Synthetic Model of the Substrate Adduct to the Reduced Active Site of Copper Nitrite Reductase

Jason A. Halfen and William B. Tolman*

Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455

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Dissimilatory reduction of NO_2^- to NO and, under certain conditions, to N_2O is carried out by bacterial copper-containing enzymes as part of the denitrification pathway.[1] Structural and spectroscopic studies on these nitrite reductases indicate that substrate binding and reduction occur at a single copper ion ligated by an approximately facial array of three histidine imidazoles.[2,3] This center lies ~12.5 Å distant from a second, type 1 (electron transfer), copper site. On the basis of mechanistic experiments and analogies drawn to data acquired for heme-iron nitrite reductases, coordination of NO_2^- to the lone "catalytic" copper ion in its reduced [Cu(I)] state has been suggested to be the first reaction step.[4] It also has been proposed that protonation and dehydration follow, yielding an electrophilic nitrosyl species which rapidly loses NO (eq 1).[4] While there are numerous reports of complexes containing the Cu^{II}–NO_2^- unit,[5] to our knowledge there exists no definitive chemical precedent in the form of structurally characterized compounds for the purported Cu^{I}–NO_2^- nitrite reductase substrate adduct. As a result of our preliminary efforts to delineate the structural and spectroscopic features of such a species, we recently isolated and characterized the novel nitrite-bridged dicopper(I) dimer $[(i\text{-}Pr_3TACN)_2Cu_2(\mu_2\text{-}(\eta^1\text{-}N{:}\eta^1\text{-}O)\text{-}NO_2)]PF_6$ (**1**, *i*-Pr_3TACN = 1,4,7-triisopropyl-1,4,7-triazacyclononane,[6] Scheme 1).[7] Here we describe the regioselective cleavage of **1** to yield a *mononuclear* Cu^{I}–NO_2^- complex that represents the first well-defined model for the reduced enzyme–substrate adduct. Moreover, in a direct demonstration of the feasibility of the sequence of enzyme reaction steps indicated in eq 1, we have observed stoichiometric release of NO upon protonation of this novel compound.

$$E\text{-}Cu^+ \xrightarrow{NO_2^-} E\text{-}Cu^+\text{-}NO_2^- \overset{2H^+ \quad H_2O}{\rightleftharpoons} E\text{-}Cu^+\text{-}NO^+ \rightleftharpoons E\text{-}Cu^{2+} + NO \qquad \text{(eq 1)}$$

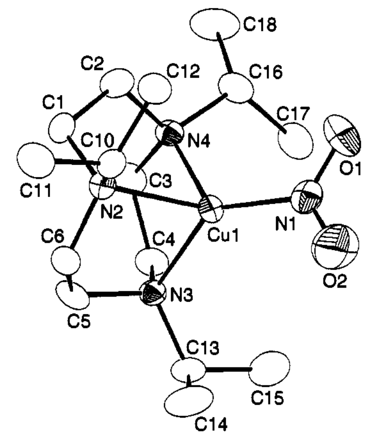
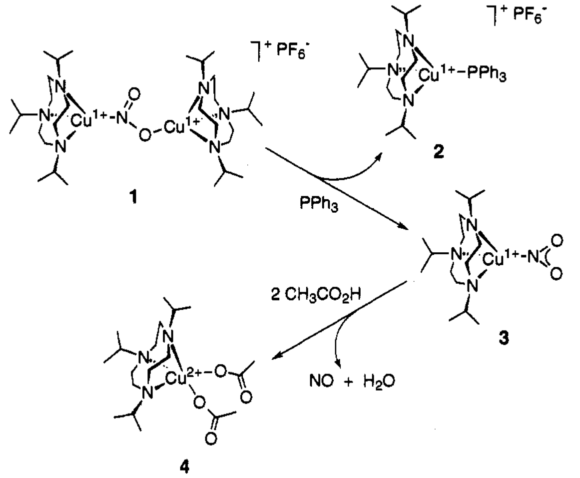


Figure 1. ORTEP representation of the X-ray structure of $[(i\text{-}Pr_3TACN)Cu((\eta^1\text{-}N)\text{-}NO_2)]$ (**3**) (50% ellipsoids, hydrogen atoms not shown for clarity). Selected intramolecular distances (Å) and angles (deg): Cu(1)–N(1), 1.903(2); Cu(1)–N(2), 2.157(3); Cu(1)–N(3), 2.133(3); Cu(1)–N(4), 2.199(4); O(1)–N(1), 1.253(5); O(2)–N(1), 1.238(5); N(1)–Cu(1)–N(2), 123.9(1); N(1)–Cu(1)–N(3), 138.4(1); N(2)–Cu(1)–N(3), 86.5(1); N(1)–Cu(1)–N(4), 124.0(1); N(2)–Cu(1)–N(4), 83.5(1); N(3)–Cu(1)–N(4), 83.2(1); Cu(1)–N(1)–O(1), 120.6(3); Cu(1)–N(1)–O(2), 122.7(3); O(1)–N(1)–O(2), 116.6(4).

Scheme 1



Addition of 1 equiv of PPh_3 to a solution of **1** in THF resulted in quantitative (^{1}H NMR) cleavage of the dimer to form $[(i\text{-}Pr_3TACN)Cu(PPh_3)]PF_6$ (**2**) and $[(i\text{-}Pr_3TACN)Cu((\eta^1\text{-}N)\text{-}NO_2)]$ (**3**), which were isolated via fractional crystallizations in 97% and 63% yields, respectively (Scheme 1).[8] A noteworthy feature of the structure of **3** (Figure 1)[9] is the η^1-*N* coordination of nitrite to the single Cu(I) ion; while such an arrangement has been identified in complexes of other transition metals,[10] in copper chemistry it has been observed in only a limited number of Cu(II) species.[5c,11] Comparison of the topological features of the coordination sphere of **3** with those of its precursor **1**[7] reveal only minor differences between them. Both exhibit similarly short $Cu\text{–}N_{nitrite}$ bond lengths [1.903(4) Å for **3** vs 1.899(2) Å for **1**]

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[8] Analytical and spectroscopic data for compounds **2–4** are reported in the supplementary material.

[9] (a) Crystal data for $[(i\text{-}Pr_3TACN)Cu((\eta^1\text{-}N)\text{-}NO_2)]$ (**3**): $C_{15}H_{33}CuN_4O_2$, fw 365.00, monoclinic, space group $P2_1/c$ (No. 14), at 177 K, a = 7.946(6) Å, b = 15.554(3) Å, c = 14.659(8) Å, β = 102.20(5)°, V = 1771(3) Å^3, Z = 4, R = 0.060 and R_w = 0.055 for 3334 reflections with $I > 2.0\sigma(I)$ and 200 parameters. (b) Crystal data for $[(i\text{-}Pr_3TACN)Cu(O_2CCH_3)_2]$ (**4**): $C_{19}H_{39}CuN_3O_4$, fw 437.09, orthorhombic, space group *Pbca* (No. 61), at 297 K, a = 16.201(6) Å, b = 16.191(5) Å, c = 16.959(5) Å, V = 4448(4) Å^3, Z = 8, R = 0.047 and R_w = 0.049 for 1805 reflections with $I > 2.0\sigma(I)$ and 244 parameters.

[10] For examples, see: (a) Nasri, H.; Wang, Y.; Huynh, B. H.; Scheidt, W. R. *J. Am. Chem. Soc.* **1991**, *113*, 717–719. (b) Yamamoto, K.; Iitaka, Y. *Chem. Lett.* **1989**, 697–698. (c) Kaduk, J. A.; Scheidt, W. R. *Inorg. Chem.* **1974**, *13*, 1875–1880.

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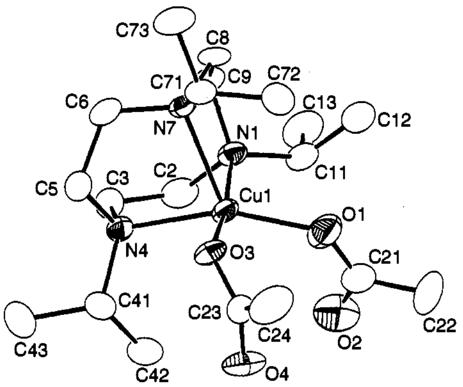

Figure 2. ORTEP representation of the X-ray structure of [(*i*-Pr_3TACN)-$Cu(O_2CCH_3)_2$] (4) (35% ellipsoids, hydrogen atoms not shown for clarity). Selected intramolecular distances (Å) and angles (deg): Cu(1)–N(1), 2.132(5); Cu(1)–N(4), 2.088(5); Cu(1)–N(7), 2.288(5); Cu(1)–O(1), 1.936(5); Cu(1)–O(3), 1.957(4); N(1)–Cu(1)–N(4), 85.3(2); N(1)–Cu(1)–N(7), 81.9(2); N(4)–Cu(1)–N(7), 83.7(2); N(1)–Cu(1)–O(1), 97.8(2); N(1)–Cu(1)–O(3), 173.3(2); N(4)–Cu(1)–O(1), 157.1(2); N(4)–Cu(1)–O(3), 92.2(2); N(7)–Cu(1)–O(1), 119.2(2); N(7)–Cu(1)–O(3), 90.6(2); O(1)–Cu(1)–O(3), 87.3(2).

and C_{3v}-distorted tetrahedral copper ion geometries. The only significant structural difference between the two is the degree of bond alternation in the coordinated nitrite ion [Δ(N–O) = 0.015 Å for **3** and 0.044 Å for **1**], an asymmetry related to the absence or presence, respectively, of a second O-bound metal ion.

When 2 equiv of glacial acetic acid were added to an air-free, yellow solution of **3** in CH_2Cl_2 at room temperature, an instantaneous color change to blue occurred and exactly 1 equiv of NO (by GC) and, we presume, H_2O evolved (Scheme 1). The colored product was identified as [(*i*-Pr_3TACN)$Cu(O_2CCH_3)_2$] (**4**, >95% yield by UV–vis)[8] on the basis of comparison of its UV–vis and EPR spectra to those of independently synthesized material. The X-ray structure of **4** (Figure 2) reveals monodentate (η^1-*O*) coordination of both acetate ions to a Cu(II) ion that adopts a distorted square pyramidal geometry. The metal ion binds to one carboxylate's *syn* lone pair, but lies outside of the other carboxylate ligand's [O(3), O(4), C(23), C(24)] plane in an unusual orientation for copper that may arise from unfavorable intramolecular steric interactions.[12] While the divergent C–O bond distances in the latter carboxylate [Δ(C–O) = 0.07 Å] are consistent with bond alternation, the same distances are equivalent in the former acetate ligand; this apparent delocalization may be a consequence of a weak interaction between Cu(1) and O(2), which resides approximately *trans* to N(7) [Cu(1)···O(2) = 3.15 Å; N(7)–Cu(1)–O(2) = 156°).[13] Evolution of NO from **3** was also promoted by addition of $HBF_4{\cdot}Et_2O$, but the yield was lower (65%), some N_2O was also produced, and we have not been able to identify the Cu(II) product(s).[14]

Several important aspects of the active site chemistry of copper nitrite reductase are replicated by the synthetic system described herein. Complex **3** is a structural model for a plausible biological Cu^I–(NO_2^-) intermediate, although in the enzyme it is still unclear whether substrate initially coordinates to the active site copper ion in its +1 oxidation state or vice versa [nitrite binding to Cu(II) prior to electron transfer]. The rapid and clean conversion of **3** to a Cu(II) species and NO, the principal enzyme product, upon addition of acid provides support for key steps in the postulated mechanism of enzyme action.[16] Future kinetic and spectroscopic work on this and other model systems should shed more light on the detailed course of the biological reaction and on the structures of possible intermediates.

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Supplementary Material Available: Analytical and spectroscopic data for **2–4** and tables of bond lengths, bond angles, atomic coordinates, and thermal parameters for **3** and **4** (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for order information.

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(13) The apparent axial interaction between a carbonyl oxygen atom and a Cu(II) ion in **4** mimics analogous contacts in certain type 1 copper proteins (e.g., azurin). See: (a) Adman, E. T. *Adv. Protein Chem.* **1991**, *42*, 145–197. (b) Lowery, M. D.; Solomon, E. I. *Inorg. Chim. Acta* **1992**, *198*–200, 233–243.

(14) Interestingly, immediately after the addition of $HBF_4{\cdot}Et_2O$ the solution of **3** flashed red before turning the final green color. The red species (λ_{max} = 442 nm, $\epsilon \sim 1200$ M^{-1} cm^{-1}) is stable for ~10 min at –70 °C, thus making it amenable to future spectroscopic investigation. We speculate that it is either a copper–nitrosyl complex analogous to others we have fully characterized[15] or a mixed-valence nitrite-bridged dimer.[7]

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(16) Dehydration of *n*-$Bu_4N(NO_2)$ by $HPF_6{\cdot}Et_2O$ in the presence of a dicopper(I) complex has been reported, but instead of NO evolution, production of a nitrosyl complex formulated as a dicopper(II)–(μ-NO^-) species was observed: Paul, P. P.; Karlin, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 6331–6332.