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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 cluster^{1–5}. 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 studies^{5,6}. 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

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Supplementary Information is available in the online version of the paper.

Author Contributions I. O. designed the iron-sulfur-carbon system for N₂ binding, performed the laboratory experiments, and analyzed data. B.Q.M. collected and interpreted crystallographic data. E.B. interpreted solid-state (SQUID) magnetic data. D.J.V. collected and fit EPR data. P.L.H. supervised the research, and I. O. and P.L.H. wrote the manuscript.

X-ray crystallographic data have been deposited in the Cambridge Crystallographic Data Centre (<http://www.ccdc.cam.ac.uk/>) with deposition numbers CCDC1402555-1402559.

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binding site (**I** to **III**, Fig. 1A)^{16,17}. N₂ 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 nitrogenase⁷, and by the observation of Fe-S cleavage upon protonation in smaller FeS clusters^{18,19}. Other N₂ binding hypotheses include side-on binding, bridging, and *endo* coordination where N₂ is positioned close to three additional iron atoms and opposite to a sulfur atom (**IV**, Fig. 1A)^{5,11,17}.

Iron-N₂ complexes supported solely by sulfur, or by sulfur and carbon supporting ligands, are likely N₂-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, N₂ does not bind to any known synthetic iron-sulfur cluster²⁰. A number of well-defined iron complexes with B, N, and P supporting ligands are known to activate N₂, and Peters has established P- and C-based systems capable of performing catalytic reduction of N₂ to ammonia^{14,15,21–24}. A few iron-N₂ 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 N₂ 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-triisopropylphenyl groups shielding the S sites. A central aromatic ring connects the two arylthiolate arms and additionally provides potential carbon based attachment sites²⁸. 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 (**1**, 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 **1** to iron(I) with potassium graphite (KC₈) results in the formation of brown-yellow **2**, with close Fe-C distances (2.04 – 2.12 Å) indicating η⁶-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 KC₈ under one atmosphere of N₂ 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 [LFeN₂]

[K(18-crown-6)(THF)₂]₂ (Fig. 2A and 3A). In **3**, N₂ 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 η² coordination of the arene. The potassium cations do not bind to the N₂ ligand.

The new N₂ complex **3** provides a structural model of the pseudotetrahedral S,S,C supported N₂ 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^{2,7}. In the fourth coordination site that has labile S and CO ligands in nitrogenase structures⁷, **3** contains an N₂ 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 L²⁻ 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 N₂-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 N₂ 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**·THF was reduced to the iron(0) oxidation level with just over two equivalents of KC₈, under conditions otherwise equivalent to those used for reduction of the iron(I) bis(thiolate) complex **2** (Fig. 2A). This yielded the same N₂ complex **3** described above, and 1.0 equiv. of free thiolate was produced. Reduction thus causes an Fe-S bond to break concomitant with N₂ binding, as in the proposed pathway for N₂ 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 N₂ 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⁻¹. These frequencies are the lowest observed for any Fe-N₂ complex with a terminal N₂ ligand²³, which shows that the thiolates are powerful electron donors that enable

substantial backbonding into the N₂ π* orbitals. The N₂ ligand in **3** exchanges with free ¹⁵N₂ (giving an ¹⁵N-¹⁵N 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 N₂ stretching vibration, further demonstrating the lability of N₂. The lability suggests that the Fe-N₂ 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^{29,30}. To our knowledge, **3** is the first high spin iron complex that contains both S and N₂ ligands^{25,26}, and shows that high-spin iron (as expected in the weak-field sulfur-dominated environment of iron atoms in the FeMoco) can activate N₂ substantially.

The preparation of an iron-N₂ complex with a sulfur-rich environment provides structural and spectroscopic precedents for FeMoco-N₂ 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 N₂ 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 N₂ activation, and that Fe-S bonds can be easily broken upon reduction to allow binding of N₂.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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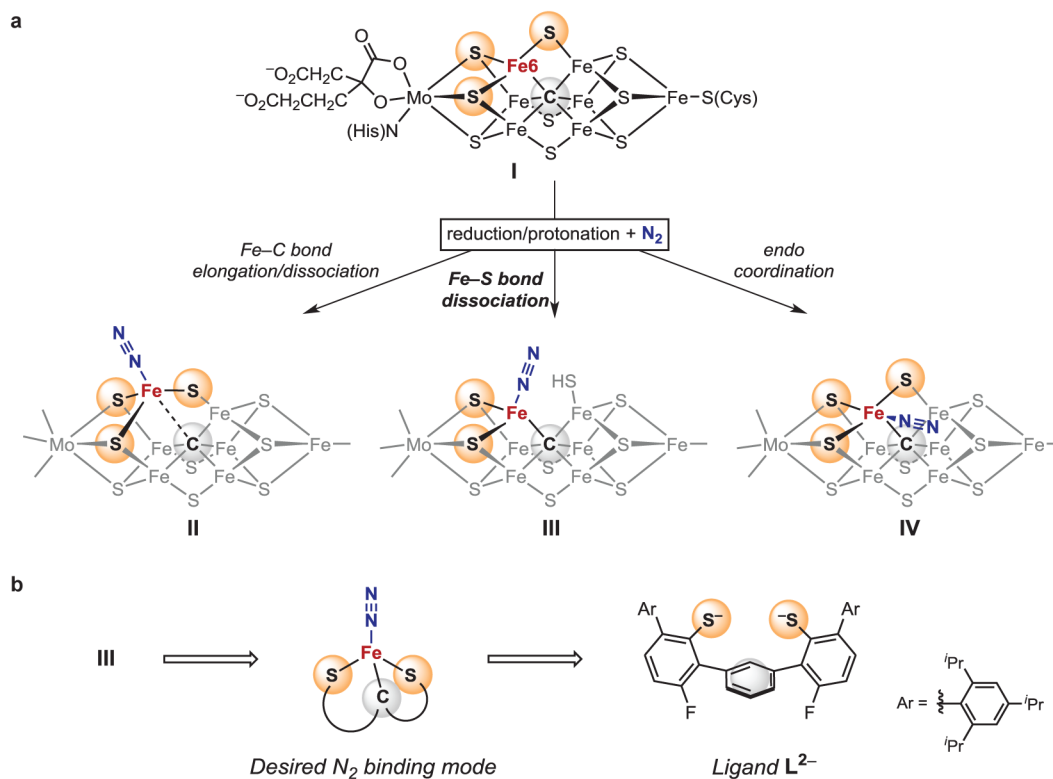


Figure 1. N_2 binding to iron in sulfur and carbon rich environments

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.

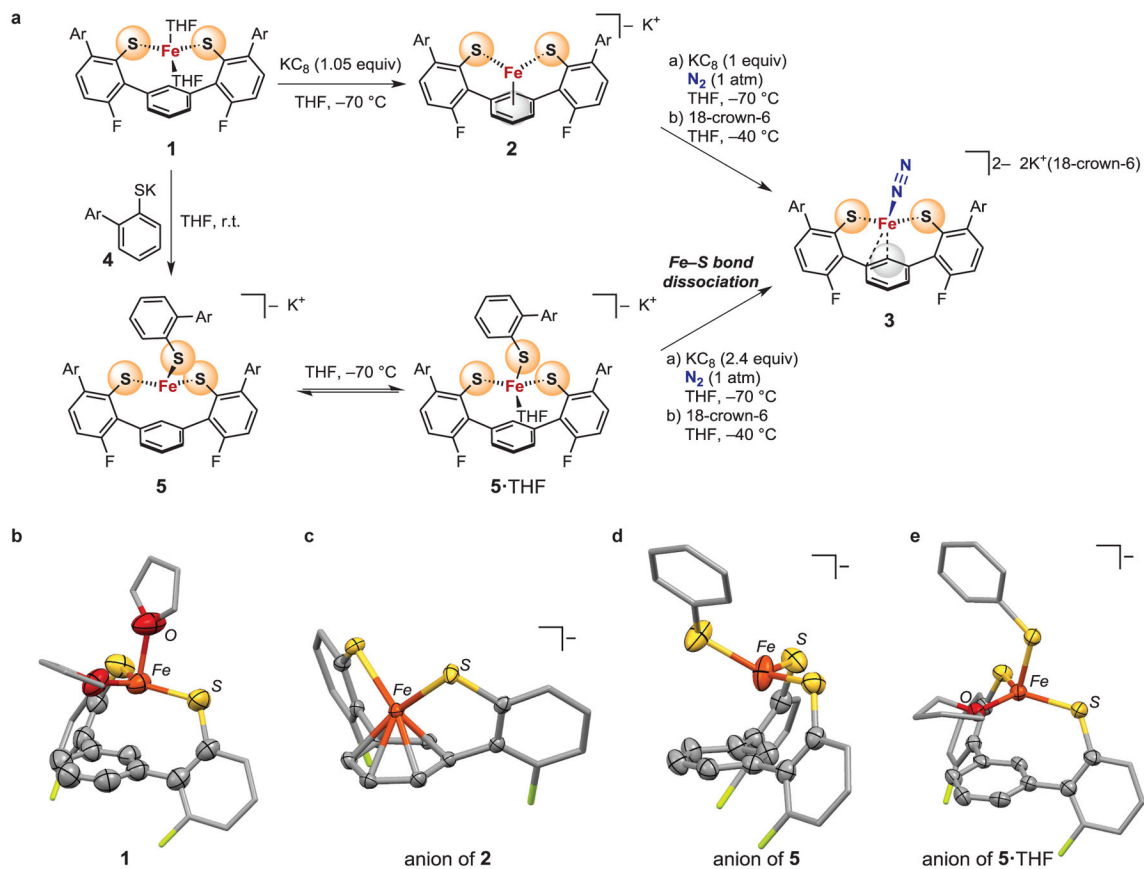


Figure 2. N₂ binding at an iron-sulfur-carbon site through Fe-S bond cleavage

a, Reactions of synthetic iron-sulfur sites leading to N₂-binding. The bottom pathway shows Fe-S cleavage with N₂ 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.

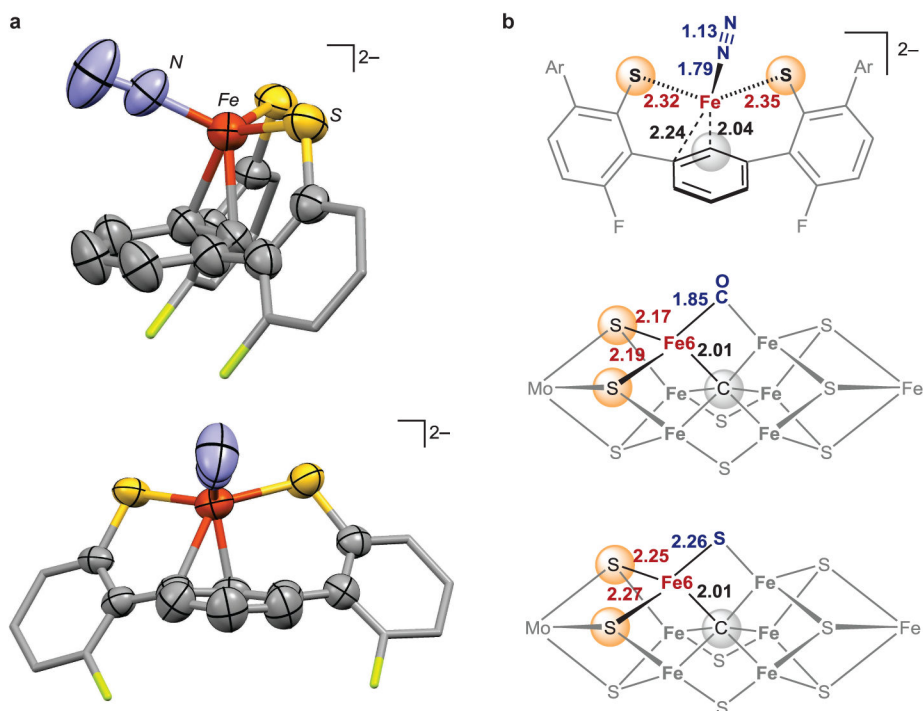


Figure 3. Iron-N₂ complex supported by sulfur and carbon ligands

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 FeMoco⁷ and resting state FeMoco². All distances are reported in Ångströms.