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Thiolate S-Oxygenation Controls Nitric Oxide (NO) Photolability of a Synthetic Iron Nitrile Hydratase (Fe-NHase) Model Derived from Mixed Carboxamide/Thiolate Ligand

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Nitrile hydratase (NHase) is an iron- or cobalt-containing enzyme that catalyzes the hydrolysis of nitriles (R−C≡N) to the corresponding amides [R−(C═O)−NH_2].[1] It contains a non-heme Fe(III) or non-corrinoid Co(III) center bound to a unique array of ligands at the active site. The crystal structure of iron NHase (Fe-NHase) revealed an Fe(III) center in an octahedral geometry. In the equatorial plane, it is coordinated to two unusual nitrogen donors derived from the peptide backbone (the deprotonated carboxamido N atoms from Cys112 and Ser113) and two cysteinato S donors that are post-translationally oxygenated to sulfenate (SO; Cys114) and sulfinate (SO_2; Cys112) moieties, while the axial site is occupied by a third cysteine S donor (unmodified Cys110). The solvent-exposed sixth site is occupied by H_2O (or OH^-) in the catalytically active enzyme. Interestingly, Fe-NHase can also be isolated in an inactive "dark form" when purified under strict low-light conditions. The 1.7 Å structure of dark-form Fe-NHase (Fe-$NHase_{dark}$) revealed a single molecule of nitric oxide (NO) bound to the active-site iron,[2] thus inhibiting enzymatic activity. Upon exposure to light, NO is photoreleased and catalytic activity is restored, suggesting that Fe-NHase is photoregulated by NO.

Synthetic work by several groups has generated small-molecule models of Fe-$NHase_{dark}$. For example, Kovacs and co-workers[3] have employed a Schiff base ligand containing imine N and thiolato S donors to generate the six-coordinate nitrosyl [$(S_2^{Me_2}N_3^{PrPr})$-Fe(NO)]$^+$, which emulates the NO-bound active site of Fe-NHase. Another model complex, namely, [(bmmp-TASN)Fe(NO)]$^+$, reported by Grapperhaus et al.,[4] also exhibits an Fe-bound NO moiety, but *neither complex exhibits NO photolability*. Artaud and co-workers[5] have incorporated carboxamido N and thiolato S donors in the nitrosyl [(N_2S_2)Fe(NO)]$^-$. However, the complex is only five-coordinate and also does not exhibit any NO photolability. No model complex to date emulates the six-coordinate {Fe−NO}^{6} active site with bound carboxamide and thiolate groups that is observed in Fe-$NHase_{dark}$. As a consequence, the chemical factors that lead to NO photolability remain unclear. In this work, we for the first time report a model of dark-form Fe-NHase with carboxamido N and thiolato S coordination. In addition, photorelease of NO from the S-oxygenated model complex is reported.

Reaction of deprotonated $Cl_2PhPepSH_4$ (NaH/DMF) with $NEt_4[FeCl_4]$ afforded a red-brown solution of the Fe(III) precursor [$(Cl_2PhPepS)Fe(Cl)]^{2-}$.[6] Addition of 3 equiv of DMAP at room temperature immediately generated a dark-green color, indicating substitution of Cl^- by DMAP. Storage of this complex in THF/Et_2O at −20 °C for several weeks afforded dichroic green/red needles of NEt_4[$(Cl_2PhPepS)$Fe(DMAP)] (**1**). The IR spectrum of **1** (KBr disk; Figure S1 in the Supporting Information) exhibits a ν_{CO} peak at 1590 cm^{-1}, which is typical for metal-bound carboxamide. The X-ray structure of **1** (Figure 1) reveals a five-coordinate structure wherein the deprotonated N_2S_2 ligand is bound to the Fe(III) center in the equatorial plane and the axial site is occupied by DMAP (ν_{CN} = 1614 cm^{-1}). Complex **1** exhibits an EPR signal with features at g = 4.39 and 1.98 (MeCN/toluene glass, 125 K;

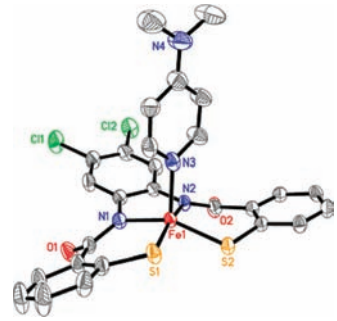

Figure 1. Thermal ellipsoid plot (50% probability level) of [$(Cl_2PhPepS)$Fe-(DMAP)]$^-$ (the anion of **1**). Selected bond distances (Å): Fe−N1, 1.949(3); Fe−N2, 1.965(3); Fe−S1, 2.1994(11); Fe−S2, 2.2065(11); Fe−N3, 2.108(4).

Figure S2) and μ_{eff} = 3.74 μ_B at 298 K, both of which are typical values for square-pyramidal $S = {}^3/_2$ Fe(III) systems.

Complex **1** displays a strong affinity for NO in solution. When a green (λ_{max} = 650 nm) solution of **1** in MeCN at −40 °C was treated with 1 equiv of NO gas, an immediate color change to pale-red was observed. Storage of the solution (1:3 MeCN/Et_2O) at −40 °C for several days afforded a red microcrystalline material. The IR spectrum of the resulting nitrosyl exhibits a strong ν_{NO} stretch at 1849 cm^{-1} (KBr disk; Figure S3) characteristic of an {Fe−NO}^{6} system. The IR spectrum also exhibits a feature at 1624 cm^{-1} due to bound DMAP. The X-ray structure (Figure 2) confirms that the resulting nitrosyl is indeed the monomeric species NEt_4[$(Cl_2Ph-PepS)$Fe(NO)(DMAP)] (**2**). The iron center of **2** is coordinated to the two carboxamido N and two thiolato S donors in the equatorial plane. The extreme distortion of the planar ligand frame results in asymmetric Fe−N bond distances [Fe−N1 = 1.944(9) Å; Fe−N2 = 2.007(11) Å], while the Fe−S bond distances are quite similar [Fe−S1 = 2.273(5) Å; Fe−S2 = 2.279(3) Å]. The nearly linear Fe−N−O unit [173.2(8)°] resembles that observed in the native dark-form enzyme (av 165°), and the corresponding bond distances [Fe−N3 = 1.612(10) Å; N3−O3 = 1.167(11) Å] are typical of an

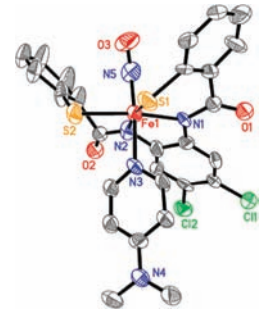

Figure 2. Thermal ellipsoid plot (50% probability level) of [$(Cl_2Ph-PepS)$Fe(NO)(DMAP)]$^-$ (the anion of **2**). Selected bond distances (Å) and bond angles (deg): Fe−N1, 1.944(9); Fe−N2, 2.007(11); Fe−S1, 2.273(5); Fe−S2, 2.279(3); Fe−N3, 2.065(8); Fe−N5, 1.612(10); N5−O3, 1.167(11); Fe−N5−O3, 173.2(8).

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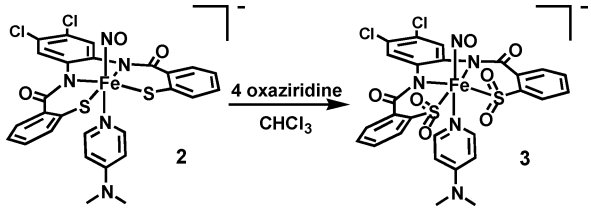
{Fe−NO}^{6} core. The bound DMAP is trans to NO and exhibits a shorter Fe−N bond distance [Fe−N3 = 2.065(8) Å] than that observed in **1** [Fe−N3 = 2.108(4) Å].

The nitrosyl **2** exhibited interesting NO reactivity in solution *under dark conditions*. When red crystals of **2** were redissolved in MeCN (at room temperature or −40 °C), the solution immediately turned green, indicating formation of **1**. This was confirmed by the electronic absorption, EPR, and IR spectra of the green product. Repeated NO addition followed by evacuation at −40 °C cleanly cycled between **1** (λ_{max} = 590 nm) and **2** (λ_{max} = 610, 950 nm; Figure S4). This shows that the bound NO in **2** is *labile* and readily dissociates in MeCN and other coordinating solvents such as THF or DMF. In contrast, **2** can be dissolved in noncoordinating solvents (e.g., $CHCl_3$ or CH_2Cl_2), where the red solutions are stable even at room temperature.

Next, we tested the photoactivity of **2**. When red solutions of **2** in noncoordinating solvents were exposed to visible or UV light, no change in the electronic absorption spectrum was observed. This clearly indicates that *NO is not photolabile* from **2**, despite its structural similarity (octahedral, carboxamido N, thiolato S) to the active site of Fe-NHase$_{dark}$. Also, the red solution of **2** (λ_{max} = 610, 950 nm) bore little resemblance to that of the orange enzyme (Fe-NHase$_{dark}$: $\lambda_{max} \approx$ 400 nm). The lack of NO photolability suggested that further modification of **2** was required to accurately model the photoactive {Fe−NO}^{6} core of Fe-NHase$_{dark}$.

It has been proposed by us[7] and others[2,4] that S-oxygenation of cysteine thiolates may regulate the photolability of NO from Fe-NHases. In the present work, we explored this possibility by attempting to oxygenate the thiolato S donors of **2**. Treatment of **2** with a variety of standard oxygenating agents (O_2, H_2O_2, and dimethyldioxirane) at −40 °C led only to decomposition of the reaction mixture (no isolable species). However, we found that treatment of **2** with 4 equiv of (1*S*)-(+)-(10-camphorsulfonyl)oxaziridine in $CHCl_3$ *in the dark* afforded a pale-orange solution with λ_{max} = 440 nm (compare to $\lambda_{max} \approx$ 400 nm for Fe-NHase$_{dark}$; see Figure 3). This species was stable only for limited periods of time in MeCN, CH_2Cl_2, or $CHCl_3$ at temperatures as low as −40 °C.



Unlike **2**, the oxygenated species *exhibits no lability of NO* under vacuum. The resulting IR spectrum (Figure S5) displays a slightly shifted ν_{NO} value of 1854 cm^{-1} (enzyme ν_{NO} = 1853 cm^{-1}) as well as features characteristic of S-oxygenation (ν_{SO} = 1078, 1046, 1007 cm^{-1}), similar to those observed in Fe− and Co−sulfinates such as $[(Cl_2N_2\{SO_2\}_2)Fe(CN)_2]^{3-}$, $[(PyP\{SO_2\}_2)Fe(CN)]^{2-}$, and $[(LN_2\{SO_2\}_2)$-$Co(t\text{BuNC})_2]^-$ (all of which exhibit ν_{SO} = 1000−1200 cm^{-1}).[8−10] Also, the ν_{CO} stretch at 1599 cm^{-1} and ν_{CN} at 1625 cm^{-1} indicate that the deprotonated $Cl_2PhPepS^{4-}$ ligand and DMAP remain tightly bound to the Fe−NO unit. On the basis of the spectroscopic (Figure S5) and mass spectral (*m*/*z* = 721.1) data, we assign this species as $[(Cl_2PhPep\{SO_2\}_2)Fe(NO)(DMAP)]^-$ (**3**).[11]

Most interestingly, exposure of a fresh solution of **3** in MeCN or $CHCl_3$ at −40 °C to low-intensity (10 mW) visible light immediately generated a pale-green solution with a new peak at 650 nm, similar to the behavior of the light-activated enzyme. Quantitative photochemistry in the visible region (λ_{irr} = 450 nm) afforded a quantum yield value of ϕ_{450} = 0.55, which is close to the quantum efficiency observed for Fe-NHase$_{dark}$ (ϕ_{355} = 0.48).[12] The fact that the color change was due to photorelease of NO from **3** was confirmed by measurement with a NO-sensitive electrode (Figure S6). The IR spectrum of irradiated **3** also confirmed the loss of NO from the complex. EPR measurements revealed the formation of low-spin $[(Cl_2PhPep\{SO_2\}_2)Fe(DMAP)_2]^-$ (g = 2.23, 2.03, and 2.02 and *m*/*z* = 823.1; Figure S7) as the photoproduct in the irradiated solution (generated at −40 °C in presence of 2 equiv of DMAP).

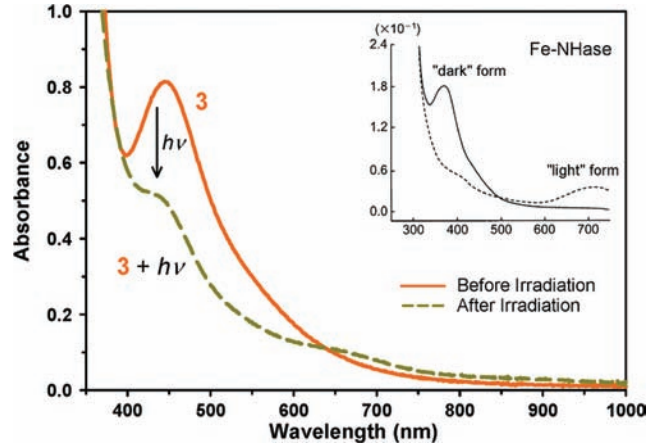


Figure 3. Electronic absorption spectrum of S-oxygenated {Fe−NO}^{6} nitrosyl **3** (MeCN, −40 °C), indicating its similarity to the spectrum of native Fe-NHase$_{dark}$ (shown in the inset). Solid lines are spectra taken under dark conditions, while dashed lines represent spectra after exposure to light.

In summary, we have isolated a new monomeric iron nitrosyl derived from a mixed carboxamide/thiolate ligand that bears a unique resemblance to the "dark-form" Fe-NHase bound to NO. While NO is not photolabile from unmodified **2**, NO is photolabile from the S-oxygenated species **3**, suggesting an important role for S-oxygenation in controlling NO photorelease from Fe-NHase. Structural characterization of **3** is in progress.

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Supporting Information Available: IR spectra of **1** (Figure S1), **2** (Figure S3), and **3** (Figure S5); X-band EPR spectra of **1** (Figure S2) and the photoproduct of **3** (Figure S7); electronic absorption spectra showing the reversible formation of **1** and **2** (Figure S4); NO amperogram of **3** (Figure S6); and X-ray crystallographic data (CIF) for **1**·THF·Et$_2$O and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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