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The Catalytic Reduction of Hydrazine to Ammonia by the $MoFe_3S_4$ Cubanes and Implications Regarding the Function of Nitrogenase. Evidence for Direct Involvement of the Molybdenum Atom in Substrate Reduction

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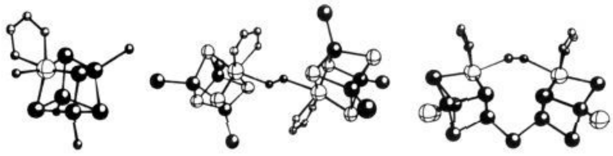
The vast majority of the Fe/Mo/S clusters that have been synthesized and proposed as structural models for the nitrogenase active site[1] suffer from the drawback that they do not mediate chemistry related to dinitrogen reduction.[2] An important exception is the $[Mo_2Fe_6S_8(SPh)_9]^{3-}$ cluster that, in an electrochemically generated reduced form, has been found effective in the reduction of nitrogenase substrates.[3] Also important from *a fundamental chemical point of view are the protonation/* reduction reactions reported for the dinitrogen or hydrazine ligands in certain molybdenum or tungsten complexes.[4–8]

In this communication we report our studies on the catalytic reduction of hydrazine to ammonia by synthetic Fe/Mo/S clusters that structurally resemble the Fe/Mo/S site of nitrogenase. The recently determined structure of the Fe/Mo protein of nitrogenase[9,10] has shown the Fe/Mo/S active center to contain Fe_4S_3 and $MoFe_3S_3$ cuboidal subunits bridged by two[9] or three[10] sulfide ligands. The location of the coordinatively saturated Mo atom, in the periphery of the Fe/Mo/S cluster, has raised doubts concerning its direct involvement in catalysis.

The syntheses in our laboratory of singly[11] (Figure 1, **II**) and doubly[12] (Figure 1, **III**) bridged "double cubanes" have made available clusters that contain cuboidal subunits with two $MoFe_3S_4$ cores and a N_2H_4 molecule bridging the two Mo atoms. The extent to which the latter is activated toward N–N bond cleavage was explored by allowing the "double cubanes" to react with reducing agents in the presence of protons in either stoichiometric or catalytic reactions. Of these, **III** did not promote the reduction of hydrazine to ammonia and only low concentrations of ammonia were detected when **II** was used as a catalyst. By far the most active catalysts were the $[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3{\cdot}L]^{n-}$ (Figure 1, **Ia**, L = CH_3CN,[16] $n = 2$) and $[(citr)MoFe_3S_4(Cl)_3]^{3-}$ (Figure 1, **Ib**) single cubanes.



I II III

Figure 1. Molecular structures of **I**, the $[(cat)MoFe_3S_4(Cl)_3{\cdot}L]^{n-}$ cubane clusters (L = a neutral, $n = 2$, or anionic, $n = 3$, ligand; cat = a substituted catecholate dianion; ref 1b); **II**, the $\{[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3]_2(N_2H_4)\}^{4-}$, singly-bridged double cubane (ref 11); **III**, $\{[(Cl_4\text{-cat})MoFe_3S_4(Cl)_2]_2\text{-}(\mu\text{-}N_2H_4)(\mu\text{-}S)\}^{4-}$ (refs 1a, 12a). In all structures, for clarity, only the two oxygen donors and the attached carbon atoms of the Cl_4-cat ligand are shown.

Catalytic reductions were investigated (eq 1) with various N_2H_4:cubane ratios (10:1, 20:1, and 40:1) with cobaltocene, $Co(Cp)_2$, as a reducing agent and 2,6-lutidine hydrochloride, Lut·HCl, as a source of protons. In the absence of $Co(Cp)_2$ and Lut·HCl, **Ia** slowly catalyzed the disproportionation of hydrazine at a rate approximately an order of magnitude slower than that of the catalytic reductions (eq 1). This disproportionation reaction[14] (eq 2) was not catalyzed by **Ib**.

$$N_2H_4 + 2e^- + 4H^+ \rightarrow 2NH_4^+ \tag{1}$$

$$3N_2H_4 \rightarrow 4NH_3 + N_2 \tag{2}$$

The results of the catalytic reductions obtained at ambient temperature in CH_3CN solution show that the single cubanes serve as catalysts with good turnover values[15] (Table I). The data show that in solutions 1.25×10^{-3} M in N_2H_4 and having $[N_2H_4]$:[catalyst] ratios of 10, 20, or 40 the catalytic reduction of hydrazine to ammonia (eq 1) is nearly quantitative after ca. 2 h. *The characteristics* $S = {}^3/_2$ *EPR spectra of these solutions, obtained after the catalytic reduction of* N_2H_4 *is complete, are virtually identical to those obtained from solutions in the pure catalyst prior to catalysis and show that the* $MoFe_3S_4$ *core retains its integrity and is directly involved in the catalysis.*

In more concentrated solutions (5×10^{-3} M in N_2H_4) the catalytic reduction of N_2H_4 stops before all of the hydrazine is reduced to ammonia. This is due to the precipitation ("salting-out") of $N_2H_4{\cdot}HCl$ that ensues after ca. ${}^1/_2$ h of reaction time and depletes the solution from substrate. At this concentration a significant amount of the Fe/Mo/S cluster also precipitates out of solution as a NH_4^+ salt.[16] The insoluble precipitates that form concomitantly with the production of NH_3 almost entirely dissolve in CH_3CN upon addition of Bu_4NI, and the resulting solution shows an electronic spectrum and a $S = {}^3/_2$ EPR signal

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[1] (a) Coucouvanis, D. *Acc. Chem. Res.* **1991**, *24*, 1. (b) Holm, R. H.; Simhon, E. D. In *Molybdenum Enzymes*; Spiro, T. G., Ed.; Wiley Interscience: New York, 1985; Chapter 1.

[2] Leigh, G. J. *J. Mol. Catal.* **1988**, *47*, 363.

[3] (a) Tanaka, M.; Tanaka, K.; Tanaka, T. *Chem. Lett.* **1982**, 767. (b) Tanaka, K.; Hozumi, Y.; Tanaka, T. *Chem. Lett.* **1982**, 1203. (c) Tanaka, K.; Imasaka, Y.; Tanaka, M.; Honjo, M.; Tanaka, T. *J. Am. Chem. Soc.* **1982**, *104*, 4258.

[4] (a) Chatt, J.; Dilworth, J. R.; Richards, R. L. *Chem. Rev.* **1982**, *78*, 589. (b) George, T. A.; Koczon, L. M.; Tisdale, R. C.; Gebreyes, K.; Ma, L. *Inorg. Chem.* **1991**, *30*, 883. (c) Cummins, C. C.; Baxter, S. M.; Wolczanski, P. K. *J. Am. Chem. Soc.* **1988**, *110*, 8731.

[5] Schrauzer, G. N.; Robinson, P. R.; Moorehead, E. L.; Vickrey, T. M. *J. Am. Chem. Soc.* **1976**, *98*, 2815 and references therein.

[6] Shilov, A. E. *J. Mol. Catal.* **1987**, *41*, 221.

[7] Schrock, R. R.; Glassman, T. E.; Vale, M. G.; Kol, M. *J. Am. Chem. Soc.* **1993**, *115*, 1760.

[8] Block, E.; Ofori-Okai, G.; Kang, H.; Zubieta, J. *J. Am. Chem. Soc.* **1992**, *114*, 758.

[9] (a) Kim, J.; Rees, D. C. *Science* **1992**, *257*, 1677. (b) Kim, J.; Rees, D. C. *Nature* **1992**, *360*, 553. (c) Chan, M. K.; Kim, J.; Rees, D. C. *Science* **1993**, *260*, 792.

[10] (a) Bolin, J. T.; Campobasso, N.; Muchmore, S. W.; Minor, W.; Morgan, T. V.; Mortenson, L. E. In *New Horizons in Nitrogen Fixation*; Palacios, R., Mora, J., Newton, W. E., Eds.; Kluwer Academic Publishers: Dordrecht, 1993; pp 89–94.

[11] Mosier, P. E.; Kim, C. G.; Coucouvanis, D. *Inorg. Chem.* **1993**, *32*, 2620.

[12] (a) Challen, P. R.; Koo, S.-M.; Kim, C. G.; Dunham, W. R.; Coucouvanis, D. *J. Am. Chem. Soc.* **1990**, *112*, 8606. (b) Challen, P. R. Thesis, University of Michigan, 1990. (c) Coucouvanis, D.; Challen, P. R.; Koo, S.-M.; Davis, W. M.; Butler, W. M.; Dunham, W. R. *Inorg. Chem.* **1989**, *28*, 4181.

[13] Coucouvanis, D.; Demadis, K. D.; Kim, C. G.; Dunham, R. W.; Kampf, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 3344. Cl_4-cat = the tetrachlorocatecholate dianion, and citr = the citrate trianion.

[14] The N_2 that forms in this reaction was detected as ${}^{15}N^{15}N$ by mass spectrometry in a catalytic disproportionation experiment that used ${}^{15}N_2H_4$ as a reagent.

[15] It was established that ammonia did not form in appreciable amounts from N_2H_4 or NH_2NHPh in the absence of the cubanes and in the presence of $Co(Cp)_2$ and Lut·HCl. It was also demonstrated that the CH_3CN that was used as a solvent did not undergo reduction under the reaction conditions.

[16] Solutions of Et_4N^+ **Ia** do not give precipitates upon addition of $LutH^+$, $[Co(Cp)_2]^+$, or $N_2H_5^+$ ions. Insoluble precipitates of **Ia** or **Ib** are obtained, however, with NH_4^+ in combinations with the above cations.

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Table I. Production of NH_3[a] by the Catalytic Reduction of N_2H_4 by **Ia**, $[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3(CH_3CN)]^{2-}$, or **Ib**, $[(citr)MoFe_3S_4(Cl)_3]^{3-}$, in the Presence of $Co(Cp)_2$ and Lut·HCl

catalyst	$[N_2H_4]$: [catalyst]	NH_3 yield,[b] equiv[c,d]			NH_3 max[e]	% conversion		
		1/2 h	1 h	2 h		1/2 h	1 h	2 h
Ia[f]	1	1.4(2)	1.5(2)	1.6(2)	2	68	74	80
	10	13.2(2)	14.6(2)	19.2(2)	20	66	73	96
	20	32.8(3)	37.2(3)	40.0(3)	40	82	93	100
	40	61.6(3)	69.6(3)	73.9(3)	80	77	87	92
Ib[f]	10	20.0(2)	20.0(2)	20.0(2)	20	100	100	100
	20	38.0(2)	38.4(2)	na[g]	40	95	96	na
	40	48.8(2)	56.8(2)	na	80	61	71	na
II[f]	1:2	0.6(2)	0.8(2)	1.0(2)	2	29	40	50
III[f]	1:2	*h*	*h*	*h*	2			
IV[b]	1[i,j]	0.5(2)	0.7(2)	0.8(2)	1	50	67	80
	10[i,j]	4.5(1)	5.8(1)	10.0(1)	10	45	58	100

[a] Reactions were carried out in CH_3CN, at ambient temperature under N_2 using the indicated catalyst and various amounts of N_2H_4 as shown in the second column. The initial concentrations of N_2H_4, $Co(Cp)_2$, and Lut·HCl were 1.25×10^{-3}, 2.50×10^{-3}, and 5.00×10^{-3} 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 N_2H_4 disproportionation reaction have been deposited as supplementary material. [b] The NH_3 was quantified by the indophenol method (Chaney, A. L.; Marbach, E. P. *Clin. Chem.* (*Winston-Salem, N.C.*) **1962**, *8*, 130), and the N_2H_4 was quantified with *p*-(dimethylamino)benzaldehyde (Watt, G. W.; Chrisp, J. D. *Anal. Chem.* **1952**, *24*, 2006). The protocol used in sample collection and treatment prior to analysis is identical to the one described in ref 7. [c] The average of *n* independent experiments is reported, where *n* is the number in parentheses. [d] Analyses for N_2H_4 were carried out randomly for experiments where the yield of NH_3 indicated less than 100% conversion. In general there was a nitrogen atom balance to 100 ± 5%. The results are included in a table deposited as supplementary material. [e] Maximum yield of NH_3 possible. [f] **Ia**: $[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3(CH_3CN)]^{2-}$. **Ib**: $[(citr)MoFe_3S_4(Cl)_3]^{3-}$. **II**: $\{[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3]_2(\mu\text{-}NH_2H_4)\}^{4-}$. **III**: $\{[(Cl_4\text{-cat})MoFe_3S_4(Cl)_2]_2(\mu\text{-}N_2H_4)(\mu\text{-}S)\}^{4-}$. **IV**: $\{[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3(NH_2NHPh)\}^{2-}$. [g] Not available, due to the precipitation of the catalyst from solution. [h] Only a small trace of ammonia was detected after 12 h. [i] Phenylhydrazine to cubane ratio. [j] Aniline was detected by a GC–mass spectroscopic measurement.

characteristic of **Ia**. This solution is active in the catalytic generation of NH_3 upon the addition of N_2H_4, Lut·HCl, and $Co(Cp)_2$.[17] Quantitative analytical studies of this system demonstrate convincingly that the cubane cluster retains its integrity through the course of the reaction.[20] The slow rate of ammonia formation in the stoichiometric reduction of **II** and the absence of NH_3 as a product in attempts to reduce **III** (Table I) indicate that *the hydrazine molecule is activated by coordination to one $MoFe_3S_4$ cubane, I, and the presence of an additional 1 equiv of cubane (that is known to give II) inhibits the reduction.* Apparently, 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 reactivity of **II** (Figure 1) may well be attributed to **I** that likely exists in small amounts in equilibrium with **Ia**. Additional evidence that the interaction of hydrazine with only one single cubane is necessary and sufficient for the catalytic reduction is available in studies with phenylhydrazine, $PhHNNH_2$. The replacement of the CH_3CN molecule in **Ia** by $PhHNNH_2$ occurs readily.[21] 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[22] and shows the η^1-phenylhydrazine ligand coordinated to the Mo atom. The stoichiometric and catalytic reductions of the terminally coordinated $PhHNNH_2$ in **IV** were carried out as described for **I** and resulted in the formation of NH_3 and aniline (Table I).

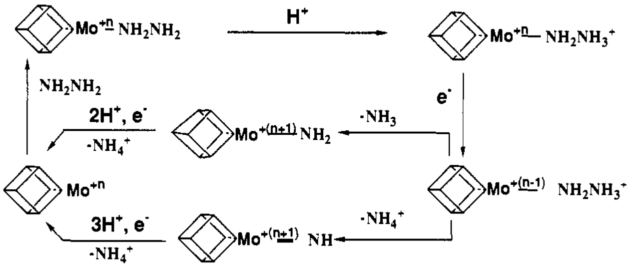

Figure 2. A proposed pathway for the catalytic reduction of N_2H_4 to NH_3 by the $[(citr)MoFe_3S_4(Cl)_3]^{3-}$ and the $[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3{\cdot}L]^{2-}$ cubanes ($L = CH_3CN$).

The catalytic activity of **Ib** is intriguing and can be rationalized if the hapticity of the tridentate citrate ligand changes upon protonation and generates a coordination site for the hydrazine molecule prior to reduction. A similar event may be envisioned for the Mo-bound homocitrate ligand in the Fe/Mo/S center in nitrogenase. The significant differences in the rates of hydrazine reduction with the two different Fe_3MoS_4 cubane catalysts, **Ia** and **Ib**, the lack of catalytic activity with the Fe_4S_4 cubanes, and the inability of **Ib** to catalyze the disproportionation of hydrazine (in the absence of acidic protons capable of protonating the citrate ligand) indicate that the molybdenum atom may be directly involved in the catalytic process.

The structural similarity of the $MoFe_3S_4$ clusters to the Fe/Mo/S center in nitrogenase and the competence of the former in the catalytic reduction of N_2H_4, a nitrogenase substrate,[23] *support the suggestion that the catalytic reductions by the Fe/Mo/S center in nitrogenase also may directly involve the Mo atom.* A possible pathway for the catalytic reduction of N_2H_4 to ammonia (Figure 2) is similar to the single-metal mechanism proposed previously for the reduction of N_2H_4 on the $(Me_5Cp)W(CH_3)_3$ unit[7] and consistent with models for the action of nitrogenase derived by Thorneley and Lowe and others.[24] A further understanding of this system should become available from kinetic studies presently under way in our laboratory.

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Supplementary Material Available: Experimental procedures, detailed analytical studies, and additional data of stoichiometric and catalytic reduction and disproportionation reactions and tables containing listings of positional parameters, thermal parameters, and selected distances and angles of **IV** (21 pages), listing of calculated and observed structure factors for **IV** (14 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(17) The $[(Cl_4\text{-cat})Mo(O)(\mu\text{-}S)_2FeCl_2]^{2-}$ and $[(S)Mo(O)(\mu\text{-}S)_2FeCl_2]^{2-}$ dimers[18] (possible oxidative degradation products of the $[(Cl_4\text{-cat})MoFe_3S_4(Cl)_3{\cdot}CH_3CN]^{2-}$ cubane) have been tested as potential catalysts and have been found totally ineffective in the catalytic reduction of hydra zine under identical reaction conditions. The $[Fe_4S_4Cl_4]^{2-}$ cluster also is inactive as a catalyst. In contrast, the tungsten analog of **Ia** is comparably effective as a catalyst.[19]

(18) Coucouvanis, D.; Al-Ahmad, S.; Kim, C. G.; Mosier, P. E.; Kampf, J. W. *Inorg. Chem.* **1993**, *32*, 1533.

(19) Work currently in progress.

(20) A detailed account of these studies has been deposited as supplementary material.

(21) Palermo, R. E.; Singh, R.; Bashkin, J. K.; Holm, R. H. *J. Am. Chem. Soc.* **1984**, *106*, 2600.

(22) Details of the crystal structure investigation including crystal data, diffraction data, and a table of bond distances and angles have been deposited as supplementary material.

(23) Thorneley, R. N. F.; Lowe, D. J. *Biochem. J.* **1984**, *224*, 887.

(24) (a) Thorneley, R. N. F.; Lowe, D. J. *Biochem. J.* **1983**, *215*, 393. (b) Cleland, W. W. *Methods Enzymol.* **1979**, *63*, 112. (c) Guth, J. H.; Burris, R. H. *Biochemistry* **1983**, *22*, 5111.