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Facile Nitrite Reduction in a Non-heme Iron System: Formation of an Iron(III)-Oxo

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Supporting Information

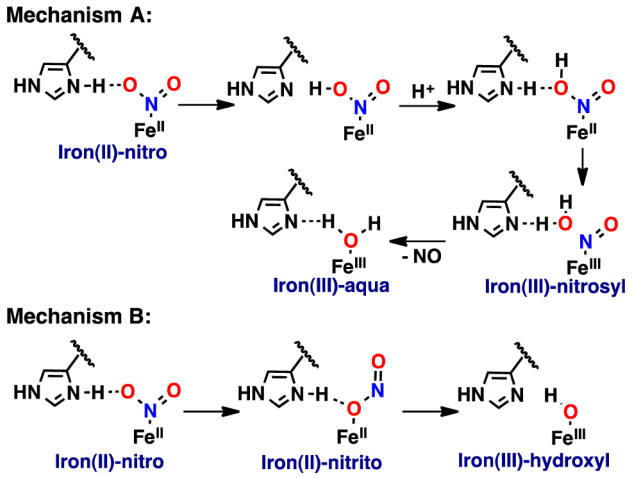
ABSTRACT: Reaction of tetrabutylammonium nitrite with $[N(afa^{Cy})_3Fe(OTf)](OTf)$ cleanly resulted in the formation of an iron(III)-oxo species, $[N(afa^{Cy})_3Fe(O)](OTf)$, and NO(g). Formation of NO(g) as a byproduct was confirmed by reaction of the iron(II) starting material with half an equivalent of nitrite, resulting in a mixture of two products, the iron-oxo and an iron-NO species, $[N(afa^{Cy})_3Fe(NO)](OTf)_2$. Formation of the latter was confirmed through independent synthesis. The results of this study provide insight into the role of hydrogen bonding in the mechanism of nitrite reduction and the binding mode of nitrite in biological heme systems.

Inorganic nitrite (NO_2^-) is one of the simplest naturally occurring anions.[1] Prior to 2001, nitrite's only utility in the body was thought to be as a marker for endogenously produced nitric oxide (NO).[1,2] In 2003, when supra-physiological concentration of NO_2^- caused a significant increase in the blood flow within a human forearm, it was proposed that nitrite is a physiological source of NO.[3] Known to be a bioregulatory molecule, NO has multiple mammalian functions, including vasodilation and neurotransmission.[1,2,4] Enzymatic nitrite reduction has been achieved by a host of proteins including heme-associated globins (e.g., hemoglobin,[3,5,6] myoglobin,[7−9] and mitochondrial proteins, including cytochrome *c* and cytochrome *c* oxidase, nitric oxide synthase, and cytochrome P450[2,4]).

Although much is known about the capability of these metalloproteins to reduce nitrite, two questions remain about the process: (1) Does nitrite bind to the metal center via the nitrogen (M-nitro) or oxygen (M-nitrito) atom? (2) What is the mechanism for nitrite reduction? A large majority of crystallographically characterized iron(II) or iron(III) porphyrin complexes favor nitro bonding.[10−15] However, DFT studies have indicated that the energy difference between the nitro and nitrito isomers may be overcome by hydrogen bonding from a pendant histidine.[16] Additionally, two reported mechanisms of nitrite reduction have been investigated (Scheme 1) and present the formation of either a weakly bound {FeNO}^{6}, supported by crystallography,[17−20] kinetics,[3,21,22] and computation,[23] or an Fe(III)-OH species, cited as a result of DFT[16] and experimental investigations.[24]

Recently our research group has reported the synthesis and characterization of a family of late, first-row transition metal complexes featuring a ligand platform, tris(5-cycloiminopyrrol-2-ylmethyl)amine ($H_3[N(pi^{Cy})_3]$), that is capable of either hydrogen bond donating or accepting.[25] Upon dative coordination to $Fe(OTf)_2(MeCN)_2$, formation of the amino-azafulvene tautomer $(N(afa^{Cy})_3)$ of the ligand framework is observed, giving $[N(afa^{Cy})_3Fe(OTf)](OTf)$ (**1**), as evidenced by distortions in intraligand bond distances as well as the presence of an amino-derived, secondary coordination sphere.[18]

Scheme 1. Proposed Mechanisms of Biological Nitrite Reduction



Interested in examining these complexes in the context of the development of mimetic systems for metalloenzymes with redox-active amino acids in the secondary coordination sphere, we explored their reactivity toward a variety of substrates. Herein, we communicate an investigation of nitrite reduction utilizing a non-heme system that features a ligand scaffold with a secondary coordination sphere that is capable of hydrogen bonding. The results of this study address the two main questions regarding nitrite reduction: binding mode and mechanism.

Upon exposure of $[N(afa^{Cy})_3Fe(OTf)](OTf)$ (**1**) to an equivalent of $[(^nBu)_4N]NO_2$, an immediate color change from yellow to brown-red was noted, consistent with oxidation of the trigonal bipyramidal, high-spin iron(II) starting material (Scheme 2).[26,27] Following workup, the product (**2**) was isolated as a crystalline solid in good yields (63%). Characterization of **2** by ^{1}H NMR spectroscopy revealed formation of a

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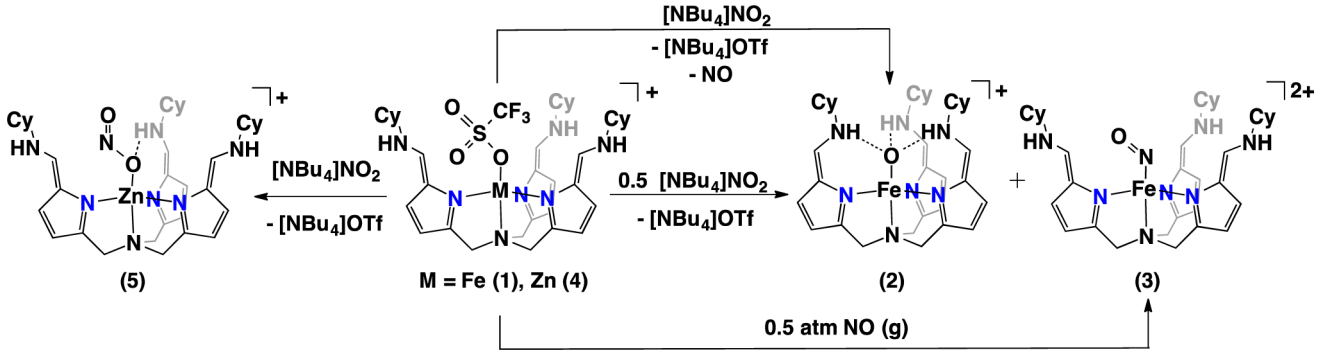
Scheme 2. Preparation of Complexes 2, 4, and 5



paramagnetic product with four distinct, broad resonances ranging from 5.41 to 79.52 ppm, indicative of a C_3-symmetric molecule in solution (Supporting Information, Figure S1). A cluster of signals located between 1.41 and 2.96 ppm were assigned to the protons of the cyclohexyl rings. The IR spectrum of the product revealed no features in the N−H or O−H region. The single, broad band at 1667 cm^{-1} is assigned to the C═N stretches of the ligand platform, a value blue-shifted by 30 cm^{-1} from that for **1** (1637 cm^{-1}) and consistent with azafulvene ligation of the ligand.[25]

To elucidate the molecular structure of the product, crystals suitable for X-ray analysis were grown from a concentrated solution of acetonitrile and benzene layered with diethyl ether. Upon refinement, an Fe(III)-oxo complex with a distorted trigonal bipyramidal geometry was noted (Figure 1, Table 1).

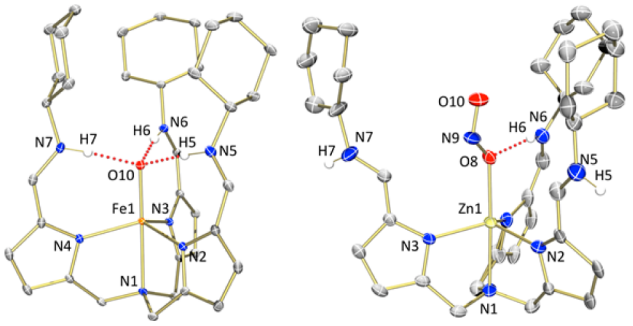


Figure 1. Molecular structures of **2** and **5** shown with 50% probability ellipsoids. Outer-sphere triflate molecules, selected hydrogen atoms, and solvent molecules have been omitted for clarity.

Table 1. Structural Parameters for Complexes 2 and 5

bond	**2** (M = Fe; *X* = 10)	**5** (M = Zn; *X* = 8)
M1−N1	2.3082(11) Å	2.256(3) Å
M1−N(pyr)	2.0388(11)−2.0539(11) Å	2.055(3)−2.067(6) Å
M1−O*X*	1.8079(9) Å	2.073(4) Å
O*X*···H5	1.709(19) Å	
O*X*···H6	1.750(19) Å	2.04(5) Å
O*X*···H7	1.87(2) Å	
O8−N9		1.184(5) Å
N9−O10		1.209(7) Å
O8−N9−O10		121.2(7)°

In contrast to the structure of **1**, all three arms of the ligand platform are now rotated inward, with the pendant amino functional groups pointing toward a newly installed terminal oxo moiety bound *trans* to the apical nitrogen (N1). The equatorial plane of $[N(afa^{Cy})_3Fe(O)](OTf)$ (**2**) is composed of the azafulvene nitrogen atoms, with Fe−N bond distances ranging from 2.0388(11) to 2.0539(11) Å. As a result of the appendage of the strong Fe−O bond, the interaction of the metal center with the apical nitrogen of the ligand platform (Fe1−N1: 2.3082(11) Å) has been elongated from that in complex **1** (2.268(2) Å).[25]

The Fe1−O10 distance of 1.8079(9) Å is within error of the Fe−O distance reported by Borovik and co-workers for $[Fe^{III}H_3buea(O)]^{2-}$ (1.813(3) Å), where the terminal oxo is supported by the secondary coordination sphere of a urea-derived ligand platform.[27] Futhermore, this distance is significantly shorter than those reported for terminal Fe(III)-OH complexes, which range from 1.876 to 1.926 Å.[28−31] The secondary coordination sphere of **2**, composed of the amino moieties, has reorganized to stabilize the Fe(III)-O moiety, resulting in hydrogen bonding of all three amino groups (N*X*−H*X*; *X* = 5, 6, 7) with the terminal oxygen atom. The N*X*···O10 distances, ranging from 2.6420(15) to 2.6451(15) Å, are slightly shorter than those reported for the intramolecular H-bonds between the oxo and the NH groups of $[Fe^{III}H_3buea(O)]^{2-}$ (2.686(7)−2.732(5) Å).[27]

Formation of an iron-oxo complex from nitrite has previously been reported in both iron and manganese porphyrin systems; however, photolysis was required to cleave the O−N bond, resulting in a M(IV)-oxo species and NO(g).[32] Previous work, related to nitrite reduction by discrete inorganic complexes, has noted the formation of nitric oxide upon the addition of weak acids to the isolated Fe-NO_2 complexes, invoking Mechanism A (Scheme 1) as the mode of degradation of the anion.[4] The nitrite anion is proposed to bind to the iron center through the nitrogen center; subsequent protonations result in the release of an equivalent of water and formation of an {FeNO}^{7} complex.

Notably, the formation of complex **2** proceeds without addition of excess acid and instead is supported by the secondary coordination sphere of the ligand platform. The single example of reductive nitrite degradation resulting in the formation of an Fe(III)−O bond was reported within the past year.[24] Reductive cleavage of the N−O bond of $[(Bim)_3Fe(\kappa^2\text{-}ONO)](BF_4)$ was observed only upon addition of acetic acid. In analogy to the work presented here, those authors invoked secondary interactions between an equivalent of acetic acid bound to the iron center and the nitrito oxygen atom bound to the metal center.

Similarly, the reduction of nitrite by **1** results in the formation of the $Fe^{III}(O)$ moiety with loss of NO (Mechanism B, Scheme 1). The formation of complex **2** suggests

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progression through an alternative mechanism: NO_2^- binds to the iron center as the iron-nitrito isomer. Hydrogen bonding from the amino moieties (analogous to the histidine located in the secondary coordination sphere of the active site of the enzyme) promotes reductive N−O bond cleavage, utilizing an electron from the reduced metal center. This results in the formation of **2** with the concurrent loss of an equivalent of NO.

To confirm the production of NO during the reduction of nitrite, compound **1** was reacted with 0.5 equiv of $[(^nBu)_4N]NO_2$ (Scheme 2). The ^{1}H NMR spectrum of the crude reaction mixture revealed quantitative formation of **2** and another paramagnetic species. The IR spectrum of this mixture of products clearly revealed a N═O stretch at 1744 cm^{-1}, a feature not observed in either the starting material, **1**, or the iron-oxo product, **2**. The NO stretch at 1744 cm^{-1} is within range previously reported for iron−nitrosyl complexes (1667−1831 cm^{-1}).[33−35] Based on the IR spectrum, we tentatively assigned this new species as $[N(afa^{Cy})Fe(NO)](OTf)$ (**3**). To confirm the formation of the nitrosyl adduct, independent synthesis of complex **3** was attempted from the reaction of **1** with 0.5 atm of NO(g) (Scheme 2). The 4 K EPR spectrum of **3** is typical of a slightly rhombic S = 3/2 system (Supporting Information, Figure S4). The ^{1}H NMR and IR spectra match those of the new paramagnetic species identified in the previous reaction mixture, confirming formation of the iron-NO adduct in the absence of excess nitrite.

As the formation of complex **2** is proposed to proceed through reduction of the transient intermediate, $[N(afa^{Cy})_3Fe(ONO)](OTf)$, synthesis of the redox-inert zinc derivative was attempted to gain structural insight into hydrogen bonding within the proposed intermediate. Addition of an equivalent of $[(^nBu)_4N]NO_2$ to $[N(afa^{Cy})_3Zn(OTf)](OTf)$ (**4**) yielded no obvious color change (Scheme 2). However, following workup and recrystallization, a new product (**5**) was isolated as a crystalline, colorless solid in good yields (82%). Analysis of **5** by ^{1}H NMR spectroscopy revealed a diamagnetic product with an asymmetric distribution of resonances (Supporting Information, Figure S5). The infrared spectrum of **5** included two bands for the N−H asymmetric stretches (3230, 3295 cm^{-1}) and a single feature assigned to the C═N stretches of the azafulvene ligand (1640 cm^{-1}). These IR features are extremely similar to those of the starting material, indicating only minor changes in the electronic environment of the ligand framework. The complex fingerprint region of the IR spectrum prevented definitive assignment of the ν(N═O) and ν(N−O) stretches in order to differentiate between the nitro or nitrito linkage isomers.

Analysis by X-ray crystallography of a single crystal confirmed formation of the Zn-nitrito adduct, $[N(afa^{Cy})Zn(ONO)](OTf)$ (**5**) (Figure 1, Table 1). Complex **5** has a pseudo trigonal-bipyramidal geometry about the metal center, with the nitrite group bound trans to the axial nitrogen atom. A single arm of the ligand is oriented with the amino moiety pointed toward the substrate, engaging in a hydrogen-bonding interaction with the oxygen atom bound directly to the metal center, structurally analogous with the proposed iron(II) nitrito intermediate invoked in Mechanism B (Scheme 1). The H6···O8 distance of 2.04(5) Å is significantly longer than that in **2**.

This crystal structure is in accordance with previously described computational studies on nitrite reduction from the nitrito isomer.[16] These studies suggested that hydrogen bonding from the pendant histidine favors the nitrito isomer. The minor energy difference associated between the nitro and nitrito linkage isomers (4 kcal/mol) is easily overcome by the presence of a single hydrogen bond, as illustrated in our system. This single hydrogen bond in our ligand scaffold favors the formation of the zinc-nitrito species. Furthermore, DFT studies[16] suggested that N−O bond cleavage from the nitrito isomer is even more facile than that from the nitro isomer, which is accurately demonstrated in our iron system.

The aforementioned results demonstrate the influence hydrogen bonding has on the reduction of nitrite. Consistent with previously reported DFT studies on nitrite reduction,[16] our results illustrate that a single hydrogen bond in the secondary coordination sphere results in the preferential binding of nitrite through the oxygen atom to form the iron-nitrito species. This linkage isomer was clearly depicted in the X-ray crystal structure of the zinc complex, $[N(afa^{Cy})Zn(ONO)](OTf)$ (**5**). Reaction of nitrite with our iron(II) starting material solely resulted in the formation of an iron(III)-oxo species concomitantly with NO(g). The presence of NO(g) as a byproduct was confirmed by reactivity of our iron(II) starting material and a substoichiometric amount of nitrite. Independent synthesis of the resultant iron(II)-NO species was also completed. These findings not only support the preferred formation of the iron-nitrito isomer in the presence of H-bonds but also provide a facile route to nitric oxide through the reduction of nitrite. Investigations into other NO_x derivatives as well as a more detailed understanding of this reaction sequence, including the electronic structure of the Fe(III)-oxo, are ongoing in our laboratories.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectral data, synthesis, details for complexes **2**-**5** and selected crystallographic data and CIF files for complexes **2** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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