Functional Modeling of Copper Nitrite Reductases: Reactions of NO_2^- or NO with Copper(I) Complexes

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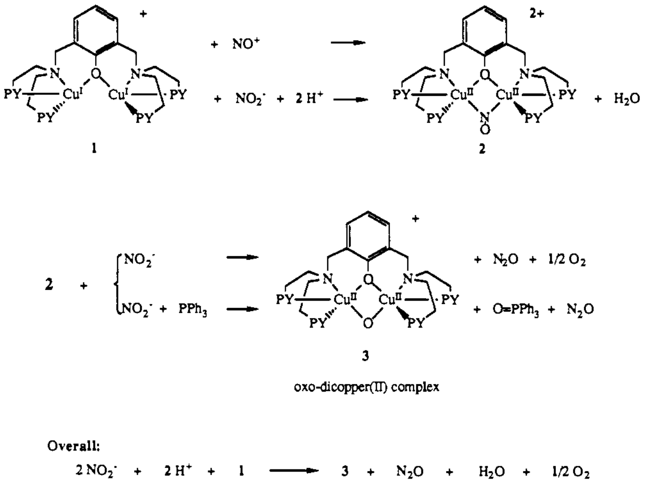
From an environmental chemistry perspective, transition-metal-mediated reactions of nitrogen oxides (NO_x) are of interest,[1] while anaerobic denitrifying bacteria use NO_x species as electron acceptors through metalloenzyme-catalyzed conversions.[2] The reduction of NO_2^- to nitrous oxide (N_2O) (eq 1) is catalyzed by either Cu or Fe enzymes,[2-5] although there also exist separate NO-reductases that effect the N–N coupling reaction of intermediate NO(g) that may be produced.[2a,4c,5] Following our efforts

$$2NO_2^- + 4e^- + 6H^+ \rightarrow N_2O + 3H_2O \qquad (1)$$

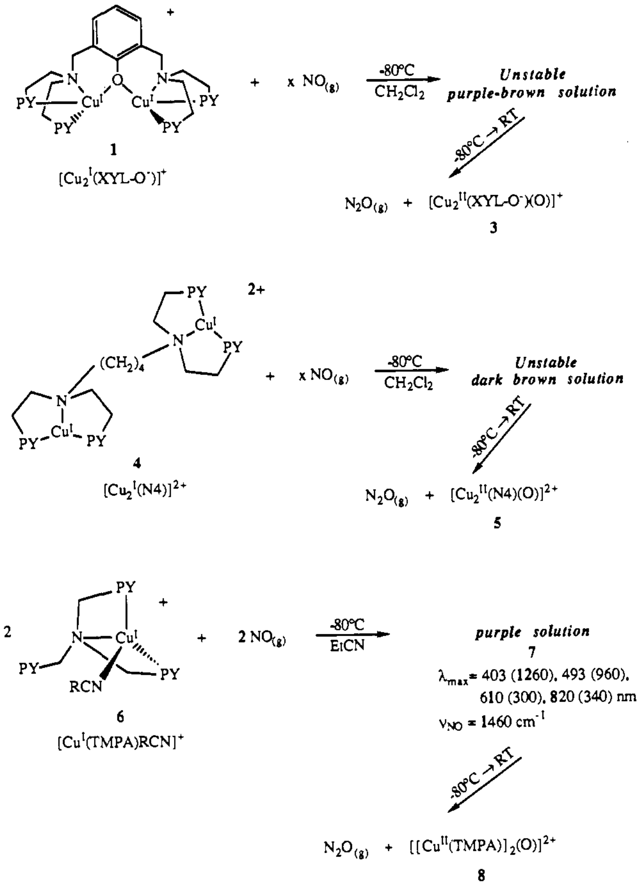
in Cu ion mediated reduction of O_2,[6] we have developed an interest in biomimetic NO_x–copper reactivity, especially considering the few examples of {Cu–NO} species.[7,8] Here, we report the functional modeling of copper nitrite reductases,[9] providing examples where reactions of NO_2^- *or* NO with Cu(I) complexes lead to the stoichiometric productions of N_2O with concomitant oxo–dicopper(II) compound formation.

We recently reported that the reaction of dicopper(I) complex **1** (PY = 2-pyridyl) with nitrosonium cation (NO^+) gives a stable $[Cu_2^{II}-(NO^-)]$ complex **2** (ν_{NO} = 1536 cm^{-1}), which was structurally characterized (Scheme I).[7] We now find that **2** can also be generated by the reaction of *n*-$Bu_4N(NO_2)$ with **1** in the presence of stoichiometric amounts of acid ($HPF_6 \cdot Et_2O$).[10] This dehydration of NO_2^- (an NO^+ equivalent)[11] to produce **2** is reminiscent of the enzyme reaction since, in *Achromobacter cycloclastes* nitrite reductase, there is evidence for a copper–nitrosyl intermediate ($[E-Cu-NO]^+$) being produced from NO_2^-.[3,12]

Scheme I



Scheme II



We can also mimic the proposed enzymic reaction of NO_2^- with the $[E-Cu-NO]^+$ intermediate (giving N_2O),[3] resulting in an overall copper-mediated conversion of 2 mol of NO_2^- to give nitrous oxide (eq 1 and Scheme I). Here, further reaction of *n*-$Bu_4N(NO_2)$ (1 equiv) with **2** produces N_2O (79% yield),[13] oxo–dicopper(II) complex **3** (60%), and O_2 gas.[14] If the reaction is carried out in the presence of PPh_3 (1 equiv), the yield of N_2O

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[1] (a) Mingos, D. M. P.; Sherman, D. J. *Adv. Inorg. Chem.* **1989**, *34*, 293–377. (b) Bottomley, F. In *Reactions of Coordinated Ligands*; Braterman, P. S., Ed.; Plenum Publishing Corporation: New York, 1989; Vol. 2, pp 115–222.

[2] (a) Hochstein, L. I.; Tomlinson, G. A. *Annu. Rev. Microbiol.* **1988**, *42*, 231–261. (b) Payne, W. J. *Denitrification*; Wiley-Interscience: New York, 1981.

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[4] (a) Goretski, J.; Hollocher, T. C. *J. Biol. Chem.* **1990**, *265*, 889–895. (b) Goretski, J.; Zafiriou, O. C.; Hollocher, T. C. *J. Biol. Chem.* **1990**, *265*, 11535–11538. (c) Hoglen, J.; Hollocher, T. C. *J. Biol. Chem.* **1989**, *264*, 7556–7563.

[5] Carr, G. J.; Ferguson, S. J. *Biochem. J.* **1990**, *269*, 423–429. (b) Heiss, B.; Frunzke, K.; Zumft, W. G. *J. Bacteriol.* **1989**, *171*, 3288–3297.

[6] Tyeklár, Z.; Karlin, K. D. *Acc. Chem. Res.* **1989**, *22*, 241–248 and references cited therein.

[7] Paul, P. P.; Tyeklár, Z.; Farooq. A.; Karlin, K. D.; Liu, S.; Zubieta, J. *J. Am. Chem. Soc.* **1990**, *112*, 2430–2432 and references cited therein.

[8] Previous reports including reactions of NO with Cu salts include the following: (a) Fraser, R. T. M.; Dasent, W. E. *J. Am. Chem. Soc.* **1960**, *82*, 348–351. (b) Mercer, M.; Fraser, R. T. M. *J. Inorg. Nucl. Chem.* **1963**, *25*, 525–534. (c) Yordanov, N. D.; Terziev, V.; Zhelyakowa, G. G. *Inorg. Chim. Acta* **1982**, *58*, 213–216. (d) Doyle, M. P.; Siegfried, B.; Hammond, J. J. *J. Am. Chem. Soc.* **1976**, *98*, 1627–1629.

[9] A X-ray crystallographic study on *Achromobacter cycloclastes* nitrite reductase discloses an active site with two separate but apparently electronically linked copper ion centers (type 1 and 2, Cu···Cu ~ 12.5 Å). There is also evidence that nitrite substrate binds at the type 2 site. Godden, J. W.; Turley, S.; Teller, D. C.; Adman, E. T.; Liu, M.-Y.; Payne, W. J.; LeGall, J. *Science*, in press.

[10] The reaction was performed in the presence of activated molecular sieves (4 Å), because **2** is hydrolytically unstable.

[11] Glidewell, C. *J. Chem. Educ.* **1989**, *66*, 631–633 and references cited therein.

[12] Suzuki, S.; Yoshimura, T.; Kohzuma, T.; Shidara, S.; Masuko, M.; Sakurai, T.; Iwansaki, H. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 1366–1372.

[13] N_2O was determined by sampling of the reaction flask headspace gas and using GC (Porapak Q (80/100) column (12 ft × 1/8 inch)) using a TC detector (He and H_2, carrier and reference gases, respectively). GC yields were determined by using a calibration curve made by using known amounts of N_2O.

[14] $[Cu_2(XYL-O^-)O](PF_6)$ (**3**-(PF_6)): Anal. Calcd for $C_{36}H_{39}Cu_2F_6N_6O_2P$: C, 50.29; H, 4.57; N, 9.77. Found: C, 50.86; H, 4.03; N, 9.34. UV–vis (CH_2Cl_2): λ_{max} = 339 (ϵ 3450), 618 (180). IR (Nujol) ν (PF) = 843 (s) cm^{-1}. μ_{RT} = 1.16 μ_B/Cu. Λ_m (CH_3CN) = 153 Ω^{-1} cm^2 mol^{-1}. **3**-(PF_6) reacts with 3 PPh_3 to give $O{=}PPh_3$ and $[Cu_2(XYL-O^-)(PPh_3)_2]$-(PF_6). Dioxygen gas was qualitatively determined by passing the gases evolved through an alkaline pyrogallol test solution.[6]

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is 97%, O_2 evolution is not observed, and $O{=}PPh_3$ is produced stoichiometrically. An oxidizing equivalent ($^1/_2O_2$) is produced in these reactions since only two electrons (from **1**) of the four needed (eq 1) have been provided.

We also find that nitric oxide (NO(g)) reacts with Cu(I) complexes to give copper–nitrosyl intermediates, which convert to oxo–dicopper(II) complexes and N_2O (Scheme II). These reactions further demonstrate (cf. Scheme I) the use of Cu^{II} ion (rather than H^+) to help drive oxo-transfer reactions, trapping O^{2-} and producing Cu–O–Cu complexes. Thus, exposure of **1** to NO(g) at −80 °C in CH_2Cl_2 gives an unstable purple-brown solution (λ_{max} = 380 nm). Standing or warming produces N_2O (96%) and oxo complex **3** (55%, isolated). A similar reactivity is observed for **4**; the unstable intermediate (λ_{max} = 358 nm) decomposes to give a 56% yield of N_2O and oxo complex **5**.[15] The reaction of NO(g) with mononuclear Cu(I) complex **6** is more interesting because a deep purple −80 °C stable intermediate (**7**) forms in EtCN (Scheme II). This arises from the stoichiometric addition of 1 NO per copper ion complex **6** (manometry in EtCN at −80 °C; 1.09 ± 0.11 NO/Cu, three trials). Direct evidence for a copper–nitrosyl species was obtained by using FT-IR spectroscopy via in situ low-temperature monitoring.[16] A 1460-cm^{-1} absorption is assigned to ν_{NO} on the basis of its shifting to 1446 cm^{-1} when labeled ^{15}NO (99%) is used. This particularly low energy ν_{NO}[1a] value and the observed EPR silence of these purple solutions suggest a dinuclear $Cu^{II}–(NO^-)_2–Cu^{II}$ formulation for intermediate **7**,[17] perhaps facilitating the N–N coupling reaction to give the N_2O product (98%) observed. A dinuclear oxo–dicopper(II) complex (**8**) (78%) is again formed.[18]

Thus, we have shown that copper ion complexes can mediate N–N coupling reactions starting with NO_2^- *or* NO(g). The latter reactions appear to proceed via dinitrosyl intermediates, as is observed in the chemistry of other transition metals.[1b,19] The present study provides the first example of a reaction of a metal–nitrosyl compound with nitrite (Scheme I);[1b] N_2O generation makes this reaction notably reminiscent of Averill and Tiejde's proposal for N_2O production in the iron and copper enzymes.[3,20] We have also provided the first authenticated example (by IR spectroscopy) of a {Cu–NO} complex (i.e., dinuclear **7**) generated from a Cu(I) precursor and NO(g).

On the basis of the copper-mediated coupling of NO(g) we observe here (i.e., Scheme II), we suggest an alternate possible reaction pathway for N_2O production by protein nitrite reductases, not previously considered. Free NO(g)[2-5] may be formed via a prior one-electron reduction and dehydration of NO_2^-, and release. Attack of NO(g) on a separately formed enzyme (type 2)[9] {Cu–NO} intermediate[3] could proceed through a dinitrosyl {Cu–$(NO)_2$} intermediate,[1b,19] leading to N–N coupling. Additional reduction (via type 1 Cu?)[9] and protonation of a putative Cu–(N_2O_2) intermediate[3] would complete the stoichiometric reaction (eq 1), giving nitrous oxide and water. In our model system, the chemistry is mediated and an extra electron supplied by the second copper ion, the latter being a role probably reserved for type 1 copper in the protein.

Further studies will deal with other aspects of Cu–NO_x chemistry and mechanistic investigations of the systems described here.

Acknowledgment. We are grateful to the National Institutes of Health for support of this research.

Note Added in Proof. Recent studies with *A. cycloclastes* nitrite reductase[3d] are consistent with a NO(g) rebound mechanism, which may well be analogous to the chemistry suggested here.

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[15] $[Cu_2(N4)O](PF_6)_2$ (**5**-$(PF_6)_2$): Anal. Calcd for $C_{32}H_{40}Cu_2F_{12}N_6OP_2$: C, 40.81; H, 4.28; N, 8.92. Found: C, 40.71; H, 4.19; N, 9.38. UV–vis (CH_3CN): λ_{max} = 370 (ϵ 2040), 619 (130). IR (Nujol) ν (PF) = 840 (s) cm^{-1}. μ_{RT} = 1.01 μ_B/Cu. Λ_m (CH_3CN) = 297 Ω^{-1} cm^2 mol^{-1}.

[16] At −75 °C by addition of NO(g) over a solution of **6**, FT-IR spectra were recorded using an internal-reflection probe incorporated as part of the low-temperature glass reaction vessel; Spectra-Tech Applied Systems, Inc., ReactIR Reaction Analysis System.

[17] (a) A $\{Cu–NO\}^+$ adduct formed from $1Cu^I$ + NO(g) would be expected to be paramagnetic and thus EPR active. The low ν_{NO} and low-energy vis absorption (d–d transition) suggest a $Cu^{II}–NO^-$ description, with a dinuclear formulation accounting for EPR silence through an antiferromagnetic coupling interaction. (b) **7** is unlikely to be a Cu–N_2O species; we observe that N_2O does not react with any of the Cu complexes used in these studies, and M–N_2O species are rare.[1b] See: Vaughan, G. A.; Rupert, P. B.; Hillhouse, G. L. *J. Am. Chem. Soc.* **1987**, *109*, 5538–5539.

[18] $[\{Cu(TMPA)\}_2O](PF_6)_2{\cdot}CH_2Cl_2$ (**8**-$(PF_6)_2{\cdot}CH_2Cl_2$): Anal. Calcd for $C_{37}H_{38}Cl_2Cu_2F_{12}N_8OP_2$: C, 40.45; H, 3.49; N, 10.20. Found: C, 40.47; H, 3.43; N, 10.64. UV–vis (C_2H_5CN): λ_{max} = 403 (ϵ 530), 736 (220), 900 (320). IR (Nujol) ν(PF) = 845 (s) cm^{-1}. μ_{RT} = 1.73 μ_B/Cu. Λ_m (CH_3CN) = 259 Ω^{-1} cm^2 mol^{-1}.

[19] (a) Eisenberg, R.; Meyer, C. D. *Acc. Chem. Res.* **1975**, *8*, 26–34. (b) Gwost, D.; Caulton, K. G. *Inorg. Chem.* **1974**, *13*, 414–417. (c) Haymore, B. L.; Ibers, J. A. *J. Am. Chem. Soc.* **1974**, *96*, 3325–3327.

[20] Reaction of NO_2^- with **2** may generate a $[Cu^{II}–(N_2O_3^{2-})–Cu^{II}]$ (e.g., attack on NO^-) or $[(NO^-)–Cu^{II}\cdots Cu^{II}–(NO_2^-)]$ (e.g., attack on Cu^{II}) species.[3b]

New Chiral Ligand for the Asymmetric Conjugate Addition of Organocopper Reagents to Enones

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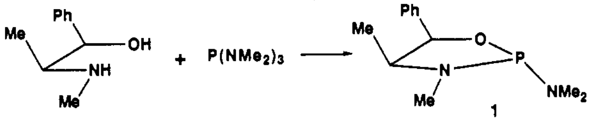
The asymmetric version of the conjugate addition reaction of organocopper reagents to enones is a long-pending problem. In the case of *achiral* enones this reaction was attempted either with chiral heterocuprates[1] or with chiral ligands.[2] Among the ligands of copper, trivalent phosphorus derivatives are the best ones,[3] and we thought that chirality on the phosphorus atom itself would induce asymmetry during the conjugate addition. We present herein our results with the chiral phosphorus derivative **1**, which demonstrate, for the first time, the effectiveness of this class of ligands in the conjugate addition of organocopper reagents. This compound[4] is easily and quantitatively prepared from (−)-ephedrine and HMPT (eq 1) and is configurationally homogeneous at the phosphorus stereogenic center. In the same manner were also prepared the phosphorus derivatives **2**, **3**, **4**,[5] **5**,[6] and **6**[7] (Scheme I).



(1)

Derivatives **1–6** form soluble complexes with a CuX (X = Br, I) salt in Et_2O or THF. Reaction with an organolithium or Grignard reagent gives rise to an organocopper or cuprate derivative, according to the stoichiometry. The transfer of the nBu

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[1] Among many other papers, see the following ones and the references therein: (a) Corey, E. J.; Naef, R.; Hannon, F. J. *J. Am. Chem. Soc.* **1986**, *108*, 7114. (b) Dieter, R. K.; Tokles, M. *J. Am. Chem. Soc.* **1987**, *109*, 2040. (c) Bertz, S. H.; Dabbagh, G.; Sundararajan, G. *J. Org. Chem.* **1986**, *51*, 4953. (d) Leyendecker, F.; Jesser, F.; Laucher, D.; Ruhland, B. *New J. Chem.* **1985**, *9*, 7. (e) Tanaka, K.; Ushio, H.; Suzuki, H. *J. Chem. Soc., Chem. Commun.* **1990**, 795.

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[6] Alexakis, A.; Mutti, S.; Mangeney, P.; Normant, J. F. *Tetrahedron: Asymmetry* **1990**, *1*, 437.

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