compatible reduction potentials not far beyond the thermodynamical limit of N_2 reduction at -280 mV.

Acknowledgements

Support of our investigations by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

References

[1] (a) R. Sunningham, Chemistry (ACS) October (1998) 5. (b) A.J. Bard, G.M. Whitesides, R.N. Zare, F.W. McLafferty, Acc. Chem. Res. 28 (1995) 91.
[2] For a more recent summary of all aspects of nitrogen fixation, cf. I.A. Tikhonovich, N.A. Provorov, V.I. Romanov, W.E. Newton (Eds.), Nitrogen Fixation: Fundamentals and Application, Kluwer, Dordrecht, 1995.
[3] J.B. Howard, D.C. Rees, Chem. Rev. 96 (1996) 2965.
[4] (a) B.K. Burgess, D.J. Lowe, Chem. Rev. 96 (1996) 2983. (b) R.N.F. Thorneley, D.J. Lowe, J. Biol. Inorg. Chem. 1 (1996) 576. (c) C.J. Pickett, J. Biol. Inorg. Chem. 1 (1996) 601. (d) G.J. Leigh, Eur. J. Biochem. 14 (1995) 229.
[5] R.R. Eady, Chem. Rev. 96 (1996) 3013.
[6] (a) P.E. Bishop, D.M.L. Jarlenski, D.R. Hetherington, J. Bacteriol. 150 (1982) 1244. (b) P.E. Bishop, R. Premakumar, D.R. Dean, M.R. Jacobson, J.R. Chisnell, T.M. Rizzo, P. Kopzcynski, Science 92 (1986) 232. (c) K. Schneider, U. Gollan, M. Dröttboom, S. Selsemaier-Voigt, A. Müller, Eur. J. Biochem. 244 (1997) 789. (d) E. Krahn, K. Schneider, A. Müller, Appl. Microbiol. Biotechnol. 46 (1996) 285.
[7] (a) M. Ribbe, D. Gadkari, O. Meyer, J. Biol. Chem. 272 (1997) 26 627. (b) D. Gadkari, G. Mörsdorf, O. Meyer, J. Bacteriol. 174 (1992) 6840.
[8] G.E. Hoch, K.C. Schneider, R.H. Burris, Biochim. Biophys. Acta 37 (1960) 273.
[9] B.K. Burgess, S. Wherland, W.E. Newton, E.I. Stiefel, Biochemistry 20 (1981) 5140.

[10] B.K. Burgess, J.L. Corbin, J.F. Rubinson, J.-G. Li, M.J. Dilworth, W.E. Newton, in: C. Veeger, W.E. Newton (Eds.), Advances in Nitrogen Fixation Research, Nijhoff-Junk, The Hague, 1984, p. 146.
[11] R.N.F. Thorneley, D.J. Lowe, in: Th.G. Spiro (Ed.), Molybdenum Enzymes, Wiley, New York, 1985, p. 221.
[12] T.A. Bazhenova, A.E. Shilov, Coord. Chem. Rev. 69 (1995) 144.
[13] (a) D. Sellmann, A. Fürsattel, Angew. Chem. Int. Ed. Engl. 38 (1999) 2023. (b) T. Gottschalk-Gaudig, Ph.D. Thesis, University of Erlangen–Nürnberg, 1997.
[14] D. Sellmann, T. Gottschalk-Gaudig, F.W. Heinemann, in press.
[15] D. Sellmann, J. Sutter, Acc. Chem. Res. 30 (1997) 460 and literature cited therein.
[16] (a) D. Sellmann, T. Gottschalk-Gaudig, F.W. Heinemann, Inorg. Chem. 37 (1998) 3982. (b) D. Sellmann, G.H. Rackelmann, F.W. Heinemann, Chem. Eur. J. 3 (1997) 2071.
[17] D. Sellmann, M. Geck, M. Moll, J. Am. Chem. Soc. 113 (1991) 5259.
[18] (a) D. Sellmann, J. Sutter, J. Biol. Inorg. Chem. 1 (1996) 587. (b) D. Sellmann, J. Utz, N. Blum, F.W. Heinemann, Coord. Chem. Rev. 190–192 (1999) 607.
[19] B.K. Burgess, Chem. Rev. 90 (1990) 1377.
[20] D. Sellmann, Th. Becker, F. Knoch, Chem. Eur. J. 2 (1996) 1092.
[21] (a) H. Deng, R. Hoffmann, Angew. Chem. Int. Ed. Engl. 32 (1993) 1062. (b) I.G. Dance, Aust. J. Chem. 47 (1994) 979. (c) I.G. Dance, J. Biol. Inorg. Chem. 1 (1996) 581. (d) K.K. Stavrev, M.C. Zerner, Chem. Eur. J. 2 (1996) 83. (e) T.H. Rod, J.K. Norskov, B. Hammer, Phys. Rev. Lett. 82 (1999) 4054.
[22] D. Sellmann, W. Soglowek, F. Knoch, M. Moll, Angew. Chem. Int. Ed. Engl. 28 (1989) 1271.
[23] D. Sellmann, H. Friedrich, F. Knoch, M. Moll, Z. Naturforsch. 48b (1993) 76.