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N₂ Binding to an Iron-Sulfur-Carbon Site

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Abstract

Nitrogenases are found in some microorganisms, and these enzymes convert atmospheric N₂ to ammonia, thereby providing essential nitrogen atoms for higher organisms. Some nitrogenases reduce atmospheric N₂ at the FeMoco, a sulfur-rich iron-molybdenum cluster¹–⁵. The iron centers that are coordinated to sulfur and carbon atoms in FeMoco have been proposed as the substrate binding sites, based on kinetic and spectroscopic studies⁵,⁶. Studies on the enzyme indicate that iron atom Fe6 and possibly also adjacent belt iron sites are involved.⁵–⁸ In the resting state, the central Fe sites (including Fe6) have identical environments consisting of three sulfides and a carbide. Addition of electrons to the resting state causes the FeMoco to react with N₂, but the geometry and bonding environment of N₂-bound species remain unknown⁵. In this manuscript, we describe a synthetic complex with a sulfur-rich coordination sphere that, upon reduction, breaks an Fe-S bond and binds N₂. The product is the first synthetic Fe–N₂ complex in which iron has bonds to sulfur and carbon atoms, providing a model for N₂ coordination in the FeMoco. Our results demonstrate that breaking an Fe-S bond is a chemically reasonable route to N₂ binding in the FeMoco, and show structural and spectroscopic details for weakened N₂ on a sulfur-rich iron site.

N₂ binding to FeMoco is believed to take place at an iron center with three sulfur ligands following Fe-C bond elongation or dissociation (I to II, Fig. 1A)⁹–¹⁵. These sites could accommodate N₂ binding by breaking an Fe-S or an Fe-C bond, but data on the enzyme do not yet distinguish between these possibilities. The likely enzymatic intermediates, iron-N₂ species supported either solely by sulfur, or by sulfur and carbon ligands, are unprecedented in isolated coordination complexes. Here, we focus on an alternative hypothesis where one of the Fe-S bonds at the active site is broken upon reduction/protonation to expose the N₂.
binding site (I to III, Fig. 1A)\(^{16,17}\). \(N_2\) would thus bind at a pseudotetrahedral S,S,C-bound iron site. The feasibility of Fe-S bond cleavage in FeMoco is experimentally supported by the loss of this S atom in the structure of CO-inhibited nitrogenase\(^7\), and by the observation of Fe-S cleavage upon protonation in smaller FeS clusters\(^{18,19}\). Other \(N_2\) binding hypotheses include side-on binding, bridging, and *endo* coordination where \(N_2\) is positioned close to three additional iron atoms and opposite to a sulfur atom (IV, Fig. 1A)\(^5,11,17\).

Iron-\(N_2\) complexes supported solely by sulfur, or by sulfur and carbon supporting ligands, are likely \(N_2\)-bound species in the nitrogenase catalytic cycle, but they are experimentally unprecedented. Though chemists have prepared complex iron-sulfur clusters inspired by the multimetallic structure of FeMoco, \(N_2\) does not bind to any known synthetic iron-sulfur cluster\(^20\). A number of well-defined iron complexes with B, N, and P supporting ligands are known to activate \(N_2\), and Peters has established P- and C-based systems capable of performing catalytic reduction of \(N_2\) to ammonia\(^{14,15,21–24}\). A few iron-\(N_2\) complexes have thioether/thiolate donors on the same iron center, and each is additionally supported by P- or N-donors\(^25–27\). To the best of our knowledge, there are no examples of terminal \(N_2\) complexes of any metal having immediate ligand environments similar to those in II-IV, which hinders scientists’ ability to predict the behavior of the FeMoco.

For this work, we designed bis(thiolate) ligand \(L^{2-}\), which offers only sulfur and carbon based coordination sites (indicated by yellow and grey spheres in Fig. 1B). Our approach was guided by the proposed binding mode III in Fig. 1A, which requires the presence of two coordinating sulfur atoms. These are provided by two chelating arylthiolate donors with bulky 2,4,6-trisopropylphenyl groups shielding the S sites. A central aromatic ring connects the two arylthiolate arms and additionally provides potential carbon based attachment sites\(^28\). Although carbide is electronically different than the arene ring in \(L^{2-}\), each could provide flexible bonding for stabilization of various intermediates during ammonia production\(^14,15\).

Iron(II) ions were installed in the ligand framework by treating \(LH_2\) with iron(II) bis(bis(trimethylsilyl)amide) in tetrahydrofuran (THF), which yielded the bright yellow, high spin iron(II) complex LFe(THF)\(_2\) (I, Fig. 2A). Its crystal structure reveals that it is four-coordinate, and that all Fe-C distances are at least 2.59 Å (Fig. 2B). Reduction of I to iron(I) with potassium graphite (KC\(_8\)) results in the formation of brown-yellow 2, with close Fe-C distances (2.04 – 2.12 Å) indicating \(\eta^6\)-binding of the central arene ring (Fig. 2A, 2C). Comparison of the molecular structures of 1 and 2 reveals that rotation of the arylthiolate arms enables the central aryl ring to move closer to the iron atom. Compound 2 has a rhombic EPR (electron paramagnetic resonance) spectrum with \(g = [2.180, 2.020, 1.989]\) and a solution magnetic moment of 2.1 μ\(B\), which indicate a low-spin (\(S = 1/2\)) iron(I) center.

Encouraged by the ability of the ligand to stabilize low-valent iron sites, we further reduced the iron site to the iron(0) oxidation state. Reduction of a brown-yellow solution of 2 with one equivalent of KC\(_8\) under one atmosphere of \(N_2\) at \(-70\) °C resulted in an immediate color change to deep red. After addition of 18-crown-6 to sequester potassium cations, dark red-brown crystals of 3 grew at \(-40\) °C. X-ray diffraction analysis shows that 3 is [LFeN\(_2\)]

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In 3, N\textsubscript{2} is bound as a terminal ligand at a pseudotetrahedral iron(0) site, which is further bound to two S atoms and the arene of the supporting ligand. The closest Fe-C distance in 3 is 2.04 Å, and there is a second carbon atom within bonding distance (Fe-C = 2.24 Å), indicating asymmetric ƞ\textsuperscript{2} coordination of the arene. The potassium cations do not bind to the N\textsubscript{2} ligand.

The new N\textsubscript{2} complex 3 provides a structural model of the pseudotetrahedral S,S,C supported N\textsubscript{2} binding mode III proposed for FeMoco (Fig. 1A). It is compared to the experimental structures of resting state FeMoco and CO-inhibited FeMoco in Fig. 3B\textsuperscript{7}. In the fourth coordination site that has labile S and CO ligands in nitrogenase structures\textsuperscript{7}, 3 contains an N\textsubscript{2} ligand. The Fe-S bond distances in 3 (2.32–2.35 Å) are somewhat longer than the Fe-S bonds in resting state FeMoco (2.25–2.27 Å), due to either the lesser negative charge of the thiolate or the greater steric hindrance. Remarkably, the Fe-C distance in 3 at 2.04 Å is very close to the Fe6-carbide distance of 2.01 Å in FeMoco structures. Overall, the relatively simple ligand L\textsuperscript{2} is capable of arranging appropriate atoms around iron and imparting a geometry that resembles the likely active iron site in FeMoco structures. However, the electronic structure of the iron(0) complex 3 may be different than the iron site in the N\textsubscript{2}-binding form of the FeMoco (for which the structure and iron oxidation state are unknown).

Next, we designed a compound (5) intended to test the idea that Fe-S bond dissociation could provide a coordination site for N\textsubscript{2} binding (I to III in Fig. 1A). The bis(thiolate) complex 1 reacted with thiolate 4 to give the iron tris(thiolate) complex 5 (Fig. 2A). This orange high-spin iron(II) complex contains three S ligands, like Fe6 in the FeMoco resting state (I in Fig. 1). The interaction of iron with the central arene ring is weak, with the closest Fe-C distance at 2.48 Å (Fig. 2D). Thus we view this site as three-coordinate and unsaturated, which is supported by the reversible binding of one THF molecule at low temperature (Fig. 2E and Supplementary Information show the X-ray crystal structure of 5·THF and temperature-dependent UV-vis spectra).

The tris(thiolate) iron(II) site in 5/5·THF was reduced to the iron(0) oxidation level with just over two equivalents of KC\textsubscript{8}, under conditions otherwise equivalent to those used for reduction of the iron(I) bis(thiolate) complex 2 (Fig. 2A). This yielded the same N\textsubscript{2} complex 3 described above, and 1.0 equiv. of free thiolate was produced. Reduction thus causes an Fe-S bond to break concomitant with N\textsubscript{2} binding, as in the proposed pathway for N\textsubscript{2} binding to FeMoco in Fig. 1A (I to III). We note that tris(thiolate) 5 contains all the nearby atoms to support alternative binding modes II and IV in Fig. 1A, but Fe-S dissociation takes place instead.

We return to describe the further characterization of 3, which gives insight into potential properties of N\textsubscript{2} after binding at FeMoco. Though complex 3 is very thermally sensitive, it was possible to isolate pure samples of 3 in >80% yield from reduction of 5 at low temperature and washing the crystals with cold butane at −70 °C. Analysis of these crystals by Mössbauer spectroscopy confirms the presence of a single iron species. Infrared spectroscopy (IR) analysis of single crystals of 3 revealed a strong N-N stretching band at 1880 cm\textsuperscript{-1}. These frequencies are the lowest observed for any Fe-N\textsubscript{2} complex with a terminal N\textsubscript{2} ligand\textsuperscript{23}, which shows that the thiolates are powerful electron donors that enable
substantial backbonding into the N$_2$ π* orbitals. The N$_2$ ligand in 3 exchanges with free $^{15}$N$_2$ (giving an $^{15}$N-$^{15}$N stretching band at 1813 cm$^{-1}$) at −70 °C in the solid state. Samples of 3 kept at room temperature for a few hours lack the N$_2$ stretching vibration, further demonstrating the lability of N$_2$. The lability suggests that the Fe-N$_2$ interaction, though strong as judged by IR spectroscopy, may be compensated with tighter binding to the arene ring.

Compound 3 has a high spin ($S = 1$) electronic configuration, as determined by SQUID (superconducting quantum interference device) magnetometry on a crystalline sample. This experimental observation was confirmed with density functional theory calculations on a truncated model of 3. Optimization with $S = 1$ gave a model close to the experimental geometry, but optimization with $S = 0$ gave significantly different bond lengths and angles, and a Gibbs free energy (ΔG°) that was higher by 37 kJ/mol (see Supplementary Information). High spin iron(0) dinitrogen complexes are rare, and have been seen mainly in cases where high symmetry makes the frontier orbitals nearly degenerate. To our knowledge, 3 is the first high spin iron complex that contains both S and N$_2$ ligands, and shows that high-spin iron (as expected in the weak-field sulfur-dominated environment of iron atoms in the FeMoco) can activate N$_2$ substantially.

The preparation of an iron-N$_2$ complex with a sulfur-rich environment provides structural and spectroscopic precedents for FeMoco-N$_2$ binding, and also gives insight into the nitrogenase mechanism. Reduction of complex 5 breaks an Fe-S bond as in the hypothetical conversion of I to III in the FeMoco (Fig. 1), and binds N$_2$ in a form where the N-N bond is greatly weakened. In this way, the results support the idea that the sulfur-rich iron site in the FeMoco is particularly well-suited for N$_2$ activation, and that Fe-S bonds can be easily broken upon reduction to allow binding of N$_2$.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. \( N_2 \) binding to iron in sulfur and carbon rich environments

a. Schematic representations of FeMoco and three potential \( N_2 \) binding modes. Potentially protonated sulfur ligands are not specified. b. Ligand design for a synthetic sulfur-carbon site.
Figure 2. $N_2$ binding at an iron-sulfur-carbon site through Fe-S bond cleavage

a. Reactions of synthetic iron-sulfur sites leading to $N_2$-binding. The bottom pathway shows Fe-S cleavage with $N_2$ binding. Ar = 2,4,6-triisopropylphenyl. b–e. Molecular structures of the synthetic mononuclear iron-sulfur sites presented here. Hydrogen atoms and Ar groups are omitted for clarity.
Figure 3. Iron-N$_2$ complex supported by sulfur and carbon ligands

a. Two views of the molecular structure of the anionic part of 3. Hydrogen atoms and Ar groups are omitted. b. Comparison of geometric parameters with CO-inhibited FeMoco$^7$ and resting state FeMoco$^2$. All distances are reported in Ångströms.