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Copper(II) Activation of Nitrite: Nitrosation of Nucleophiles and Generation of NO by Thiols

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Supporting Information

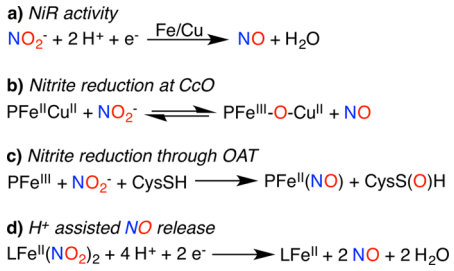
ABSTRACT: Nitrite (NO_2^-) and nitroso compounds (E-NO, E = RS, RO, and R_2N) in mammalian plasma and cells serve important roles in nitric oxide (NO) dependent as well as NO independent signaling. Employing an electron deficient β-diketiminato copper(II) nitrito complex $[Cl_2NN_{F6}]Cu(\kappa^2\text{-}O_2N)\cdot THF$, thiols mediate reduction of nitrite to NO. In contrast to NO generation upon reaction of thiols at iron nitrite species, at copper this conversion proceeds through nucleophilic attack of thiol RSH on the bound nitrite in $[Cu^{II}](\kappa^2\text{-}O_2N)$ that leads to *S*-nitrosation to give the *S*-nitrosothiol RSNO and copper(II) hydroxide $[Cu^{II}]$-OH. This nitrosation pathway is general and results in the nitrosation of the amine Ph_2NH and alcohol ^tBuOH to give Ph_2NNO and ^tBuONO, respectively. NO formation from thiols occurs from the reaction of RSNO and a copper(II) thiolate $[Cu^{II}]$-SR intermediate formed upon reaction of an additional equiv thiol with $[Cu^{II}]$-OH.

Nitrite plays a critical role in cell signaling, both directly as well as through its biochemical connectivity with other important signaling molecules such as NO and *S*-nitrosothiols (RSNOs).[1,2] Nitrite can serve as a storage pool of NO activity under oxygen deficient conditions, thereby complementing the O_2 dependent NO-synthase (NOS) pathway.[2] Nitrite reduction to NO occurs in vivo through transition-metal-assisted processes at deoxymyoglobin, cytochrome *c* oxidase (C*c*O), and xanthine oxidase.[3] Cytochrome *c* oxidase (C*c*O), a O_2 reducing heterodinuclear heme-Fe/Cu functionality in the mitochondrial respiratory chain, is capable of reducing nitrite to NO, thereby inhibiting the reduction of O_2 and allows for cellular O_2 accumulation under hypoxic conditions.[3c] Notably, the oxidized form of C*c*O oxidizes NO to nitrite under normoxia. On the other hand, the reactivity of nitrite toward deoxyhemoglobin reduces the oxygen carrying capacity of hemoglobin and causes abnormalities like methemoglobinemia.[3a] Furthermore, the type 1 Cu sites of ceruloplasmin in mammalian plasma exhibit NO oxidase activity, converting NO to NO_2^-.[4]

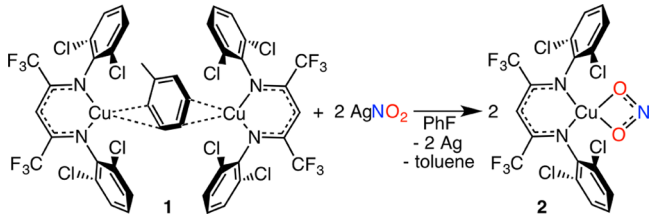
A number of Fe- and Cu-nitrite complexes[5,6] have been reported as functional models for microbial Fe- and Cu-nitrite reductase (NiR)[7] enzymes that reduce nitrite to NO with $2H^+/e^-$ (Scheme 1a). Karlin has developed a novel C*c*O model comprising a heme-Fe/Cu assembly that illustrates reversible interconversion of nitrite and NO (Scheme 1b).[8] Moreover, the availability of thiols[9] in the biological milieu create opportunities for thiol/nitrite crosstalk[10] that lead to *S*-nitrosothiols and other modified thiol species. Ford and co-workers demonstrated that at ambient pH, a porphyrin-Fe(III)-nitrite complex reacts with thiols such as cysteine to yield the corresponding Fe(II)-NO complex along with cysteine-sulfenic acid through oxygen atom transfer (OAT) (Scheme 1c).[11] Harrop and co-workers have reported NO release from a nonheme-dinitro complex through protonation by thiols ($pK_a = 9-10$) (Scheme 1d).[12] A report by Filipovic and Ivanović-Burmazović also described the generation of HNO and HSNO from the reduction of nitrite by H_2S at a Fe(II) heme site.[13] While thiol/H_2S mediated reduction of nitrite at iron sites has received significant attention, comparatively little is known concerning analogous reactions at other biologically relevant metal centers. For instance, thiols RSH react with zinc-bound nitrite to generate *S*-nitrosothiols RSNO via acid-base exchange with $[Zn](\kappa^2\text{-}O_2N)$ that produces zinc thiolates [Zn]-SR along with HONO.[14] Herein, we report a novel mechanistic pathway for thiol-mediated reduction of nitrite at copper(II) that proceeds via initial nucleophilic attack on an electrophilic nitrite ligand bound to a copper(II) center.

Nitration of the β-diketiminato copper(I) complex $\{[Cl_2NN_{F6}]Cu\}_2(\mu\text{-toluene})$[15] (**1**) by $AgNO_2$ in fluorobenzene affords air-stable, dark green $[Cl_2NN_{F6}]Cu(\kappa^2\text{-}O_2N)$ (**2**) (Scheme 2). The X-ray structure of **2** (Figure 1a) shows square-

Scheme 1. Nitrite Reduction Pathways at Iron and Copper Sites



Scheme 2. Synthesis of $[Cl_2NN_{F6}]Cu(\kappa^2\text{-}O_2N)$ (2)



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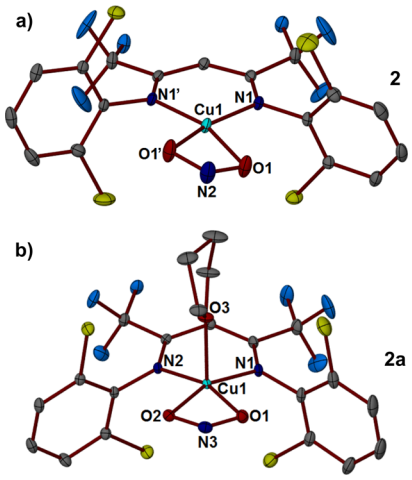
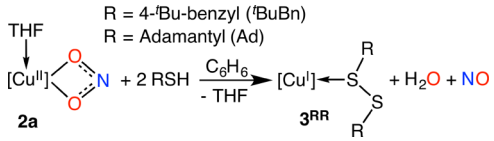


Figure 1. X-ray structures of **2** and **2a**.

planar coordination of the Cu^{II} center that possesses crystallographic C_2 symmetry to give symmetric κ^2-*O,O′* binding of nitrite (Cu-O, 2.0140(12) Å). Performing this reaction in THF leads to dark green $[Cl_2NN_{F6}]Cu(\kappa^2\text{-}O_2N)\cdot$THF (**2a**). The addition of THF to **2** leads to square pyramidal coordination at copper along with nearly unchanged κ^2-*O,O′* binding of nitrite (Cu-O = 2.0350(8), 2.0335(8) Å) (Figure 1b). Binding of THF demonstrates the high Lewis acidity of the copper center in $[Cl_2NN_{F6}]Cu(\kappa^2\text{-}O_2N)$ (**2**). The UV−vis absorption spectrum of **2a** exhibits a low intensity broad feature centered at λ_{max} = 720 nm (120 M^{-1} cm^{-1}). The X-band EPR spectrum of **2a** recorded at 25 °C in pentane exhibits a four line signal centered at g_{iso} = 2.106 with A_{iso}(Cu) = 189 MHz, confirming its formulation as a copper(II), $S = {}^1/_2$ species (Figure S20). The cyclic voltammogram of **2a** in THF (Figure S2) at room temperature shows a quasi-reversible reduction wave centered with $E_{1/2}$ = −470 mV vs Fc^+/Fc.

Reaction of 2 equiv thiol (RSH) with $[Cu](\kappa^2\text{-}O_2N)\cdot$THF (**2a**) in benzene cleanly provides NO, water, and RS-SR along with reduction to copper(I) (Scheme 3). Treatment of a benzene

Scheme 3. Thiol Mediated Conversion of Nitrite to NO at Copper(II)



solution of **2a** with 2 equiv 4-*tert*-butylbenzylthiol (^{t}BuBnSH) or 1-adamantanethiol (AdSH) at room temperature leads to an immediate color change from green to bright orange. ^{1}H NMR analysis indicates the formation of Cu^I-bound disulfide complexes $[Cl_2NN_{F6}]Cu(RSSR)$ ($\mathbf{3^{RR}}$) in 80% (R = ^{t}BuBn) and 85% (R = Ad) yield; water is also identified in 53% yield (R = ^{t}BuBn; Figure S5). X-ray crystallography provides confirmation of the molecular structures of disulfide adducts $\mathbf{3^{RR}}$ (Figures 2, S27−S28). The X-ray structure of the ^{t}BuBn derivative shows κ^1-*S* coordination at $[Cl_2NN_{F6}]Cu$ (Cu-S = 2.1614(4) Å) in which the disulfide possesses an *anti* conformation (S-S = 2.0477(6) Å; C-S-S-C = 82.68°). The sum of three angles around the Cu center in $\mathbf{3^{BnBn}}$ is 358.77(4)°, thus confirming its trigonal planar geometry. The molecular structure of $\mathbf{3^{AdAd}}$ (Cu-S = 2.1882(18) Å; S-S =

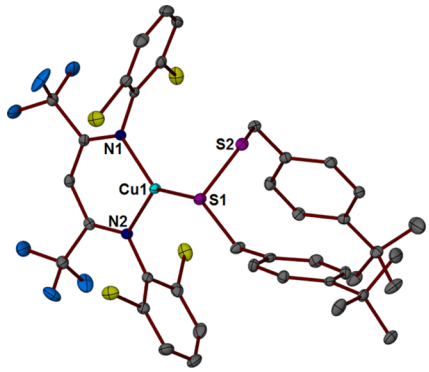


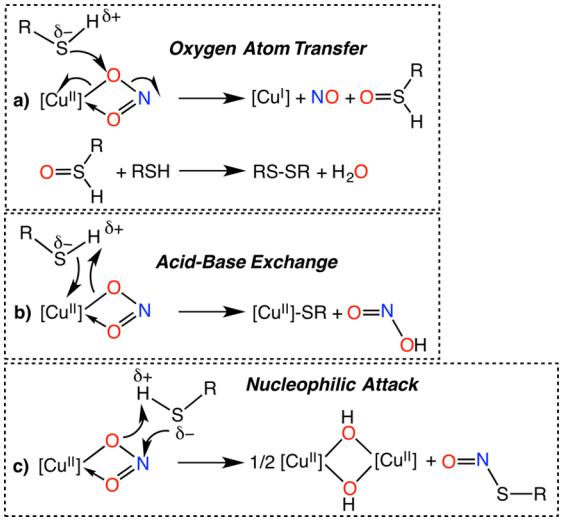
Figure 2. X-ray structure of $\mathbf{3^{BnBn}}$.

2.070(2) Å; C-S-S-C = 117.87°) shows similar geometric features (Figure S28).

Furthermore, the generated nitric oxide gas in the headspace of the reactions of **2a** and thiols (^{t}BuBnSH or AdSH) can be trapped with $(TPP)Co^{II}$ or $(dtc)_2Fe^{II}$ complexes (TPP = tetraphenylporphyrin, dtc = *N,N*-diethyldithiocarbamate).[12,14] The characteristic three line isotropic EPR spectrum of $(dtc)_2Fe(NO)$ clearly reveals that NO is formed in the reaction of **2a** with thiols (Figure S11) and trapping by $(TPP)Co^{II}$ demonstrates the formation of NO in almost quantitative yield for both ^{t}BuBnSH and AdSH (Figure S9).

We envisioned three possibilities for the initial $[Cu^{II}](\kappa^2\text{-}O_2N)$/RSH interaction in this thiol-promoted NO_2^- to NO transformation. Inspired by mechanistic findings at a Fe(III) heme model complex (Scheme 1c),[11] we considered copper(II)-nitrite mediated oxygen atom transfer (OAT) to sulfur that leads to the corresponding sulfenic acid (RSOH) (Scheme 4a). Alternatively, the acidity of thiols could lead to acid-base

Scheme 4. Mechanistic Pathways Considered for Thiol Reactivity with $[Cu^{II}](\kappa^2\text{-}O_2N)$



exchange releasing HONO with formation the copper(II) thiolate $[Cu^{II}]$-SR (Scheme 4b). This pathway has been suggested for related $[Zn](\kappa^2\text{-}O_2N)$ complexes.[14] Finally, simple nucleophilic attack by the thiol on an electron-deficient nitrite anion in $[Cu^{II}](\kappa^2\text{-}O_2N)$ could result in *S*-nitrosation of the thiol to give RSNO and the copper(II) hydroxide $[Cu^{II}]$-OH subject to dimerization to give $[Cu^{II}]_2(\mu\text{-}OH)_2$ (Scheme 4c).

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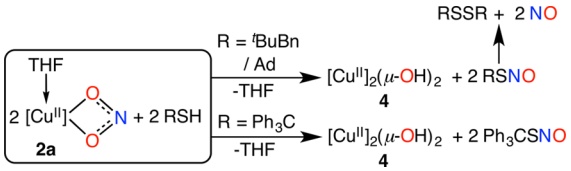
Careful inspection of the reaction mixture provides no evidence for sulfenic acid formation in the reaction of **2a** with 2 equiv RS-H (R = ^{t}BuBn or Ad) using dimedone, a sulfenic acid trap.[11] Because additional equivalents of thiol may accelerate the conversion of any in situ generated sulfenic acid to disulfide and water (Scheme 4a), we investigated the 1:1 reaction of RSH with $[Cu^{II}](\kappa^2\text{-}O_2N)\cdot$THF (**2a**). Interestingly, addition of 1 equiv ^{t}BuBnSH or AdSH to **2a** in benzene-d_6 at room temperature leads to an instantaneous change in color from dark green to light orange with concomitant formation of brownish-yellow precipitate **4** (Scheme 5). ^{1}H NMR

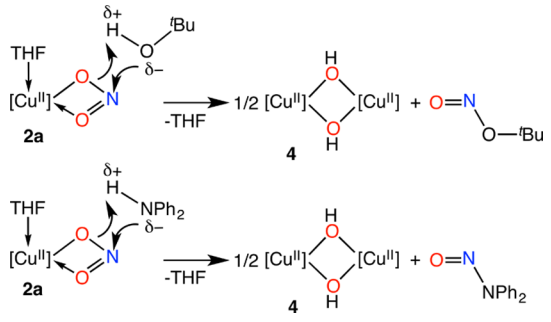
Scheme 5. Nitrite to NO via S-Nitrosothiol



analysis on the yellow filtrate indicates the formation of free disulfide ^{t}BuBnS-SBn^{t}Bu or AdS-SAd, respectively, in near quantitative yield (Figure S12). FT-IR spectroscopic analysis on **4** shows a sharp vibrational feature at 3650 cm^{-1} (KBr, ν_{OH}), consistent with the formation of the dicopper bis(μ-hydroxo) complex $\{[Cl_2NN_{F6}]Cu\}_2(\mu\text{-}OH)_2$ (**4**) (85%, based on gravimetric analysis) (Figure S13). This assignment has been confirmed by X-ray analysis on single crystal isolated from a solution of **4** in fluorobenzene (Figure S29).[16] Formation of $[Cu^{II}]_2(\mu\text{-}OH)_2$ (**4**) rules out an acid-base pathway (Scheme 4b) and provides strong support for direct nucleophilic attack of the thiol on the N atom of the bound nitrite in **2** (Scheme 4c). Moreover, reaction of **2a** with the very bulky thiol Ph_3CSH leads to $[Cu^{II}]_2(\mu\text{-}OH)_2$ complex **4** in 78% yield along with formation of a green solution containing Ph_3CSNO in >95% spectroscopic yield (Scheme 5).[17]

Nitrosation of organic substrates by nucleophilic attack on $[Cu^{II}](\kappa^2\text{-}O_2N)\cdot$THF (**2a**) is not limited to thiols. Reaction of **2a** with equimolar amounts of ^{t}BuO-H or Ph_2N-H in benzene-d_6 provides ^{t}BuO-NO or Ph_2N-NO in 45 and 58% yield, respectively, precipitating the insoluble copper(II) hydroxide **4** (71−75% yield) (Scheme 6). Use of $[Cl_2NN_{F6}]Cu(\kappa^2\text{-}O_2{}^{15}N)\cdot$THF

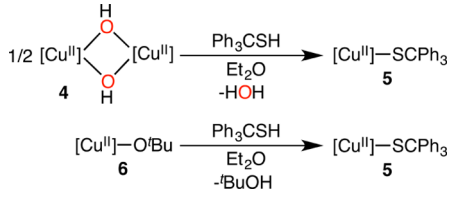
Scheme 6. Conversion of Nitrite to O-/N-Nitroso Compounds



(**2a**-^{15}**N**) (prepared from **1** and $Ag^{15}NO_2$) results in ^{15}N-labeled organonitroso species ^{t}BuO-^{15}NO (δ 585.1 ppm) and Ph_2N-^{15}NO (δ 554.6 ppm) (Figures S15 and S16). Unlike thiols, ^{t}BuOH and Ph_2NH are mild nucleophilies that are neither oxophilic nor particularly acidic. Thus, the *S*-, *O*-, and *N*-nitrosation reactions that occur at $[Cu^{II}](\kappa^2\text{-}O_2N)$ with thiols, alcohols, and amines likely proceed via direct attack on the nitrite N atom made quite electrophilic though ligation to copper(II).

While the mechanisms of thiol assisted nitrite to NO conversions are of paramount interest,[11,12] understanding thiol to disulfide formation may also provide vital insights into the modifications[18] of proteins containing cysteine as well as mobile thiols such as glutathione under oxidative stress. To address the pathway for both NO and disulfide formation in the reaction of $[Cu^{II}](\kappa^2\text{-}O_2N)$ with 2 equiv RSH, we added a second equiv RSH to $[Cu^{II}]_2(\mu\text{-}OH)_2$ (**4**) formed upon reaction of RSH with $[Cu^{II}](\kappa^2\text{-}O_2N)$ (**2a**) (Scheme 7). Monitoring the reaction of **4**

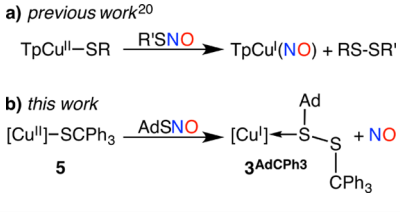
Scheme 7. Formation of $[Cu^{II}]$-SCPh$_3$ (5)



with 2 equiv Ph_3CSH at 25 °C by UV−vis spectroscopy shows the decay of the hydroxo species **4** with a concomitant growth of a new absorption feature at 760 nm (Figure S17). This low energy, intense absorbance is very similar to that observed for previously reported β-diketiminato copper(II) thiolates $[Cu^{II}]$-SCPh$_3$,[19] suggesting this new intermediate as $[Cl_2NN_{F6}]$Cu-SCPh$_3$ (**5**). Although the thermal sensitivity of the thiolate complex **5** hampers its isolation, we additionally verified its assignment via independent synthesis in the acid-base reaction between Ph_3CSH and $[Cl_2NN_{F6}]$Cu-O^{t}Bu (**6**) (Scheme 7). Thus, reaction between $[Cu^{II}]_2(\mu\text{-}OH)_2$ (**4**) and thiols RSH generates copper(II) thiolate intermediates $[Cu^{II}]$-SR.

Using tris(pyrazolyl)borate complexes, some of us have shown that copper(II)-thiolate species ^{iPr2}TpCu^{II}-SR can directly react with R′SNOs to generate directly the disulfide RSSR′ and a copper-nitrosyl ^{iPr2}TpCu(NO) (Scheme 8a).[20] In a related

Scheme 8. Formation of Disulfide from Copper(II)-Thiolate and *S*-Nitrosothiol



experiment, addition of an equimolar amount of AdSNO with in situ generated $[Cl_2NN_{F6}]$Cu-SCPh$_3$ (**5**) leads to the copper(I) adduct of the mixed disulfide $[Cu^I](\kappa^1\text{-}S(Ad)SCPh_3)$ ($\mathbf{3}^{AdCPh3}$) (Scheme 8b). Single crystal X-ray diffraction of $\mathbf{3}^{AdCPh3}$ (Figure 3) reveals κ^1-*S* coordination (Cu-S1 2.2088(8) Å) through the AdS-end of the unsymmetrical disulfide AdS-SCPh$_3$ (S-S 2.0601(9) Å) that possesses an *anti* conformation (C-S-S-C = −100.86°). This reaction also generates NO (detected via $(dtc)_2Fe^{II}$, vide supra) and adds to the generality of direct disulfide formation with release of NO upon reaction of *S*-nitrosothiols with copper(II) thiolates.[20]

These model studies illustrate that the nitrite anion in $[Cu^{II}](\kappa^2\text{-}O_2N)$ (**2**) is especially activated toward nitrosation reactions with RSH, ROH, and R_2NH nucleophiles leading to nitrosated species

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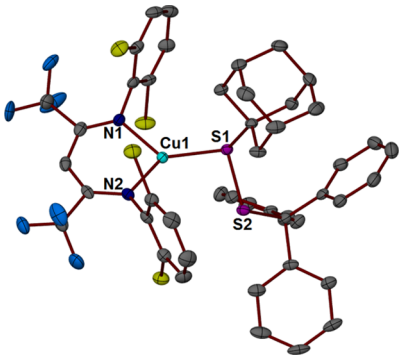

Figure 3. X-ray structure of $\mathbf{3}^{\mathbf{AdCPh3}}$.

RSNO, RONO, and R_2NNO along with the formation of $[Cu^{II}]$-OH. Moreover, NO is released through the reaction of the RSNO formed with $[Cu^{II}]$-SR intermediates generated upon reaction of $[Cu^{II}]$-OH species with RSH.[20] These new findings shed light on reactivity pathways available to nitrite at biological copper sites that may interconnect nitrite with RSNOs and NO, complementary molecular signals in nitric oxide biology.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b11332.

UV−vis, EPR, NMR, X-ray crystallographic, and experimental details (PDF)
X-ray crystallographic data for **2** (CIF)
X-ray crystallographic data for **2a** (CIF)
X-ray crystallographic data for $\mathbf{3}^{\mathbf{BnBn}}$ (CIF)
X-ray crystallographic data for $\mathbf{3}^{\mathbf{AdAd}}$ (CIF)
X-ray crystallographic data for **4** (CIF)
X-ray crystallographic data for **6** (CIF)
X-ray crystallographic data for $\mathbf{3}^{\mathbf{AdCPh3}}$ (CIF)

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Notes

The authors declare no competing financial interest.

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