Catalytic Reduction of Hydrazine to Ammonia with $MoFe_3S_4$−Polycarboxylate Clusters. Possible Relevance Regarding the Function of the Molybdenum-Coordinated Homocitrate in Nitrogenase

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The catalytic function of the previously synthesized and characterized $[(L)MoFe_3S_4Cl_3]^{2-,3-}$ clusters (L = tetrachlorocatecholate, citrate, citramalate, methyliminodiacetate, nitrilotriacetate, thiodiglycolate) and of the $[MoFe_3S_4Cl_3(thiolactate)]_2^{4-}$ and $[(MoFe_3S_4Cl_4)_2(\mu\text{-oxalate})]^{4-}$ clusters in the reduction of N_2H_4 to NH_3 is reported. In the catalytic reduction, which is carried out at ambient temperature and pressure, cobaltocene and 2,6-lutidinium chloride are supplied externally as electron and proton sources, respectively. In experiments where the N_2H_4 to the $[(L)MoFe_3S_4Cl_3]^{n-}$ catalyst ratio is 100:1, and over a period of 30 min, the reduction proceeds to 92% completion for L = citrate, 66% completion for L = citramalate, and 34% completion for L = tetrachlorocatecholate. The $[Fe_4S_4Cl_4]^{2-}$ cluster is totally inactive and gives only background ammonia measurements. Inhibition studies with PEt_3 and CO as inhibitors show a dramatic decrease in the catalytic efficiency. These results are consistent with results obtained previously in our laboratory and strongly suggest that N_2H_4 activation and reduction occur at the Mo site of the $[(L)MoFe_3S_4Cl_3]^{2-,\ 3-}$ clusters. A possible pathway for the N_2H_4 reduction on a single metal site (Mo) and a possible role for the carboxylate ligand are proposed. The possibility that the Mo-bound polycarboxylate ligand acts as a proton delivery "shuttle" during hydrazine reduction is considered.

Introduction

The total amount of N_2 fixed on a global basis is estimated to be about 2.4×10^8 tons annually. Some 65% of this amount is carried out by nitrogen fixing microorganisms, while the industrial Haber process accounts for about 25%. The latter occurs at high temperatures (300−400 °C) and pressures (350−1000 atm), whereas the biological fixation proceeds under ambient conditions. In biology, the enzyme nitrogenase in symbiotic or free living bacteria is responsible for the fixation of atmospheric N_2 to NH_3[1] with the concomitant reduction of $2H^+$ to H_2 (eq 1).

$$N_2 + 8e^- + 10H^+ + 16MgATP \rightarrow 2NH_4^+ + H_2 + 16MgADP + 16PO_4^{2-} \quad (1)$$

Spectroscopic data,[2] accumulated over a period of two decades, have revealed many structural and electronic features of the Fe/Mo/S center essential for substrate binding and activation. This remarkable polymetallic Mo/Fe/S aggregate, also known as the iron−molybdenum cofactor (FeMo-co)[3] was structurally outlined in its entirety in the crystal structure determination of the MoFe protein of *Azotobacter vinelandii* and *Clostridium pasteurianum* by two individual groups.[4,5] The current model of the cofactor, based on the interpretation of electron density maps, shows an elongated $MoFe_7S_9$ cluster composed of $MoFe_3(\mu_3\text{-}S)_3$ and $Fe_4(\mu_3\text{-}S)_3$ cuboidal subunits linked by three μ_2-S bridges (Figure 1). The Mo atom occupies a unique position at the *pole* of the cluster. In addition, it

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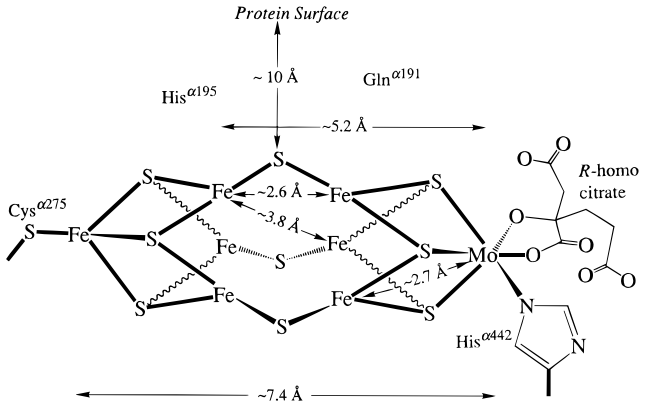

Figure 1. Structure of the Fe/Mo/S center in nitrogenase.[4,5]

appears coordinatively saturated surrounded by three inorganic sulfides, a histidine imidazole, and a bidentate homocitrate ((*R*)-2-hydroxy-1,2,4-butanetricarboxylic acid) molecule in an approximately octahedral environment.

The presence of homocitrate as an integral constituent of the FeMo-co was detected and reported previously by Ludden *et al.*, who established its identity by a combination of techniques.[1ag,6] The exact site on the cofactor involved in N_2 coordination and activation is not yet known although a variety of possibilities have been proposed,[1a,6j,7] some of them based on theoretical calculations.[7] Experimental results suggest, but do not prove, that the Mo site may be involved directly or indirectly in some stage(s) of substrate reduction.[8] Two "alternative" forms of nitrogenase, neither of which contains Mo, have been reported. The first has V in the place of Mo and shows similar substrate selectivity but much lower efficiency than the Mo prototype.[9] The second is an "all-Fe" nitrogenase, and its functional details still are under investigation. The latter shows even lower activity than the V nitrogenase.[10]

A large number of coordination compounds have been proposed as possible structural or functional models for nitrogenase. Included among these are: (a) mononuclear[11] and binuclear[12] transition metal complexes, with reactivity features relevant to the nitrogenase function,[11e,12c] and (b) polynuclear Mo/Fe/S aggregates many of which contain "cubane" type structural subunits and a Fe:Mo ratio of 3:1[13] or 4:1.[13j] The cubane $[MoFe_3S_4]^{3+}$ structural units are partial structural models for the nitrogenase FeMo-co[13,14] and show very similar first and second coordination spheres around the Mo atoms. The characteristic EPR signature[15] ($S = {}^3/_2$) of the single-cubane clusters that contain the $[MoFe_3S_4]^{3+}$ core also is very similar to those obtained for the semireduced state of the Fe−Mo protein of nitrogenase and of the extruded FeMo-co.[16] Recently, we reported the catalytic reduction of hydrazine to ammonia[17] and of acetylene to ethylene[18] using the $[MoFe_3S_4Cl_3(Cl_4\text{-cat})(CH_3CN)]^{2-}$ and $(Et_4N)_3[MoFe_3S_4Cl_3(Hcit)]^{3-}$ cubanes as catalysts (L = Cl_4-cat = tetrachlorocatecholate; Hcit = the citrate trianion). Similar studies have been carried out utilizing the $[VFe_3S_4]^{2+}$ cubanes[19] for the catalytic reduction of hydrazine. For these reactions evidence has been presented that strongly supports the involvement of the heterometal in the catalytic process. The catalytic reduction of hydrazine (eq 2) has been

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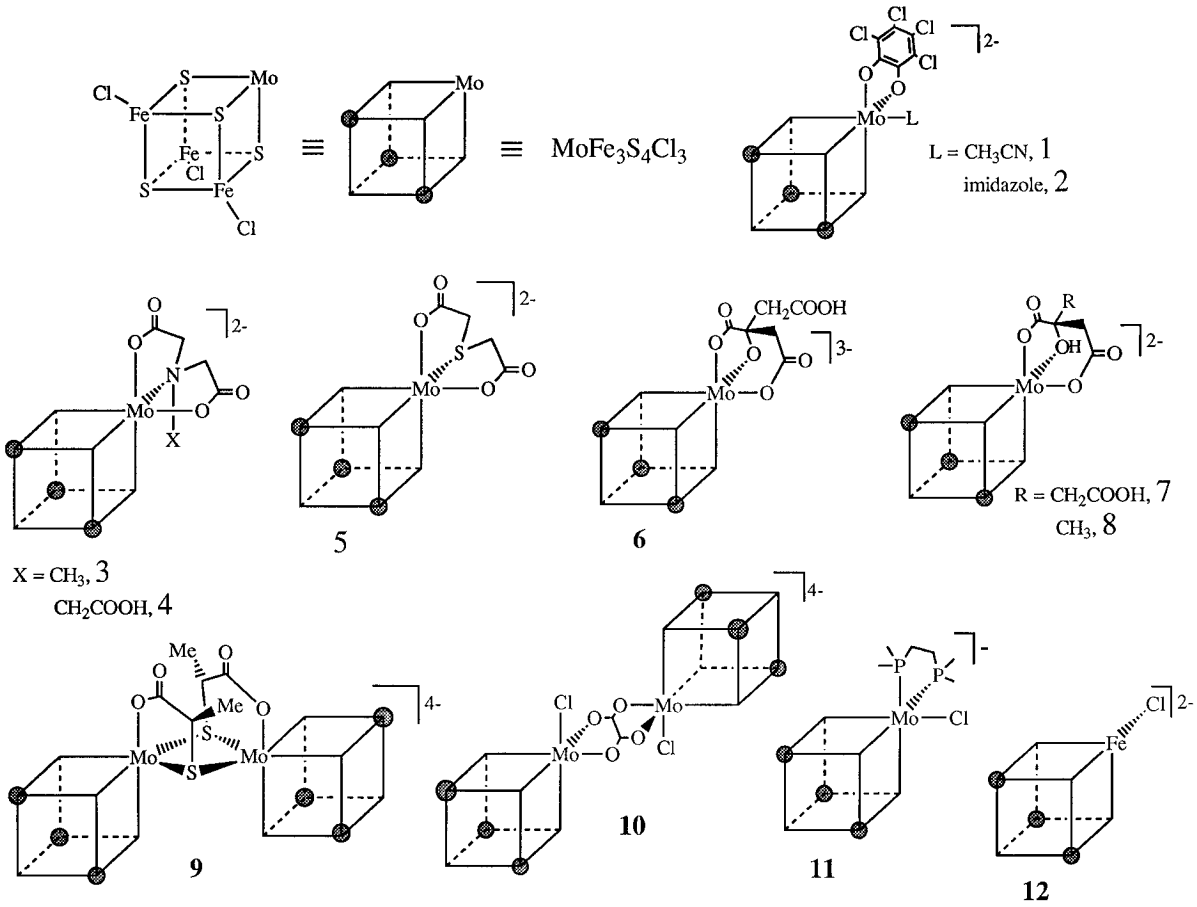

Figure 2. Schematic structures of the [(L)$MoFe_3S_4$ Cl_3] cubanes used in this study with L = carboxylate group containing ligands. The structures of compounds **1**, **3**, **5**, and **9**−**12** have been crystallographically determined.

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

thoroughly studied.[20] Hydrazine is a substrate[21a−c] as well as a product of functioning nitrogenase and has been isolated by quenching the enzyme.[21d−g]

In this paper, we report results of our studies on the behavior of the (L)$MoFe_3S_4$ cubanes (L = polycarboxylate anions) as catalysts in the reduction of hydrazine to ammonia. Preliminary reports of this study have appeared in the literature.[17,22,23]

Experimental Section

General Considerations. Abbreviations used: Cl_4-cat = tetrachlorocatecholate, maa = mercaptoacetate, tla = thiolactate, tdga = thiodiglycolate, Hcit = citrate trianion, H_2cit = citrate dianion, Hcmal = citramalate dianion, mida = methyliminodiacetate, Hnta = nitrilotriacetate dianion, ox = oxalate, im = imidazole, dmpe = dimethylphosphinoethane, Lut·HCl = 2,6-lutidine hydrochloride. Details on the purification of solvents and instrumentation used for the spectroscopic and electrochemical studies have been given elsewhere.[14b] All of the carboxylic acids, cobaltocene, hydrazine, and a 1.0 M solution of HCl in Et_2O were purchased from Aldrich and were used as received. The clusters $(Et_4N)_2[MoFe_3S_4Cl_3(Cl_4\text{-cat})(CH_3CN)]$ (**1**),[24] $(Et_4N)_2$-$[MoFe_3S_4Cl_3(mida)]$ (**3**),[14b] $(Et_4N)_2[MoFe_3S_4Cl_3(tdga)]$ (**5**),[23] $(Et_4N)_3$-$[MoFe_3S_4Cl_3(Hcit)]$ (**6**),[14] $(Et_4N)_2[MoFe_3S_4Cl_3(H_2cit]$ (**7**),[14] $(Et_4N)_2$-$[MoFe_3S_4Cl_3(Hcmal)]$ (**8**),[14] $(Et_4N)_4[MoFe_3S_4Cl_3(tla)]_2$ (**9**),[22,23] $(Et_4N)_4$-$[(MoFe_3S_4Cl_4)_2(\mu\text{-ox})]$ (**10**),[14] $(Et_4N)[MoFe_3S_4Cl_4(dmpe)]$ (**11**),[25] and $(Bu_4N)_2(Fe_4S_4Cl_4)$ (**12**)[26] were synthesized by published procedures. Also, Lut·HCl was prepared as previously described.[19] The structures of the above clusters are depicted schematically in Figure 2.

$(Et_4N)_2[MoFe_3S_4Cl_3(Cl_4\text{-cat})(im)]$ (2). A 0.30 g amount of **1** (0.29 mmol) was mixed with imidazole (0.02 g, 0.29 mmol) in 30 mL of CH_3CN, and the homogeneous solution was stirred overnight. The color turned to brown-orange. At this point, filtration (negligible amount of black residue), addition of Et_2O (100 mL) to the filtrate, and overnight standing gave a highly crystalline black solid (yield 0.27 g, 90%). Anal. Calcd for $MoFe_3S_4Cl_7O_2C_{25}H_{44}N_4$ (**2**, MW 1072.57): C, 27.98; H, 4.10; N, 5.22. Found: C, 28.31; H, 4.33; N, 5.25. Electronic spectrum (CH_3CN): lacking prominent features. Far-infrared spectrum (in CsI disks, cm^{-1}): 299 (m), 314 (w), 350 (vs), 373 (sh), 392 (w), 410 (m), 423 (m). Mid-infrared spectrum (in CsI disks, cm^{-1}): peaks assigned to the imidazole ligand ν(C=N + C=C) 1650 (m), 1600 (m); ν(N−H) 3128 (m).

$(Et_4N)_2[MoFe_3S_4Cl_3(Hnta)]$ (4). A 0.30 g amount of **1** (0.29 mmol) was dissolved in 30 mL of CH_3CN. Nitrilotriacetic acid (N(CH_2-

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$COOH)_3$, 0.083 g, 0.44 mmol, 1.5-fold excess) was partially dissolved in 5 mL of MeOH, and this suspension was added dropwise to the cluster solution under vigorous stirring. While the temperature was kept at ~50 °C, the mixture was stirred overnight, after which it was allowed to cool at room temperature. A black insoluble residue was removed by filtration and was discarded. Et_2O (~70 mL) was layered onto the brown-reddish filtrate, and after 6 h of standing, a microcrystalline solid precipitated. It was isolated by filtration and washed thoroughly with MeOH, acetone, and Et_2O. Finally, it was recrystallized from CH_3CN/Et_2O and dried *in vacuo* to afford analytically pure material (yield 0.15 g, 55%). Anal. Calcd for $MoFe_3S_4Cl_3O_6N_3C_{22}H_{47}$ (**4**, MW 947.73): C, 27.90; H, 5.00; N, 4.43. Found: C, 28.25; H, 5.07; N, 4.19. Electronic spectrum (CH_3CN, nm): 470, 314. Far-infrared spectrum (cm^{-1}): 324 (s), 353 (sh), 362 (vs), 397 (w), 414 (m). Mid-infrared spectrum (cm^{-1}): ν_{asym}(C═O) 1625 (vs), 1658 (vs), 1746 (m, due to uncoordinated −COOH); ν_{sym}(C−O) 1393; ν(O−H) 3440 (m).

Catalytic Reductions of Hydrazine. Reactions were performed under strict anaerobic conditions in CH_3CN. The final volume of the reaction mixture was ~40 mL, and the substrate:catalyst ratio was 100:1. The concentration of the catalyst was 1.26×10^{-5} M, and all the other reagent concentrations were scaled accordingly. The catalytic reductions were carried out by two methods.

Method A. To an Erlenmeyer flask was added 0.5 mL of a 1 mM stock solution of the cubane catalyst in CH_3CN. Then a volume of <40 mL of CH_3CN was added. Hydrazine (0.25 mL of a 0.2 M stock solution in CH_3CN) was injected via syringe into the flask under continuous stirring, and the the volume was adjusted to 40 mL. The concentrations of the catalyst and hydrazine at this stage were 1.25×10^{-5} and 1.25×10^{-3} M, respectively. Solid cobaltocene (0.02 g) and Lut·HCl (0.03 g) were added to the reaction mixture, which was vigorously stirred. Samples (3.0 mL) were obtained at 5 min, 30 min, 1 h, and 12 h.

Method B. To a 125 mL flask was added 20.0 mL of a 0.25 M solution of cobaltocene in CH_3CN, followed by 20.0 mL of a 0.38 M CH_3CN solution of LutHX (X = Cl, BPh_4) and 0.50 mL of a 0.10 M CH_3CN solution of hydrazine. Immediately after the addition of hydrazine, 0.10 mL of a 0.005 M solution of the catalyst in CH_3CN was added ($t = 0$). Samples (3.0 mL) were obtained at 1, 2, 4, and 6 min (all ±3 s).

Ammonia and Hydrazine Analysis. The addition of solid cobaltocene and Lut·HCl (requiring some time to dissolve; method A) makes it difficult to sample and analyze the reaction mixture at the very early stages (<5 min). The rapid mixing of LutHX and N_2H_4 solutions (method B) generates $N_2H_5^+X^-$, which at times precipitates (X = Cl^-) or may serve as a $N_2H_5^+$ source for the catalyst, which itself precipitates. The latter method, however, allows for sampling in the earlier stages of the reaction, often prior to the onset of precipitation.

Samples (3.0 mL) were removed from the reaction solution at predetermined times, were immediately quenched with an excess of HCl in Et_2O (0.2 mL), and were then taken to dryness *in vacuo*. The water-soluble species were extracted with 25 mL of deionized water. Aliquots (1 mL) of the aqueous extracts were used for ammonia determination with the indophenol test.[27] Most determinations were at least triplicate runs with a spread of approximately 7%. Periodic tests for hydrazine with the PDMAB method[28] ensured complete N atom balance (100 ± 5%).

Recovery and Identification of the $(Et_4N)_2[MoFe_3S_4Cl_3(H_2cit)]$, 7, Cluster Catalyst. In previous publications we have described in detail protocols for identifying a variety of MFe_3S_4 (M = Mo, V) cluster catalysts at the end of catalytic cycles.[19,29] Here, we briefly present analogous studies on the $(Et_4N)_2[MoFe_3S_4Cl_3(Hcit)$, **6**, cluster. For practical reasons we scaled up the amount of the cluster and performed a "pseudo-catalytic" experiment (N_2H_4:cluster 2:1). An 0.27 g sample of **6** (0.25 mmol) was dissolved in 40 mL of CH_3CN. Then, hydrazine (0.25 mL of a 0.2 M stock solution in CH_3CN, 0.50 mmol) was added dropwise, and finally $Co(Cp)_2$ (0.02 g, 1.00 mmol) and Lut·HCl (0.03 g, 2.00 mmol) were added under vigorous stirring. The solution was stirred for ~3 h, during which all of the cluster precipitated, leaving a yellow supernatant liquid (due to $Co(Cp)_2Cl$, identified from its characteristic electronic spectrum). The brown-black residue was isolated by filtration and tested for ammonia. The residue was washed thoroughly with CH_3CN, THF, EtOH, and Et_2O (to remove the byproducts) and finally dried *in vacuo*. An 0.25 g amount of the final product was isolated, which corresponds to 92% recovery of the catalyst. This material is totally insoluble in all solvents, so solution studies proved impossible. The far-infrared spectrum (302, 318, 346, 374, 383, 412 cm^{-1}), is consistent with a cubane structural core.[14] Mid-infrared spectrum (cm^{-1}): ν_{asym}(C═O) 1552, 1606, 1638, 1712. Vibrations assigned to $Co(Cp)_2^+$:[30] 460, 866, 1414, 3100 cm^{-1}. Anal. Calcd for $MoFe_3S_4Cl_3Co_2C_{26}H_{26}$ (**7**, MW 1085.85), $[Co(Cp)_2]_2[MoFe_3S_4Cl_3(H_2cit)]$: C, 28.73; H, 2.39; N, 0.0. Found: C, 28.43; H, 2.59; N, 0.0. It should be noted that actually compound **7** (the protonated form of **6**) is isolated at the end of the reaction (*vide infra*). Previous studies on the identification of recovered catalyst **1** included elemental analyses, electronic spectroscopy, infrared spectroscopy, and quantification of the characteristic $S = 3/2$ EPR signal. Those results unequivocally demonstrated that there is little or no loss of the catalyst (by decomposition) during catalytic cycles and the $[MoFe_3S_4]^{3+}$ core remains intact after the completion of the reaction.[29]

Results and Discussion

The X-ray structure of the Fe/Mo/S aggregate of nitrogenase[4,5] has revealed the Mo atom to be coordinatively saturated and located on the periphery of the $MoFe_6S_9$ core. These observations have led to the suggestion that perhaps the Mo atom is not *directly* involved in the activation and reduction of N_2 or other substrates.[4] The merits of this conclusion may be explored by reactivity studies on synthetic Mo/Fe/S clusters in which the Mo coordination closely resembles the Mo environment in the nitrogenase cofactor. The Fe/Mo/S "cubane" clusters that contain the $[MoFe_3S_4]^{3+}$ cores are well suited for such studies. In these compounds the first and second coordination spheres around the Mo atoms are nearly identical to those found in the Fe/Mo/S center in nitrogenase.

Reactivity studies of the $MoFe_3S_4$ single cubanes have shown these clusters to be unreactive toward dinitrogen in either the oxidized (core charge = +3) or the reduced (core charge = +2) form.[13a] In contrast, coordination of hydrazine and substituted hydrazines to the Mo site of the $MoFe_3S_4$ cubanes occurs readily and has been demonstrated by spectroscopic techniques and by X-ray crystallographic studies.[31] The Fe sites, mainly coordinated by thiolate or halide terminal ligands, do not undergo ligand exchange with hydrazines, and to our knowledge, no Fe−N_2H_4 adduct has ever been isolated or even transiently detected. This lack of reactivity with hydrazines is in contrast to what is observed with acetylene. The Fe sites in the $[MoFe_3S_4]^{3+}$ and $[Fe_4S_4]^{2+}$ cubanes are capable of reducing C_2H_2 to C_2H_4, as shown by results obtained in this[18] and other laboratories,[32] and very likely the reduction is preceded by acetylene coordination and activation by the Fe atoms.

The recently reported catalytic reduction of hydrazine by the $MoFe_3S_4$ single cubanes is of some relevance to the function of nitrogenase considering that HN═NH and H_2N-NH_2 are possible intermediates in biological dinitrogen fixation. Indeed, Thorneley, Lowe, and others[21d−g] have detected hydrazine upon quenching nitrogenase under turnover conditions. Furthermore,

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(28) *p*-(Dimethylamino)benzaldehyde (PDMAB) method: Watt, G. W.; Chrisp, J. D. *Anal. Chem.* **1952**, *24*, 2006.
(29) Details on these studies have been deposited as supplementary material with ref 17. Also, see ref 23.
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(31) (a) Challen, P. R.; Koo, S. M.; Kim. C. G.; Dunham, W. R.; Coucouvanis, D. *J. Am. Chem. Soc.* **1990**, *112*, 8606. (b) Mosier, P. E.; Kim, C. G.; Coucouvanis, D. *Inorg. Chem.* **1993**, *32*, 3620. (c) Challen, P. R. Ph.D. Thesis, The University of Michigan, 1990.
(32) (a) Tanaka, K.; Honjo, M.; Tanaka, T. *J. Inorg. Biochem.* **1984**, *22*, 187. (b) Tanaka, T.; Nakamoto, M.; Tashiro, Y. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 316. (c) McMillan, R. S.; Renaud, J.; Reynolds, J. G.; Holm, R. H. *J. Inorg. Biochem.* **1979**, *11*, 213.

Table 1. Production (%) of NH_3[a] from the Catalytic Reduction of NH_2NH_2[b] by Various $MoFe_3$−Carboxylate Cubanes Using $Co(Cp)_2$ as the Reducing Agent and Lut·HCl as the Proton Source (Method A; $[NH_2NH_2]$:[Catalyst] = 100:1)[c]

cluster catalyst[d]	no. of trials	5 min	30 min	1 h	12 h
$[MoFe_3S_4Cl_3(Cl_4\text{-cat})(CH_3CN)]^{2-}$ (**1**)	3	30	34	38	61
$[MoFe_3S_4Cl_3(Cl_4\text{-cat})(im)]^{2-}$ (**2**)	1	31	34	35	48
$[MoFe_3S_4Cl_3(mida)]^{2-}$ (**3**)	2	55	64	70	79
$[MoFe_3S_4Cl_3(Hnta)]^{2-}$ (**4**)	3	48	53	59	65
$[MoFe_3S_4Cl_3(tdga)]^{2-}$ (**5**)	3	38	45	47	65
$[MoFe_3S_4Cl_3(Hcit)]^{3-}$ (**6**)	4	83	92	96	98
$[MoFe_3S_4Cl_3(H_2cit)]^{2-}$ (**7**)	3	80	86	94	95
$[MoFe_3S_4Cl_3(Hcmal)]^{2-}$ (**8**)	3	62	66	68	78
$[MoFe_3S_4Cl_3(tla)]_2^{4-}$ (**9**)[e]	2	58	58	66	71
$[(MoFe_3S_4Cl_4)_2(\mu\text{-ox})]^{4-}$ (**10**)[e]	2	63	71	77	85
$[MoFe_3S_4Cl_4(dmpe)]^{-}$ (**11**)	1	12	13	14	14
$(Fe_4S_4Cl_4)^{2-}$ (**12**)	2	0	0	0	7

[a] The experiments were performed according to a protocol described in the Experimental Section. [b] Periodically, N_2H_4 quantification with the *p*-(dimethylamino)benzaldehyde method[28] confirmed complete N atom balance. In all cases, there was a balance of 100 ± 5%. [c] Sampling of the reaction mixture and treatment of the samples were performed as described previously.[19] The results were reproducible to within 5% from the mean. [d] Reactions of catalyst with $Co(Cp)_2$ and Lut·HCl (absence of N_2H_4) or of N_2H_4 with $Co(Cp)_2$ and Lut·HCl (absence of catalyst) gave NH_3 within background limits (<3%). [e] For clusters **9** and **10** two molecules of N_2H_4 per double cubane (or one N_2H_4 per Mo) were used in the experiments.

the presence of N_2H_4 has also been detected by Dilworth and Eady[33] in the reduction of N_2 to NH_3 by the alternative V nitrogenase.[9]

A cofactor essential for nitrogenase function is the homocitrate molecule coordinated to the Mo atom of the FeMo-co. It has been reported that the nif*V* gene which encodes for the synthesis of homocitrate is needed for the biosynthesis of all three (FeMo-co, FeV-co, and FeFe-co) cofactors.[34] The discovery of the Mo-bound homocitrate in the FeMo-co led to exhaustive biochemical studies.[6] The catalytic competence of the FeMo-co was investigated in the presence of various polycarboxylate ligands in experiments designed to delineate the possible role of the homocitrate ligand in N_2 fixation. A number of interesting results have derived from this sructure/function study: (a) a minimum of two −COOH groups and one −OH (in some cases a keto) group is required for activity; (b) the D acids are preferred over the L acids; (c) small changes on various parts of the homocitrate ligand cause dramatic effects on the N_2 fixing ability of the FeMo-co. Citrate, the acid anion structurally closest to homocitrate, effected the biosynthesis of a FeMo-co which could fix N_2, albeit at very low yields and at much higher concentrations than homocitrate.[6g] The apparent reactivity differences tunable by slight modifications of the Mo coordination environment and/or alterations in the polycarboxylate chain[6f,j] prompted us to explore the reactivity characteristics of the synthetic $MoFe_3S_4$−polycarboxylate cubanes. The methodology for the synthesis of these compounds has been developed in our laboratory[14,22,23] and has made them available for the studies reported herein.

The catalytic reduction of hydrazine was attempted with a variety of these clusters, and the results are compiled in Table 1 and displayed in Figure 3. All of the polycarboxylate clusters have a coordinatively saturated Mo site, and the coordination and activation of N_2H_4 must be preceded by at least partial dissociation of the carboxylate ligand. With the high proton concentrations of acid that prevail under the reaction conditions (100-fold excess of LutH·HCl), in addition to hydrazine

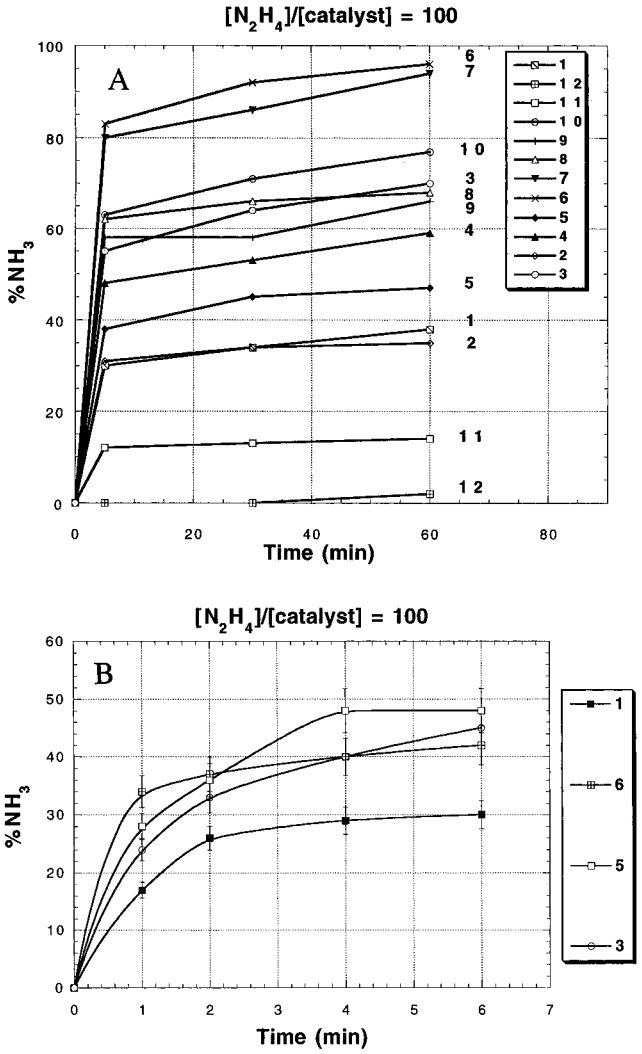

Figure 3. (**A**) Ammonia production (%) *vs* time in CH_3CN solvent, at ambient temperature, from the reduction of N_2H_4 in the presence of $Co(Cp)_2$ and Lut·HCl under initially heterogeneous conditions using various catalysts, as indicated. This graph was based on data taken from Table 1 using method A (see Experimental Section). (**B**) Ammonia production (%) *vs* time in CH_3CN solvent, at ambient temperature, from the reduction of N_2H_4 in the presence of $Co(Cp)_2$ and Lut·$HBPh_4$ under initially homogeneous conditions using various catalysts, as indicated (method B, Experimental Section).

protonation, facile protonation and partial dissociation of the bound carboxylate functions in compounds **3**−**8** are expected to occur. The yields of ammonia, obtained for all clusters after 5 min of reaction time (Table 1, Figure 3), represent 80%−90% of the ammonia produced after 1 h. This rapid leveling off of the amount of NH_3 produced indicates that the anionic cluster catalysts rapidly precipitate as insoluble $[Co(Cp)_2]^+$, $N_2H_5^+$, or NH_4^+salts. A reaction of **8** with 2 equiv of hydrazine in the presence of $[Co(Cp)_2]$ and Lut·HCl has resulted in the formation of the very insoluble $[Co(Cp)_2]_2[(CitH_2)MoFe_3S_4(Cl)_3]$ product (see Experimental). The onset of precipitation for each cluster is difficult to ascertain, and consequently meaningful initial rates (at <5% of substrate consumption) are nearly impossible to measure. As a result, the amount of NH_3 obtained after 5 min (Figure 3) for each of the clusters cannot be used as an indicator of relative reactivity.

In an attempt to obtain meaningful kinetic data, conditions were adopted that made it possible to sample the reaction mixture at times less than 5 min in the "initial rate" linear region. These conditions consist of the initial dissolution of the $[Co(Cp)_2]$ and Lut·HX reagents in CH_3CN followed by the addition of hydrazine and finally a solution of the catalyst that

(33) Dilworth, M. J.; Eady, R. R. *Biochem. J.* **1991**, *277*, 46.
(34) Kennedy, C.; Dean, D. *Mol. Gen. Genet.* **1992**, *231*, 494.

defines $t = 0$. The choice of counterion in Lut·HX had a profound influence in the results. For $X = Cl^-$, upon addition of hydrazine, a white precipitate was observed, presumably $N_2H_5^+Cl^-$. The percent conversion of hydrazine to ammonia was low after 6 min (~16%) for a selected group of clusters (Cl_4-cat, mida) probably limited by the rate of dissolution of $N_2H_5^+Cl^-$. Reactions with Lut·HCl were therefore discontinued. For $X = BPh_4^-$, the reactions were considerably faster and the results are shown in Figure 3B for four cubanes (Cl_4-cat, mida, Hcit, tdga). These results indicate little or no difference between the relative rates of hydrazine reduction between the clusters. All show an initial burst to about 25% which then slowly levels off to about 50% in 6 min. Due to the lack of precision in aqcuiring data in times less than 1 min, initial rates cannot be obtained reliably. Nevertheless, the curvature in the lines (Figure 3B) may indicate relative rates in addition to the onset of catalyst precipitation. It appears likely that, by having all components soluble initially, the "true" rate of reaction can be witnessed. Unfortunately reliable data cannot be obtained in times less than 1 min from $t = 0$. An examination of Figure 3 shows that the carboxylate cubanes are more efficient catalysts than the Cl_4-cat cubane, **1**. The rapid leveling off of the rate for the citrate cubane, **6**, after only 1 min of reaction time indicates that this cluster precipitates very rapidly and may have a faster initial rate than indicated. The overall greater yields of ammonia over the same period of time obtained with method A (Figure 3A) very likely reflect a control of the rate (slowing down) of precipitation of the catalyst by the slow release of the reagents in solution and the smaller ionic strength of the solution.

In addition to generating a coordination site on the Mo atom, protonation causes anodic shifts of the electrochemical reduction potentials of the clusters. For example, addition of a 5-fold excess of Lut·HCl to **5** shifts the reduction potential from −750 to −670 mV.[35] Such shifts are at times needed to bring the reduction potential of the clusters within the reducing capacity of cobaltocene (under identical conditions,[35] $E_{1/2}$ of cobaltocene = −940 mV). In catalytic studies with the $[VFe_3S_4]^{2+}$ cubanes,[19] Lut·HCl, effectively used in the catalytic reaction, is sufficiently acidic ($pK_a(CH_3CN) = 14.1$) to protonate hydrazine. Acids weaker than $N_2H_5^+$ ($pK_a(CH_3CN) = 16.6$) such as Et_3NH^+ ($pK_a(CH_3CN) = 18.3$) however are not effective, and as a consequence the use of Et_3NH^+ as a source of protons shuts off the catalytic process.

Hypotheses concerning the possible role of the unique homocitrate ligand have been presented in the literature and include possible modulation of the electrochemical potentials or participation in proton transfer steps.[1e,g,6] The latter seems more reasonable, taking into account the fact that the bidentate coordination mode of the homocitrate ligand allows two uncoordinated −COOH (or −COO−) groups to become involved in protonation/deprotonation steps.[36]

The qualitative results obtained with the majority of the carboxylate $MoFe_3S_4$ clusters **3**−**10** show that the catalytic reduction of hydrazine is occurring generally with a higher efficiency than that of either **1** or **2.** Furthermore, the apparent coordination saturation of the Mo atom in these clusters does not interfere with the catalytic process.

Cluster **2** has the same core structure and Mo coligands as **1** except that it has an imidazole coordinated to the Mo instead of an CH_3CN (a histidine imidazole has been found coordinated to the Mo of the FeMo-co). Its catalytic efficiency is almost identical to that **1** and indicates that the imidazole ligand dissociates from the Mo (very likely as a result of protonation[37,38]), thus generating a vacant site on the Mo for N_2H_4 coordination and reduction.

In the $MoFe_3S_4$ cubanes, the Mo−O (catecholate or carboxylate) moieties are located in positions that allow for hydrogen-bonding interactions with the Mo-coordinated hydrazine molecule. Such interactions are evident in the structures of the $[MoFe_3S_4Cl_3(Cl_4\text{-cat})(NH_2NHPh)]^{2-}$ [39] and $\{[MoFe_3S_4Cl_2(Cl_4\text{-cat})]_2(\mu\text{-}N_2H_4)(\mu\text{-}S)\}^{4-}$ clusters.[13d,31a] Although the H atoms of the $PhNHNH_2$ or N_2H_4 molecules were not located, the distances between the tetrachlorocatecholate oxygen atoms and the hydrazine nitrogens (2.73−3.85 Å) are indicative of hydrogen-bonding interactions. Not unlike the possible involvement of the carboxylate ligands in H^+ transfer, the catecholate oxygen atoms also may be important in the protonation of the substrate during the catalytic process.

The lack of reactivity of cluster **11** (Figures 2 and 3) may be attributed to the following factors: (a) the nonlability of the Mo-bound Cl, which already has been established[25] for this cluster, and (b) the inability of the dmpe ligand to serve as an effective proton transfer site. The "all-Fe" cluster **12** was found totally inactive in the reduction of N_2H_4 to NH_3, showing only a 7% conversion after 12 h.[40] A possible explanation for this lack of reactivity could be the inability of N_2H_4 to displace the Fe-bound Cl^- ligands.[41]

Inhibition Studies. To further explore the importance of the direct Mo involvement in the catalytic process, attempts were made to inhibit hydrazine reduction with ligands that are known to bind exclusively and irreversibly to the Mo atom in the $[MoFe_3S_4]^{3+}$ cubanes. Triethylphosphine is one such ligand,[43] and in its presence the reduction of hydrazine is strongly inhibited (Figure 4). The observed low (above background) levels of NH_3 production can be explained by (a) the dissociation of some of the bound PEt_3 in the presence of a large excess of N_2H_4 or (b) the H^+-promoted dissociation of one "arm" of the Cl_4-cat ligand that generates a vacant coordination site on the Mo atom.

(35) Voltammetric measurements in CH_3CN solution vs a Ag/AgCl reference electrode using Pt working and auxiliary electrodes and Bu_4NClO_4 as supporting electrolyte.

(36) It is very interesting to notice that the closely related citrate binds to an Fe of an Fe_4S_4 cluster in the active site of aconitase, in the same bidentate fashion: (a) Glusker, J. P. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1971; p 413. (b) Lauble, H.; Kennedy, M. C.; Beinert, H.; Stout, C. D. *Biochemistry* **1992**, *31*, 2735. (c) Kennedy, M. C.; Stout, C. D. In *Advances in Inorganic Chemistry*, *Vol. 38*; Cammack, R., Sykes, A. G., Eds.; Academic Press: New York, 1992; p 413.

(37) In dimethylformamide, DMF, for Lut·HCl, $pK_a \approx 5.0$; for citric acid, $pK_{a1} = 7.2$, $pK_{a2} = 10.9$, and $pK_{a3} = 12.2$: Izutsu, K. *Acid Base Dissociation Constants in Dipolar Aprotic Solvents*; IUPAC Chemical Data Series, No. 35; Blackwell Scientific Publications: Oxford, U.K., 1990.

(38) (a) Benoit, R. L.; Lefebvre, D.; Frechette, M. *Can. J. Chem.* **1987**, *65*, 996. (b) Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456.

(39) Coucouvanis, D.; Patton, S.; Kim, C. K. Unpublished results.

(40) This cluster has been found effective in the reduction of acetylene at a rate much slower than that of the Mo cubanes.[18]

(41) Tanaka *et al*. have reported N_2H_4 reductions to NH_3 using electrochemically generated forms of the $[Fe_4S_4(SR)_4]^{2-}$ cluster (R = SPh, SCH_2CH_2OH) as the active center (Hozumi, Y.; Imasaka, Y.; Tanaka, K.; Tanaka, T. *Chem. Lett.* **1983**, 897). These systems differ from ours in that they are substoichiometric (noncatalytic) and employ thiolates as Fe ligands. It is well established that Fe-bound thiolates cathodically shift the electrochemical potentials by least 200 mV;[42] therefore, the reduced cluster is "charged" with higher reducing power.

(42) (a) Cambray, J.; Lane, R. W.; Wedd, A. G.; Johnston, R. W.; Holm, R. H. *Inorg. Chem.* **1977**, *16*, 2565. (b) Hill, C. L.; Renaud, J.; Holm, R. H.; Mortenson, L. E. *J. Am. Chem. Soc.* **1977**, *99*, 2549.

(43) (a) Holm, R. H. In *Biomimetic Chemistry*; Yoshida, Z., Ise, N., Eds.; Elsevier: New York, 1983; p 79−99. (b) Mascharak, P. K.; Armstrong, W. H.; Mizobe, Y.; Holm, R. H. *J. Am. Chem. Soc.* **1983**, *105*, 475.

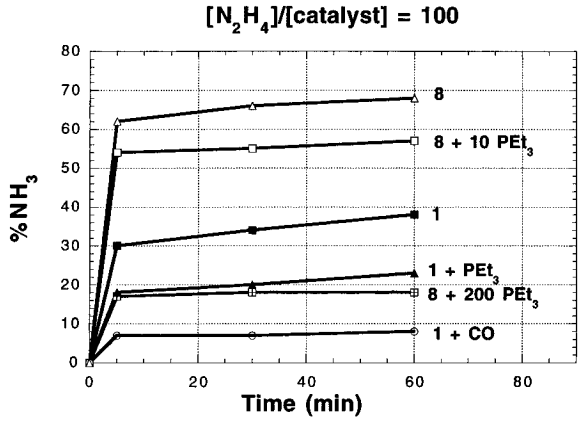

Figure 4. Ammonia production (%) *vs* time in CH_3CN solvent, at ambient temperature, from the reduction of N_2H_4 in the presence of PEt_3 or CO as inhibitor, $Co(Cp)_2$, and Lut·HCl using various catalysts, as indicated. This graph was based on data taken from Table 2.

Table 2. Production (%) of NH_3 by the Catalytic Reduction of NH_2NH_2 by Various $MoFe_3$−Carboxylate Cubanes in the Presence of Inhibitor PEt_3 or CO (Experimental Conditions as in Table 1; $[NH_2NH_2]$:[Catalyst] = 100:1)

cluster catalyst	no. of trials	5 min	30 min	1 h	12 h
$[MoFe_3S_4Cl_3(Cl_4\text{-cat})(CH_3CN)]^{2-}$ (**1**)[a]	3	30	34	38	61
$[MoFe_3S_4Cl_3(Cl_4\text{-cat})(CH_3CN)]^{2-}$ (**1**) + PEt_3	2	18	20	23	37
$[MoFe_3S_4Cl_3(Cl_4\text{-cat})(CH_3CN)]^{2-}$ (**1**)/CO[b]	2	7	7	8	9
$[MoFe_3S_4Cl_3(Hcmal)]^{2-}$ (**8**)[a]	3	62	66	68	78
$[MoFe_3S_4Cl_3(Hcmal)]^{2-}$ (**8**) + PEt_3 (1:10)	1	54	55	57	66
$[MoFe_3S_4Cl_3(Hcmal)]^{2-}$ (**8**) + PEt_3 (1:200)	1	17	18	18	19

[a] For comparison reasons NH_3 yields (%) from Table 1 using clusters **1** and **8** as catalysts have been also included. [b] In this experiment, the reaction flask was evacuated and immediately refilled with CO (Johnson & Mathey) as soon as all the reagents were mixed.

Carbon monoxide is known to inhibit nitrogenase reactivity;[44] furthermore, CO can bind to the $MoFe_3S_4$ cubanes *only* at their reduced (2+) oxidation level.[43b] In both cases, the precise site of CO coordination (Fe, Mo, or both) is not entirely clear. A hydrazine reduction experiment using cluster **1** as a catalyst was performed under a CO atmosphere. The ammonia production yields (Table 2), although slightly above background, were much lower than the corresponding yields under a N_2 atmosphere (Figure 4). This result suggests that the $[MoFe_3S_4]^{3+}$ core undergoes reduction to the 2+ oxidation level during the catalytic process, where it is attacked and irreversibly inactivated by CO (very likely at the Mo site). The fact that hydrazine protonation precedes cluster reduction further suggests that the CO molecules compete with the $N_2H_5^+$ ligand for binding sites in the Mo coordination sphere. The PEt_3 and CO ligands previously have been used successfully in our laboratory as inhibitors in N_2H_4 reduction by the $[VFe_3S_4]^{2+}$ cores[19] and in C_2H_2 reduction by the $[MoFe_3S_4]^{3+}$ cores.[18]

Mononuclear *vs* Binuclear Activation of N_2H_4. Recently we reported that the $\{[MoFe_3S_4Cl_2(Cl_4\text{-cat})]_2(\mu\text{-}N_2H_4)(\mu\text{-S})\}^{4-}$ doubly bridged double cubane[31a] did not reduce the end-on bridging hydrazine to ammonia in the presence of $Co(Cp)_2$ and Lut·HCl.[17] This result was explained by the unavailability of lone pairs on the bridging hydrazine molecule for the essential initial protonation and subsequent reduction. These data are consistent with results reported for many other *binuclear* transition metal complexes that contain end-on bridging N_2. These complexes upon reduction/protonation give high yields of N_2H_4[45] but little or no NH_3.[46] In the case of the $MoFe_3S_4$−carboxylate cubanes, steric constraints preclude dimerization that would result in an end-on bridging N_2H_4 ligand and the activation/reduction of hydrazine must occur on a single metal site.

The catalytic reduction and disproportionation of N_2H_4 by a binuclear Mo complex with pyridinethiolate coligands has been reported.[47] The actual nature of the active catalyst and the coordination mode of the hydrazine ligand are not clear. In this system, N_2H_4 may be activated either by an end-on bridging fashion or (more likely) with the two Mo centers acting independently in the activation of two terminally bound N_2H_4 molecules. Kuwata *et al*. recently reported the *disproportionation* of N_2H_4 and substituted N_2H_4 with a binuclear Ru complex.[48] In this case, binuclear activation of N_2H_4 seems attractive due to the coordination unsaturation of both Ru centers.

Possible Role of the Mo-Bound Polycarboxylate Ligands in the Function of the $[MoFe_3S_4]^{3+}$ Cubanes and Their Relevance to Homocitrate in the FeMo-co. A possible pathway for the reduction of N_2H_4 to NH_3 by the polycarboxylate cubanes shown in Figure 5 is similar to that published for the reduction effected by the VFe_3S_4 cubanes[19] but also includes the possible involvement of the carboxylate ligands. The starting point (**A**) (Figure 5) is the proton-induced dissociation of a carboxylate ($-COO^-$) "arm" from the Mo atom, generating a vacant site for N_2H_4 coordination (**B**). The subsequent Mo−N_2H_4 adduct may be stabilized *via* hydrogen-bonding interactions ((hydrazine)N-H···O or (carboxylate)O−H···N). An initial protonation to the β-N of N_2H_4 occurs (**C**) which is followed by $1e^-$ reduction (**D**). An additional protonation releases the first equivalent of NH_4^+, leaving a Mo−NH_2 moiety (**E**) (or alternatively a Mo═NH (**F**) group). At this point, protonation by the uncoordinated −COOH groups and subsequent reduction will cause the release of the second equivalent of NH_4^+ and the regeneration of the starting cluster **A**.

At this stage, it is difficult to predict in which protonation step the −COOH group participates. Furthermore, it is uncertain whether the H^+ ions originate from a −COOH group, a (Mo)−OH group, or a combination of both. In contrast to intermediate **C** (Figure 5), which has been isolated and characterized with Cl_4-cat in place of citrate on the Mo,[49] **D**, **E**, and **F** have not been isolated or detected.

Proposed Pathway for Nitrogen Fixation by Nitrogenase. A plethora of binuclear complexes with bridging N_2 have been synthesized, and many of them have been stucturally character-

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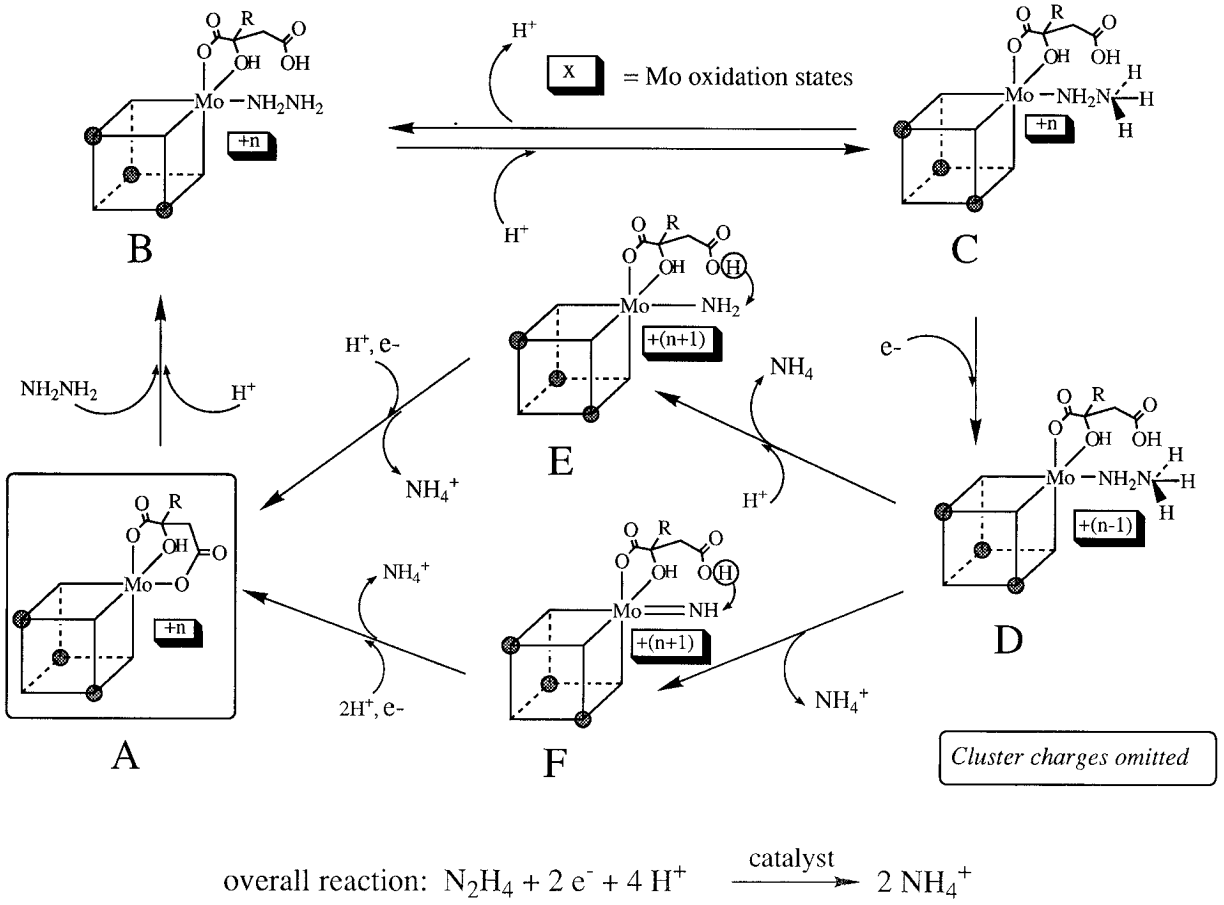

overall reaction: $N_2H_4 + 2\,e^- + 4\,H^+ \xrightarrow{\text{catalyst}} 2\,NH_4^+$

Figure 5. Proposed pathway for the catalytic reduction of N_2H_4 to NH_3 by cluster $[(H_2cit)MoFe_3S_4Cl_3]^{2-}$, **7**, with emphasis on a possible H^+ delivery role of the carboxylate function. The intra- and intermolecular electron transfer steps arbitrarily are depicted by changes in the formal oxidation level of the Mo atom.

ized.[50] Many show exceptional reactivity upon reaction with strong acids, e.g. HX (X = Cl, Br), with N_2H_4 being the product,[45] but only a small number of them yield NH_3, usually in very low yields.[46,50] Proton attack on the μ-N_2 (or on the μ-HN=NH at a subsequent step[51,52]) ligand can be easily visualized given the π-electron density of the bridging unit. Once the μ-N_2H_4 level is reached however, protonation is impossible because neither π-electron density nor lone pairs on the N atoms are available any longer. As a consequence, reduction of μ-N_2 ceases at the hydrazine level.

In mononuclear complexes, terminal N_2 ligands are reductively transformed to terminally bound hydrazines with lone pairs on the unbound nitrogen atoms available for protonation and subsequent reduction. As a result, the N_2 ligands in these complexes are reduced readily to NH_3.[21e,50d,53] In these reductions, several metal−hydrazido ($N_2H_x^{n-}$; $x = 1-3$; $n = 1-3$) intermediates have been isolated and some have been structurally characterized.[11c,20b,54]

A proposed pathway (Figure 6) for the reduction of N_2 to NH_3 consistent with the available data on the reduction of N_2 and N_2H_4 in model complexes, the structure of the nitrogenase cofactor, and previously suggested mechanisms[7] is as follows: (a) The N_2 molecule initially coordinates to one of the Fe_4 square faces[7b] of the Fe_6 prismatic unit of the cofactor (step 1). (b) The bridging N_2 undergoes proton attack followed by a two-electron reduction to diazene. Subsequent protonation followed by reduction leads to hydrazine (or hydrazide).[45] Protonation steps during the first two steps of reduction ($N_2 \rightarrow HN{=}NH \rightarrow NH_x{-}NH_y$[55]) may be facilitated by the μ_2-S ligands in the center of the FeMo-co, perhaps forming μ-S···H species (steps 2 and 3). (A N−H···S hydrogen bond between a histidine-195 imidazole and one of the central, doubly bridging, S ligands of the FeMo-co was recently reported.[56]). (c) At the hydrazine stage, the bridging N_2H_4 cannot be protonated and further reduced due to the unavailability of lone pairs on the N atoms. It is likely, then, that the hydrazine molecule either changes to a monodentate binding mode or *migrates* from the Fe site(s) to the Mo site and coordinates to it in a terminal fashion (step 4).

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(51) In fact, such a pathway has been proposed to take place upon protonation of the thermally unstable $[Fe(^iPr)(PPh_3)_2]_2(\mu\text{-}N_2)$ complex. The final product of this transformation is N_2H_4: Yu, G.; Borodko, M. O.; Boitman, L. M.; Kachapina, A. E.; Shilov, A. E.; Yu, L. *Chem. Commun.* **1971**, 1185.

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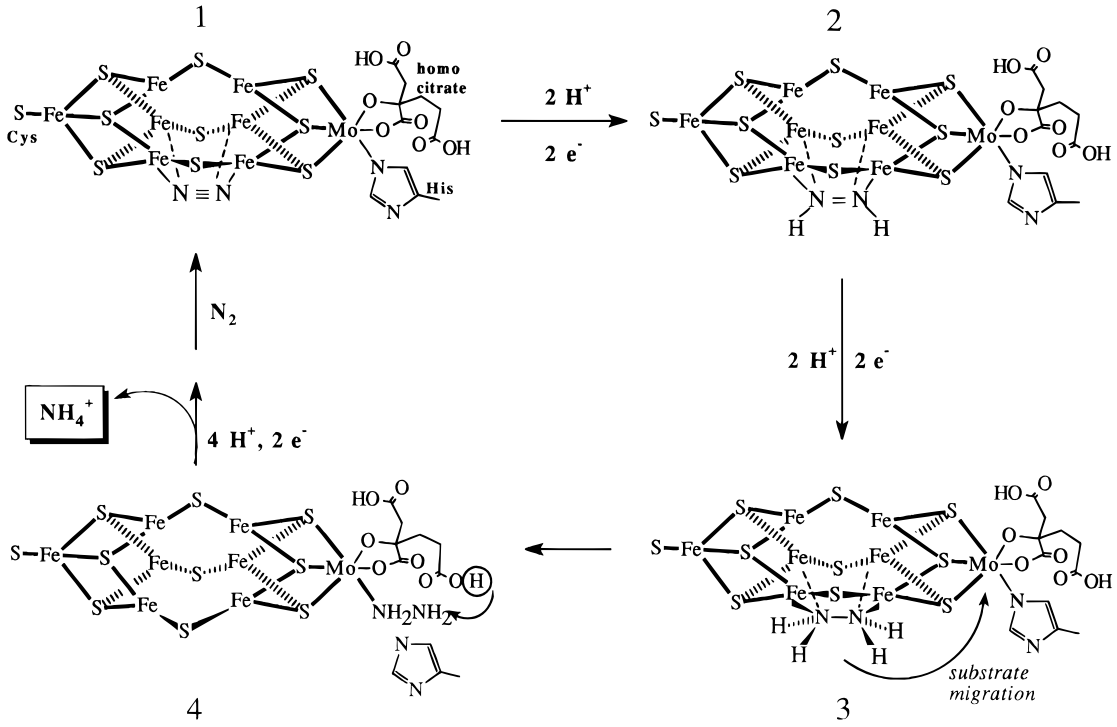

Figure 6. Possible mechanism for the binding, activation, and reduction of N_2 to NH_3 by the FeMo-co based on a combination of available literature data and the present study. Diazene (HN═NH) and hydrazine (NH_2NH_2) as possible intermediates are also shown.

These events possibly are accompanied by positional, conformational, and electronic changes in the vicinity of and/or within the FeMo-co. The protein environment surrounding the FeMo-co plays an important role in the substrate reduction process, since the *isolated* FeMo-co does not reduce N_2. Other substrates such as acetylene and cyclopropene are reduced by the cofactor, albeit at a small fraction (8%) of the enzymatic rate.[57,58] (d) During the last reduction step the Mo-bound homocitrate ligand may participate in proton transfers (see also the proposed pathway in Figure 5) to the substrate (hydrazine).

Abiological nitrogen fixation systems that involve carboxylate coligands have been reported. Early reports include studies by Folkesson and Larsson[59] on N_2 reduction to N_2H_4 utilizing V^{II} species in the presence of α,ω-dicarboxylates ($^-OOC(CH_2)_n$-COO^-, $3 \le n \le 10$) and the photocatalytic reduction of N_2 to NH_3 by Ru(EDTA) complexes.[60] Similarly, $Ru^{III}(EDTA)(H_2O)$ compounds catalyze the reduction of N_2H_4 to NH_3 in aqueous acidic solution.[20a] The above studies emphasized the positive effects of the polycarboxylate ligands in the process. However, their possible role as proton transfer devices was not invoked. In a recent report, N_2 binding to $[MH_2(\eta^2\text{-}OOCCH_3)(dppe)]^+$ (M = Mo, W) was reported to require initial dissociation of the acetate ligand.[61]

The pathway proposed in Figure 6 does not include or attempt to explain the H_2 evolution concomitant with N_2 reduction (eq 1). It has been proposed[62] that binding of a H^+ to the FeMo-co (possibly involving μ_3-S ligands) and its $2e^-$ reduction to H^-, can be envisioned as a step that prepares the enzyme to bind N_2, and dihydrogen, H_2, is displaced when N_2 binds to the enzyme.[62] The participation of the Mo-bound core μ_3-S ligands in the FeMo-co during the last protonation steps is possible and may play an important role. Indeed, the presence of hydrogen bonds between the μ_3-S ligands in the $[Fe_4S_4]^{2+}$ centers and N−H groups (from neighboring amino acids) is well documented.[63]

Summary

The principal findings and conclusions of this investigation are the following: (a) The $[MoFe_3S_4]^{3+}$ cores catalytically reduce N_2H_4 to NH_3 with Lut·HCl as the proton source and $Co(Cp)_2$ as the reducing agent. (b) The Mo-bound carboxylate ligands under acidic conditions partially dissociate and allow coordination of hydrazine to the Mo atom. They appear to promote rather than hinder the catalytic reduction of hydrazine to ammonia. (c) Control experiments with the Fe_4S_4 cubanes and inhibition studies, with ligands that are known to coordinate to the Mo atom, have shown total or pronounced loss of catalytic activity. These results support the suggestion that reduction occurs at the Mo site. (d) The possible involvement of the carboxylate functions as "built-in", local, H^+ delivery agents may play an important role in catalysis. The homocitrate molecule in the FeMo-co may very well function in a similar manner, liberating a site on the coordinatively saturated Mo and subsequently providing the protons to the substrate(s).

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