A Synthetic Model for the NO-Inactivated Form of Nitrile Hydratase

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Nitric oxide (NO)[1] has been shown to perform a number of important functions in medicine and biology, including blood pressure control, neurotransmission, and inhibition of tumor growth.[2,3] More recently, NO was found to play a regulatory role in the metalloenzyme nitrile hydratase (NHase)[4,5] by reversibly binding to its iron active site.[6] Nitrile hydratases[7–12] are non-heme, low-spin Fe(III)-containing microbial enzymes which promote the hydration of nitriles to amides. Three *facial* cysteinates, two cis peptide nitrogens,[11] and a hydroxide[13] ligate the iron in its active form (NH_{lt}). An inactive, NO-bound form (NH_{dk}) is generated in whole cell bacterial samples stored in the dark.[14] Activity is restored upon exposure to light.[4–6] The presence of an IR band at 1853 cm^{-1} suggests that NO coordinates to the iron site.[14] Spin-trapping[4] and quantitative NO titration experiments[6] show that one NO is released per iron during photoactivation. Initial studies[5,14] suggested that NH_{dk} contained an Fe(II)–NO species. The EPR silence[15] of inactivated NH_{dk} was, however, inconsistent with this proposal. A recent X-ray structure[16] of NH_{dk} from *Rhodococcus sp.* N-771 showed that two of the ligated cysteinates had been oxidized to sulfenate and sulfinate groups. However, it was not mentioned whether the crystals used in this study (a) could be photolytically reactivated or (b) displayed the ν_{NO} characteristic of NH_{dk}. Sulfinate groups are not detected, by resonance Raman, in NH_{lt} from *Rhodococcus sp.* R312.[17] Herein, we report a synthetic, NO-bound, bis-(thiolate)-ligated iron complex which closely matches the properties of the NH_{dk} iron site, despite the absence of sulfinate groups.

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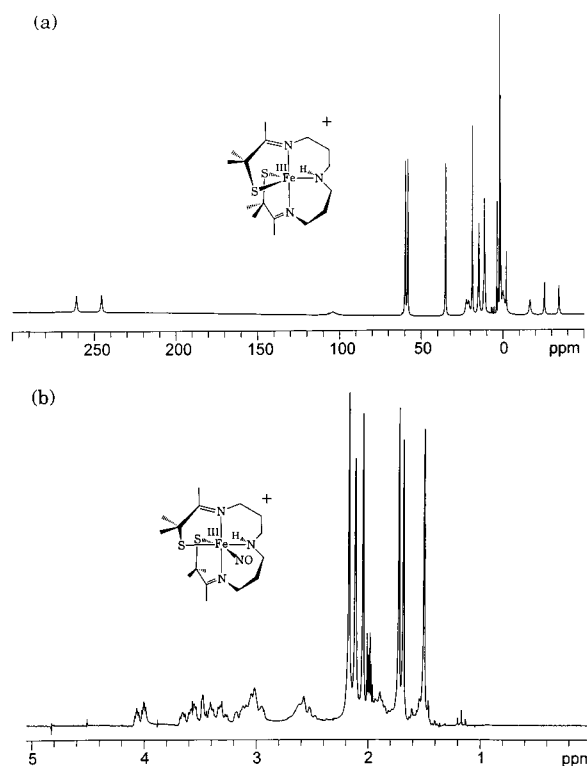

Figure 1. (a) ^1H NMR spectrum (200 MHz) of $[Fe(III)S_2^{Me2}N_3(Pr,Pr)]^+$ (**1**) vs (b) $[Fe(III)S_2^{Me2}N_3(Pr,Pr)(NO)]^+$ (**3**) in CD_3CN solution at 297 K.

This represents the first structure of an Fe(III)–NO compound containing thiolate ligands.

Five-coordinate $[Fe(III)S_2^{Me2}N_3(Pr,Pr)]^+$ (**1**)[18] is coordinatively unsaturated with an open S(1)–Fe–N(2) angle (132.3(1)°) suggesting that the metal ion should be accessible to small molecules. Azide binds to this site to afford $[Fe(III)S_2^{Me2}N_3(Pr,Pr)-(N_3)]$ (**2**),[18] which displays an $S = 1/2$ EPR signal ($g = 2.23$, 2.16, 1.99) nearly identical to that of azide-inhibited NHase ($g = 2.23$, 2.14, 1.99).[7] This suggests that the iron site of our model closely resembles that of the enzyme. Addition of $NO\bullet_{(g)}$ causes the paramagnetic NMR signal of **1** (Figure 1) to collapse to a diamagnetic signal, indicating that the two $S = 1/2$ radicals[19] couple. The product of this reaction,[20] $[Fe(III)S_2^{Me2}N_3(Pr,Pr)-(NO)]^+$ (**3**), displays an ν_{NO} IR stretch at 1822 cm^{-1} close to that ($\nu_{NO} = 1853$ cm^{-1}) of NH_{dk},[5,14] in the range (1857–1937 cm^{-1}) expected for an $\{FeNO\}^6$ complex,[21–26] but well outside the range

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(19) Although five-coordinate **1** is mixed-spin at ambient temperature, addition of a sixth ligand causes the higher spin-states to become thermally inaccessible.
(20) On a high-vacuum line, 1.15 equiv (in a calibrated 50-mL bulb) of NO gas was added to a frozen degassed MeCN solution (10 mL) of **1**. The resulting solution was allowed to warm to ambient temperature and then overlayed with 90 mL of Et_2O to afford single crystalline **3** after 2 days at –35 °C. Anal. Calcd for $FeC_{16}H_{31}ON_4S_2PF_6$: C, 34.29; H, 5.58; N, 10.00; S, 11.45; O, 2.86. Found: C, 33.83; H, 5.88; N, 9.63; S, 12.01; O, 5.93. IR: $\nu(cm^{-1}) = 1822$ (NO), 1615 (C≡N).
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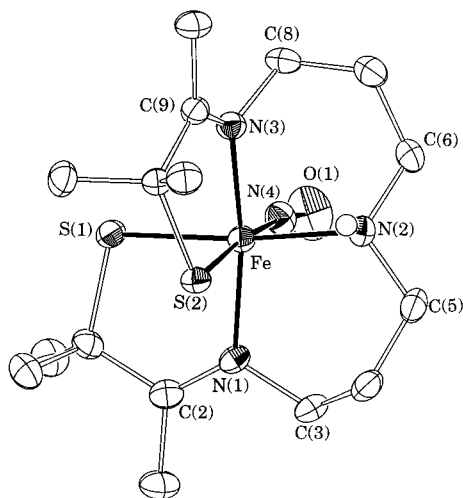
Figure 2. ORTEP plot of NO-bound $[Fe(III)S_2^{Me2}N_3(Pr,Pr)(NO)]^+$ (**3**) showing 50% probability ellipsoids and atom labeling scheme. All H atoms, except for the N(2)−H proton, have been omitted for clarity. Selected distances (Å) and angles (deg): Fe−S(1), 2.2603(9); Fe−S(2), 2.2604(9); Fe−N(1), 1.993(3); Fe−N(2), 2.108(3); Fe−N(3), 1.979(3); Fe−N(4), 1.676(3); N(4)−O(1), 1.161(4); S(1)−Fe−S(2), 91.09(3); S(1)−Fe−N(2), 174.29(8); S(2)−Fe−N(2), 89.02(7); S(2)−Fe−N(4), 173.8(1); N(2)−Fe−N(4), 88.9(1); N(1)−Fe−N(3), 165.9(1); Fe−N(4)−O(1), 172.3(3).

$(1620-1675\ cm^{-1})$ for an $\{FeNO\}^7$ complex.[21,27−30] An $\{FeNO\}^6$ description[22] is consistent with Fe(III)−NO•, Fe(II)−NO$^+$, and Fe(IV)−NO$^-$, but not the Fe(II)−NO• electronic arrangement initially suggested[14] for NH$_{dk}$. The ν_{NO} stretch of both **3** and NH$_{dk}$ fall closer to that of free NO• $(1875\ cm^{-1})$, than to free NO$^+$ $(2377\ cm^{-1})$ or NO$^-$ $(1470\ cm^{-1})$.[1]

An ORTEP of **3** is shown in Figure 2.[31] Nitric oxide binds approximately linearly and inserts into the more open S−Fe−N angle of **1** opposite to the N(2)−H proton, and trans to one of the thiolate sulfurs (S(2)). The mean Fe−S distance increases dramatically, from 2.15(1) Å in **1**[18] to 2.260(1) Å in **3**, making it longer than any other low-spin, Fe(III) thiolate bond,[32−34] including those in $[Fe(III)S_2^{Me2}N_3(Pr,Pr)(N_3)]$ (**2**) (2.202(7) Å)[18] but significantly shorter than in reduced $[Fe(II)S_2N_3(Pr,Pr)]$ (**4**) (2.35(1) Å),[35] implying that NO does not reduce the metal ion. Surprisingly, this change in Fe−S distance is not limited to the sulfur which is trans to the NO. A similar lengthening of Fe−S bonds is observed upon conversion of NH$_{lt}$ (2.21(1) Å)[9] to NO-bound NH$_{dk}$ (2.27(2) Å).[36] The Fe−N(2) distance, on the other hand, is noticeably shorter in **3** (2.108(3) Å) relative to **2** (2.157(3)

Å).[18] The Fe−NO distance is approximately the same as that of NH$_{dk}$[16,36] and significantly shorter than the other Fe−N distances in **3**, indicating that there is a considerable amount of Fe−NO double bond character. This is typical of $\{FeNO\}^6$ systems.[23−26] The N−O distance in **3** is slightly elongated, relative to most $\{FeNO\}^6$ systems and free NO (1.15 Å), but far from NO$^-$ (1.26 Å),[1] suggesting that π-back-donation to NO may be slightly greater in **3** as a consequence of having π-donor thiolates in the coordination sphere. An ∼+1 eV shift in the XAS edge[36] of **3** vs. **2** provides additional evidence for π-back-donation to NO in **3**. The resultant decrease in electron density at iron is most likely responsible for the shorter Fe−N(2) distance.

The electronic spectra of **3** (Figure S-1) and NH$_{dk}$[4] both lack the low-energy charge-transfer band near 700 nm characteristic of six-coordinate, low-spin, thiolate-ligated Fe(III)[18,32,34] and instead display charge-transfer bands at 420(1700) and 370 (∼4300) nm, respectively. This is additional evidence that NO significantly perturbs the electronic structure of both **3** and NH$_{dk}$, most likely as a consequence of significant mixing between the metal d and π^*(NO) orbitals. The redox potential[37] shifts from −400 mV (**1**) to −455 mV (**3**) (vs SCE) upon binding NO, implying that NO and/or the six-coordinate structure stabilizes the Fe(III) oxidation state.

Photolysis of **3** (6 h, 25 °C, Hg lamp)[38] in MeCN solution under N$_{2(g)}$ and slightly reduced pressure causes NO to dissociate, as determined by mass spectral analysis of headspace gases. This reaction is, however, complicated by the subsequent photodecomposition of the initial product **1**. Photolysis of **3** in the presence of trapping agents is currently being investigated.

The model compound **3** described herein lacks the sulfinate groups recently shown[16] to be present in the NO-inactivated form (NH$_{dk}$) of nitrile hydratase from *Rhodococcus sp.* N-771, yet it closely matches its properties, as well as those of NH$_{dk}$ from *Rhodococcus sp.* R-312. The somewhat longer Fe−S distances in NH$_{dk}$ vs typical low-spin Fe(III) thiolates could be attributed to the presence of ligated sulfinate groups. However, our work shows that the observed increase in this distance can be induced by NO binding alone, and does not require that the sulfurs be oxidized. Increased population of antibonding orbitals, rather than the trans influence of NO,[36] is likely responsible for this lengthening. Calculations aimed at determining the factors responsible for the bond-length changes and electronic spectral band shifts observed upon introducing NO to the coordination sphere of both our model and NHase will be pursued soon.

Supporting Information Available: Additional tables include crystallographic data, atomic positional and thermal parameters, bond distances and angles, calculated hydrogen atom positional parameters, and torsional angles for **3**. Supplementary figures include an electronic spectrum and IR of $[Fe(III)S_2^{Me2}N_3(Pr,Pr)(NO)](PF_6)$ (**3**) and cyclic voltammograms of **3** and $[Fe(III)S_2^{Me2}N_3(Pr,Pr)]^+$ (**1**) (17 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA981117E

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(31) Crystallographic Data. (Mo Kα (λ = 0.710 69 Å) radiation, Nonius Kappa CCD diffractometer, 161(2) K) for **3**: $C_{16}H_{31}F_6PN_4OS_2Fe$, red crystal, monoclinic space group $P2_1/n$, a = 12.863(1) Å, b = 14.149(1) Å, c = 13.621(1) Å, β = 109.379(1) °, V = 2338.6(3) Å^3, Z = 4; 5011 observed reflections, R = 0.0568, R_w = 0.1594, GOF = 1.09. The structure was solved by direct methods (SIR92) and refined with SHELX97.

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(38) Photolysis reactions were monitored by ^{1}H NMR and UV/vis.