

## COMMENTARY

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**Functional analogs for the reduction of certain nitrogenase substrates. Are multiple sites within the Fe/Mo/S active center involved in the  $6e^-$  reduction of  $N_2$ ?**

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**Abstract** Reactivity studies of clusters that contain the  $MFe_3S_4$  cores ( $M = Mo, V$ ) with catecholate, multicarboxylate (or DMF) ligands coordinated to the Mo (or V) atoms, and Cl ligands coordinated to the Fe atoms have been carried out. These studies show the  $M/Fe/S$  single cubane clusters to be effective catalysts in the reduction of nitrogenase substrates such as hydrazine, acetylene and protons to give ammonia, ethylene and dihydrogen respectively. The same molecules do not activate or catalyze the reduction of dinitrogen. The results indicate that the observed catalyses are occurring at the Mo (V) sites by a process that, in the case of hydrazine, involves substrate protonation prior to reduction. The facile catalytic reduction of hydrazine by clusters that contain coordinatively saturated polycarboxylate-bound Mo atoms is rationalized in terms of a possible protonation/proton delivery function of the coordinated polycarboxylate ligands. The reactivity characteristics of the  $M/Fe/S$  clusters (structurally quite similar to the nitrogenase cofactor) have led to the suggestion that the Mo (V) atoms may be involved in the reduction of hydrazine in the later stages of dinitrogen reduction.

**Key words** Nitrogenase · Analogs · Function · Catalysis · Reduction

**Introduction**

The commentary by Thorneley and Lowe [1] presents in detail an overview of what is presently known about the structure and function of nitrogenase. Their account also summarizes results of recent independent single-crystal X-ray diffraction studies [2–15] of the MoFe proteins of the nitrogenase systems from *Clostridium pasteurianum* and *Azotobacter vinelandii*,

which have revealed the structures of the “P” clusters and the Fe/Mo/S centers at a molecular level.

The determined structure of the Fe/Mo/S center defines the specific, albeit formidable, task associated with the synthesis of structurally analogous clusters. It also presents a challenge in understanding the mode of substrate activation and reduction. As a result of these recent developments, emphasis in the bioinorganic chemistry of nitrogenase has shifted from the synthesis of speculative models to (a) attempts toward the designed synthesis and isolation of a well-defined (probably metastable) target molecule and (b) reactivity studies of clusters that contain chromophores similar to those found in the nitrogenase cofactor.

In this account we present reactivity studies that were undertaken in our laboratory in an attempt to explore the following important unanswered questions regarding nitrogenase function:

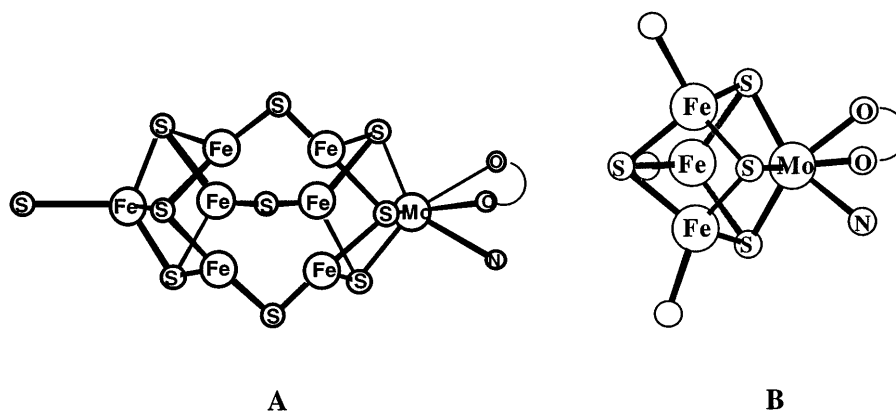
- Is there a direct involvement of the Mo (or V) atom in catalysis, and does the Fe/M/S center have multiple reaction sites?
- What is the function of the homocitrate ligand?
- How important is bi(multi)-metallic activation of substrates in the catalytic process, and is proton transfer coupled with the reduction process?
- Is the Mo (or V) atom directly involved in the activation (reduction) of  $N_2$ ?

**Catalytic studies with synthetic analogs**

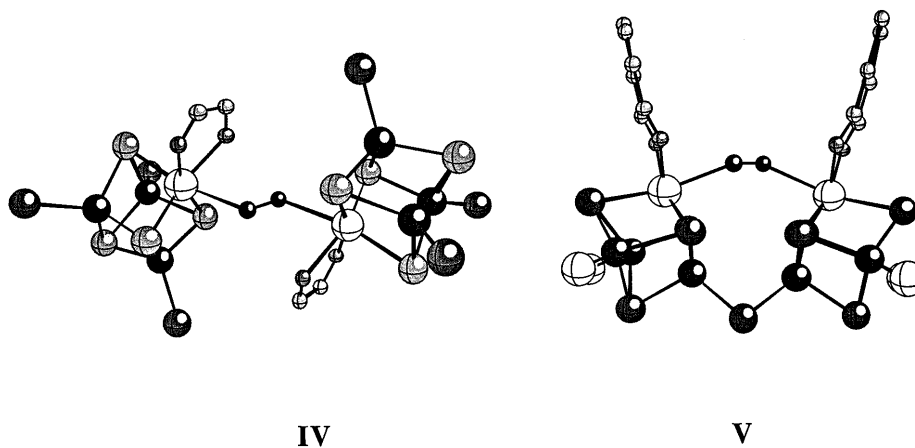
Various synthetic models for the Fe/Mo/S center in nitrogenase have become available over the years [16–31], and in each case their constitution and structure were based on the available spectroscopic and analytical information. Of these structures, the ones we have employed in substrate reduction studies contain as a common structural feature the  $MFe_3S_4$  “cubane” unit ( $M = Mo, V$ ). In these clusters, the coordination environment around the heteroatom, extending over two coordination shells, is almost identical to that found in

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**Fig. 1** Structures of **A** the Fe/Mo/S center in nitrogenase that contains the  $\text{Fe}_7\text{MoS}_9$  core and of **B** the  $\{[(\text{Cl}_4\text{-cat})(\text{CH}_3\text{CN})\text{MoFe}_3\text{S}_4(\text{Cl})_3]^{2-}\}$  cubane that contains the  $\text{Fe}_3\text{MoS}_4$  core



**Fig. 2** Molecular structures of the  $\{[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4(\text{Cl})_3]_2(\mu\text{-N}_2\text{H}_4)\}^{4+}$ , singly bridged double cubane, **III** and the  $\{[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4(\text{Cl})_2]_2(\mu\text{-N}_2\text{H}_4)(\mu\text{-S})\}^{4+}$  doubly bridged double cubane, **IV**



nitrogenase. (Fig. 1). A structural feature not present in the synthetic  $\text{MoFe}_3\text{S}_4$  clusters (Fig. 1B) is the unique  $\text{Fe}_3(\mu\text{-S})_3\text{Fe}_3$  core found in the nitrogenase cofactor and characterized by three coordinate Fe atoms and short Fe–Fe distances. The trigonal prismatic array of the six iron atoms in this core (Fig. 1A) may be a site of exceptional reactivity where the initial two- or four-electron reduction of  $\text{N}_2$  takes place (see commentary by Dance [32]).

The reactivity characteristics of the  $\text{MoFe}_3\text{S}_4$  clusters and their effectiveness in the catalytic reduction of nitrogenase substrates are partially relevant to nitrogenase function. Comparable relevance is difficult to find in the chemistry of organometallic mononuclear dinitrogen complexes that contain phosphine-type ligands and undergo reduction under severe biologically incompatible conditions (see commentary by Pickett [33]).

The specific clusters we have used in our catalytic studies are: (a) the  $\{[(\text{L})\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{CH}_3\text{CN})]^{n-}\}$  single cubane clusters, (L =  $\text{Cl}_4\text{-catecholate}$  [34],  $n = 2$ , **Ia**, Fig. 1B; L = citrate [29, 30], **Ib<sub>5</sub>**, **Ib<sub>6</sub>**; L = citramalate [29, 30], **Ib<sub>7</sub>**; L = methyl-iminodiacetate [30], **Ib<sub>2</sub>**; L = nitrilo-triacetate [31], **Ib<sub>3</sub>**; L = thio-diglycolate [35], **Ib<sub>4</sub>**; ( $n = 2, 3$ );  $[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{thiolactate})]_2^{4+}$  [35–37], **II**;  $[(\text{MoFe}_3\text{S}_4\text{Cl}_4)_2(\mu\text{-oxalate})]^{4+}$  [29, 30], **III**), (b) the singly bridged double cubane [19, 20, 24, 27],  $\{[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4\text{Cl}_3]_2(\mu\text{-NH}_2\text{NH}_2)\}^{4+}$  clusters, SBDC, **IV**,

and (c) the doubly bridged double-cubanes [19, 20, 24, 26, 28],  $\{[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4\text{Cl}_2]_2(\mu\text{-L})(\mu\text{-S})\}^{4+}$ , DBDC, **V** ( $\mu\text{-L} = \text{NH}_2\text{NH}_2$  and  $\text{CN}^-$ ), (Fig. 2).

Thus far, various clusters with the  $\text{MFe}_3\text{S}_4$  core have been found to be effective in the catalytic reductions of hydrazines, (M = Mo, V) acetylene, nitrite, hydroxylamine and protons at ambient temperature in  $\text{CH}_3\text{CN}$  or, in the case of acetylene, in DMF solution. In these reductions, cobaltocene is employed as an outer sphere electron reductant and lutidine hydrochloride as a source of protons. In the reduction of hydrazine, the anionic cluster catalysts precipitate out of solution as the concentrations of the cationic reaction byproducts  $[\text{NH}_4^+, \text{Co}(\text{Cp})_2^+]$  increase. As a result, the catalytic reactions slow down and at times stop prior to quantitative substrate reduction to  $\text{NH}_3$ . In the catalysis of acetylene reduction, which was studied in DMF solution, the onset of catalyst precipitation is slower, and the resilience of the catalyst during the reaction can be studied. The presence of the catalyst  $\{[(\text{L})\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{DMF})]\}$  has been monitored quantitatively during the course of the catalytic reaction by periodic integration of the characteristic  $S = 3/2$  EPR signal. Within the error of integration, the intensity of the signal does not change, and at the end of the reaction the catalyst can be recovered. The recovered cluster shows the same characteristic EPR spectrum and can be re-used for continuing the reaction.

The results of the catalytic studies will be summarized with reference to the questions raised previously regarding the function of nitrogenase.

### Is there direct involvement of the Mo (or V) atom in catalysis?

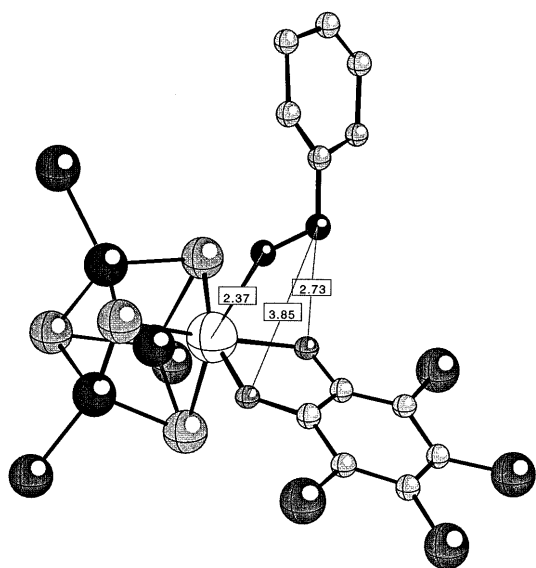
The affirmative response to this question is based on various results and observations:

(1) The  $[\text{VFe}_3\text{S}_4]^{2+}$  and  $[\text{MoFe}_3\text{S}_4]^{3+}$  cores [38–40], are effective catalysts [38–40] in the reduction of hydrazine to ammonia (Table 1). In the catalytic reduction of hydrazine by the  $\text{MFe}_3\text{S}_4$  cubanes, the heteroatoms (Mo, V) rather than the Fe are directly involved in the catalytic process.

The initial encounter of the substrate with the heterometal in the cubane clusters is suggested by the results of synthetic and crystallographic studies that demonstrate coordination of substituted hydrazines to the Mo or V atom in the  $\text{MFe}_3\text{S}_4$  cubanes (Fig. 3) [38, 41] (also S.M. Malinak and S. Patton, work in progress).

In these studies, the labile  $\text{CH}_3\text{CN}$  ligand in the  $[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{CH}_3\text{CN})]^{2-}$  cubane and the DMF ligand in  $[(\text{DMF})_{3-n}(\text{L})\text{VFe}_3\text{S}_4\text{Cl}_3]^-$ , ( $\text{L}=\text{PEt}_3$ ,  $n=1$ ,  $\lambda=\text{bpy}$ ,  $n=2$ ),  $\text{Fe}_3\text{S}_4\text{Cl}_3]^-$  have been replaced by hydrazines. Attempts to introduce hydrazines as terminally coordinated ligands in the  $\text{Fe}_4\text{S}_4$  cubanes have not been successful, and the reduction of hydrazines is not catalyzed by the  $\text{Fe}_4\text{S}_4$  cubanes.

The  $[\text{VFe}_3\text{S}_4]^{2+}$  clusters with identical terminal ligands on the Fe atoms and similar reduction potentials show a decrease in the relative rate of hydrazine reduction, with a decrease in the number of labile sites at the V atom (Table 1). The same clusters with identical terminal ligands on the V atom and similar reduction potentials but different terminal ligands on the Fe atoms



**Fig. 3** The structure of the  $[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4(\text{Cl})_3(\text{NH}_2\text{NHC}_6\text{H}_5)]^{2-}$  cubane

**Table 1** Production of  $\text{NH}_3^a$  by the catalytic reduction of  $\text{N}_2\text{H}_4$  by clusters that contain  $\text{MoFe}_3\text{S}_4$  or  $\text{VFe}_3\text{S}_4$  units in the presence of  $\text{CO}(\text{Cp})_2$  and  $\text{Lut}:\text{HCl}$

Catalyst	$\text{NH}_3$ yield <sup>b</sup> , equivalent <sup>c,d</sup> (% conversion)				
	$[\text{N}_2\text{H}_4]$ [catalyst]	1/2 h	1 h	2 h	$\text{NH}_3$ max <sup>e</sup>
<b>Ia<sup>f</sup></b>	10	13.2(2) (66)	14.6(2) (73)	19.2(2) (96)	20
<b>Ib<sub>5</sub><sup>f</sup></b>	10	20.0(2) (100)	20.0(2) (100)	20.0(2) (100)	20
<b>IV<sup>f</sup></b>	1/2	0.6(2) (29)	0.8(2) (40)	1.0(2) (50)	2
<b>V</b>	1:2	trace	trace	trace	2
<b>VII<sup>f</sup></b>	10 <sup>h,i</sup>	4.5(1) (45)	5.8(1) (58)	10.0(1) (100)	10
<b>VIII<sup>j</sup></b>	10	15.2(3) (76)	17.8(3) (89)	20.0(3) (100)	20
<b>IX<sup>k</sup></b>	10	5.6(2) (28)	7.6(2) (38)	9.4(2) (47)	20
<b>X<sup>l</sup></b>	10	N.A. <sup>g</sup>	2.2(2) (11)	3.4(2) (17)	20

<sup>a</sup> Reactions were carried out in  $\text{CH}_3\text{CN}$  at ambient temperature under  $\text{N}_2$  using the indicated catalyst and various amounts of  $\text{N}_2\text{H}_4$  as shown in the second column. The initial concentrations of  $\text{N}_2\text{H}_4$ ,  $\text{CO}(\text{Cp})_2$  and  $\text{Lut}:\text{HCl}$  were  $1.25 \times 10^{-3}$  M,  $2.50 \times 10^{-3}$  M and  $5.00 \times 10^{-4}$  M, respectively. The concentration of the catalyst was scaled accordingly to achieve the indicated ratios. A table with results obtained with more concentrated solutions and data showing the catalytic but much slower  $\text{N}_2\text{H}_4$  disproportionation reaction has been deposited with the supplementary material

<sup>b</sup> The  $\text{NH}_3$  was quantified by the indophenol method [63] and the  $\text{N}_2\text{H}_4$  was quantified with *p*-(dimethylamino)benzaldehyde [64]. The protocol used in sample collection and treatment prior to analysis is identical to the one described in ref. [35]

<sup>c</sup> The average of  $n$  independent experiments is reported where  $n$  is the number in parentheses

<sup>d</sup> Analyses for  $\text{N}_2\text{H}_4$  were carried out randomly for experiments where the yield of  $\text{NH}_3$  indicated less than 100% conversion. In general there was a nitrogen atom balance to  $100 \pm 5\%$ . The results are included in a table deposited with the supplementary material

<sup>e</sup> Maximum yield of  $\text{NH}_3$  possible

<sup>f</sup> Abbreviations for the compounds are: **Ia**  $[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4(\text{Cl})_3(\text{CH}_3\text{CN})]^{2-}$ , **Ib<sub>5</sub>**  $[(\text{Citr})\text{MoFe}_3\text{S}_4(\text{Cl})_3]^{3-}$ , **IV**  $[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4(\text{Cl})_3(\mu_2\text{-N}_2\text{H}_4)]^{4-}$ , **V**  $[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4(\text{Cl})_2(\mu_2\text{-N}_2\text{H}_4)(\mu_2\text{-S})]^{4-}$ ; and **VII**:  $[(\text{Cl}_4\text{-cat})\text{MoFe}_3\text{S}_4(\text{Cl})_3(\text{NH}_2\text{NHPh})]^{2-}$

<sup>g</sup> N.A.

<sup>h</sup> Phenylhydrazine to cubane ratio

<sup>i</sup> Aniline was detected by a GC-mass spectroscopic measurement

<sup>j</sup>  $[(\text{DMF})_3\text{VFe}_3\text{S}_4\text{Cl}_3]^-$

<sup>k</sup>  $[(\text{DMF})_2(\text{PEt}_3)\text{VFe}_3\text{S}_4\text{Cl}_3]^-$

<sup>l</sup>  $[(\text{DMF})(\text{Bipy})\text{VFe}_3\text{S}_4\text{Cl}_3]^-$

showed little change in the relative rates of hydrazine reduction. These results taken together strongly suggest the direct involvement of the V atom in substrate activation and reduction. In the alternative V-nitrogenase [42–50], which contains a V/Fe/S cofactor structurally similar to the Mo/Fe/S center, reduction of  $\text{N}_2$  to ammonia also occurs but is not as effective as that ob-

served with the Mo-nitrogenase. Furthermore, it has been found that hydrazine is a product of N<sub>2</sub> reduction by the isolated V-nitrogenase [42–50].

(2) The [MoFe<sub>3</sub>S<sub>4</sub>]<sup>3+</sup> cores are effective catalysts in the reduction of acetylene to ethylene and small amounts of ethane [39] (Table 2).

Catalytic reductions were carried out at 20 °C using **1a** as the catalyst and cobaltocene and 2,6-lutidine hydrochloride, Lut·HCl, as sources of electrons and protons, respectively. The initial-rate method, in which [catalyst] << [substrate], was used to obtain reaction velocities,  $v_o$  (M/min), at less than 5% substrate consumption. The data obey saturation kinetics, as found for enzyme catalysis, and reaction rates remain constant for C<sub>2</sub>H<sub>2</sub>:cubane ratios <30:1, indicating zero-order substrate dependence. A double reciprocal plot,  $v_o^{-1}$  vs [C<sub>2</sub>H<sub>2</sub>]<sup>-1</sup>, is linear at optimum substrate concentration, and  $K_m = 17.9$  mM and  $V_{max} = 1.1 \times 10^{-4}$  M/min are calculated from this. Catalyst **1a** reduces acetylene with a turnover number of 0.11 mol C<sub>2</sub>H<sub>2</sub> mol<sup>-1</sup> catalyst min<sup>-1</sup>, which is approximately 0.08% of the enzymatic rate of acetylene reduction by nitrogenase.

The reaction also shows first-order dependence on proton concentration and zero-order dependence on reductant concentration. A study of the reaction at five temperatures indicates a moderate activation energy [ $E_{act} = 9(1)$  kcal mol<sup>-1</sup>] but a large entropy of activation [ $\Delta S^\ddagger = -32(2)$  cal K<sup>-1</sup> mol<sup>-1</sup>], which extrapolates to a significant Gibbs free energy of activation [ $\Delta G^\ddagger = 19(1)$ ]. In this reaction also, the Mo center appears to be the premier exponent of substrate reduction, although the Fe sites have also been implicated in the reaction process, albeit at a markedly reduced rate. Both metal sites on the [(Cl<sub>4</sub>-cat)MoFe<sub>3</sub>S<sub>4</sub>Cl<sub>3</sub>(DMF)]<sup>2-</sup> cubane catalyst may be inhibited toward acetylene re-

**Table 2** Initial velocities ( $v_o$ ) for the reduction of acetylene to ethylene catalyzed by various cubanes<sup>a</sup>

Cubane catalyst	$v_o$ (M/min) <sup>b</sup> ( $\times 10^5$ )	$V_o$ normalized to cubane <b>I</b>
<b>1a</b>	6.6(3)	1.0
[( $\eta^3$ -citrate)MoFe <sub>3</sub> S <sub>4</sub> Cl <sub>3</sub> ] <sup>3-</sup>	6.4(2)	0.97
[Fe <sub>4</sub> S <sub>4</sub> Cl <sub>4</sub> ] <sup>2-</sup>	1.3(3)	0.20
[(Cl <sub>4</sub> -cat)MoFe <sub>3</sub> S <sub>4</sub> Cl <sub>3</sub> (CN)] <sup>3-</sup>	1.6(1)	0.24
[(CO) <sub>3</sub> MoFe <sub>3</sub> S <sub>4</sub> Cl <sub>3</sub> ] <sup>3-</sup>	1.5(2)	0.23
[(CO) <sub>3</sub> MoFe <sub>3</sub> S <sub>4</sub> R <sub>3</sub> ] <sup>3-</sup> R = <i>p</i> -Cl-SPh	1.1(3)	0.17
<b>1a</b> + PEt <sub>3</sub> (1:2) <sup>c</sup>	1.3(2)	0.20
Blank (no catalyst) <sup>d</sup>	0.011	0.002

<sup>a</sup> Experiments are performed in DMF at 20 °C using CoCp<sub>2</sub> and Lut·HCl as sources of electrons and protons, respectively. The initial substrate:catalyst ratio used is 20:1

<sup>b</sup> Initial velocity,  $v_o$ , is obtained as the slope of [C<sub>2</sub>H<sub>4</sub>] (M) vs t (min), which obeys a straight-line relationship during the initial stages of the reaction

<sup>c</sup> Acetylene reduction performed using cubane **I** as the catalyst in the presence of PEt<sub>3</sub> such that Mo:PEt<sub>3</sub> = 1:2

<sup>d</sup> Typical acetylene reduction conditions except for the absence of catalyst

**Table 3** Proton reduction/hydrogen evolution catalyzed by [(Cl<sub>4</sub>-cat)MoFe<sub>3</sub>S<sub>4</sub>Cl<sub>3</sub>(CH<sub>3</sub>CN)]<sup>2-</sup> (**1a**) CH<sub>3</sub>CN in the presence of Co(Cp)<sub>2</sub> and Lut·HCl as sources of e<sup>-</sup> and H<sup>+</sup> respectively at a Co(Cp)<sub>2</sub>/Lut·HCl/Catalyst ratio of 40/40/1

Catalyst	NH <sub>2</sub> NHR	RNHNH <sub>2</sub> /catalyst	NH <sub>3</sub>	H <sub>2</sub>
no	no	0	0	1.0(5%)
<b>(1a)</b>	no	0	0	~20(100%)
<b>(1a)</b>	H	10	19(95%)	1.0(5%)
<b>(1a)</b>	CH <sub>3</sub>	10	10(50%)	10.0(50%)

duction by the use of PEt<sub>3</sub> or a saturating CO atmosphere. It has also been demonstrated that only *one* Mo coordination site on the catalyst need be vacant to ensure significant catalysis. Notwithstanding overall reaction rates and turnover numbers orders of magnitude slower than with the nitrogenase system, the present results suggest varying affinities of the Mo and Fe sites on the catalyst for the acetylene substrate. The alkene product from reduction of C<sub>2</sub>D<sub>2</sub> has been identified by gas IR as the *cis* stereoisomer.

(3) The [MoFe<sub>3</sub>S<sub>4</sub>]<sup>3+</sup> cores are effective catalysts in the reduction of H<sup>+</sup> to H<sub>2</sub>.

This reaction proceeds with first-order dependence on proton concentration and zero-order dependence on reductant concentration. It is inhibited in the presence of hydrazine (Table 3).

This inhibition suggests that the reduction of protons needs the availability of a coordination site on or near the Mo atom if the latter is indeed the site of hydrazine reduction. The available data on the catalytic reduction of hydrazines acetylene and protons by the MFe<sub>3</sub>S<sub>4</sub> cubanes thus conclusively show the direct involvement of the heteroatom (Mo, V) in the process

### What is the function of the homocitrate ligand?

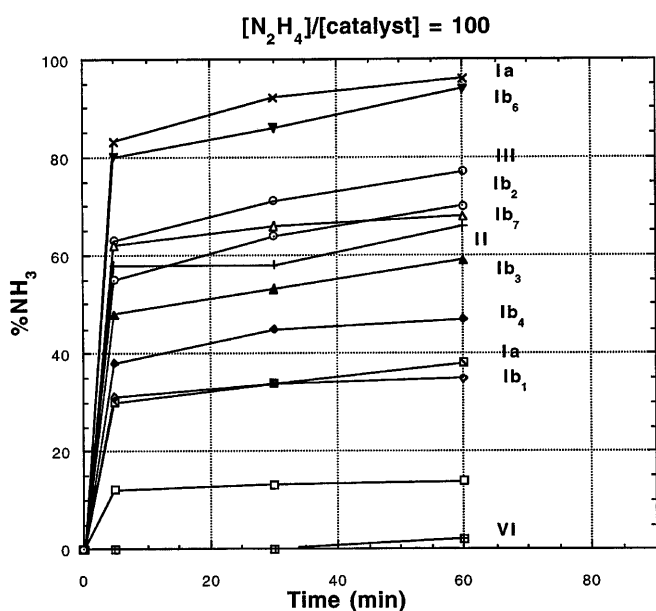
The [MoFe<sub>3</sub>S<sub>4</sub>]<sup>3+</sup> cores with Mo-bound carboxylate ligands catalytically reduce N<sub>2</sub>H<sub>4</sub> to NH<sub>3</sub> at higher rates than those obtained with the [(Cl<sub>4</sub>-cat-)MoFe<sub>3</sub>S<sub>4</sub>Cl<sub>3</sub>(CH<sub>3</sub>CN)]<sup>2-</sup> cluster (Fig. 4, Table 4). The polycarboxylate ligands, which appear to coordinatively saturate the Mo coordination sphere, promote rather than hinder the catalytic reduction of hydrazine to ammonia (Table 4). This enhancement of the rate of substrate reduction we attribute to the ability of the coordinated carboxylate groups to both liberate a coordination site under acidic conditions and concomitantly to serve as “built-in” local H<sup>+</sup> delivery agents (Fig. 5) (see also the commentary by Pickett [33]).

### How important is bi(multi)-metallic activation of substrates in the catalytic process, and is proton transfer coupled with the reduction process?

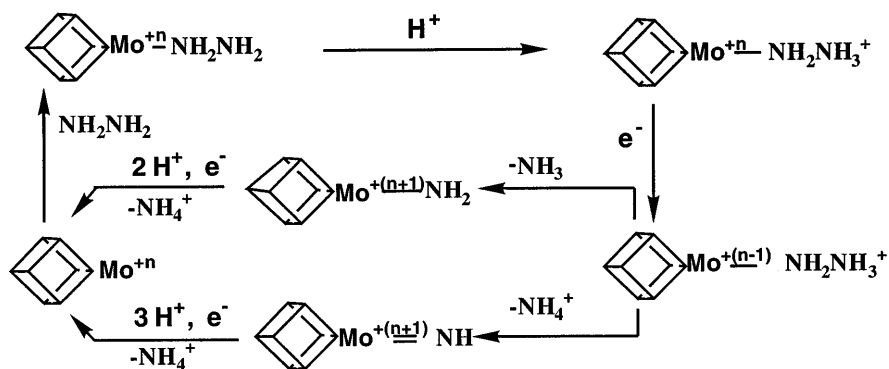
Qualitative comparative studies show that the rates of ammonia formation with **IV** (Fig. 2) as a catalyst were

**Table 4** Production (%) of  $\text{NH}_3$  from the catalytic reduction of  $\text{NH}_2\text{NH}_2$  by various  $\text{MoFe}_3\text{-cubanes}$  using  $\text{Co}(\text{Cp})_2$  as the reducing agent and  $\text{Lut}\cdot\text{HCl}$  as the proton source. The  $[\text{NH}_2\text{NH}_2]:[\text{catalyst}]$  ratio was 100:1

Cluster catalyst	No. of trials	5 min	30 min	1 h	12 h
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{Cl}_4\text{-cat})(\text{CH}_3\text{CN})]^{2-}$ ( <b>Ia</b> )	3	30	34	38	61
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{Cl}_4\text{-cat})(\text{im})]^{2-}$ ( <b>Ib<sub>1</sub></b> )	1	31	34	35	48
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{mida})]^{2-}$ ( <b>Ib<sub>2</sub></b> )	2	55	64	70	79
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{Hnta})]^{2-}$ ( <b>Ib<sub>3</sub></b> )	3	48	53	59	65
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{tdga})]^{2-}$ ( <b>Ib<sub>4</sub></b> )	3	38	45	47	65
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{Hcit})]^{3-}$ ( <b>Ib<sub>5</sub></b> )	4	83	92	96	98
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{H}_2\text{cit})]^{2-}$ ( <b>Ib<sub>6</sub></b> )	3	80	86	94	95
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{Hcmal})]^{2-}$ ( <b>Ib<sub>7</sub></b> )	3	62	66	68	78
$[\text{MoFe}_3\text{S}_4\text{Cl}_3(\text{tla})]^{2-}$ ( <b>II</b> )	2	58	58	66	71
$[(\text{MoFe}_3\text{S}_4\text{Cl}_4)_2(\mu\text{-ox})]^{4-}$ ( <b>III</b> )	2	63	71	77	85
$(\text{Fe}_4\text{S}_4\text{Cl}_4)^{2-}$ ( <b>VI</b> )	3	0	0	0	7

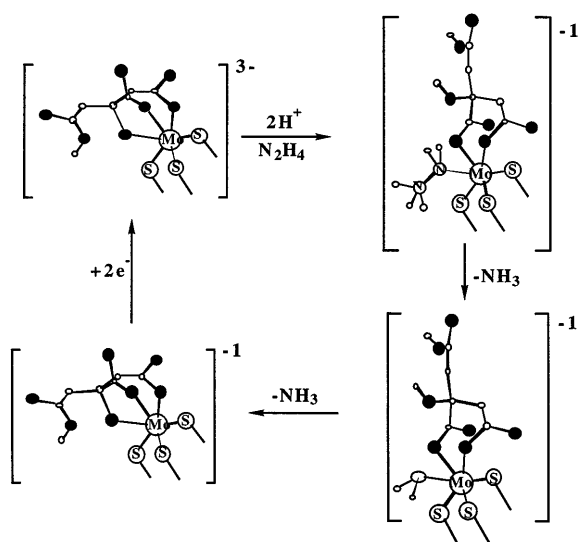
**Fig. 4** The catalytic reduction of hydrazine by the  $[(\text{L})(\text{MoFe}_3\text{S}_4(\text{Cl})_3)]^{n-}$  cubanes (L = various polycarboxylate ligands). See Table 4 for the numbering scheme

much slower than those observed with **I**, while **V** (Fig. 2) was totally ineffective as a catalyst. These results indicate that the hydrazine molecule is activated by coordination to only one  $\text{MoFe}_3\text{S}_4$  cubane and the addition of an additional equivalent of cubane (which is

**Fig. 5** A possible carboxylate protonation/proton-delivery mechanism that accounts for the facile catalytic reduction of hydrazine on what appears to be a coordinatively saturated Mo atom in the  $[(\text{L})(\text{MoFe}_3\text{S}_4(\text{Cl})_3)]^{n-}$  cubanes (L = a multicarboxylate ligand)

known to give **IV**) inhibits the reduction. Moreover, the results suggest that the availability of an uncoordinated  $\text{NH}_2$  group (and the lone pair of electrons needed for protonation) is essential for the reduction of  $\text{N}_2\text{H}_4$  to ammonia. Additional evidence that the interaction of hydrazine with a single cubane is necessary and sufficient for catalytic reduction is available in studies with phenylhydrazine,  $\text{PhHNNH}_2$ . The replacement of the  $\text{CH}_3\text{CN}$  molecule in **Ia** by  $\text{PhHNNH}_2$  occurs readily. The product (Fig. 3), which for steric reasons does not interact further with another cubane molecule to form a singly bridged double cubane (Fig. 2A), undergoes catalytic reduction to give aniline and ammonia.

That protonation is an important step that precedes reduction is supported by the synthesis and reactivity of the  $\text{I-N}_2\text{H}_5^+$  cluster. The latter has been isolated and characterized, and upon addition of  $\text{Co}(\text{Cp})_2$  (in the absence of a proton source) affords ammonia. Further support for the importance of an initial protonation step is given by observations in the catalytic reduction of hydrazine by the  $\text{VFe}_3\text{S}_4$  cubanes. Thus, in the presence of  $\text{Et}_3\text{NH}^+$ , an acid that is not able to protonate hydrazine ( $\text{Et}_3\text{N}\cdot\text{HCl}$ ,  $\text{p}K_a = 18.3$  in  $\text{CH}_3\text{CN}$ ; for  $\text{N}_2\text{H}_5^+$ ,  $\text{p}K_a = 16.6$  in  $\text{CH}_3\text{CN}$ ; for  $\text{NH}_4^+$ ,  $\text{p}K_a = 16.5$  in  $\text{CH}_3\text{CN}$ ), the reduction of hydrazine to  $\text{NH}_3$  does not occur after a 3-h period, as indicated by ammonia analysis. The reduction of hydrazine of course proceeds readily with lutidinium ion as a source of protons ( $\text{p}K_a$  of  $2,6\text{-Lut}\cdot\text{HCl}$  in  $\text{CH}_3\text{CN}$  is approximately 14.1.)



**Fig. 6** A proposed minimal pathway for the reduction of hydrazine by the  $[(L)(\text{MoFe}_3\text{S}_4(\text{Cl})_3)]^{n-}$  cubanes

This observation, coupled with the fact that protonation of the coordinated hydrazine in the  $\text{VFe}_3\text{S}_4$  cubane causes a 300-mV anodic shift in reduction potential, indicates that in the presence of protonated substrate, the V cubane can be reduced readily by  $\text{Co}(\text{Cp})_2$ . It appears certain, therefore, that, in hydrazine reduction, binding and protonation of the substrate occurs prior to the reduction of the cubane.

A simple proposed pathway (Fig. 6) for the catalytic reduction of  $\text{N}_2\text{H}_4$  to ammonia by the  $\text{Fe}_3\text{MoS}_4$  cluster involves an initial protonation step prior to reduction of the coordinated hydrazine molecule.

### Is the Mo (or V) atom directly involved in the activation (reduction) of $\text{N}_2$ , and how does the reduction take place?

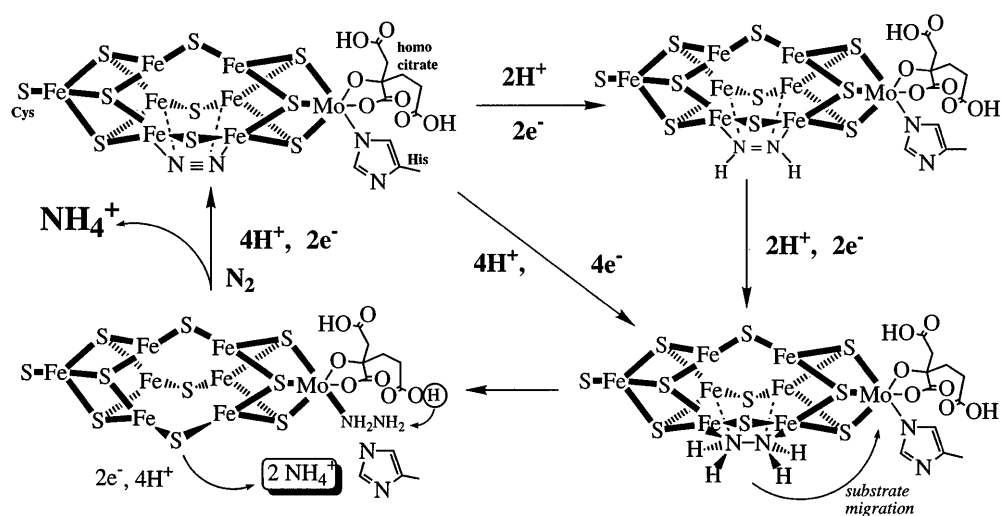
The results of our studies on the catalytic function of the  $\text{MFe}_3\text{S}_4$  cubanes clearly indicate that these mole-

cules do not catalyze the reaction of  $\text{N}_2$ . The fact that the isolated Fe/Mo cofactor also is incompetent in the reduction of  $\text{N}_2$  [51] suggests that the protein environment is of paramount importance in the binding and activation of  $\text{N}_2$  at ambient conditions. Whether the Mo (or V) atoms in the FeMo-co (or FeVa-co) centers are involved in the initial coordination and activation of  $\text{N}_2$  remains a possible albeit improbable event.

Inconclusive evidence that may implicate the Mo atom as the site of substrate activation/inhibition in the FeMo-co [51] includes (a) the profound effects in dinitrogen reduction brought about by skeletal alterations in the Mo-bound homocitrate ligand [52–61] and (b) extended X-ray absorption fine structure (EXAFS) analyses that show that  $\text{CN}^-$ , a nitrogenase inhibitor, binds to the Mo site of the nitrogenase cofactor [62].

On the basis of all the available data, we consider it very likely that, as suggested by Dance and others [2–9], the  $\text{Fe}_3(\mu\text{-S})_3\text{Fe}_3$  center is the site where the initial two- or four-electron reduction takes place. If this is indeed the case, the question regarding the role of the heterometal (Mo, V) remains unanswered. The sequence of steps as the  $\text{N}_2$  molecule eventually undergoes reduction to ammonia generates (or may generate) intermediates with different electronic structures. Indeed, the whole process may involve the reduction of ligands such as  $\text{N}_2$  and  $\text{N}_2\text{H}_4$  with drastically different coordination properties. It is quite likely that the site in the Fe/Mo/S center that readily binds and activates  $\text{N}_2$  has little affinity for the reduction products/intermediates and particularly hydrazine. At some stage therefore it is possible that other sites within the Fe/Mo/S aggregate (Mo?) may assume the role of continuing the reduction process to its conclusion. The results of our studies support a pathway where the reduction of hydrazine (a nitrogenase intermediate?) can take place on the heteroatom site. Similarly “easy” substrates such as acetylene may undergo reduction at the same site (Fig. 7). The ready reduction of acetylene, but not of dinitrogen, by the isolated cofactor supports the idea of a multisite reactive center.

**Fig. 7** A proposed multisite mechanism for the reduction of  $\text{N}_2$  by nitrogenase



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## References

1. Thorneley RNF, Lowe DJ (1996) *JBIC* 1:576–580
2. Kim J, Rees DC (1992) *Science* 257:1677
3. Kim J, Rees DC (1992) *Nature* 360:553
4. Kim J, Woo D, Rees DC (1993) *Biochemistry* 32:7104
5. Chan MK, Kim J, Rees DC (1993) *Science* 260:792
6. Kim J, Rees DC (1994) *Biochemistry* 33:389
7. Rees DC, Chan MK, Kim J (1993) In: Sykes AG (ed.); *Advances in inorganic chemistry*, vol 40. Academic Press, New York
8. Kim J, Georgiadis MM, Chan MK, Woo D, Komiya K, Rees DCJ (1993) *J Inorg Biochem* 51:2
9. Reference 2, p 170
10. Bolin JT, Ronco AE, Morgan TV, Mortenson LE, Xuong NH (1993) *Proc Natl Acad Sci USA* 90:1078
11. Bolin JT, Campobasso N, Muchmore SW, Minor W, Morgan TV, Mortenson LE (1993) In: Palacios P, Moura J, Newton WW (eds). *New horizons in nitrogen fixation*. Kluwer, Dordrecht
12. Dean DR, Bolin JT, Zheng LJ (1993) *J Bacteriol* 175:6337
13. Mortenson LE, Seefeldt LC, Morgan, TV, Bolin JT (1993) *Adv Enzymol Relat Areas Mol Biol* 67:299
14. Bolin JT, Campobasso N, Muchmore SW, Mortenson LE, Morgan TV (1993) *J Inorg Biochem* 51:356
15. Reference 2, p 186
16. Holm RH, Simhon ED (1985) In: Spiro TG (ed). *Molybdenum enzymes*. Wiley-Interscience, New York, p 1 (and references therein)
17. Holm RH (1992) In: Sykes AG (ed); *Advances in inorganic chemistry*, vol 38. Academic Press, New York, p 1
18. Holm RH (1995) *Pure and Appl Chem* 67:217
19. Coucouvanis D (1991) *Acc Chem Res* 24:1
20. Coucouvanis D In: Eichorn GL, Marzilli LG (eds) *Models in inorganic chemistry*. (Advances in inorganic biochemistry, vol 9) Elsevier, New York, p 75
21. Coucouvanis D (1993) In: Stiefel EI, Coucouvanis D, Newton WE (eds) *Molybdenum enzymes, cofactors and model systems* (ACS Symposium Series 535) American Chemical Society, Washington DC, p 304
22. Liu Q, Huang L, Liu H, Lei X, Wu D, Kang B, Lu J (1990) *Inorg Chem* 29:4131
23. Eldredge PA, Bose KS, Barber DE, Bryan RF, Sinn E, Reingold A, Averill BA (1991) *Inorg Chem* 30:2365
24. Coucouvanis D (1994) In: King BR (ed) *Encyclopedia of inorganic chemistry*, vol 5, Wiley, New York, p 2557
25. Nordlander E, Lee SC, Cen Wei, Wu ZY, Natoli CR, Di Cicco A, Filliponi A, Hedman B, Hodgson KO, Holm RH (1993) *J Am Chem Soc* 115:5549–5558
26. Challen PR, Koo SM, Kim CG, Dunham WR, Coucouvanis DJ (1990) *J Am Chem Soc* 112:8606
27. Mosier PE, Kim CG, Coucouvanis D (1993) *Inorg Chem* 32:3620
28. Challen PR (1990) PhD thesis, The University of Michigan
29. Coucouvanis D, Demadis KD, Kim C-G, Dunham RW, Kampf JW (1993) *J Am Chem Soc* 115:3344
30. Demadis KD, Coucouvanis D (1995) *Inorg Chem* 34:436
31. Demadis KD, Malinak SM, Coucouvanis D (1996) *Inorg Chem* 34:4038
32. Dance I (1996) *JBIC* 1:581–586
33. Pickett CJ (1996) *JBIC* 1:601–606
34. Palermo RE, Singh R, Bashkin JK, Holm RH (1984) *J Am Chem Soc* 106:2600
35. Demadis KD, Coucouvanis D, Demadis KD, Coucouvanis D (1995) *Inorg Chem* 34:3658
36. Demadis KD, Coucouvanis D (1994) *Inorg Chem* 33:4195
37. Demadis KD, Coucouvanis D (1995) *Inorg Chem* 34:3658
38. Coucouvanis D, Mosier PE, Demadis KD, Patton S, Malinak SM, Kim CG, Tyson MAJ (1993) *J Am Chem Soc* 115:12193
39. Laughlin LJ, Coucouvanis DJ (1995) *J Am Chem Soc* 117:3118
40. Malinak SM, Demadis KD, Coucouvanis DJ (1995) *J Am Chem Soc* 117:3126
41. Mosier PE (1995) PhD thesis, The University of Michigan
42. Robson RL, Eady RR, Richardson TH, Miller RW, Hawkins M, Postgate JR (1986) *Nature* 332:388
43. Hales BJ, Case EE, Morningstar JE, Dzeda MF, Mauterer LA (1986) *Biochemistry* 25:7251
44. Hales BJ, Langosch DJ, Case EE (1986) *J Biol Chem* 261:15301
45. Eady RR, Robson RL, Richardson TH, Miller RW, Hawkins M (1987) *Biochem J* 244:197
46. Arber JM, Dobson BR, Eady RR, Stevens P, Hasnain SS, Garner CD, Smith BE (1987) *Nature* 325:372
47. Smith BE, Eady RR, Lowe DJ, Gormal C (1988) *Biochem J* 250:299
48. Eady RR (1991) In: Sykes AG (ed) *Advances in inorganic chemistry*, vol 36; Academic Press, New York, p 77
49. Eady RR (1989) *Polyhedron* 8:1696
50. Hales BJ (1990) In: Eichorn GL, Marzilli LG (eds) *Advances in inorganic biochemistry*, vol 8; Elsevier, New York p 165 (and references therein)
51. Burgess BK (1990) *Chem Rev* 90:1377
52. Hoover TR, Shah VK, Roberts GP, Ludden PWJ (1986) *Bacteriol* 167:999
53. Hoover TR, Robertson AD, Cerny RL, Hayes RN, Imperial J, Shah VK, Ludden PW (1987) *Nature* 329:855
54. Hoover TR, Imperial J, Ludden PW, Shah VK (1989) *Biochemistry* 28:2768
55. Hoover TR, Imperial J, Liang J, Ludden PW, Shah VK (1988) *Biochemistry* 27:3647
56. Imperial J, Hoover TR, Madden MS, Ludden PW, Shah VK (1989) *Biochemistry* 28:7796
57. Madden MS, Kindon ND, Ludden PW, Shah VK (1990) *Proc Natl Acad Sci USA* 87:6517
58. Liang J, Madden MS, Shah VK, Burris RH (1990) *Biochemistry* 29:8577
59. Hoover TR, Imperial J, Ludden PW, Shah VKJ (1988) *Bacteriol* 170:1978
60. Madden MS, Paustian TD, Ludden PW, Shah VK (1991) *J Bacteriol* 173:5403
61. Reference 1a, p 196
62. Liu HI, Filipponi A, Gavini N, Burgess BK, Hedman B, Di Cicco A, Natoli CR, Hodgson KO (1994) *J Am Chem Soc* 116:2418
63. Chaney AL, Marbach EP (1962) *Clin Chem* (Winston-Salem NC) 8:30
64. Watt GW, Chrisp JD (1952) *Anal Chem* 25:2006