Catalytic Reduction of *cis*-Dimethyldiazene by the $[MoFe_3S_4]^{3+}$ Clusters. The Four-Electron Reduction of a N═N Bond by a Nitrogenase-Relevant Cluster and Implications for the Function of Nitrogenase

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Abstract: The catalytic reduction of *cis*-dimethyldiazene by the $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ cluster (Cl_4-cat = tetrachlorocatecholate) is reported. Unlike the reduction of *cis*-dimethyldiazene by the Fe/Mo/S center of nitrogenase, which yields methylamine, ammonia, and methane (the latter from the reduction of the C−N bond), the reduction of *cis*-dimethyldiazene by the synthetic cluster yields exclusively methylamine. In separate experiments, it was shown that the C−N bond of methylamine is not reduced by the $[MoFe_3S_4]^{3+}$ core, perhaps accounting for the differences observed between the biological and abiological systems. 1,2-Dimethylhydrazine, a possible partially reduced intermediate in the reduction of *cis*-dimethyldiazene, was also shown to be reduced to methylamine. Interaction of methylamine with the Mo atom of the cubane was confirmed through the synthesis and structural characterization of $(Et_4N)_2[(Cl_4\text{-cat})(CH_3NH_2)MoFe_3S_4Cl_3]$. Phosphine inhibition studies strongly suggest that the Mo atom of the $[MoFe_3S_4]^{3+}$ core, which has a Mo coordination environment very similar to that in nitrogenase, is responsible for the binding and activation of *cis*-dimethyldiazene. The reduction of a N═N bond exclusively at the heterometal site of a nitrogenase-relevant synthetic compound may have implications regarding the function of the nitrogenase Fe/Mo/S center, particularly in the latter stages of dinitrogen reduction.

Introduction

Nitrogenase is capable of reducing many small, unsaturated molecules and ions, the most important of which is atmospheric N_2. The enzymatic synthesis of ammonia by reduction of atmospheric N_2 under ambient conditions supplies nitrogen in the form needed in the biosynthesis of proteins and nucleic acids. It is not surprising therefore that much research has been undertaken in order to achieve a better understanding of this remarkable reduction process.[4] In recent X-ray structure determinations of nitrogenase the structure of the Fe/Mo/S active site has been obtained at near-atomic resolution.[5] This $MoFe_7S_9$ cluster of nitrogenase, referred to as the FeMo-cofactor, is comprised of $MoFe_3S_3$ and Fe_4S_3 site-voided cubanes linked by three bridging sulfides (Figure 1). The Mo atom of the FeMo-cofactor appears to be coordinatively saturated, with a terminal imidazole from a histidine residue and a bidentate (*R*)-homocitrate ligand completing the coordination sphere.

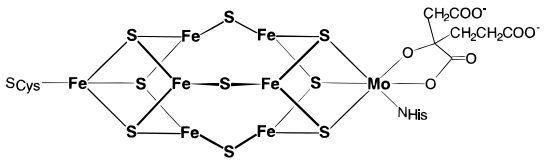

Figure 1. The FeMo-cofactor of nitrogenase.

The synthesis of exact analogs for the FeMo-cofactor has not been accomplished. Nevertheless, partial analogs exist in synthetic clusters.[6] Outstanding among these clusters are simple Fe/Mo/S cubanes that contain the $[MoFe_3S_4]$ cores[7] and a Mo atom with first and second coordination spheres nearly identical to those found for the Mo atom in nitrogenase. In addition to the structural and electronic similarities,[7] it has recently been shown that these $[MFe_3S_4]^{n+}$ clusters (M = Mo, n = 3 or M = V, n = 2, the latter of which are relevent to the alternative V-nitrogenase[8]) are also partial functional models for the enzyme. Substrate reductions that have been investigated with these clusters include the reduction of hydrazine to ammonia[9] and acetylene to ethylene and ethane.[10] The most significant results from these studies include the observation that the heterometal atom (Mo or V) in these clusters is either

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exclusively[9] or principally[10] involved in the activation of the substrate toward reduction.

The synthetic clusters most efficient in their ability to act as catalysts have been the $(Et_4N)_2[(L)(L')MoFe_3S_4Cl_3]$ single cubanes, with either a labile solvent molecule weakly coordinated to the Mo atom (L = bidentate tetrachlorocatecholate, L′ = CH_3CN[7]) or, paradoxically, a tridentate polycarboxylate ligand (L, L′ = polycarboxylato ligand[11]) that results in a coordinatively saturated Mo atom. When the structure of the Fe/Mo/S center in nitrogenase revealed that the Mo atom of the cluster was coordinatively saturated in the resting state, the direct involvement of the Mo atom in substrate binding appeared unlikely. The ability of the polycarboxylate-ligated $[MoFe_3S_4]^{3+}$ model clusters to serve as catalysts suggested a special role for the coordinated carboxylate ligands. Indeed, the observation that carboxylate-bound clusters with coordinatively saturated Mo atoms are also catalytically active in substrate reduction implies their ability to generate coordination sites for the substrate by displacing one of the "arms" of the carboxylate ligand through protonation.[9,11] In some cases,[9] these polycarboxylate-ligated clusters are actually better catalysts than the catecholate precursors, presumably due to the protonated arm of the ligand that may serve as a "shuttle", returning a proton to the reduced substrate. While N_2 is not reduced by the synthetic $[MFe_3S_4]^{n+}$ clusters, the catalytic reactivity of the latter suggests the possibility of partially reduced substrates interacting and being reduced at the heterometal atom of the Fe/M/S center in nitrogenase.

With regard to dinitrogen reduction, it has been suggested that dinitrogen is activated in the $Fe_3(\mu\text{-}S)_3Fe_3$ "cage" created by the six three-coordinate Fe atoms in the central part of the cofactor.[12] The mechanism of dinitrogen reduction is believed to proceed through diazene-like intermediates,[13] although diazene has not yet been demonstrated to interact with the nitrogenase cofactor.[14] At present, it is not known where the reduction of dinitrogen to ammonia occurs. It could take place entirely at the six-Fe "cage" or it may undergo the initial two- or four-electron reduction at the six-Fe "cage" and the final two- to four-electron reduction and cleavage of the N−N bond at the Mo atom.

Recently, it has been reported that both *cis*- and *trans*-dimethyldiazene are substrates for nitrogenase, and as such represent the first example of reduction of an unstrained N═N bond by the Fe/Mo/S center of nitrogenase.[15] Products detected included methylamine, methane, and ammonia in ratios that were dependent on both the specific isomer used as substrate and (as demonstrated for the *cis* isomer) the Fe:FeMo protein ratio. The strained-ring diazene, diazirine, is also reduced by nitrogenase to the same products.[15a] In order to investigate what role, if any, the Mo atom may play in reduction of a N═N bond, *cis*-dimethyldiazene was investigated as a potential substrate for the $[MoFe_3S_4]^{3+}$ cubanes. Herein, we report on the catalytic reduction of *cis*-dimethyldiazene by the $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ cubane. Results show that the only detectable product from the reduction of *cis*-dimethyldiazene by the synthetic cluster is methylamine. Additionally, it has been demonstrated through inhibition studies that activation and reduction of *cis*-dimethyldiazene occurs exclusively at the Mo site. The implications of these observations on the possible function of the cofactor of nitrogenase will also be discussed.

Experimental Section

General Considerations. All manipulations were performed under an inert atmosphere using standard glove box and Schlenk techniques. Solvents were distilled under N_2 from the appropriate drying agents (diethyl ether and THF from sodium/benzophenone, and CH_3CN from B_2O_3) or stored over 3 Å molecular sieves (absolute ethanol) and thoroughly degassed with N_2 or Ar prior to use. Reagent-grade chemicals were purchased from Aldrich Chemical Company (cobaltocene ($CoCp_2$), 99% 2,6-lutidine (Lut), anhydrous DMF, methylamine, ethylamine, dimethylhydrazine dihydrochloride, $NaBPh_4$, 1.0 M ethereal HCl, PEt_3) and used without further purification. Freshly prepared solutions of *cis*-dimethyldiazene[16] in distilled, degassed CH_3CN (typically 0.1−0.2 M as determined by UV spectroscopy, $\epsilon_{367} = 266$) were stored at −196 °C until immediately prior to use.

Physical Measurements. Infrared spectra (CsI disks) were obtained using a Nicolet 740 FT-IR spectrometer (far-IR 500−150 cm^{-1}) or a 5DXB FT-IR spectrometer (mid-IR 4000−400 cm^{-1}). Quantification of methylamine and ammonia was performed using an HPLC technique previously described.[17] An HP 5890 Series II gas chromatograph equipped with either a porapak N column (Supelco) or a 4% carbowax 20 m column (Supelco) was used in order to detect methane or $EtNH_2$, respectively. EPR studies and elemental analysis were performed by the Biophysics Research Division and the Analytical Services Division, respectively, at the University of Michigan. Integration of the EPR signal was accomplished according to previously discussed techniques.[18] Analysis samples were routinely kept under dynamic vacuum for 12 h before submission.

Preparation of Compounds. Analytically pure 2,6-lutidinium hydrochloride (Lut·HCl) was prepared from the reaction between lutidine and ethereal HCl. Lut·$HBPh_4$ was prepared from the metathesis reaction between Lut·HCl and $NaBPh_4$ in ethanol. $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$[19] and $(Ph_4P)_2[Fe_4S_4Cl_4]$[20] were obtained by procedures similar to those previously reported.

$(Et_4N)_2[(Cl_4\text{-cat})(RNH_2)MoFe_3S_4Cl_3]$ (R = Me or Et). An amount of RNH_2 (0.10 mL of a 2 M EtOH solution) was added to an CH_3CN solution (30 mL) of $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ (0.21 g, 0.20 mmol) in one portion. After approximately 1 h of stirring, the solution was filtered and ether (150 mL) was layered on the filtrate. After overnight standing, a near-quantitative yield of brown crystals was isolated by filtration and washed well with ether.

(A) R = Me (I). Analysis calculated for $C_{23}H_{45}N_3O_2Cl_7S_4Fe_3Mo$: C, 26.68; H, 4.38; N, 4.06. Found: C, 27.01; H, 4.22; N, 4.10. Mid-IR (CsI disks): 3290(w), 3250(w) from amine. Far-IR (CsI disks): 408(m), 351(vs). Single crystals of this complex were the subject of an X-ray structure determination.

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Table 1. Summary of Crystal Data for $(Et_4N)_2[(Cl_4\text{-cat})(CH_3NH_2)MoFe_3S_4Cl_3]$ (**I**)

chemical formula	$MoFe_3S_4Cl_7C_{23}H_{45}N_3O_2$
molecular weight, g/mol	1034.52
color, morphology	brown, needles
space group	*Pbcm*
a, Å	11.635(6)
b, Å	32.14(1)
c, Å	33.06(1)
V, Å^3	12506
Z	12
density (observed), g/mL	1.63
density (calculated), g/mL	1.65
radiation	Mo K_α
absorption coefficient	2.01
data collected	$3° \le \omega \le 45°$
unique data	4016
data used ($F_o^2 > 3\sigma(F_o^2)$)	2762
number of parameters	399
phasing techniques	direct methods
R, R_w	0.0717, 0.0840

(B) R = Et (II). Analysis calculated for $C_{24}H_{47}N_3O_2Cl_7S_4Fe_3Mo\cdot{}^1/_2CH_3CN$: C, 28.06; H, 4.58; N, 4.58. Found: C, 28.45; H, 4.53; N, 4.86. Mid-IR (CsI disks): 3297(w), 3239(w) from amine; 2249(w) from lattice CH_3CN. Far-IR (CsI disks): 421(m), 410(m), 392(w), 350(vs), 343(sh), 298(w).

X-ray Structural Determination. Crystals suitable for diffraction study were obtained by vapor diffusion of ether into an CH_3CN solution of **I**. Brown needlelike crystals were mounted in glass capillaries and flame sealed under argon. Diffraction experiments were performed at ambient temperature with a Nicolet R3m four-circle automated diffractometer using graphite-monochromatized Mo Kα radiation. The orientation and unit cell parameters were determined from 18 machine-centered reflections with $10° < 2\theta < 25°$. A total of 5234 reflections were collected in the range $3° < \omega < 45°$ with 4016 unique reflections. The structure was solved using direct methods. Anisotropic temperature factors were used for all non-carbon atoms of the anion. The final cycle full-matrix least squares refinement was based on 2762 reflections ($I > 3\sigma(I)$) and 399 variables using the SHEXTL Plus crystallographic software package. The refinement converged with $R = 0.0717$ and $R_w = 0.0840$. Crystal data are summarized in Table 1.

Reduction of *cis*-Dimethyldiazene. (A) Time-Course Studies. A 125 mL flask was charged with 0.030 g (160 μmol) of $CoCp_2$ and 0.030 g (210 μmol) of Lut·HCl. Acetonitrile was then added (35.5 mL) to form a slurry. An aliquot of a 4.8 mM CH_3CN solution of $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ (0.45 mL, 2.2 μmol) was then added, followed immediately by an aliquot of the *cis*-dimethyldiazene solution (32 μmol). The addition of the substrate marked $t = 0$ h. At $t = 0.5$, 1.0, 2.0, 3.5, and 5 h, a 100 μL sample of head space gas was obtained from the reaction flask and analyzed for CH_4. In addition, 3.0 mL samples were removed from the reaction flask and placed in 5 mL septa-capped vials. These aliquots were immediately quenched with 0.2 mL of a 1.0 M aqueous HCl solution. The vials were analyzed for methylamine, ethylamine, and ammonia. Reactions were performed under both N_2 and Ar. Also, "blanks" were run under the same conditions but only sampled at 3.0 h.

(B) Phosphine-Inhibition Studies. In a 20 mL vial fitted with a septa, 8.3 mL of a 7.2 mM $CoCp_2$ solution (CH_3CN, 60 μmol), 8.3 mL of a 10.9 mM Lut·HCl solution (CH_3CN, 90 μmol), and an appropriate amount of a 9.5 mM PEt_3 solution (CH_3CN, from 0−10 equiv based on cubane concentration) were combined. A 0.2 mL aliquot of a 4.8 mM cluster solution (CH_3CN, 0.96 μmol) was then added, followed immediately by a 0.08 mL aliquot of the substrate solution (CH_3CN, 15 μmol). Addition of the substrate marked $t = 0$ h. A 2.0 mL aliquot of the reaction solution was obtained at $t = 1$ h and immediately quenched with 0.2 mL of a 1.0 M aqueous HCl solution in a 5 mL septa-capped vial. Vials were subsequently analyzed for methylamine and ammonia. In some reactions, $(Ph_4P)_2[Fe_4S_4Cl_4]$ was used in place of the $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ cluster.

(C) Investigation of Kinetics. Reactions were prepared in 125 mL flasks. The total solution volume was brought up to 40 mL with acetonitrile. Aliquots of acetonitrile solutions of $CoCp_2$ (15.0 mL, 108 μmol), Lut·HCl (10.0 mL, 109 μmol) and cluster (0.15 mL, 0.72 μmol) analogous to those described in section B above were placed in a flask containing predetermined amounts of CH_3CN. With the concentrations of these reagents held constant, the appropriate amount of a *cis*-dimethyldiazene solution (3−167 equiv based on cluster concentration) was added to the flask, marking $t = 0$ h. At $t = 1$ h, 3.0 mL aliquots of the reaction solution were obtained and worked up as described above.

(D) Attempted Reductions of 1,2-Dimethylhydrazine, Methylamine, and Ethylamine. To a 125 mL reaction flask filled with an appropriate amount of CH_3CN (total solution volume was 40 mL) was added 4.2 mL of a 7.2 mM $CoCp_2$ solution (30 μmol), 0.03 g of Lut·$HBPh_4$ (70 μmol) and 0.2 mL of a 4.8 mM $(Et_4N)_2[(Cl_4\text{-cat})\text{-}CH_3CN)MoFe_3S_4Cl_3]$ solution. An excess of the appropriate substrate ($MeNH_2$, $EtNH_2$, or 1,2-dimethylhydrazine[21] as CH_3CN solutions, 10−15 equivalents based on cubane concentration) was then added, marking $t = 0$ h. At $t = 1$ h, aliquots were obtained and quenched with HCl as described and analyzed for products. In the case of the amines, the headspace gas in the reaction solutions was analyzed for CH_4, and the solution analyzed for ammonia by the indophenol method as previously described.[9] In the case of 1,2-dimethylhydrazine, the vials were analyzed for methylamine by HPLC.

The reactions described in sections A, B, and D were performed in duplicate, while those in section C were performed in triplicate. Additionally, each reaction aliquot was analyzed for products at least twice. While repeat measurements on each vial typically did not vary by more than 10%, absolute product yield varied slightly between identical reactions. Regardless, the trends observed for the time course of the reaction, the phosphine inhibition and the ν vs [*cis*-dimethyldiazene] curves were consistent between sets of experiments and are reported as such.

(E) Recovery and Identification of the $[MoFe_3S_4]^{3+}$ Catalyst. A 125 mL flask was charged with 23 mL of a 7.2 mM $CoCp_2$ solution, 23 mL of a 10.9 mM Lut·HCl solution, and 3.0 mL of a 4.8 mM solution of $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$. Addition of 0.42 mL of a 0.1 M *cis*-dimethyldiazene solution marked $t = 0$ min. After stirring for 90 min, the solution was taken to dryness. The resulting residue was washed well with THF to remove any unreacted $CoCp_2$, and subsequently dissolved in 10.0 mL DMF. An aliquot of this DMF solution (1.3 mM cubane) was then obtained and subjected to a quantitative EPR analysis.

Results and Discussion

Synthesis and Crystallographic Results. After some 20 years of very thorough studies, the synthesis, ligand-substitution properties, and reactivity of clusters with the $[MoFe_3S_4]^{3+}$ core are well established.[7] It has been demonstrated that (a) the catecholate moiety on the Mo atom of the cubane can be removed through protonation by acidic catechols[22] and polycarboxylic acids[11] and (b) the single labile solvent molecule on the Mo atom in $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ and related clusters can be readily replaced by any number of σ-bases[7] but generally not by π-acids except in the case of reduced cores.[23] It is not unexpected, therefore, that amines will readily coordinate to the Mo atom of the cluster through the N-lone pair, replacing the relatively poor CH_3CN ligand. The sole purpose in verifying this ligand substitution synthetically and crystallographically is to establish unambiguously the Mo−amine interaction in light of the result that neither $MeNH_2$ nor $EtNH_2$ are reduced by the $[MoFe_3S_4]^{3+}$ core (*vide infra*).

The methylamine single cubane **I**, shown in Figure 2, crystallizes with two distinct anions within the asymmetric unit, one in which all atoms are located on general positions (A)

(21) In order to prepare a standard solution of 1,2-dimethylhydrazine in CH_3CN, a slight excess of Et_3N was used to neutralize and hence solubilize the dihydrochloride salt.

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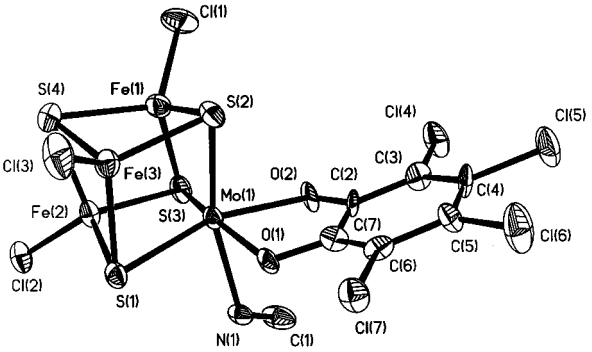

Figure 2. An ORTEP plot (30% probability ellipsoids) showing the anion of $(Et_4N)_2[(Cl_4\text{-cat})(CH_3NH_2)MoFe_3S_4Cl_3]$ (**IA**).

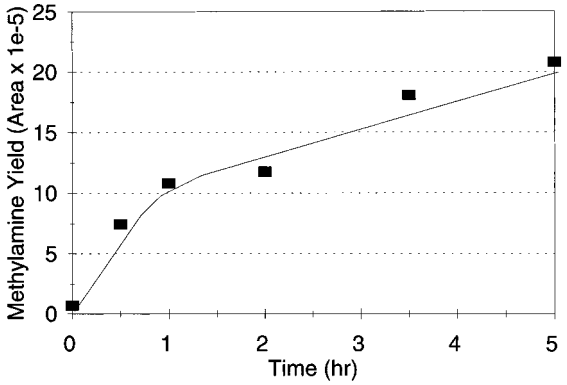

Figure 3. Time course of methylamine production from the reduction of *cis*-dimethyldiazene, plotted as HPLC response (area) vs *t*. $[(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]\ ] = 54\ \mu M$, [*cis*-dimethyldiazene]$_o = 800\ \mu M$. Conversion at 5 h is approximately 90%.

and the second which is bisected by a crystallographic mirror plane through Mo1, Fe2, S2, and S4 (B). As a result, there are 12 anions per unit cell. The structure of **I** shows the Mo single cubane ligated by methylamine (Figure 2). The nitrogen atom of methylamine in **IB** is located on the mirror plane. As a result, the methyl carbon (C1) is somewhat disordered over two positions but is modeled sufficiently by a 50% occupancy on both positions. The Mo−N distance in both anions is very nearly the same at 2.28(2) and 2.35(4) Å. The average Mo− −Fe (2.737(6) Å), Mo−S (2.346(9) Å), Mo−O (2.09(2) Å), Fe−S (2.26(1) Å), Fe−Cl (2.22(1) Å), and Fe− −Fe (2.727(7) Å) distances are unexceptional. Given the abundance of Mo−cubane structures in the literature,[7] no further description of this structure is warranted.

Substrate Reductions. As a reference point, catalytic reductions were generally performed with the substrate in approximately 15-fold excess relative to the catalyst, with externally added sources of electrons ($CoCp_2$, ≥4 equiv) and protons (Lut·HCl, ≥6 equiv). As shown in Figure 3, *cis*-dimethyldiazene is catalytically reduced to methylamine under these conditions. There is an initial burst to approximately 50% conversion (based on 2 mol of methylamine for every mole of *cis*-dimethyldiazene) in the first hour of reaction time for the 15:1 substrate/catalyst ratio, with conversion slowly leveling off. This behavior may be due to a slow precipitation of the catalyst as cations are generated in solution (i.e., $CoCp_2^+$, $MeNH_3^+$), a behavior typically observed in the reduction systems previously investigated.[9]

In order to verify that (1) the substrate was being activated and reduced by the $[MoFe_3S_4]^{3+}$ core exclusively and (2) methylamine was obtained only from reduction of *cis*-dimethyldiazene, a number of "blanks" were performed. These blanks included reaction systems with all reagents present except (a) the substrate, (b) the $[MoFe_3S_4]^{3+}$ catalyst, and (c) proton and electron sources and (d) with 10 equiv of PEt_3 added to inhibit the Mo site. As shown in Figure 4, while methylamine is present in all systems, amounts of methylamine from these blanks is typically ≤10% of the amounts detected from reactions where all reagents are present and the $[MoFe_3S_4]^{3+}$ cluster is present as a catalyst. These results taken together strongly suggest that *cis*-dimethyldiazene is catalytically reduced by the $[MoFe_3S_4]^{3+}$ cuboidal core in the presence of externally added protons and electrons.

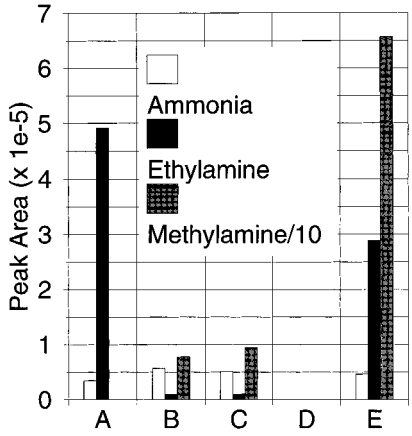

Figure 4. Product distributions (plotted as HPLC response) after 3 h for reaction systems with all reagents present except (A) *cis*-dimethyldiazene, (B) the $[MoFe_3S_4]^{3+}$ catalyst, and (C) proton and electron sources, and (D) with 10 equiv of PEt_3 added to inhibit the Mo site of the catalyst. (E) A typical product distribution for the reduction of *cis*-dimethyldiazene (15-fold excess) by the $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ catalyst. Ethylamine production results from the apparent reduction of acetonitrile. See Experimental Section for reaction conditions. (Bars are from left to right: ammonia, ethylamine, methylamine/10.)

The integrity of the $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ catalyst at the end of the reaction was verified through EPR spectroscopy. After removal of excess $CoCp_2$ ($S = {}^1/_2$) from the reaction mixture (THF washings were essentially colorless), an EPR spectrum of the reaction solution was obtained. The characteristic $S = {}^3/_2$ spectrum for the $[MoFe_3S_4]^{3+}$ core was observed, and the signal integrated to 1.1 ± 0.3 mM, suggesting no cluster decomposition during the first 90 min of reaction time. Previous studies on the identification of the recovered $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ cluster after catalytic cycles included elemental analysis, electronic and infrared spectroscopy, and demonstration of further catalysis by the recovered cubane.[9,10] These results taken together demonstrate the robust nature of $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ under reaction conditions and identifies the cubane as the active catalyst for the reduction of a variety of substrates.

Ammonia yields for the reactions shown in Figure 4 were (1) exceedingly small, only reaching approximately 1% of the total yield of methylamine in the catalytic systems, and (2) essentially equivalent for all systems, catalytic reactions and blanks alike. It was therefore concluded that the reduction of *cis*-dimethyldiazene with these synthetic clusters led to formation of methylamine but *not* ammonia, unlike what is observed in the enzyme system. No change in product distribution was observed when the reaction was performed under Ar instead of N_2. Given the inability for N_2 to serve as a ligand for the Mo cluster in either the 3+ or 2+ state, this result was expected.

The lack of reduction products derived from C−N bond reduction in *cis*-dimethyldiazene (the only route leading to NH_3) was verified in two individual experiments. First, GC analysis of the headspace gases in the catalytic systems routinely showed

no CH_4 up through a 5 h period. Methane would necessarily have to be present in a 1:1 ratio with any ammonia that was produced in the system. Second, in experiments using methylamine as a "substrate" for the $[MoFe_3S_4]^{3+}$ cluster, no ammonia or methane were observed at any time as a result of potential reduction of the C−N bond. Given that methylamine is a better ligand for the $[MoFe_3S_4]^{3+}$ cluster than CH_3CN on the basis of synthetic and crystallographic results (*vide supra*), the lack of reactivity observed with methylamine is due to its inability to be reduced by the $[MoFe_3S_4]^{3+}$ core and not to its inability to interact with the catalyst. The inability of the model systems to reduce methylamine may be due to the lack of a free lone pair needed for protonation once the substrate is coordinated to the catalyst. In model systems employing hydrazine as a substrate, protonation of the bound hydrazine was shown to be a necessary first step in making the reduction potential of the cluster accessible.[9] No turnover of hydrazine to ammonia was observed in systems which lacked sufficiently acidic protons to protonate hydrazine.

The ability of the $[MFe_3S_4]^{n+}$ (M = Mo, $n = 3$; M = V, $n = 2$) cores to catalytically reduce hydrazine to ammonia has been established previously.[9] As a matter of principle, however, it was important to verify that 1,2-dimethylhydrazine (a possible reduced intermediate in the reduction of *cis*-dimethyldiazene) reacted in a similar manner. Under catalytic conditions (15-fold excess of 1,2-dimethylhydrazine relative to catalyst), approximately 80% of the substrate was reduced to methylamine within the first hour, a yield essentially identical to that reported for the unsubstituted hydrazine.[9e] A "blank" reaction which contained no catalyst showed the typical background levels of methylamine (6%) established previously. In these reactions, Lut·$HBPh_4$ was used instead of Lut·HCl to insure that a majority of the substrate stayed soluble initially, given that the dihydrochloride salt of 1,2-dimethylhydrazine is quite insoluble in CH_3CN.

In addition to methylamine, it was established by HPLC and GC that ethylamine is also a product in these systems (Figure 4). Yields of ethylamine (a) are largest when there is no *cis*-dimethyldiazene in the system, (b) are essentially nonexistent in the absence of catalyst or protons/electrons, (c) are present in lower amounts when *cis*-dimethyldiazene is present, and (d) are not observed when 10 equiv of phosphine (per cluster) are present in the system. These results suggest that the $[MoFe_3S_4]^{3+}$ core may reduce the solvent, CH_3CN, to ethylamine at a very slow rate. When "better" ligands are present (phosphine, hydrazine, *cis*-dimethyldiazene), the yields are either decreased or nonexistent. In separate experiments, it was verified that while ethylamine binds to the Mo atom of the cluster (**II**), it is not reduced to ammonia and methane.

Role of the Mo Atom in Substrate Reduction. It has been well-established in the reduction of other nitrogenase substrates by the synthetic $[MFe_3S_4]^{n+}$ clusters that the heterometal (either Mo or V) is either exclusively (in the case of hydrazine)[9] or predominantly (in the case of C_2H_2)[10] involved in the activation of the substrate toward reduction. The role of the heterometal in the *cis*-dimethyldiazene system was investigated by (1) using the $[Fe_4S_4Cl_4]^{2-}$ as a potential catalyst in place of the $[MoFe_3S_4]^{3+}$ core and (2) observing the effect of the addition of phosphine to the catalytic system. Phosphine is known to bind strongly to the Mo atom[21] and presumably dramatically affects the ability of other, weaker σ-bases to coordinate.

By using the all-Fe cluster as a potential catalyst, the amounts of methylamine observed after 1 h were well within background limits established with the blanks, suggesting that the Fe atoms in the $[MoFe_3S_4]^{3+}$ cluster are not involved in catalysis. Additionally, as shown in Figure 5, it is clear that as phosphine is added in greater amounts to the reaction system (from 0 to 10 equiv of phosphine), the turnover of substrate to products drops dramatically. At 1 equiv of phosphine, the amount of product in 1 h drops to about half of the amount obtained when $[PEt_3] = 0$. Between 5 and 6 equiv of phosphine, the amount of methylamine present in the reaction solutions was determined to be well within background limits, indicating complete inhibition. These results taken with those results obtained previously with other substrates demonstrates the importance of the Mo atom in substrate binding and activation toward reduction.

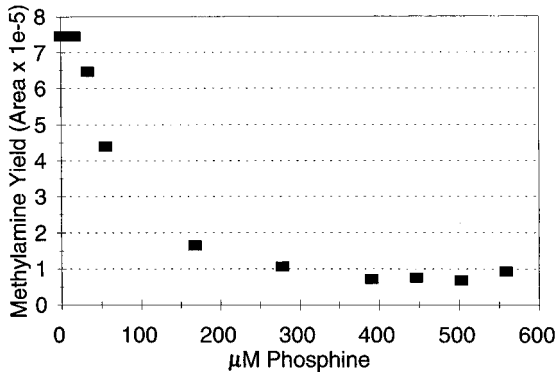

Figure 5. Inhibition of *cis*-dimethyldiazene reduction by PEt_3. See Experimental Section for reaction conditions.

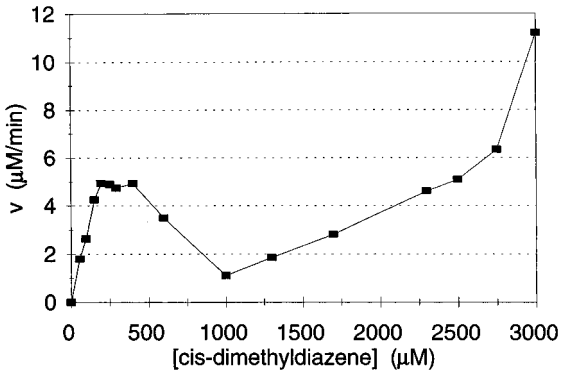

Figure 6. Reaction velocity (v) vs $[cis\text{-dimethyldiazene}]_o$. Reaction velocities are based on the first 60 min of reaction time. See Experimental Section for reaction conditions.

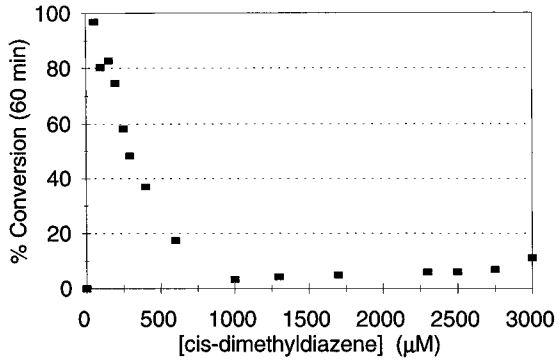

Figure 7. Percent conversion of *cis*-dimethyldiazene to methylamine vs $[cis\text{-dimethyldiazene}]_o$.

Investigation of Kinetics. From initial time-course studies, it was determined that the conversion of substrate to product with time was essentially linear within the first 60 min. Therefore, catalytic systems were performed over a number of *cis*-dimethyldiazene concentrations and analyzed at $t = 1$ h. As shown in Figure 6, it is clear that typical saturation kinetics observed for enzyme systems are not applicable in this model

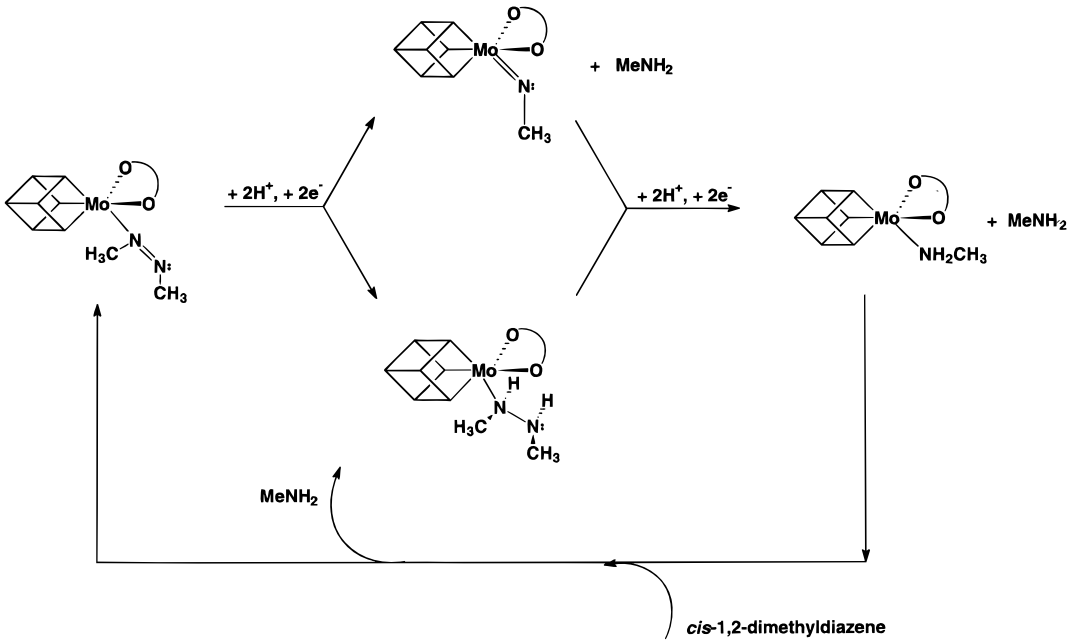

Figure 8. Possible reaction pathways for the reduction of *cis*-dimethyldiazene by synthetic [MoFe$_3$S$_4$]$^{3+}$ cuboidal cores.

system. This observed trend may be due in part to insolubility of the catalyst as cations are generated in solution ($CoCp_2^+$, $MeNH_3^+$). At lower substrate:catalyst ratios, the catalyst remains in solution to effect near quantitative reduction within the first hour. At higher concentrations of substrate, the reaction velocity drops to a minimum, followed by a gradual increase. As shown in Figure 7, however, the percent conversion of *cis*-dimethyldiazene to methylamine (based on 2 equiv of product per mole of substrate) is essentially constant at high [*cis*-dimethyldiazene].

A Comparison between the Synthetic and the Enzymatic Systems. It has been reported[15] that as a substrate for the *A. vinelandii* nitrogenase, *cis*-dimethyldiazene is reduced to NH_3, CH_4, and CH_3NH_2 in a ratio of 1.0:1.0:1.6 ± 0.1 (Fe:FeMo protein ratio of 6.6), in contrast to the synthetic system employing [MoFe$_3$S$_4$]$^{3+}$ as catalyst, where methylamine is produced exclusively. In the synthetic system, methylamine is not reduced and hence no methane or ammonia are observable as products. It seems likely that the reaction proceeds by one of two pathways (assuming mononuclear activation), as depicted in Figure 8. The coordinated *cis*-dimethyldiazene may be reduced in a "symmetric" way, forming dimethylhydrazine as an intermediate, which has been shown to yield methylamine upon protonation and reduction. Alternatively, the substrate may be reduced in an "unsymmetric" manner, forming methylamine and a Mo-bound imine after the first two-electron, two-proton step. Either way, it is clear in the model system that methylamine is the only product and that the Mo atom seems to be of primary importance.

In the enzymatic system, it seems likely that either (a) the N═N bond and the C−N bond are is some way reduced concomitantly or (b) methylamine is an initial product, which is activated in some way by the nitrogenase cofactor. If the latter, the methylamine would have to bind to a center that was capable of removing significant electron density from the C−N bond given that only one lone pair of electrons are available and, when bound to a metal center, there would be no place for protonation to occur on methylamine. Either way, it is clear that a different mechanism is occurring in the biological and abiological systems, specifically with regard to C−N bond cleavage.

Summary and Conclusions

The nitrogenase enzyme has recently been shown to catalyze the reduction of *cis*-dimethyldiazene to methane, ammonia, and methylamine. The nitrogenase model compound $(Et_4N)_2[(Cl_4\text{-cat})(CH_3CN)MoFe_3S_4Cl_3]$ also reduces the N═N bond in *cis*-dimethyldiazene, but methylamine is *the only observable product*. The inability of the model compound to reduce methylamine, even though it has been unambiguously demonstrated that methylamine binds to the Mo atom in the cluster, may account for the lack of methane and ammonia formation. Insofar as the cuboidal clusters can be considered reactivity models for the cofactor of nitrogenase, the ability of the [MoFe$_3$S$_4$]$^{3+}$ cores to reduce both N═N and N−N *exclusively at the Mo site* may suggest the Mo-atom in the cofactor is, in fact, not "innocent" as suggested in the past,[12] but rather plays a vital role in the later stages of dinitrogen reduction. We have previously considered the possibility of initial activation of dinitrogen at the unique, three-coordinate Fe atoms of the cofactor, followed by "migration" of a partially -reduced species (hydrazine) to the peripheral Mo atom where reduction to ammonia proceeds.[9a] This work not only supports this possibility, but also suggests that the Mo atom may be involved in the reduction process at the diazene level.

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Supporting Information Available: Tables of fractional atomic coordinates, calculated positions of H atoms and anisotropic displacement parameters for **I** (6 pages). See any current masthead page for ordering and Internet access instructions.

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