Catalytic and stoichiometric multielectron reduction of hydrazine to ammonia and acetylene to ethylene with clusters that contain the MFe_3S_4 cores (M = Mo, V). Relevance to the function of nitrogenase

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Abstract

A functional model for nitrogenase is currently sought in our laboratory in reactivity studies using various single cubane clusters that possess the $[MFe_3S_4]^{n+}$ core, (M = Mo, $n = 3$; M = V, $n = 2$). These clusters are used as catalysts for the reduction of enzymatically relevant substrates. Substrates such as hydrazine and acetylene are catalytically reduced by $(NEt_4)_2[(Cl_4$-cat)(CH_3CN)$MoFe_3S_4Cl_3$], I, to ammonia and ethylene respectively, in the presence of added protons and reducing equivalents. Hydrazine also is catalytically reduced by the $(NEt_4)[(DMF)_3VFe_3S_4Cl_3]$ cubane under similar conditions. Gas chromatography was employed to monitor the reduction of acetylene to ethylene and a trace of ethane. Catalysis in excess of 100 turnovers (for hydrazine reduction) and in excess of 15 turnovers (in acetylene reduction) has been demonstrated over a period of 24 h. A study of the acetylene reduction reveals saturation kinetics to be operating at high substrate concentrations. A variable temperature kinetic study of acetylene reduction shows a moderate activation energy ($E_{act} = 9(1)$ kcal mol^{-1}) but a large entropy of activation ($\Delta S^{\ddagger} = -32(2)$ cal K^{-1} mol^{-1}) which extrapolates to a significant Gibbs free energy ($\Delta G^{\ddagger} = 19(1)$ kcal mol^{-1}). These results are consistent with an ordered transition state. Considerable evidence has been amassed which directly implicates the Mo and V atoms as the primary catalytic sites. Replacement of the Mo or V bound, kinetically labile, solvent molecules with non-labile ligands acts to suppress the observed rates of reaction. The Fe sites on I are totally inactive in the reduction of hydrazine, however they have been found to effect acetylene reduction albeit at a markedly reduced rate compared to the Mo site. Catalyst integrity has also been demonstrated by a variety of techniques, primarily EPR spectroscopy which identifies the characteristic $S = 3/2$ signals of the Mo and V cubanes after at least 18 h reaction time.

1. Introduction

The Fe/Mo/S site in the nitrogenase enzymes is an extraordinary site that catalyzes the biological reduction of dinitrogen to ammonia under ambient temperature and pressure. The nitrogenase enzymes contain two proteins of which the

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Fe protein (M_r, ~ 60,000 D) binds two molecules of MgATP and serves as a specific activator and source of electrons for the FeMo protein (M_r, 220,000 D) where N_2 reduction takes place [1].

In recent single crystal X-ray structure determinations of the Fe–Mo protein of nitrogenase from *Clostridium pasteurianum* [2,3] and *Azotobacter vinelandii* [3], the structure of the Fe/Mo/S site has been revealed to near atomic
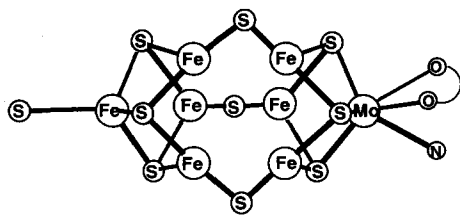
Fig. 1. The structure of the Fe/Mo/S center in nitrogenase (Refs. [2,3]).

resolution. The Fe/Mo/S cluster contains two cuboidal subunits, Fe_4S_3 and $MoFe_3S_3$, bridged by three S^{2-} ions. The cluster is anchored to the protein matrix by a cysteinyl residue coordinated to an Fe atom at one end of the cluster and by an imidazole group from a histidine residue that is bound to the Mo atom at the other end of the cluster, Fig. 1. The Mo atom also is coordinated by a homocitrate molecule that serves as a bidentate chelate. Unusual structural features include the unprecedented trigonal planar coordination geometry for the six μ-S-bridged iron atoms, and the unusually short Fe–Fe distances across the two subunits (2.5–2.6 Å). The location of the Mo atom at the periphery of the $MoFe_7S_{8-9}$ cluster was unexpected and was interpreted as an indication that the Mo atom may not be directly involved in N_2 fixation.

The clearly defined structural features of the nitrogenase Fe/Mo/S center have not led to a clear understanding of the mode of substrate activation and reduction. Indeed many questions regarding the catalytic function of nitrogenase remain unanswered. One of these questions concerns the role of the Mo atom (and of the V atom in alternate nitrogenase) in the function of the 'M' centers in nitrogenase and may be explored by reactivity studies on appropriate Fe/M/S model complexes. Numerous models for the Fe/Mo/S center in nitrogenase have been proposed over the years [4,5] and in each case their constitution and structure was based on the information available at the time of their intellectual conception.

Whereas the Fe/M/S synthetic analog clusters until now have suffered from the drawback that they do not mediate chemistry related to dinitrogen reduction [6], various other complexes of

molybdenum and tungsten undergo reactions that are of limited *direct* biological significance but are important from a fundamental chemical point of view. Among these reactions are included: (a) the protonation-assisted reduction of dinitrogen complexes of low-valent molybdenum and tungsten to the hydrazido(−2) state, [7] (b) the catalytic reduction of N_2 to NH_3 with molybdocyanide catalysts, [8] (c) the proposed bimetallic activation of N–N bonds in protic environments and the reduction of N_2 to the hydrazine level, [9] (d) the catalytic reduction of hydrazine to ammonia on pentamethylcyclopentadienyl-trimethyl tungsten hydrazine complexes [10] and (e) the catalytic reduction and disproportionation of hydrazine on certain Mo(IV) sulfur-ligated complexes [11].

For these reactions, single metal atom site mechanisms have been proposed that outline: (a) the stoichiometric reduction of N_2 to ammonia with M=NNH_2 as intermediates, [7] (b) the side-on (η^2) metal binding of N_2 followed by protonation and disproportionation [8] and (c) reduction of N_2 by one electron one proton steps where an intermediate with coordinated hydrazine is proposed to lie on the pathway to ammonia [10]. Binuclear activation mechanisms also have been proposed for some of the model reactions [5,9,11] but are not as firmly supported as the single metal atom mechanisms. The possibility of binuclear activation of N_2 (and of other nitrogenase substrates) by the Fe/Mo/S center in nitrogenase has been considered previously [12].

Indirectly, certain synthetic Fe/Mo/S [4,5] and Fe/S clusters [13] (particularly $[Mo_2Fe_6S_8(SPh)_9]^{3-}$ and $[Fe_4S_4(SPh)_4]^{2-}$ in electrochemically reduced forms) have been associated with the reduction of nitrogenase substrates in the presence of proton sources [14]. These systems are mostly non-catalytic, heterogeneous and not well defined in terms of the actual nature of the reactant clusters.

The models that we have chosen to examine in substrate reduction studies contain as a common structural feature the MFe_3S_4 'cubane' unit (M=Mo, V) and their chemistry has been the
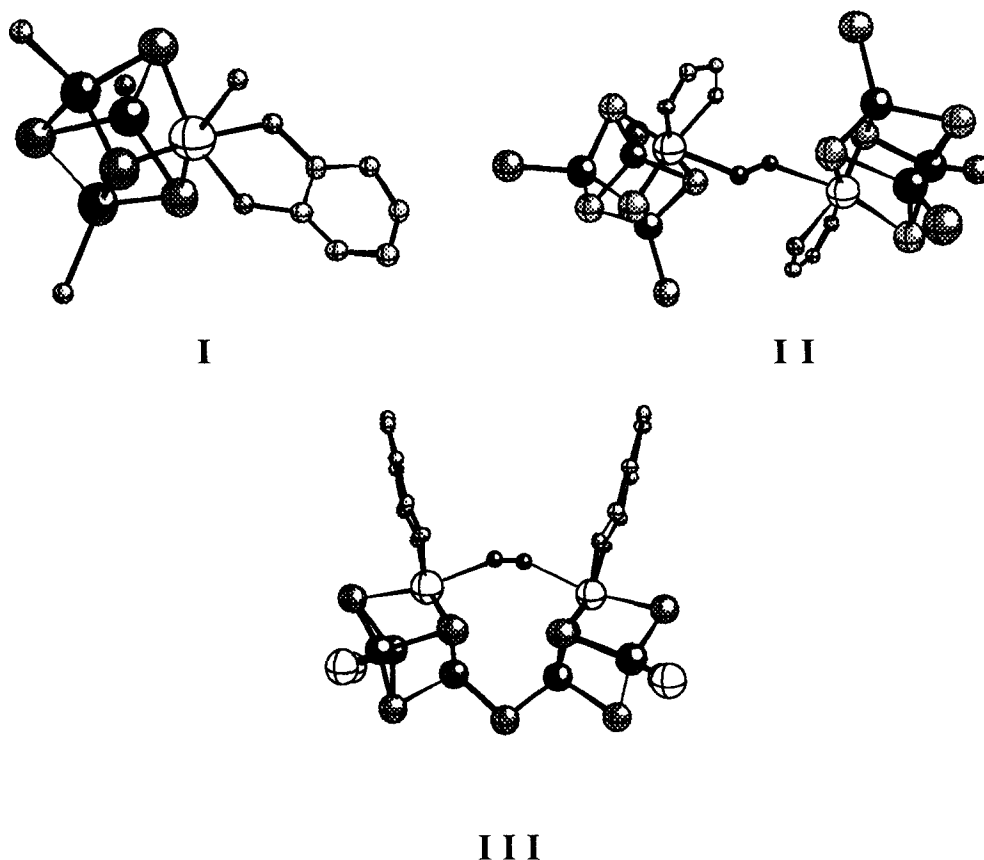
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Fig. 2. Molecular structures of: **I**, the general structure of the $[(L)MoFe_3S_4(Cl)_3X]^{n-}$ cubane clusters (X = a neutral, $n = 2$ or anionic $n = 3$ ligand, L = a substituted catecholate dianion or oxalate dianion; Refs. [15,16]). **II**, the structure of the $\{[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3]_2(\mu\text{-}N_2H_4)\}^{4-}$, singly-bridged double cubane (Ref. [17]). **III**, the structure of the $\{[(Cl_4\text{-cat})MoFe_3S_4(Cl)_2]_2(\mu\text{-}N_2H_4)(\mu\text{-}S)\}^{4-}$ (Ref. [18]).

subject of several detailed reviews [4,5]. These are (a) the $([(L)MoFe_3S_4Cl_3(CH_3CN)]^{n-}$ single cubane clusters, **SC** ($L = Cl_4$-catecholate [15], $n = 2$, **Ia**; L = citrate [16], $n = 3$, **Ib**), (b) the singly-bridged double-cubane [17], $[(Cl_4\text{-}cat)\ MoFe_3S_4Cl_3]_2\ (\mu\text{-}NH_2NH_2)]^{4-}$ clusters, **SBDC**, **II** and (c) the doubly-bridged double-cubanes [18], $\{[(Cl_4\text{-}cat)MoFe_3S_4Cl_2]_2(\mu\text{-}L)(\mu\text{-}S)]\}^{4-}$, **DBDC**, **III** ($\mu\text{-}L = NH_2NH_2$ and CN^-). The MFe_3S_4 cores in these model complexes, Fig. 2, structurally are quite similar to the MFe_3S_3 cuboidal subunits in the nitrogenase cofactor and show nearly identical first and second coordination spheres around the Mo atom.

2. Catalytic reduction of hydrazine

The extent to which the hydrazine bridging units in the bridged clusters (Fig. 2-**II**, **III**) are

activated toward N–N bond cleavage was explored by allowing these 'double cubanes' to react with reducing agents in the presence of protons.

The following processes were investigated: [19] (a) stoichiometric reactions with cobaltocene as a reducing agent and 2,6-lutidine hydrochloride, Lut·HCl, as a source of protons, or *catalytic reductions* with various $N_2H_4/MoFe_3S_4$ cubane ratios in the presence of cobaltocene and Lut·HCl, (Eq. 1)

$$N_2H_4 + 2e^- + 4H^+ \rightarrow 2NH_4^+ \qquad (1)$$

and (b) *catalytic disproportionation* reactions where N_2H_4 serves as both a reducing agent and a proton source (Eq. 2)

$$3N_2H_4 \rightarrow 4NH_3 + N_2 \qquad (2)$$

Table 1
Catalytic reduction of hydrazine by $[(Cl_4\text{-cat}) MoFe_3S_4Cl_3 (CH_3CN)]^{2-}$ in CH_3CN in the presence of $Co(Cp)_2$ and $Lut \cdot HCl$ as sources of e^- and H^+, respectively

N_2H_4/catalyst [a]	Time (min)	NH_3 [b,c]	% reduction [e]
10 [d]	30	20	100
10	30	13.2(7)	66
	60	14.6(1.1)	73
20	30	32.8(1.5)	82
	60	37.2(1.7)	93
40	30	61.6(2.5)	77
	60	69.6(3.1)	87
100	30	60(4.0)	30
	60	68	34
	120	104	52

[a] Concentration of catalyst $\sim 1 \times 10^{-4}$ M.
[b] Ammonia reported as equivalents produced per equivalent of catalyst, quantified by the indophenol method (Ref. [22]).
[c] Numbers in parentheses represent the standard deviation from the mean when more than three measurements were made.
[d] Results obtained with the $[(citrate)MoFe_3S_4Cl_3]^{3-}$ catalyst, **Ib**.
[e] Ambient temperature.

It was established that ammonia did not form in appreciable amounts from N_2H_4 in the presence of $Co(Cp)_2$ and $Lut \cdot HCl$ alone. Also it was demonstrated that the CH_3CN (a poor substrate of nitrogenase) [20] that was used as a solvent did not undergo reduction under the reaction conditions. Of the Fe/Mo/S clusters studied the doubly-bridged double-cubane, **III**, (Fig. 2) and the $[(Cl_4\text{-cat})Mo(O)(\mu\text{-S})_2FeCl_2]^{2-}$ dimer [21] (one possible oxidative degradation product of the $[(Cl_4\text{-cat}) MoFe_3S_4 (Cl)_3 CH_3CN]^{2-}$ cubane) did not promote the reduction or disproportionation of hydrazine to ammonia. Somewhat slow activity was detected when **II** [17] was used as a

Table 2
Catalytic disproportionation of N_2H_4, $(3N_2H_4 \rightarrow 4NH_3 + N_2)$ in CH_3CN by $[(Cl_4\text{-cat})MoFe_3S_4Cl_3(CH_3CN)]^{2-}$ at ambient temperature

N_2H_4/catalyst [a]	Time (min)	NH_3 [b]	% reduction [c]
10	30	6.2	46
20	30	7.1	27
40	30	11.0	21

[a] Cubane concentration 2×10^{-4} M.
[b] Ammonia yields reported as equivalents of catalyst and were quantified by the indophenol method (Ref. [22]).
[c] Maximum yield is 4/3 of NH_3 per N_2H_4.

catalyst. By far the most active catalyst in these reactions was the $[(Cl_4\text{-cat}) (CH_3CN) MoFe_3S_4 (Cl)_3]^{2-}$ single cubane [15] **Ia**, (Cl_4-cat = tetrachlorocatecholate). The use of **Ia** in all of the reactions outlined above at ambient temperature resulted in the catalytic disproportionation, and the stoichiometric or catalytic reduction of N_2H_4 to NH_3 (Tables 1 and 2). In all *catalytic disproportionation* reactions (in the absence of $Co(Cp)_2$ or $Lut \cdot HCl$) the concentration of **Ia** after 12 h of reaction time was found nearly the same as it was in the beginning of the reaction [1], the production of ammonia was reproducible and the yields of repetitive experiments fell within a relatively narrow range. In contrast the *catalytic reduction* of N_2H_4 with $Co(Cp)_2$ as a source of electrons and $Lut \cdot HCl$ as a source of protons stops before all of the hydrazine is reduced to ammonia. This may be attributed to the precipitation of the **Ia** anion by nascent counterions [2] that include $Lut \cdot H^+$, $[Co(Cp)_2]^+$, NH_4^+ and $N_2H_5^+$. The onset of precipitation within ≈ 0.5 h is not exactly predictable and as a result the yields of ammonia between successive runs show some variation. In DMF solution the onset of precipitation is longer (≈ 12 h) however the substitution of the DMF ligand by hydrazine in the coordination sphere of the molybdenum atom is not as facile as the substitution of CH_3CN [23] and consequently the catalytic reduction is slower.

Comparative studies show that the rates of ammonia formation with **II** as a catalyst were much slower than those observed with **I** while **III** was totally ineffective as a catalyst. These results indicate that *the hydrazine molecule is activated by coordination to only one $MoFe_3S_4$ cubane and*

[1] In a catalytic disproportionation reaction with a N_2H_4 to **Ib** ratio of 20, the conversion of N_2H_4 to NH_3 after 72 h was found to be 32%. At this time a spectroscopic determination of the amount of **Ib** in solution showed that the concentration of the cluster was lower by 40%. The results indicate that, over extended periods of time, **Ib** decomposes.
[2] In the reactions with **Ia** the suspension of the insoluble precipitate in CH_3CN solution dissolves upon the addition of Bu_4NI and shows electronic and EPR spectra nearly identical to those of **Ia**. This solution was found active in the catalytic generation of NH_3 upon the addition of N_2H_4, $Lut \cdot HCl$ and $Co(Cp)_2$.
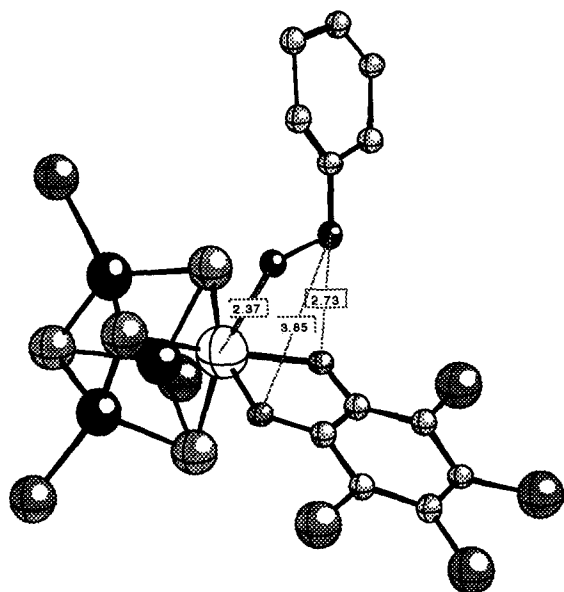
Fig. 3. The structure of the $[(Cl_4\text{-cat}) MoFe_3S_4 (Cl)_3 (NH_2 NHC_6 H_5)]^{2-}$ cubane (Ref. [19]).

the addition of an additional equivalent of cubane (*that is known to give* **II**) *inhibits the reduction*. In addition the results suggest that the availability of an uncoordinated NH_2 group (and the lone pair of electrons needed for protonation) is essential for the reduction of N_2H_4 to ammonia. The lack of reactivity of **III** almost certainly derives from the robust nature of the double cubane [18] that precludes rupture of the N_2H_4 bridge and consequently prevents the generation of an available lone pair. The reactivity of **II** (Fig. 2) may well be attributed to **I** that very likely exists in small amounts in equilibrium with **II**. Indeed addition of N_2H_4 to a solution of **II** leads to the formation of the $[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3(N_2H_4)]^{2-}$ cubane. Additional evidence that the interaction of hydrazine with only one single cubane is necessary and sufficient for catalytic reduction is available in studies with phenyl hydrazine, Ph-$HNNH_2$. The replacement of the CH_3CN molecule in **I** by $PhHNNH_2$, by a known substitution reaction [15], occurs readily. The product, **IV**, which for steric reasons does not interact further with another cubane molecule to form a bridged double cubane similar to **II**, has been structurally characterized [19], Fig. 3. The stoichiometric or catalytic reductions of the terminally coordinated

$PhHNNH_2$ in **IV** proceed with the formation of NH_3 and aniline [19].

3. Importance of the Mo atom

With the effectiveness of **I** in the catalytic reduction of hydrazine to NH_3 well established, the question regarding the identity of the metal site involved in catalysis remained to be answered. Toward this goal the reduction of N_2H_4 was attempted with the $[(Cl_4\text{-cat})MoFe_3S_4 (Cl)_3(PEt_3)]^{2-}$, **V**, [24] and $[(L) MoFe_3S_4 (Cl)_3]^{3-}$, L = citrate [16], **Ib**; Fig. 2-**I**, clusters as potential catalysts. The PEt_3 ligand in **V** is known [24] to be substitutionally inert and so is the tridentate citrate ligand in **Ib**. The results show that under identical conditions the hydrazine *disproportionation* reactions (reactions that do not need the addition of external H^+ and are not complicated by catalyst precipitation) catalyzed with either **V** or **Ib** are very much slower than the same reactions catalyzed with **Ia**. In the *catalytic* reactions however, Table 1, (with Lut·HCl added as a source of H^+) **Ib** is a better catalyst than **Ia**. The Mo-bound tridentate citrate ligand, in the citrate–cubane catalyst, rather than blocking access of hydrazine (and inhibiting subsequent reduction) promotes the catalytic process. One can speculate that protonation of the citrate ligand causes dissociation of one of the oxygen donor atoms and provides the N_2H_4 molecule access to the Mo coordination sphere. The availability of the added proton on the citrate ligand conveniently facilitates the transfer of this proton to the hydrazine molecule as the reduction takes place. The results suggest that the homocitrate ligand bound the Mo atom in the nitrogenase cofactor may play a similar, proton transfer role.

The catalytic *disproportionation* of N_2H_4, Table 2, is not observed when substitutionally inert ligands are coordinated to the Mo atom in the $[(Cl_4\text{-cat})MoFe_3S_4Cl_3(L)]^{n-}$ cubane catalyst (L = CN^-, PEt_3) or in **Ib**. In contrast, the *catalytic reduction* by the same cubanes is not

Table 3

Catalysis of hydrazine disproportionation and reduction reactions with the $[(Cl_4\text{-cat})MoFe_3S_4\ Cl_3(L)]^{n-}$ cubanes

Catalyst (L) [a]	N_2H_4/catalyst	Reductions [b,c]	Disproportion [b,c]
CH_3CN	10	14.6 (73%)	8.6 (64%)
CN^-	10	8.1 (40%)	1.1 (8%)
CH_3NH_2	10	8.6 (43%)	1.2 (9%)
Cl^-	10	7.2 (36%)	2.4 (18%)
PEt_3	10	8.0 (40%)	0.7 (5%)

[a] Concentration of catalyst was 2×10^{-4} M.

[b] All reactions were analyzed for NH_3 after 60 min.

[c] Ammonia quantified by the indophenol method (Ref. [22]).

significantly affected by the substitutionally inert ligands.

The results show, Table 3, that in the *disproportionation* reaction, where no external source of protons is added, the Mo atom is effectively blocked and the coordination/reduction of N_2H_4 cannot take place to a significant extent. The *catalytic reduction* of N_2H_4 however is possible with the same clusters if the externally added acidic protons add to the O atoms of the Mo-bound Cl_4-catecholate ligand (and/or the citrate ligand in **Ib**) and in the process generate a coordination site on the Mo atom thus allowing the N_2H_4 molecule to coordinate and undergo reduction.

The site of N_2H_4 reduction also is the site of H^+ reduction. Indeed N_2H_4 is an inhibitor of H^+ reduction, Table 4. This inhibition is less pronounced with substituted hydrazines that are bound to the Mo atom with the R substituted N atom. Steric interactions of the R group with the catecholate ligand promote dissociation of the $RNHNH_2$ ligand and allow for the reduction of H^+ that undoubtedly hydrogen-bonds to the Mo-bound O atoms of the catecholate ligand. The importance of the Mo atom, rather than the Fe atoms, in catalysis is supported further by the observation that the $[Fe_4S_4Cl_4]^{2-}$ cluster [13] is not active as a N_2H_4 reduction or disproportionation catalyst over a period of 12 h. After extended periods of time (~ 36 h) some hydrazine is converted to NH_3, however the electronic spectrum of the solution at this stage was different than the expected spectrum of the $[Fe_4S_4Cl_4]^{2-}$ cluster

Table 4

Proton reduction/hydrogen evolution catalyzed by $[(Cl_4\text{-cat})MoFe_3S_4Cl_3\ (CH_3CN)]^{2-}$ (**I**) in CH_3CN in the presence of $Co(Cp)_2$ and Lut·HCl as sources of e^- and H^+, respectively

Catalyst	NH_2NHR	$RNHNH_2$/catalyst	NH_3 [a]	H_2 [b]
no	no	0	0	1.0 (5%)
(**I**)	no	0	0	~ 20 (100%)
(**I**)	H	10	19 (95%)	1.0 (5%)
(**I**)	CH_3 [c]	10	10 (50%)	10.0 (50%)

[a] Yields reported as equivalents of ammonia per equivalent of **I**.

[b] Hydrogen yields are reported as equivalents per equivalent of **I** and were quantified by gas chromatography.

[c] The crystal structure of the $(CH_3)HNNH_2$–SC cluster [25] has been determined and shows the methyl hydrazine ligand coordinated to the Mo atom by the CH_3 substituted (more basic) N atom.

and very likely the reaction is catalyzed by a different, as yet unidentified, species [3]. The structural similarity of the $MoFe_3S_4$ clusters (Fig. 2) to the Fe/Mo/S center in nitrogenase (Fig. 1) and the competence of the former in the catalytic reduction of N_2H_4, a nitrogenase substrate [26], raise the possibility that the activation and reduction of dinitrogen by the Fe/Mo/S center in nitrogenase takes place on the Mo atom by a single metal site mechanism.

4. Possible pathway of hydrazine reduction

A simple proposed pathway, Fig. 4, for the catalytic reduction of N_2H_4 to ammonia by the $MoFe_3S_4$ cluster is similar to a portion of the mechanism proposed previously for the reduction of N_2 on the $(Me_5Cp)W(CH_3)_3$ fragment [10]. An initial protonation step prior to reduction of the coordinated hydrazine molecule, Fig. 4, is supported by the synthesis and reactivity of the $[(Cl_4\text{-}cat)(N_2H_5)MoFe_3S_4Cl_3]^-$ cluster. The latter has been isolated and characterized and upon addition of $Co(Cp)_2$ (in the absence of Lut·HCl) affords ammonia. For the action of nitrogenase, a mathematical model has been derived by Thorneley and Lowe [27] and is based on detailed analyses

[3] The decomposition of the $[Fe_4S_4Cl_4]^{2-}$ cluster under the reaction conditions with the formation of new Fe/S clusters is presently under investigation.
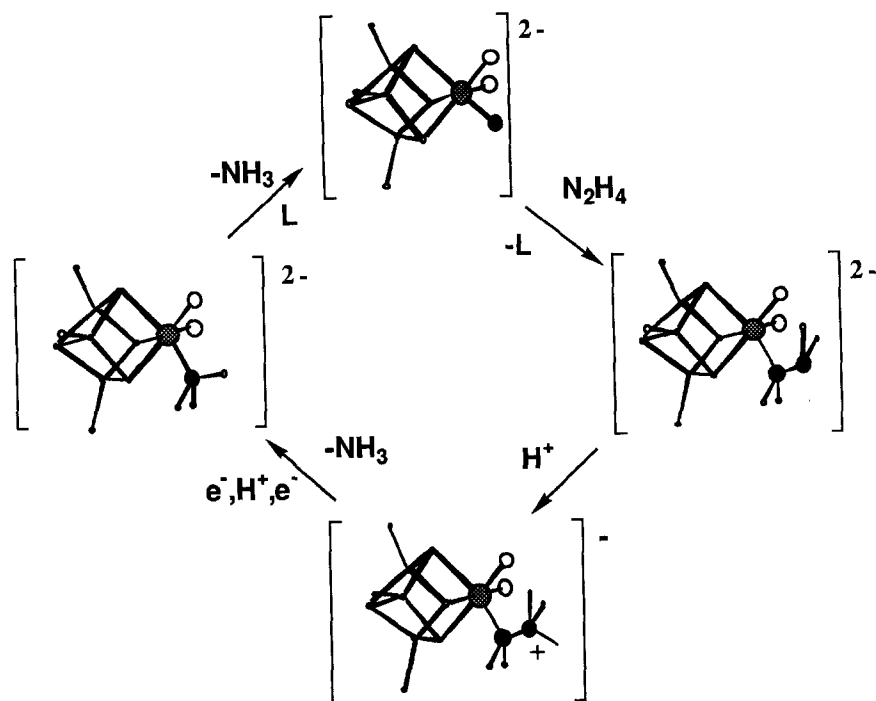
Fig. 4. A proposed mechanism for the catalytic reduction of N_2H_4 to NH_3 by the $[(Citr)MoFe_3S_4(Cl)_3]^{3-}$ and the $[(Cl_4\text{-cat}) MoFe_3S_4 (Cl)_3 \cdot L]^{2-}$ cubanes ($L = CH_3CN$, PEt_3).

of pre-steady state kinetics of hydrazine and ammonia formation and of hydrogen evolution as a function of various reagent concentrations and component ratios. The model is based on eight individual electron transfers on a mononuclear molybdenum species as required for the reduction of one N_2 molecule and two H^+ ions to H_2. Similar but less complete mechanisms also have been suggested by Cleland [28] and by Guth and Burris [29].

5. Catalysis of hydrazine reduction with the V/Fe/S clusters

Nitrogenase systems that contain V in place of Mo have been isolated from *A. vinelandii* [30] and *A. chroococcum* [30] and function under conditions of molybdenum deficiency. The structural similarity of the Fe/V/S center in the V–Fe protein from *A. chroococcum* to the Fe/Mo/S centers in the 'conventional' Mo-nitrogenases has been established by a V-EXAFS analysis [30].

The catalytic behavior of synthetic Fe/V/S clusters that structurally resemble the Fe/V/S site of nitrogenase was investigated [31] with the $[(L)(L')(L'')VFe_3S_4Cl_3]^{n-}$ clusters [37] (L, $L',L'' = DMF$, **VI**; Fig. 5; $L = PEt_3, L',L'' = DMF$, **VII**; L, $L' = 2,2'$-bipyridyl, $L'' = DMF$, **VIII**; $n = 1$). These clusters that contain the $[VFe_3S_4]^{2+}$ cuboidal core, also are effective catalysts in the reduction of hydrazine to ammonia
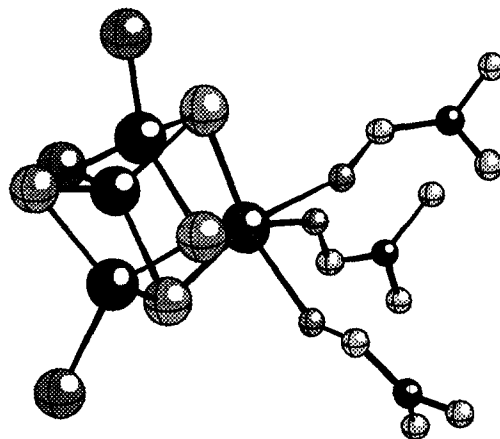

Fig. 5. Molecular structure of the $[(DMF)_3VFe_3S_4(Cl)_3]^-$ cubane cluster (Ref. [32]).

Table 5
Catalytic reduction of hydrazines with the vanadium cubanes in CH_3CN solution

Catalyst	Substrate	Substrate/Catalyst	NH_3 (equiv.)			NH_3 (Max)	Conversion %		
			0.5 h	1.0 h	2.0 h		0.5 h	1.0 h	2.0 h
VI	N_2H_4	1	0.9 (3)	1.0 (4)	1.1 (2)	2	45	51	54
		10	15.2 (3)	17.8 (3)	20.0 (3)	20	76	89	100
		10 (DMF)	–	–	2.0 (2)	20	trace	trace	10
		20	23.2 (3)	28.8 (3)	35.6 (2)	40	58	72	89
		40	36.8 (3)	48.0 (3)	64.8 (2)	80	46	60	81
VII	N_2H_4	10	5.6 (2)	7.6 (2)	9.4 (2)	20	28	38	47
VI	N_2H_3Ph	1	0.5 (1)	0.5 (2)	0.6 (1)	1	51	54	63
		10	1.1 (3)	1.5 (3)	2.4 (3)	10	11	15	24
VIII	N_2H_4	10	–	2.2 (2)	3.4 (2)	20	trace	11	17

VI $(L=L'=L''=DMF)$.
VII $(L=L'=DMF; L''=PEt_3)$.
VIII $(L=DMF; L', L''=bipy)$.

in the presence of cobaltocene and 2,6-lutidine hydrochloride as sources of electrons and protons, respectively, Table 5. The catalytic reduction of phenylhydrazine to ammonia and aniline also is effected by **VI**, **VII** and **VIII**. An important result, that demonstrates the ability of hydrazine-like substrate molecules to interact directly with the V atom, has been obtained in the synthesis of the $(Me_4N)[(PhHNNH_2)(bpy)VFe_3S_4Cl_3]$ single cubane [31].

The importance of the heteroatom (V) in the reduction of hydrazine was demonstrated by the discovery that V-coordinated terminal ligands have a profound effect on the relative rates of hydrazine reduction. Specifically, as the number of labile solvent molecules coordinated to the V atom decreases, the relative rate of hydrazine

Table 6
Electrochemical data of the substituted $[VFe_3S_4]^{2+}$ cores

Cluster	Oxidation [a]	Reduction	Ref.
[VI]	+0.17(qr) [b,c]	−1.20(irr)	[32]
[VII]	+0.13(qr)	−1.20(irr)	[32]
[VIII]	+0.30(qr)	−1.22(qr)	[32]
[IX]	0.00(qr)	−0.68(irr)	[31]
[VI]–Br	+0.12(qr)	−1.18(irr)	[31]
[VI]–I	+0.14(qr)	−1.12(irr)	[31]

[a] vs. Ag/AgCl in CH_3CN solution.
[b] qr = quasireversible process, irr = irreversible process.
[c] E_{pc} or E_{pa} is reported.

reduction decreases, Table 5. This behavior is pushed to the limit with the $[(HBpz_3)VFe_3S_4Cl_3]^{2-}$ cubane (L, L', L″ = hydrotris(pyrazolyl)borate, **IX**; $n=2$), where all coordination sites on the V atom are 'blocked'. The latter, for which the structure has been determined [31], shows no catalytic or stoichiometric hydrazine reduction.

Unlike **VI**, **VII**, and **VIII** that do not show significant changes in the $1-/2-$ reduction potential as the DMF ligands are substituted by other ligands, Table 6, **IX** shows a less negative $2-/3-$ reduction potential and this difference in reduction potential may indeed be the reason for the lack of catalytic activity. The role of the Fe sites in the $[VFe_3S_4]^{2+}$ cubanes during catalysis was investigated using the $[(DMF)_3VFe_3S_4X_3]^-$ cubanes [31] (X = Cl, Br, or I). The relative rates of hydrazine reduction with each of these clusters as catalysts were virtually identical, indicating little or no involvement of the Fe atoms during catalysis. The results of these reactivity studies are comparable to those obtained with the Mo cubanes and it appears likely that the reduction with the V cubanes follow a similar reaction pathway.

6. Catalysis of acetylene reduction

Previous studies on the abiological reduction of C_2H_2 [33–42] suffer from major drawbacks

Table 7

Initial velocities (v_0) for the reduction of acetylene to ethylene catalysed by various cubanes [a]

Cubane catalyst	v_0 (M/min) [b] ($\times 10^5$)	n_0 normalized to cubane **I**
Ia	6.6(3)	1.0
$[(\eta^3\text{-citrate})MoFe_3S_4Cl_3]^{3-}$	6.4(2)	0.97
Recovery cubane [c]	2.7(3)	0.41
$[Fe_4S_4Cl_4]^{2-}$	1.3(3)	0.20
$[(Cl_4\text{-cat})MoFe_3S_4Cl_3(CN)]^{3-}$	1.6(1)	0.24
$[(CO)_3MoFe_3S_4Cl_3]^{3-}$	1.5(2)	0.23
$[(CO)_3MoFe_3S_4R_3]^{3-}$	1.1(3)	0.17
$R = p\text{-Cl-SPh}$		
$\mathbf{I} + PEt_3$ (1:2) [d]	1.3(2)	0.20
Blank (no catalyst) [e]	0.011	0.002

[a] Experiments are performed in DMF solvent at 20°C using $CoCp_2$ and Lut·HCl as sources of electrons and protons, respectively. The initial substrate:catalyst ratio used is 20:1.

[b] Initial velocity, v_0, is obtained as the slope from $[C_2H_4]$ (M) vs. t (min) which obeys a straight-line relationship during the initial stages of the reaction.

[c] 'Recovered cubane' is the form of the active catalyst isolated following acetylene reduction.

[d] Acetylene reduction performed using cubane **I** as the catalyst in the presence of PEt_3 such that $Mo:PEt_3 = 1:2$.

[e] Typical acetylene reduction conditions except for the absence of catalyst.

including incomplete catalyst identification [33,34,36], the use of mononuclear or binuclear Mo complexes of little relevance to the nitrogenase problem [35–41] and sub-stoichiometric, non-catalytic, substrate reduction [42]. Our studies have provided substantial evidence that identifies the $[MoFe_3S_4]^{3+}$ cubanes as catalysts in the reduction of acetylene to ethylene and implicates both the Mo and Fe sites in acetylene reduction [43].

Catalytic reductions of C_2H_2 to C_2H_4 and traces of C_2H_6 were carried out at 20°C using **Ia** as the catalyst and cobaltocene and 2,6-lutidine hydrochloride, Lut·HCl, as sources of electrons and protons, respectively. Initial catalyst concentration was maintained at 0.96 mmol while substrate excess over catalyst was varied from 5-fold to 80-fold. In addition, a $C_2H_2:H^+:e^-$ ratio of 1:4:4 was employed in the initial stages of the reaction.

The initial-rate method, whereby [catalyst] ≪ [substrate] was used to obtain reaction velocities, v_0(M/min), at less than 5% substrate consumption, Table 7. The data obey saturation kinetics, as found for enzyme catalysis. Reaction rates remain constant for C_2H_2:cubane ratios < 30:1, indicating zero-order substrate dependence. At $[C_2H_2] < 6$ mmol the reaction is first-order in substrate. A double reciprocal plot, v_0^{-1} vs. $[C_2H_2]^{-1}$, is linear at optimum substrate concentration from which $K_m = 17.9$ mmol and $V_{max} = 1.1 \times 10^{-4}$ M/min are calculated. Catalyst **Ia** reduces acetylene with a turnover number of 0.11 mol C_2H_2/mol catalyst/min which is approximately 0.08% 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^{-1}) but a large entropy of activation ($\Delta S^{\ddagger} = -32(2)$ cal K^{-1} mol^{-1}) which extrapolates to a significant Gibbs free energy ($\Delta G^{\ddagger} = 19(1)$). The large negative ΔS is consistent with an ordered transition state, expected if the substrate and perhaps the protons were to assume a specific arrangement as the reaction proceeds.

The characteristic EPR spectrum of **Ia** ($S = 3/2$) remains unchanged as the reaction progresses and spectral line-shape and g-values of pure cubane compared with that used in substrate reduction are indistinguishable. Quantitation of the EPR spectra obtained before and after reaction with acetylene shows no loss of signal intensity.
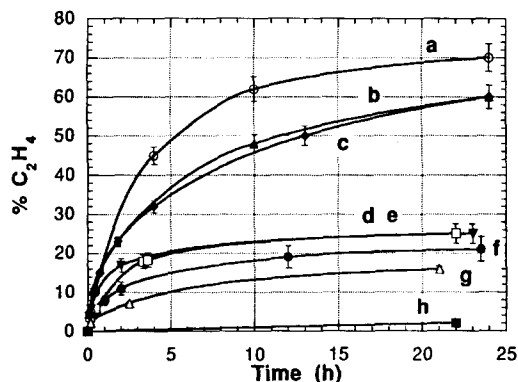
Fig. 6. Ethylene production in DMF solvent at 20°C from the reaction, $20C_2H_2 + 80Lut \cdot HCl + 80CoCp_2 + 1catalyst$. (a): catalyst = cubane **I**. (b): catalyst = η^3-citrate cubane. (c): catalyst = 'recovery cubane' cubane. (d): catalyst = CN–cubane (e): catalyst = $[Fe_4S_4Cl_4]^{2-}$. (f) (R = Cl) and (g) (R = p-Cl-SPh): catalyst = $[R_3Fe_3S_4Mo(CO)_3]^{3-}$. (h): Blank reaction (no catalyst).

These results strongly suggest that the $[MoFe_3S_4]^{3+}$ cubane core remains structurally intact during the reaction and that **Ia** may be considered the true catalyst in the reduction of acetylene.

The active catalyst isolated from the reaction mixture following reduction of acetylene has been characterized as $[CoCp_2]_2[(Cl_4\text{-cat})MoFe_3S_4Cl_3 (DMF)]$ and has been found active

in the catalytic reduction of acetylene, albeit at a somewhat slower rate compared to pure catalyst **Ia**, Fig. 6.

The stereochemistry of addition across the substrate triple bond was investigated using gaseous FT-IR spectroscopy using deuterated acetylene (C_2D_2) as a substrate. The formation of solely cis-1,2-$C_2D_2H_2$ was revealed by the characteristic IR absorption, $\nu_7 = 842$ cm^{-1} [44]. No evidence for trans-substituted ethylene was found (IR absorption at 988 cm^{-1}). This result is consistent with a transition state intermediate in which substrate acetylene is side-on bonded to the Mo atom of **Ia**. Nitrogenase catalysed reduction of acetylene also proceeds with a high degree of stereoselectivity resulting in cis addition across the triple bond [45].

The involvement of the Mo atom in acetylene reduction has been confirmed by modification of the coordination sphere around the heterometal, Fig. 6. The rate of ethylene production varies considerably depending on the availability of Mo coordination sites, Table 8. Replacing the bidentate Cl_4-cat ligand in **Ia** with η^3-citrate [16] effects a slight reduction of reaction rate. This is

Table 8
Kinetic data for the reduction of acetylene to ethylene catalysed by cubane **Ia** [a]

C_2H_2:**I**	v_0 (M/min) [b] ($\times 10^5$)	$[C_2H_2]$ (mM) [c]	% reaction completion [d]	$1/v_0 \times 10^{-4}$ (optimum $[C_2H_2]$ range) [e]
5:1	1.7(1)	1.91	6.4	–
10:1	3.4(2)	3.83	4.6	–
15:1	5.3(1)	5.74	4.0	1.9(1)
20:1	6.6(3)	7.65	5.2	1.5(3)
25:1	7.4(3)	9.56	5.2	1.4(3)
30:1	7.7(2)	11.5	4.2	1.3(2)
40:1	8.0(1)	15.3	4.8	1.2(1)
50:1	7.9(2)	19.1	3.6	1.2(2)
60:1	7.8(2)	23.0	2.9	–
70:1	7.8(2)	26.8	2.8	–
80:1	7.9(3)	30.8	2.9	–

[a] Experiments are performed in DMF solvent at 20°C using $Co(Cp)_2$ and Lut · HCl as sources of electrons and protons, respectively.
[b] Initial velocity, v_0, is obtained as the slope from $[C_2H_4]$ (M) vs. t (min) which obeys a straight-line relationship during the initial stages of the reaction.
[c] Calculated using the total vial volume of 25 ml.
[d] Consumption of C_2H_2 as a percentage of the initial $[C_2H_2]$. The initial rate method for determining v_0 is optimum to approx. 5% reaction completion.
[e] The double reciprocal plot (DRP), v_0^{-1} vs. $[C_2H_2]^{-1}$, is obtained in the optimal $[C_2H_2]$ range, typically, 0.33–2.0 K_m. At high $[C_2H_2]$, 3.3–20 K_m, the reaction is zero-order in C_2H_2 and the DRP is essentially horizontal. At low $[C_2H_2]$, 0.033–0.2 K_m, the reaction is first-order in C_2H_2 and the DRP intercepts both axes too close to the origin to allow K_m and V_{max} to be determined accurately.

in contrast with the reduction of hydrazine with the same catalyst (vide supra). A significant decrease in the reaction rate is observed when CN^- is used as the terminal ligand in place of CH_3CN or DMF (Fig. 6). The non-labile cyanide ligand precludes binding of substrate acetylene to the Mo atom thereby restricting substrate reduction to the Fe sites (vide infra) and slowing ethylene production to a rate comparable with $[Fe_4S_4Cl_4]^{2-}$ cubanes. A similar result is obtained when the $[(CO)_3MoFe_3S_4R_3]^{3-}$ (R = Cl, p-Cl-SPh) cubanes [46] are used as catalysts.

Catalytic C_2H_2 reduction by cubane **Ia** is effectively prevented if the reaction is carried out under a saturating CO atmosphere and the overall yield of C_2H_4 and C_2H_6 products is considerably diminished to approximately that of the background level (no catalyst present). This suggests that CO precludes C_2H_2 binding to the Mo atom of **Ia**, probably by virtue of its own binding to the heterometal. Interestingly, a saturated CO atmosphere also appears to block substrate reduction at the secondary (Fe) catalytic sites (vide infra) on **Ia**.

In attempts to block the Mo site from catalysis it was discovered that the Fe atoms on cubane **Ia** also are competent in substrate reduction albeit at a slower rate, Table 8. Typically all the Fe sites combined completed 2–3 turnovers in a 24 h period, while the entire cluster, with the Mo site free for substrate binding, was capable of at least 16 turnovers in the same time. The use of $[NBu_4]_2[Fe_4S_4Cl_4]$ as the catalyst effected no appreciable increase in reaction rate or overall yield compared to cubane **Ia** with the Mo atom 'blocked' from substrate binding/reduction. The rate of acetylene reduction varies considerably depending on the reaction site, Mo vs. Fe, the former being decidedly more competent in catalyzing the reaction.

An inhibition study of the Mo and Fe substrate binding sites using PEt_3 revealed that both centers are adversely affected by the phosphine. The affinity of PEt_3 for the heterometal in cubane **I** is well documented [24] and our work now shows that

at sufficiently high concentrations PEt_3 also serves to block the Fe sites on **I** from reducing acetylene.

An important contrast can be made between the catalytic reduction of acetylene and the catalytic reduction of N_2H_4 by **Ia**. In the latter the $[Fe_4S_4Cl_4]^{2-}$ cubanes were shown to be totally ineffective and no ammonia was found. The results imply that the Mo and Fe sites in the Fe/Mo/S cubanes have varying affinities for different nitrogenase substrates and also that both metal sites may be important at specific times during the step-wise reduction of a given substrate. This has been suggested by Dance [47] in his ab initio study of dinitrogen binding to the Fe/Mo/S center of nitrogenase. Our modeling studies suggest that the 'Fe' sites of the $[Fe_4S_4Cl_4]^{2-}$ and $[MoFe_3S_4]^{3+}$ cubanes are capable of mediating *all* stages of C_2H_2 reduction to C_2H_4 (and trace C_2H_6), whereas reduction of N_2H_4 (one or more stages) cannot occur with the $[Fe_4S_4Cl_4]^{2-}$ cubane but does occur in the presence of the heterometallic cubane, **I**. The heterometal (Mo, V), advantageous in C_2H_2 reduction, seems to be a necessary requirement for the reduction of hydrazine to ammonia.

7. Summary and conclusions

The results of our studies demonstrate that the molybdenum atom in **I**, and the V atom in **VI**, in environments very similar to those in the Fe/M/S centers of the nitrogenases, are catalytically active in the reduction of N_2H_4 to ammonia. It remains to be established whether the molybdenum atom in **I** also is involved in the activation and reduction of N_2 to the hydrazine level. At present we have been unable to show any reactivity of **I** with N_2 and as reported earlier the CH_3CN ligand in **I** is not replaced by N_2 although the possibility still exists that **I** may bind to N_2 through the Mo atom at a different (lower) oxidation level. It should be emphasized nevertheless that, as suggested recently [3,47,48], the direct involvement of the unique coordinatively unsaturated Fe atoms cannot be ruled out. Indeed the early stages of N_2

reduction on the Fe/Mo/S center of nitrogenase may involve binuclear (Fe–Mo) activation prior to reduction to the hydrazine level. The binuclear activation of N_2 by initial binding to Fe and participation of the Mo atom in a Fe–μ-S–Mo bridging unit has been proposed previously [12].

Earlier studies in our laboratory have shown that cuboidal subunits with the Fe_3S_4 core can serve as ligands for Mo(O) when the latter is stabilized by π acceptor ligands such as CO. At present we are exploring the possibility that the $[(CO)_3MoFe_3S_4(L)_3]^{3-}$ clusters [46] and derivatives will bind and activate N_2 and/or other nitrogenase substrates. *Our studies thus far have shown that the synthetic Fe/Mo/S clusters are versatile in the activation and reduction of nitrogenase substrates. Within these clusters distinct, substrate specific, reactivity sites have been identified in catalytic reductions of N_2H_4 and C_2H_2.*

Acknowledgements

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