Partial synthetic models of FeMoco with sulfide and carbyne ligands: Effect of interstitial atom in nitrogenase active site

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Nitrogen-fixing organisms perform dinitrogen reduction to ammonia at an Fe-M (M = Mo, Fe, or V) cofactor (FeMco) of nitrogenase. FeMco displays eight metal centers bridged by sulfides and a carbide having the MFe_7S_8C cluster composition. The role of the carbide ligand, a unique motif in protein active sites, remains poorly understood. Toward addressing how the carbon bridge affects the physical and chemical properties of the cluster, we isolated synthetic models of subsite MFe_3S_3C displaying sulfides and a chelating carbyne ligand. We developed synthetic protocols for structurally related clusters, $[Tp^*M'Fe_3S_3X]^{n-}$, where $M' = Mo$ or W , the bridging ligand $X = CR, N, NR, S$, and $Tp^* = Tris(3,5\text{-dimethyl-1-pyrazolyl})\text{-hydroborate}$, to study the effects of the identity of the heterometal and the bridging X group on structure and electrochemistry. While the nature of M' results in minor changes, the chelating, μ_3 -bridging carbyne has a large impact on reduction potentials, being up to 1 V more reducing compared to nonchelating N and S analogs.

FeMoco model | nitrogenase | carbide clusters | reduction potential | iron-sulfur clusters

Biological dinitrogen conversion to ammonia is performed by nitrogenases, a class of enzymes displaying several complex iron-sulfur clusters (1). The site of N_2 reduction in the most efficient nitrogenase is a heterometallic cluster displaying Fe and Mo, the iron-molybdenum cofactor (FeMoco) (1). Two other nitrogenases are known where Fe or V are found at the Mo position. FeMoco consists of Fe_4S_3C and $MoFe_3S_3C$ cubanes with μ_3 -sulfides joined together by a shared interstitial μ_6 -carbide and three additional sulfides that bind in μ_2 -fashion (Fig. 1) (2). The impact of the carbide ligand on the electronic structure and reactivity of the cofactor, and therefore its role in the catalytic cycle of N_2 -to- NH_3 conversion, is unclear (3). The carbide ligand is not lost during catalysis, and it has been suggested that it becomes protonated before N_2 activation (3). To address the effect of carbon-based ligands for N_2 activation, such as providing electronic stabilization and structural flexibility to accommodate multi-electron redox processes, synthetic models have included arene (4), *N*-heterocyclic carbene (5), aryl (6), and alkyl (7, 8) donors in mononuclear iron complexes.

Bi- and multimetallic synthetic analogs focused on interrogating the role of the interstitial atom and multimetallic effects have been targeted (7, 9–23), but complexes that display bridging carbide (11, 24–27) or even carbyne (9, 10, 28) ligands are rare. Carbide-containing Fe clusters display four to six metal centers but invariably are rich in CO ligands (24, 25, 27). The presence of this strong field donor limits the comparison to FeMoco given the significantly different electronic structure conferred by the weak field sulfides. Moreover, the formal oxidation state of the Fe centers is significantly more reduced, between Fe^0 and Fe^{II} , than in the protein, between Fe^{II} and Fe^{III} (2). Recent promising advances have been made toward the incorporation of sulfide ligands into carbide-containing iron carbonyl clusters (10, 11). In order to gain a more accurate understanding of the impact of the carbide on the properties

of clusters related to FeMoco, metal complexes structurally related to the biological active site that are multimetallic, have multiple sulfide ligands and few CO ligands, and display bridging carbon-based ligands and oxidation states of Fe^{II} - Fe^{III} are desirable.

Toward developing synthetic methodologies to structures analogous to FeMoco that include a bridging carbon donor, we focus our initial efforts on the cubane subsite, $MoFe_3S_3C$ (Fig. 1, *Top Row*). Because the nature of the μ_2 -bridging ligands in FeMoco is variable, with sulfide, selenide (29), CO (30), or NH (31) (for FeVco) moieties at these positions as characterized by crystallography, the primary target was to match the composition of the cubane core. In this work, we present the preparation of a series of heterometallic iron-sulfur cubane-type clusters containing Mo or W with biologically relevant μ_3 bridging ligands X ($X = N, NR, CR$, and S) incorporated at the Fe_3 face—including examples bearing a bridging CR ligand. These variations in the bridging ligand result in a large shift in the biologically relevant $M'Fe_3^{11+}/M'Fe_3^{10+}$ redox couple of up to 2 V, with the most reducing system occurring for the cluster bearing a bridging carbyne. These results suggest an important role of the interstitial carbide ligand in FeMoco in modulating the electronic properties of the cluster toward rendering it more reducing and potentially more reactive in N_2 activation and conversion into NH_3 .

Significance

Nitrogen, a common component of biomolecules, is sourced from abundant dinitrogen in the atmosphere through conversion to ammonia. Organisms capable of fixing N_2 employ nitrogenases, metalloproteins that display metal-sulfide clusters that facilitate electron transfers and substrate activation. The site of N_2 conversion to NH_3 is FeMco (M = Mo, Fe, or V), a cluster of notable complexity in bioinorganic chemistry, which displays an unusual interstitial carbon ligand. The function of this bridging ligand remains unclear, and systematic structure-function studies with bridging C-donors are challenged by a lack of synthetic methods for analogous clusters. Herein, we report the first synthetic cluster that models a cubane moiety of FeMco bearing a chelating carbyne ligand and related structure-property studies.

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Fig. 1. (Top) Structure of FeMoco in Mo-dependent nitrogenase from the Protein Data Bank structure 3U7Q with a blue circle emphasizing the cubane subsite and its schematic representation highlighting in color the subsite of focus in this study. (Bottom) Carbyne and carbide-containing model complexes (9–11).

Results and Discussion

To rationally incorporate different ligands at the μ_3 -bridging position corresponding to the carbide, a WFe_3S_3 cluster supported by a W-coordinated Tp^* ligand, **1-W**, was selected as precursor bearing a μ_3 -Cl at the carbide position (Fig. 2) (32). Although heterometallic iron-sulfur clusters of the MFe_3 types have been reported with $M = V, Mo$, and W , they typically display a μ_3 -S vertex opposite the heterometal that is difficult to substitute with other donor types relative to chloride (33, 34). Indeed, starting from a μ_3 -Cl precursor offers a versatile route to incorporating biologically relevant light atoms at the bridging position by ligand metathesis reactions (32). As an example, the μ_3 -Cl ligand can be substituted with μ_3 -S or μ_3 -NSiMe₃ by oxidative metathesis with S_8 or Me_3SiN_3 , respectively (32).

For the installation of a carbon-based ligand at the μ_3 position, we were inspired by the utilization of the strained carbene bis(diisopropylamino)cyclopropenylidene (BAC) (35) for promoting C-atom transfer to the $Fe\equiv N$ bond of the iron(IV) nitride [$\{PhB(iPr_2Im)_3\}Fe(N)$] ($iPr_2Im = 1,2$ -diisopropylimidazolydene) (36). This ultimately generated a cyanide ligand, with the release of alkyne $iPr_2NC\equiv CNiPr_2$ as the side product. Mixing **1-W** with 3 equivalents of BAC in tetrahydrofuran

(THF) in the presence of $NaBPh_4$ as a chloride abstracting agent results in the gradual disappearance of the insoluble **1-W** to form a dark red solution, along with the precipitation of a colorless solid, assigned as $NaCl$ (Fig. 2). Upon filtration, the vapor diffusion of pentane into the filtrate over one day leads to the formation of dark purple needles. A single crystal X-ray diffraction (XRD) study of these crystals confirmed the structure of the product, where the three terminal chlorides have been substituted with BAC to give a monocationic cluster, **2-W**, with a BPh_4^- counteranion (Fig. 2). Although MFe_3S_3 clusters supported by carbene ligands have not been structurally characterized, the Fe-C distances are in the range of Fe_4S_4 clusters supported by NHC ligands (15, 37).

In order to promote the delivery of a C atom or CR group, at least one C-C bond has to be cleaved, which can be achieved by methods such as heating (38), photolysis (38), or reduction (36, 39). While **2-W** remains unchanged when irradiated with a 75-W Xe lamp and decomposes when heated at reflux in THF under an inert atmosphere, reduction with one equivalent of a strong reducing agent like potassium naphthalenide leads to the new cluster **3**. Instead of generating a neutral, one-electron reduced form of **2-W** and $KBPh_4$ as byproduct, product **3** loses

Fig. 2. Synthesis of carbyne-containing clusters.

the μ_3 -Cl ligand as KCl likely driven by precipitation, leaving an open triangular Fe_3 face, as demonstrated by XRD characterization (Fig. 2).

Cluster **3** possesses a rare incomplete cubane geometry for iron-sulfur clusters. The related $[\text{Fe}_4\text{S}_3]$ geometry has only been reported in the anion $[\text{Fe}_4\text{S}_3(\text{NO})_7]^-$ of Roussin's black salt (40) in inorganic compounds, and an oxygen-tolerant $[\text{NiFe}]$ -hydrogenase in biology (41). Incomplete heterometallic cubanes of the form $\text{M}'\text{Fe}_3\text{S}_3$ have only been observed for $\text{M}' = \text{Mo}$ in a synthetic system, where the Fe atoms are ligated by multiple CO ligands (42). The open-face Fe_3 triangle resembles the sulfide-free triiron systems supported by multinucleating trisamide ligands, which can bind μ_3 -nitride or μ_3 -imide moieties (14, 43). Thus, the open nature of the Fe_3 cluster face in **3** makes it a promising platform for the rational installation of various bridging ligands in a μ_3 mode.

Cluster **3** can further be reduced with an excess of KC_8 to form the neutral, Et_2O soluble cluster **4-W**. Gratifyingly, under these highly reducing conditions, the C-C bond in the BAC ligand is cleaved and the cyclopropene ring opens, delivering a carbyne ligand to the bridging position. The cluster loses its C_3 symmetry, resulting in two Fe atoms ligated by BAC and a unique Fe center, to which the rest of the ring-opened BAC ligand anchors as a vinyl fragment. This is an example of a synthetic iron-sulfur cluster without CO ligands that displays a carbyne donor. Aside from the bridging carbyne ligand, the terminal hydrocarbyl ligand is also notable, given the role of such ligands in SAM enzymes (44) and their scarcity in synthetic iron-sulfur cluster chemistry (45). Conveniently, **4-W** can also be synthesized directly from **2-W** using an excess of KC_8 or potassium naphthalene without isolating **3**. This reaction stops at **3** if conducted at -78°C for 1 h, while appreciable conversion to **4-W** can only be achieved at room temperature over longer reaction times, suggesting that the ring opening and rearrangement of the BAC ligand is rate-limiting. The vinyl ligand in **4-W** can be alkylated with MeOTf , leading to a five-membered amine-carbyne chelate with $\text{N}'\text{Pr}_2$ bound to the unique Fe (Fig. 2). This cluster is reminiscent of a putative NH_3 -bound form of FeMoco, as it displays a bridging C-based ligand and a nitrogen donor at one of the Fe centers.

Toward preparing structural analogs of the μ_3 -carbyne ligand, **3** was investigated as a precursor to a cluster bearing N or S at the bridging position. Treatment of **3** with NBu_4N_3 , Me_3SiN_3 , and PPh_3S (or S_8) leads to the formation of the corresponding nitride- (**6**), imide- (**7**), and sulfide- (**8**) bridged clusters (Fig. 3). Complexes **6**, **7**, and **8** are isostructural, with a $\text{WFe}_3\text{S}_3\text{X}$ ($\text{X} = \text{N}$ or S) cubane supported by Tp^* at W and one BAC ligand bound to each iron center (Fig. 4). The presence of three BAC ligands is a distinct feature relative to **4-W**. Targeting a carbyne analog with the same number of BAC donors, compound **5** was treated with BAC; however, no reaction was observed, likely due to a combination of steric constraints and stability of the chelate.

For closer similarity to FeMoco, a Mo variant of the above clusters was targeted. The Mo-containing precursor **1-Mo** was conveniently synthesized from $[\text{NEt}_4][\text{Tp}^*\text{MoS}_5]$ via $[\text{NEt}_4][\text{Tp}^*\text{MoS}_3]$ (46) generated by sulfur abstraction with PPh_3 (*SI Appendix*). Adapting the synthetic protocol developed for **2-W**, chloride substitution with BAC from **1-Mo** allowed for the isolation of **2-Mo**. Ring opening upon reduction with KC_8 resulted in the formation of **4-Mo** (Fig. 2). Notably, the $\text{MoS}_3\text{Fe}_3\text{C}$ cluster core of **4-Mo** reproduces one half of the structure of FeMoco, including the bridging carbon donor. Furthermore, the geometry of the unique Fe in the C-bridged clusters **4-W**, **4-Mo**, and **5** reproduces the four-coordinate, distorted trigonal pyramidal geometry found in the belt sites of FeMoco (Fig. 2). The S-Fe-S-C and S-Fe-S-N torsion angles in **4-W** (173.2°) and **5** (154.2°) approach 180° , bringing these four atoms close to coplanar, which corresponds to a distorted trigonal pyramidal geometry at Fe, leaving

Fig. 3. Synthesis of nitride, imide, and sulfide-containing clusters.

the axial site open for potential substrate coordination, as has been previously invoked for N_2 binding in FeMoco (17). In addition, the Fe-N distance in **5** is 2.16 Å, close to the Fe-N bond length in the previously characterized NH-bound FeVco (2.01 ± 0.04 Å) (31). Further studies are being conducted to investigate reactivity at this site.

A comparison of the structural aspects of the reported clusters and the corresponding subsite of FeMoco is informative (*SI Appendix, Table S3*). The W/Mo-S distances vary modestly (2.36 to 2.39 Å) in the series of cubane complexes, suggesting that the metal oxidation state remains unchanged. Although the total redox state of the metal core varies from $(\text{M}'\text{Fe}_3)^{8+}$ to $(\text{M}'\text{Fe}_3)^{11+}$, it is likely that the formal oxidation state for M' lies within the $3+/4+$ range, based on literature assignments for MoFe_3S_4 (47) and WFe_3S_4 (48) in two redox states, $(\text{M}'\text{Fe}_3)^{10+}$ and $(\text{M}'\text{Fe}_3)^{11+}$, as well as the trend in M' -S bond length as a function of oxidation states of M' from related species (*SI Appendix, Table S4*). Comparison of bond lengths within the organic fragment supporting the carbyne ligand reveals notable differences in **4-W/4-Mo** versus **5**. In **4-W/4-Mo**, the C10-N11 (average 1.36 Å) and C9-N18 (average 1.46 Å) distances are significantly different, suggesting multiple bonding character in C10-N11, while in **5** C10-N11 and C9-N12 are more similar [1.47 (1) and 1.43 (1) Å, respectively]. The orientation of N11 in **4-W/4-Mo** is such that the lone pair can engage in delocalization within the olefin π bond, increasing the N-C bond order and lowering the C9-C10 bond order (*SI Appendix, Fig. S33*). Because the carbyne is directly bonded to the olefin, its character is linked to the propensity of the amine lone pair to delocalize, therefore rendering **4-W/4-Mo** more Fischer-like than **5** (49). The possible changes in the character of the carbyne makes oxidation states ambiguous, but for consistency, herein the carbyne is assigned in the same way in all of the compounds. It is worth noting that the nature of the carbide ligand in FeMoco may also vary as a function of changes in the interactions with the other, remote metal centers. Additional experiments will be necessary to determine the overall redox states and distribution between metals; nevertheless, these compounds are in the range assigned for FeMoco (2). The structural parameters for the W and Mo analogs **4-W** and **4-Mo** are very similar, which suggests analogous redox distribution within the cluster despite different heterometals M' . The Fe-C distances in **4-W**, **4-Mo**, and **5** are in the range of 1.94 to 1.95 Å, which are close to the average Fe-C bond length in FeMoco of 2.00 Å

Fig. 5. CV scans for compounds **4** to **8**. Each voltammogram starts from the open-circuit potential, and the boxed peaks correspond to the $(M'Fe_3)^{11+}/(M'Fe_3)^{10+}$ couples of interest. The redox assignment is indicated above each wave in terms of the charge of the $(M'Fe_3)$ metal core. Conditions: ~ 2.5 mM cluster in MeCN with 0.2 M TBAPF₆, scan rates of 200 mV s⁻¹ (**4-W**, **5** to **8**) or 250 mV s⁻¹ (**4-Mo**). The asterisk indicates small amounts of **4-W** impurity in samples of **6**.

character of the ligand. Osmium compounds supported by dppe versus dppm ligands show a relatively small change of 30 mV (52), but we could not find specific precedent for the potential range of such effect for carbynes.

The $(M'Fe_3)^{11+}/(M'Fe_3)^{10+}$ couple for the C-containing clusters **4-W**, **4-Mo**, and **5** appear at potentials below -2 V, significantly more negative than for **6** to **8** at -0.87 V to -1.16 V. While **6** to **8** contain three BAC ligands and **4-W/5** only have two, complicating comparison, compound **5** displays a weaker donor in the tertiary amine compared to BAC in **6** to **8**. Although a less ambiguous analysis would benefit from an analog of **5** with a BAC ligand instead of the amine, which could not be accessed (*vide supra*), the greater electron donation ability of the NHC ligand compared to the tertiary amine is expected to render that hypothetical version of **5** even more reducing. Compounds **4-W**, **4-Mo**, and **5** all display chelates, unlike **6** to **8**. Multidentate phosphines and pyridines show shifts of less than 0.3 V in redox potentials relative to monodentate variants (52). Although this difference is much smaller than the differences observed here, because the carbyne interactions may be more strongly impacted by changes in bond angles, we cannot rule out that changes in reduction potentials are primarily due the presence of chelates in **4-W**, **4-Mo**, and **5**. Overall, the combination of carbyne and chelate results in a remarkable redox potential difference. Compounds **5** and **6** maintain the same formal charge for the bridging ligand (3-) as carbyne versus nitride. Still, a difference in the $(M'Fe_3)^{11+}/(M'Fe_3)^{10+}$ redox potentials of 1.12 V is observed, a substantial

impact of the chelating C- versus N-based, though nonchelating, ligands. Changing the donor from nitride (**6**) to imide (**7**) or sulfide (**8**) shifts the redox potential less than 200 mV, highlighting the similar effect of S and N donors on the redox chemistry, in contrast to the chelating carbyne.

These electrochemical results suggest that the interstitial carbon ligand in FeMoco may play an important role in increasing the reductive power of the clusters. While the specific oxidation states of the metal centers cannot be verified without additional spectroscopic studies, the reduced form, $(M'Fe_3)^{10+}$, corresponds to an average metal oxidation state of 2.5 (or 2 if a Fischer carbyne resonance is considered), close to the resting state of FeMoco as $Fe^{II}_3Fe^{III}_4Mo^{III}$ (average metal oxidation state 2.6) (2). Additional reduction steps lower the average formal oxidation state of FeMoco, but not below 2, and do not bring it in the range typically observed for mononuclear Fe complexes studied for N₂ activation (53). Therefore, the ability of the biological cofactor to perform N₂ activation at high oxidation states is unusual. A possible explanation is charge redistribution within the cluster to increase reducing equivalents at the site of substrate binding or electronic communication between different metal sites (21–23, 54). We find here that the chelating carbyne ligand has a remarkable impact on the cluster reduction potentials, with very reducing potentials for relatively high, biologically relevant metal oxidation states. Moreover, the chelating carbyne clusters are significantly more reducing for the same redox state compared to N and S analogs. It is important to note that the chelation present in all carbynes reported here and the delocalization of amine lone pair in some of them may have a substantial effect on the redox chemistry by tuning the electronic properties of the carbon ligand; conceptually related, changes in the coordination environment of distal iron centers in FeMoco may have similar effects on the carbide.

Toward addressing the effect of the interstitial ligands of FeMoco, we have reported studies of tetranuclear Fe clusters with μ_4 -fluoride and oxide ligands (21). In those cases, the oxide makes the cluster about 1 V more reducing compared to fluoride for the same redox state while also promoting NO activation. Additionally, remote metal centers affect reactivity through interactions with the bridging moiety (O or F). In those systems, the charge of the interstitial atom could play a role in changing the potential. Here, this series of clusters accounts for changes in ligand charge. Chelating carbyne (**5**) and nitride (**6**) ligands have the same formal charge but result in ~ 1 V difference in reducing power, in contrast with the nitride (**6**), imide (**7**), and sulfide (**8**) species that have reduction potentials within 300 mV. The ability of the chelating carbyne to increase the reduction power is likely a consequence of its stronger interaction with the metal centers, an aspect that will be pursued in a separate study, including nonchelated targets.

In the context of N₂ reduction, the redox tuning observed here suggests that the interstitial carbon may allow FeMoco to access higher reducing power, enabling more facile transfer of electrons to the N₂ substrate for conversion to NH₃. This parallels the results from experiments using synthetic iron catalysts for N₂ reduction, many of which require strong external reducing agents like KC₈ (8, 55). Considering the potential impact of replacing the bridging carbyne with a more biologically inexpensive sulfide, a much less reducing cluster (**8**) is generated for the same redox state, $(M'Fe_3)^{10+}$, and even an additional reducing equivalent in $(M'Fe_3)^{9+}$ does not match with the $(M'Fe_3)^{10+}$ carbyne system. Therefore, an interstitial sulfide may not provide sufficient reducing power to efficiently convert N₂ into NH₃, leading instead to the preference for the unusual bridging carbide motif.

In summary, we have described the synthesis of a series of heterometallic iron-sulfur clusters of the form $M'Fe_3S_3X$ ($M' =$

Mo or W and X = CR, N, NR, and S) with the cubane geometry matching the structure of the $\text{MFe}_3\text{S}_3\text{C}$ subsite of FeMoco. These include examples of iron-sulfur clusters containing a chelating carbon-based ligand bridging the Fe_3 face. Importantly, electrochemical studies indicate that the presence of a bridging C-donor in combination with electronic tuning (by chelation and amine lone pair delocalization) allows the clusters to reach highly reducing states, with potential implications for N_2 reduction chemistry. These studies shed light on possible structural and electrochemical roles of the interstitial carbide ligand in nitrogenase.

Materials and Methods

All reactions were performed at room temperature in a N_2 -filled MBraun glovebox or using standard Schlenk techniques unless otherwise specified. Solvents were dried by sparging with N_2 for at least 15 min and then passing through a column of activated alumina under positive N_2 pressure and stored over molecular sieves prior to use. All experimental conditions and procedures, spectroscopic data, and details of characterization of compounds $[\text{Net}_4][\text{Tp}^*\text{MoS}_3]$ and **1** to **8** are given in *SI Appendix*. CV experiments were

performed with a Pine Instrument Company AFCBP1 bipotentiostat in a three-electrode cell, which consisted of glassy carbon (working; $\phi = 3.0$ mm), Ag wire (reference), and bare Pt wire (counter), in a N_2 -filled MBraun glovebox at room temperature; dry CH_3CN that contained 0.2 M $[\text{Bu}_4\text{N}][\text{PF}_6]$ was used as the electrolyte solution.

Data Availability. Atomic coordinates and structure factors data have been deposited in the Cambridge Structural Database (CSD) of the Cambridge Crystallographic Data Centre (<https://www.ccdc.cam.ac.uk/structures/>). CSD reference numbers are as follows: **2084246** (**1-Mo**), **2081620** (**2-W**), **2084269** (**2-Mo**), **2081619** (**3**), **2081616** (**4-W**), **2084247** (**4-Mo**), **2081621** (**5**), **2081617** (**6**), **2081622** (**7**), and **2084054** (**8**). All other study data are included in the article and/or *SI Appendix*.

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