

Cu^I and Cu^{II} Complexes Containing Nitrite and Tridentate Aromatic Amine Ligand as Models for the Substrate-Binding Type-2 Cu Site of Nitrite Reductase

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Cu^I and Cu^{II} complexes containing a tridentate aromatic amine compound [bis(6-methyl-2-pyridylmethyl)amine, Me₂bpa] in the absence and presence of nitrite have been prepared as models for the active site of dissimilatory copper-containing nitrite reductase (CuNIR). [Cu^{II}(Me₂bpa)(H₂O)(ClO₄)][ClO₄] (**1**), [Cu^{II}(Me₂bpa)(NO₂)(ClO₄)] (**2**), [Cu^I(Me₂bpa)(CH₃CN)]PF₆ (**3**) and [Cu^I(Me₂bpa)(NO₂)₂][(Ph₃P)₂-NPF₆] (**4**) were prepared. The X-ray crystal structural analyses of **1**, **2**, and **4** reveal that the geometries of the Cu centers are distorted square pyramidal, distorted octahedral, and tetrahedral, respectively. The coordination modes of the nitrite ligands in **2** and **4** depend on the oxidation state of the copper ion: nitrite is coordinated to Cu^{II} and Cu^I through two oxygen atoms (O,O'-coordination mode) and one nitrogen atom (N-coordination mode), respectively. A comparison of the absorption spectra of **1** and **2** in acetone solution indicates that the 387-nm absorption band ($\epsilon = 780 \text{ M}^{-1} \text{ cm}^{-1}$) of **2** is

a charge-transfer transition. The broad absorption band at around 320 nm ($\epsilon \approx 5000 \text{ M}^{-1} \text{ cm}^{-1}$) of **4** in dichloromethane is also due to a charge-transfer transition. Functional modeling of CuNIR has been accomplished by treating solutions of **4** with acid; the nitrite-binding Cu^I complex quantitatively gives the one-electron reduction product, NO. The nitrite reduction of **4** in dichloromethane obeys a second-order rate law, suggesting that the rate-determining step would be protonation of the nitrite ligand of **4**. Electronic structure calculations of the Cu^I and Cu^{II} complexes containing Me₂bpa and nitrite by the density functional theory method demonstrate that the net charge of nitrite in Cu^I(Me₂bpa)(nitro) is larger than that in Cu^I(Me₂bpa)(nitrito). The mechanism of the nitrite reduction is discussed in comparison with the enzyme reaction mechanism of CuNIR.

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Introduction

The global inorganic nitrogen cycle sustained by bacteria plays an important role in the animal and plant kingdoms. Dinitrogen is introduced into the biosphere by the biological fixation of dinitrogen, and is removed from there into the atmosphere again by denitrification. Denitrification is the dissimilatory reduction of nitrate or nitrite to produce dinitrogen by prokaryotic organisms. Nitrite reductase (NIR), a key enzyme of denitrification, catalyzes the reduction of nitrite to nitrogen monoxide. Cu-containing NIR (CuNIR) is a 110-kDa homotrimer in which a monomer contains one each of type-1 Cu (blue copper) and type-2 Cu (nonblue copper).^[1–3] The interatomic distance between the two Cu sites is 12.5 Å. The type-2 Cu site shows a distorted tetrahedral geometry and is bound by three His

residues and one solvent water; the active site pocket containing the Cu site lies at the bottom of a 16.5 Å cavity formed at the interface between two adjacent monomers. X-ray crystal structure analyses of nitrite-soaked oxidized NIRs have demonstrated that the substrate is coordinated to type-2 Cu^{II} in an asymmetric bidentate fashion through two oxygen atoms instead of the water ligand,^[4–6] and an enzymatic mechanism has been proposed.^[7,8] In the very recent X-ray crystal structure of nitrite-soaked oxidized *Alcaligenes faecalis* CuNIR at 1.4 Å resolution, NO₂[−] coordinates to the Cu primarily by one O atom, whereas the other O atom interacts more weakly with the metal center.^[9] Moreover, the plane defined by the Cu and the two O atoms and the plane defined by the N and the two O atoms form a 75° angle and the NO₂[−] binds to the Cu with an almost face-on interaction. Several nitrite adducts of Cu^{II} complexes have been reported as model complexes for the active site of CuNIR.^[10–14] Tolman et al. have reported the nitrite-binding copper(I) complexes L^{iPr}₃Cu(NO₂) (L^{iPr}₃ = 1,4,7-triisopropyl-1,4,7-triazacyclononane) and [(L^{iPr}₃Cu)₂(μ-NO₂)]²⁺ as structural and functional models for the nitrite-binding type-2 Cu site and have discussed a mechanism for NO generation involving these Cu^I complexes.^[15,16]

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We report here the spectral and structural characterization of Cu^{I} and Cu^{II} complexes containing nitrite and a tridentate aromatic amine [bis(6-methyl-2-pyridylmethyl)amine (Me_2bpa)] as a substrate-binding type-2 Cu site model of CuNIR. Moreover, the nitrite reduction mechanism of the Cu^{I} complex is discussed based on the parameters of activation of the transition state. The enzymatic reduction mechanism of nitrite at the type-2 Cu is also proposed in comparison with the present model complexes and the binding mode of nitrite observed in the native and mutant CuNIRs from *Alcaligenes faecalis* S-6 by X-ray crystal structure analysis.^[9]

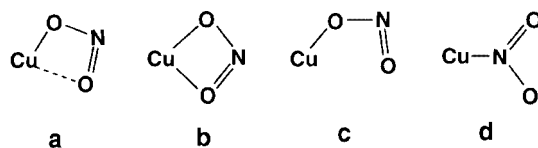
Results and Discussion

Structural and Spectral Aspects of the Cu^{II} Model Complex Containing NO_2^-

The reaction of $[\text{Cu}^{\text{II}}(\text{Me}_2\text{bpa})(\text{H}_2\text{O})(\text{ClO}_4)]\text{ClO}_4$ (**1**) with one equivalent of NaNO_2 in H_2O at room temperature gives $[\text{Cu}^{\text{II}}(\text{Me}_2\text{bpa})(\text{NO}_2)(\text{ClO}_4)]$ (**2**), which was recrystallized from aqueous methanol solution as green crystals. Figure 1 shows the X-ray crystal structures of **1** and **2**.

The geometry of Cu^{II} in **1** is distorted square pyramidal with three nitrogen atoms of the Me_2bpa ligand and one oxygen atom from each of H_2O and ClO_4^- ; a 15.0° upside deviation of the O(1) ligating atom of H_2O from the N(1)N(2)N(3) plane of Me_2bpa is observed. The structure of **2** reveals the replacement of the H_2O ligand by nitrite and asymmetric O, O' -nitrite chelation to the distorted octahedral Cu^{II} ion along with the Me_2bpa and ClO_4^- ligands. The equatorial O(2) coordinating atom deviates from the

N(1)N(2)N(3) plane of Me_2bpa by 19.6° and the O(1) ligating atom binds axially to the Cu^{II} ion. The structural studies of nitrite-binding Cu^{II} complexes have shown that nitrite has a variety of coordination modes to a Cu^{II} center (Scheme 1).^[17] Complex **2** exhibits two distinct $\text{Cu}^{\text{II}}\text{--O}_{\text{nitrite}}$ bond lengths [$\text{Cu}(1)\text{--O}(1) = 2.47(3) \text{ \AA}$, $\text{Cu}(1)\text{--O}(2) = 1.98(2) \text{ \AA}$] and the coordination mode of NO_2^- (**a** in Scheme 1) is similar to those of the $\text{Cu}^{\text{II}}\text{--NO}_2^-$ model complexes^[10–14] and the nitrite-binding type-2 Cu^{II} site in nitrite-soaked NIR at $1.4 \text{ \AA}$ resolution ($\text{Cu}\text{--O} = 2.29\text{--}2.38$, $2.04\text{--}2.08 \text{ \AA}$).^[9] The Cu, N(4), and two O(1 and 2) atoms lie in the same plane. The N–O bond lengths [$\text{N}(4)\text{--O}(1) = 1.24(4) \text{ \AA}$, $\text{N}(4)\text{--O}(2) = 1.28(4) \text{ \AA}$] and the O(1)–N(4)–O(2) angle [$115.8(27)^\circ$] are similar to those in the free nitrite ion [$\text{O}\text{--N}\text{--O} = 114.9(5)^\circ$ and $\text{N}\text{--O} = 1.240(3) \text{ \AA}$].^[18]



Scheme 1.

The electronic absorption spectrum of **1** in acetone solution (Figure 2) shows one band at 675 nm ($\epsilon = 190 \text{ M}^{-1} \text{ cm}^{-1}$), which is assigned to a ligand field transition. Complex **2** exhibits two bands at 378 nm ($\epsilon = 780 \text{ M}^{-1} \text{ cm}^{-1}$) and 671 nm ($\epsilon = 200 \text{ M}^{-1} \text{ cm}^{-1}$), which are ascribed as a nitrite $\rightarrow \text{Cu}^{\text{II}}$ charge transfer (LMCT) and a d-d transition, respectively. The small blue shift of the d-d band from 675 nm to 671 nm is due to the O -nitrite coordination of nitrite. Moreover, the 77-K EPR spectra of **1** and **2** in ace-

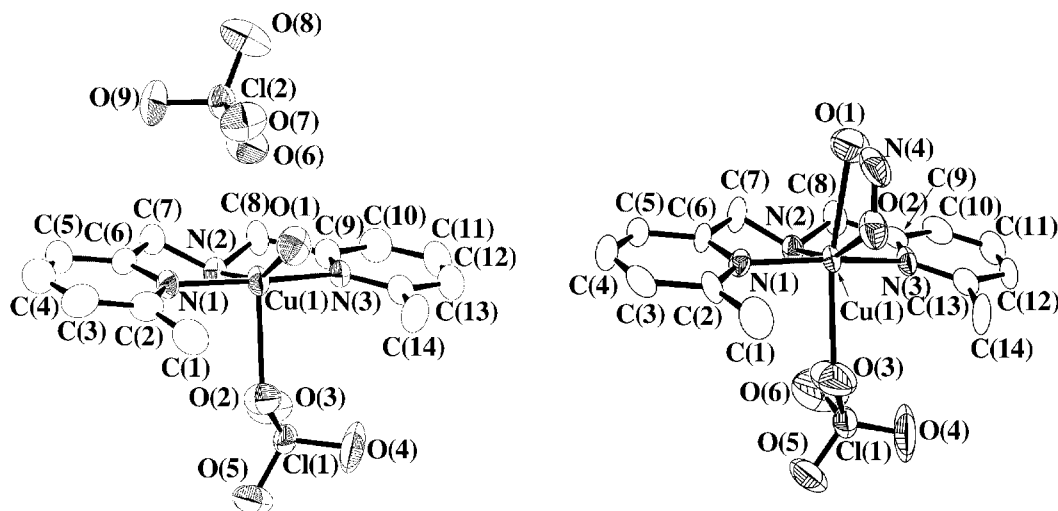


Figure 1. ORTEP plots of **1** (left) and **2** (right) with 50% probability thermal ellipsoids. The hydrogen atoms have been omitted for clarity. Selected distances [$\text{\AA}$] and angles [$^\circ$] for **1** are follows: $\text{Cu}(1)\text{--O}(1) 2.017(7)$, $\text{Cu}(1)\text{--O}(2) 2.441(7)$, $\text{Cu}(1)\text{--N}(1) 2.001(6)$, $\text{Cu}(1)\text{--N}(2) 1.957(7)$, $\text{Cu}(1)\text{--N}(3) 2.011(6)$; $\text{O}(1)\text{--Cu}(1)\text{--O}(2) 111.6(3)$, $\text{O}(1)\text{--Cu}(1)\text{--N}(1) 93.0(3)$, $\text{O}(1)\text{--Cu}(1)\text{--N}(2) 153.0(3)$, $\text{O}(1)\text{--Cu}(1)\text{--N}(3) 97.4(3)$, $\text{O}(2)\text{--Cu}(1)\text{--N}(1) 90.5(3)$, $\text{O}(2)\text{--Cu}(1)\text{--N}(2) 95.2(3)$, $\text{O}(2)\text{--Cu}(1)\text{--N}(3) 91.7(3)$, $\text{N}(1)\text{--Cu}(1)\text{--N}(2) 83.4(3)$, $\text{N}(1)\text{--Cu}(1)\text{--N}(3) 167.8(3)$, $\text{N}(2)\text{--Cu}(1)\text{--N}(3) 84.4(3)$. Selected distances [$\text{\AA}$] and angles [$^\circ$] for **2** are follows: $\text{Cu}(1)\text{--O}(1) 2.47(3)$, $\text{Cu}(1)\text{--O}(2) 1.98(2)$, $\text{Cu}(1)\text{--O}(3) 2.52(3)$, $\text{Cu}(1)\text{--N}(1) 2.012(18)$, $\text{Cu}(1)\text{--N}(2) 1.99(3)$, $\text{Cu}(1)\text{--N}(3) 2.003(18)$, $\text{O}(1)\text{--N}(4) 1.20(4)$, $\text{O}(2)\text{--N}(4) 1.28(4)$; $\text{O}(1)\text{--Cu}(1)\text{--O}(2) 54.9(10)$, $\text{O}(1)\text{--Cu}(1)\text{--O}(3) 159.4(9)$, $\text{O}(2)\text{--Cu}(1)\text{--N}(2) 155.6(10)$, $\text{N}(1)\text{--Cu}(1)\text{--N}(2) 82.8(8)$, $\text{N}(2)\text{--Cu}(1)\text{--N}(3) 82.1(9)$, $\text{N}(1)\text{--Cu}(1)\text{--O}(3) 87.9(8)$, $\text{Cu}(1)\text{--O}(1)\text{--N}(4) 83.7(19)$, $\text{Cu}(1)\text{--O}(2)\text{--N}(4) 105.6(19)$, $\text{O}(1)\text{--N}(4)\text{--O}(2) 115.8(27)$.

tone solution reveal the typical axial signals ($g_{\parallel} > g_{\perp}$) of a tetragonal Cu^{II} chromophore having a $d_{x^2-y^2}$ ground state (Figure S1, Supporting Information). The parameters for **2** ($g_{\parallel} = 2.25$, $g_{\perp} = 2.09$, $A_{\parallel} = 16.8$ mT) exhibit a slight decrease in the $g_{\parallel}$ and $A_{\parallel}$ values with respect to the precursor complex **1** ($g_{\parallel} = 2.27$, $g_{\perp} = 2.07$, $A_{\parallel} = 17.2$ mT). The difference between the parameters of these Cu^{II} complexes suggests the weak apical coordination [Cu(1)–O(3)] of nitrite in **2**.

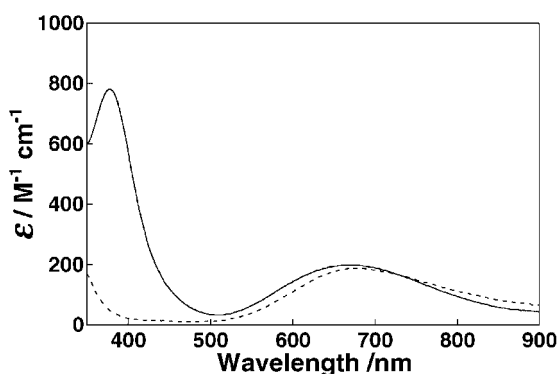


Figure 2. Electronic absorption spectra of **1** (broken line) and **2** (solid line) in acetone at room temperature.

Structural and Spectral Aspects of the Cu^I Model Complex Containing NO₂[−]

The reduced complex of **1**, the pale-yellow crystalline solid [Cu^I(Me₂bpa)(CH₃CN)]PF₆ (**3**), was prepared by treatment of [Cu(CH₃CN)]PF₆ with Me₂bpa in methanol. The nitrite-binding copper(I) complex, [Cu(Me₂bpa)(NO₂)₂][(Ph₃P)₂NPF₆] (**4**) was obtained as a yellow powder from an equimolar mixture of [Cu(CH₃CN)]PF₆, Me₂bpa, and [(Ph₃P)₂N]NO₂ in acetone under nitrogen. The X-ray crystal structure of **4** (Figure 3) reveals the *N*-nitrite coordination (**d** in Scheme 1) to the Cu^I center that adopts a distorted tetrahedral geometry. Although such a binding mode of nitrite in **4** is similar to that of L^{iPr₃}Cu(NO₂), some important structural aspects are different.^[15,16] The N–O bond lengths [1.253(5) and 1.238(5) Å] and the O–N–O angle [116.6(4)°] of nitrite in L^{iPr₃}Cu(NO₂)^[16] are almost the same as those of free nitrite ion,^[18] but the nitrite N–O distances [O(1)–N(4) = 1.076(8) Å and O(2)–N(4) = 1.039(8) Å] and the O(1)–N(4)–O(2) angle [133.6(8)°] of **4** are different from the corresponding values of free nitrite. The Cu(1)–N(4) bond length of **4** is 2.053(4) Å, which is longer than the value of 1.903(4) Å in L^{iPr₃}Cu(NO₂).^[16] Unfortunately, the large temperature factor of the O-atoms of the nitrite in **4** suggest the rotation/vibration of the NO₂[−] ligand and lead to serious errors in the parameters of nitrite bound to Cu^I ion.^[19] Therefore, it is difficult to discuss the structure of nitrite-binding copper(I) complex in detail on the basis of our present results.

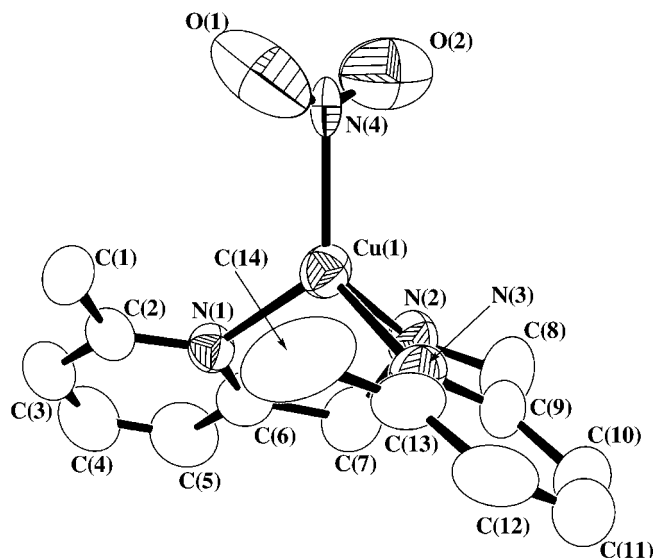


Figure 3. ORTEP plots of the Cu^I complex in **4** with 50% probability thermal ellipsoids. The hydrogen atoms and [(Ph₃P)₂NPF₆] have been omitted for clarity. Selected distances [Å] and angles [°] for **4** are follows: Cu(1)–N(1) 2.053(4), Cu(1)–N(2) 2.168(5), Cu(1)–N(3) 2.033(5), Cu(1)–N(4) 2.087(5), O(1)–N(4) 1.076(8), O(2)–N(4) 1.039(8); N(1)–Cu(1)–N(2) 82.4(2), N(1)–Cu(1)–N(3) 111.7(2), N(2)–Cu(1)–N(3) 81.0(2), N(1)–Cu(1)–N(4) 121.9(2), N(2)–Cu(1)–N(4) 118.8(2), N(3)–Cu(1)–N(4) 124.2(2), Cu(1)–N(4)–O(1) 111.5(5), Cu(1)–N(4)–O(2) 114.8(5), O(1)–N(4)–O(2) 133.6(8).

The electronic absorption spectra of **3** and **4** in CH₂Cl₂ are shown in Figure 4. These Cu^I complexes have no absorption band in the visible region. Complex **4** exhibits an intense broad band around 320 nm ($\epsilon \approx 5000$ M^{−1} cm^{−1}), which is assigned to a Cu^I → NO₂[−] MLCT transition.^[10–14] Tolman et al. have reported that L^{iPr₃}Cu(NO₂) exhibits an intense MLCT band at 308 nm ($\epsilon = 3800$ M^{−1} cm^{−1}) in CH₂Cl₂. The shift of the MLCT band could be due to the different electronic structures of **4** and L^{iPr₃}Cu(NO₂).

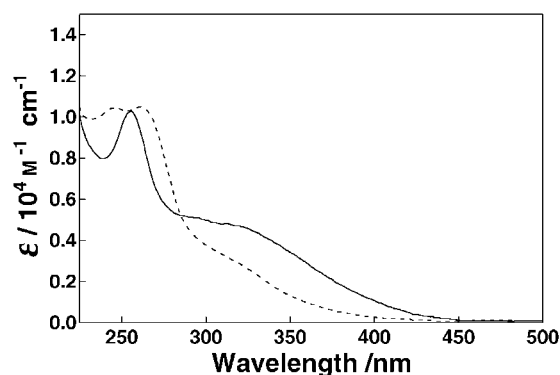


Figure 4. Electronic absorption spectra of **3** (broken line) and **4** (solid line) in CH₂Cl₂ at room temperature.

Nitrite Reductase Reactivity of the Cu^I Model Complex Containing NO₂[−]

The cyclic voltammogram of **2** in H₂O shows a response with $E_{1/2} = -95$ mV vs. Ag/AgCl and a peak-to-peak separation of 100 mV.

ration, ΔE , of 74 mV in the presence of 500 mM NaNO_2 (a in Figure 5). The ΔE value indicates a quasi-reversible electron-transfer process. Addition of acid to the aqueous solution of **2** brings about a strong catalytic current due to the reduction of NO_2^- (b in Figure 5). Thus, **2** serves as an electrocatalyst in the reduction of NO_2^- at pH 5.5.

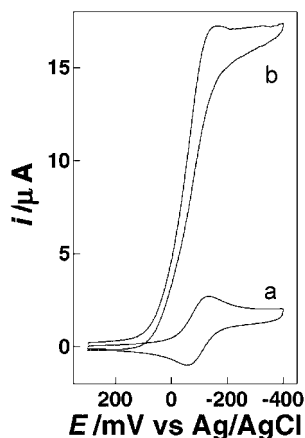


Figure 5. Voltammetric behavior of **2** (a) and after addition of HClO_4 (b) in 0.1 M TBAClO_4 aqueous solution at 25.0 °C (**2**: 0.5 mM; NaNO_2 : 500 mM; scan rate: 10 mV s^{-1}).

When four equivalents of trifluoroacetic acid was added to a CH_2Cl_2 solution of **4** at room temperature, the color of the solution was changed from pale yellow to blue and the stoichiometric evolution of NO gas (>95% vs. **4**) was observed by gas chromatography (Figure S2, Supporting Information). No N_2O gas was detected, and $[\text{Cu}(\text{Me}_2\text{bpa})(\text{OCOCF}_3)_2]$ was isolated from the reaction mixture. The reaction of **4** with proton was monitored either by recording the transient absorption spectra at one-second intervals or by recording absorbance vs. time at a fixed wavelength (320 nm). At 213.0 K, the 320-nm band of **4** was observed to be a sharp peak as shown in Figure 6. Introduction of four equivalents of trifluoroacetic acid to the solution under an Ar atmosphere induced a decrease in the absorbance at 320 nm and a slight increase in the absorbance at about 700 nm. As shown in the inset of Figure 6, the reaction of **4** with proton was found to be first-order both in $[\text{4}]$ and in $[\text{acid}]$ ($v = k_{\text{obs}}[\text{4}][\text{acid}]$) at 213.0, 223.0, and 231.8 K. The rate constants (k_{obs}) are listed in Table 1. The activation parameters (Table 2) were estimated from Arrhenius–Eyring plots of the data ($\ln k_{\text{obs}} = \ln A - E_a/RT$, $\ln(hk_{\text{obs}}/k_B T) = \Delta S^\ddagger/R - \Delta H^\ddagger/RT$; Figure S3, Supporting Information).

The mechanism of nitrite reduction of **4** with proton is illustrated in Scheme 2. The kinetic data of the reaction of **4** with trifluoroacetic acid suggest that the rate-determining step is the first protonation of the nitrite ligand. Since a negative value of $\Delta S^\ddagger$ generally corresponds to the additional loss of freedom of motion by the solvating molecule, the negative $\Delta S^\ddagger$ value ($-51 \text{ J K}^{-1} \text{ mol}^{-1}$) means that protonated **4** will be an active intermediate. The positive $\Delta H^\ddagger$ suggests that **4** is more stable than protonated **4**. The resonance structure of the nitrite ligand in **4** ($\text{O}=\text{N}-\text{O}^- \leftrightarrow ^-\text{O}-\text{N}=\text{O}$) might contribute to the stability of **4**.

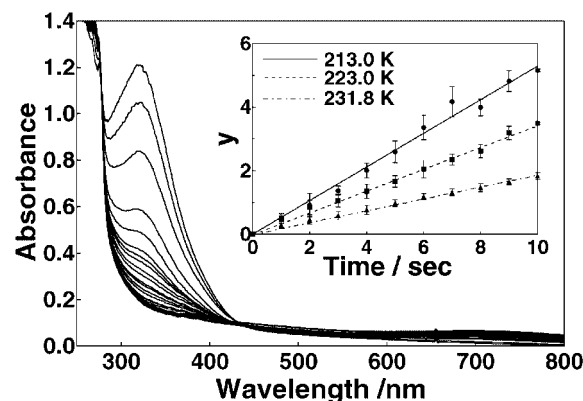


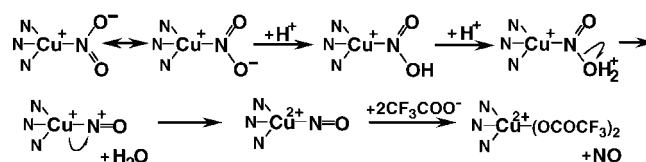
Figure 6. Spectral changes observed during the anaerobic reaction of **4** with trifluoroacetic acid in CH_2Cl_2 at 213.0 K. The subsequent traces, with progressively decreasing absorbance at 320 nm, refer to spectra taken every second. Inset: plots of the second-order rate law for the reactions of **4** with CF_3COOH at 213.0, 223.0, and 231.8 K. The y -axis is $(1/[\text{4}]_0[\text{acid}]_0) \ln([\text{acid}]_0[\text{4}]_x/[\text{4}]_0[\text{acid}]_x)$.

Table 1. Second-order rate constants for nitrite reduction of **4**.

T [K]	$k_{\text{obsd.}}$ [$\text{M}^{-1} \text{s}^{-1}$]
231.8	0.53 ± 0.04
223.0	0.34 ± 0.03
213.0	0.18 ± 0.02

Table 2. Activation parameters for nitrite reduction of **4**.

E_a [kJ mol^{-1}]	$\Delta G^\ddagger$ [kJ mol^{-1}]	$\Delta H^\ddagger$ [kJ mol^{-1}]	$\Delta S^\ddagger$ [$\text{J K}^{-1} \text{ mol}^{-1}$]
23 ± 1	40 ± 2	25 ± 1	-51 ± 3



Scheme 2.

Electronic-Structure Calculations of the Cu^{I} and Cu^{II} Complexes Containing Me_2bpa and Nitrite by the Density Functional Theory (DFT) Method

Electronic-structure calculations of the Cu^{I} and Cu^{II} complexes containing Me_2bpa and nitrite were carried out with the DFT method using the Gaussian 98 program.^[20] The X-ray crystal data of **2** and **4** were employed for initial geometries. The $\text{Cu}^{\text{II}}(\text{Me}_2\text{bpa})$ complex binding nitrite asymmetrically through two oxygen atoms ($\text{Cu}^{\text{II}}\text{-ONO}^-$, O,O' -nitrite coordination) is stabilized by $13.1 \text{ kcal mol}^{-1}$ relative to that binding nitrite through one nitrogen atom ($\text{Cu}^{\text{II}}\text{-NO}_2^-$, N -nitrite coordination), while $\text{Cu}^{\text{I}}\text{-NO}_2^-$ is less stable than $\text{Cu}^{\text{I}}\text{-ONO}^-$ ($1.0 \text{ kcal mol}^{-1}$). Therefore, nitrite is preferentially coordinated to Cu^{II} through two oxygen atoms ($\text{Cu}^{\text{II}}\text{-ONO}^-$), whereas the Cu^{I} complex could have two nitrite-binding forms ($\text{Cu}^{\text{I}}\text{-ONO}^-$ and $\text{Cu}^{\text{I}}\text{-NO}_2^-$). These findings are consistent with the experimental data

that the two L^{Pr3}-containing Cu^I complexes are bridged by nitrite through both nitrogen and oxygen atoms in the dicopper(II) complex [(L^{Pr3}Cu)₂(μ-NO₂)]PF₆.^[16] The Cu–nitrite bond energies of Cu^I–NO₂[−] and Cu^I–ONO[−] were estimated to be 103.2 and 104.2 kcalmol^{−1}, respectively, whereas those of Cu^{II}–NO₂[−] and Cu^{II}–ONO[−] were calculated to be 200.7 and 213.8 kcalmol^{−1}, respectively. The binding of nitrite to Cu^{II} in both the *N*-nitrite and the *O,O'*-nitrite coordination is energetically favored over that of nitrite to Cu^I in the two forms. The net charges of the nitrite ligands of Cu^I–NO₂[−], Cu^I–ONO[−], Cu^{II}–NO₂[−] and Cu^{II}–ONO[−] were evaluated to be −0.649, −0.562, −0.314, and −0.403, respectively, suggesting that the *N*-coordinated nitrite ligand of Cu^I–NO₂[−] will be most easily attacked by proton to produce NO and H₂O.

Proposed Mechanism of Nitrite Reductases

According to the crystallographic studies of the nitrite-binding Cu^{II} complexes^[10–16] and the nitrite-soaked oxidized CuNIR,^[4–6] nitrite preferentially binds to the oxidized type-2 Cu by asymmetric *O,O'*-coordination. We have proposed a catalytic mechanism for CuNIR incorporating roles for Asp98 hydrogen-bonded to the substrate and His255 hydrogen-bonded to Asp98 through H₂O, of which both the residues form a hydrogen-bonding network around the type-2 Cu.^[8] In an intermediate species during nitrite reduction, one of the two oxygen atoms of nitrite coordinated to type-2 Cu is probably hydrogen-bonded by both the protonated Asp98 and His255 residues. Very recently, Murphy et al. have reported that the nitrite-bound CuNIR crystal structure shows an angular orientation of nitrite bound to the type-2 Cu; the N atom is out of the plane defined by the two O atoms and the Cu atom, which forms a 75° angle with the plane defined by the N and the two O atoms.^[9] Therefore, they proposed that electron transfer (ET) from the type-1 Cu reduces the type-2 Cu to trigger a rearrangement of NO₂[−] (from *O,O'*-coordination to *N*-coordination) to release water and form a Cu^I–NO⁺ intermediate, that is, the proximity of the N atom of nitrite to the Cu in the substrate-soaked structure will facilitate this rearrangement step. The present studies of the X-ray crystal structure, nitrite reduction kinetics, and electronic-structure calculation of **4** also suggest that nitrite is initially coordinated to the oxidized type-2 Cu by the *O,O'*-coordination and then is converted into *N*-coordination by the reduction of the type-1 Cu. As shown in Figure 7, nitrite displaces a water ligand, which leaves a proton on Asp98 and is released as OH[−] (I → II). When the type-2 Cu is reduced by an electron from the type-1 Cu, the binding mode of nitrite is changed from *O,O'*-coordination to *N*-coordination and the proton of His255, which has an imidazolium group, is donated to the oxygen of *N*-coordinated nitrite (II → III → IV → V). *N*-Coordination of nitrite to the Cu^I ion and protonation to the nitrite ligand are supported by the kinetic analysis of **4** and the electronic-structure calculations of **2** and **4**. Finally, the electron transfer from the reduced type-2 Cu to NO₂H

would facilitate the N–O bond cleavage to produce NO gas through a Cu^I–NO⁺ intermediate (V → VI).

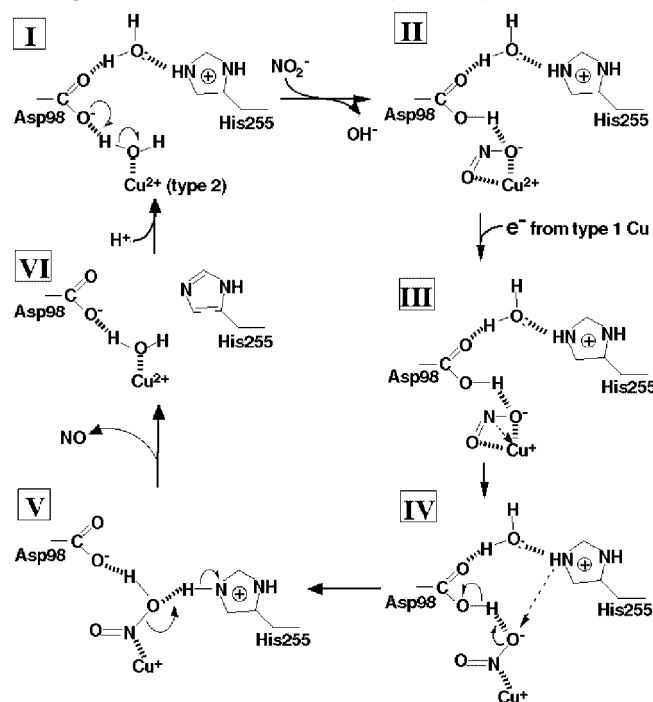


Figure 7. Revised enzymatic reaction mechanism of CuNIR containing type-1 Cu and type-2 Cu (cf. ref.^[7]).

Conclusions

Cu^I and Cu^{II} complexes containing a tridentate aromatic amine compound (Me₂bpa) and nitrite have been prepared as models for the active site of dissimilatory copper-containing nitrite reductase (CuNIR). The X-ray crystal structural analyses of **2** and **4** reveal that the geometries of the Cu centers are distorted octahedral and tetrahedral, respectively, and the coordination modes of nitrite are *O,O'*-coordination and *N*-coordination, respectively. The spectroscopic data of these complexes are consistent with their structural data. The reduction of nitrite by proton donation to **4** gives NO. These findings imply that both structural and functional modeling of CuNIR are achieved with Cu^I and Cu^{II} complexes containing the same aromatic amine ligand, Me₂bpa. The kinetic results of the reaction of **4** with acid suggest that the rate-determining step is protonation of the nitrite ligand of **4** and the resultant protonated species is the active intermediate. Moreover, electronic-structure calculations of the Cu^I and Cu^{II} complexes containing Me₂bpa and nitrite demonstrate that nitrite is preferentially coordinated to Cu^{II} through two oxygen atoms, but could bind to Cu^I by both *O,O'*- and *N*-coordination. Since the *N*-coordinated nitrite ligand of **4** will be more easily attacked than the *O,O'*-coordinated one by proton, the nitrite reduction of **4** probably occurs through the *N*-coordination of nitrite to Cu^I. According to the model complex studies for the active site of CuNIR, we have proposed a revised catalytic mechanism^[7] for CuNIR.

Experimental Section

General Remarks: All reagents used were of the highest grade available. Solvents were dried and distilled under N₂. Bis(6-methyl-2-pyridylmethyl)amine (Me₂bpa),^[21] [Cu(CH₃CN)₄]PF₆, and [(Ph₃P)₂-N]NO₂^[22] were synthesized according to the published procedures. All air-sensitive reactions were performed by using standard Schlenk and vacuum-line techniques. Electronic absorption spectra were recorded on a Shimadzu UV-2200 spectrophotometer. Low-temperature absorption spectra were obtained with a Shimadzu Multi-Spec 1500 spectrophotometer. X-band EPR spectra were recorded on a JEOL JES-FE1X spectrometer at 77 K. Gas chromatography was performed by using a Shimadzu GC14B analyzer with TCD detector (3-m molecular sieve 13X column, 23.5 mL min⁻¹ flow rate, helium carrier gas, and at 50 °C). Cyclic voltammetric analyses were carried out using a Bioanalytical Systems Model CV-50W voltammetric analyzer with a three-electrode system consisting of a Ag/AgCl reference electrode, a gold wire counter electrode, and a glassy carbon working electrode under an Ar atmosphere at 25 °C.

[Cu^I(Me₂bpa)(H₂O)(ClO₄)]ClO₄ (1): A solution of Cu(ClO₄)₂·6H₂O (3.71 g, 10 mmol) in methanol (5 mL) was added to a solution of Me₂bpa (2.27 g, 10 mmol) in methanol (50 mL). The resultant solution was stirred for 1 h and stored for a few days at room temperature to give blue crystals of **1** (4.39 g, 86.5%). C₁₄H₁₉Cl₂CuN₃O₉ (507.8): calcd. C 33.11, H 3.78, N 8.28; found C 33.11, H 3.68, N 8.30.

[Cu^{II}(Me₂bpa)(NO₂)(ClO₄)] (2): A solution of NaNO₂ (0.069 g, 1 mmol) in H₂O (10 mL) was added to a solution of **1** (0.51 g, 1 mmol) in H₂O (25 mL). The resultant solution was stirred for 1 h at room temperature to give **2** as a green precipitate. The green complex **2** was recrystallized from methanol/H₂O (0.362 g, 83.0%). C₁₄H₁₇ClCuN₄O₆ (436.3): calcd. C 38.54, H 3.93, N 12.84; found C 38.37, H 3.95, N 12.83.

[Cu^I(Me₂bpa)(CH₃CN)]PF₆ (3): [Cu(CH₃CN)₄]PF₆ (0.167 g, 0.5 mmol) was added as a solid, under an N₂ atmosphere, to a solution of Me₂bpa (0.114 g, 0.5 mmol) in methanol (5 mL). After stirring at 60 °C for 10 min, the resultant solution was stored at -20 °C. The pale-yellow crystalline solid thus obtained was filtered off, washed with dry diethyl ether, and dried under N₂ (0.196 g, 82.2%). C₁₆H₂₀CuF₆N₄P (476.9): calcd. C 40.29, H 4.24, N 11.75; found C 40.09, H 4.23, N 11.89.

[Cu^I(Me₂bpa)(NO₂)₂·[(Ph₃P)₂NPF₆]] (4): [Cu(CH₃CN)₄]PF₆ (0.167 g, 0.5 mmol) was added as a solid, under an N₂ atmosphere, to a solution of Me₂bpa (0.114 g, 0.5 mmol) in acetone (3.5 mL). [(Ph₃P)₂N]NO₂ (0.292 g, 0.5 mmol) was then added to the resultant solution, under an N₂ atmosphere. After stirring for 1 h **4** precipitated as a yellow powder (0.301 g, 88.7%). For X-ray analysis, yellow crystals of **4** were obtained from CH₂Cl₂/acetone. C₆₄H₆₄Cu₂F₆N₉O₄P₃ (1357.3): calcd. C 56.63, H 4.76, N 9.29; found C 56.64, H 4.90, N 9.41.

Measurement of NO Generated from 3: A solution of **4** (10 mg, 7.5 μmol) in CH₂Cl₂ (0.45 mL) was prepared in a small vial capped with a rubber septum. A degassed solution of CF₃COOH (6 μL) in CH₂Cl₂ (0.05 mL) was then introduced with a syringe at room temperature. The solution changed immediately from yellow to blue. Analysis of the head-space gas by a GC analyzer indicated that NO had been generated (7.3 ± 0.2 μmol).

Kinetics: The kinetic studies of nitrite reduction of **4** in CH₂Cl₂ were carried out by monitoring the intensity decrease of the 320-nm band. The absorbance was detected by a fiber-optics quartz

probe (0.2-cm path-length) immersed in a small vessel containing a 5 mL CH₂Cl₂ solution of **4** (3.4 mg, 2.5 μmol) under an Ar atmosphere. The reaction was started with the addition of 100 μL of a CH₂Cl₂ solution containing CF₃COOH (10 μmol), which had been degassed with N₂ beforehand.

X-ray Crystallographic Study of [Cu^{II}(Me₂bpa)(H₂O)(ClO₄)]ClO₄ (1) and [Cu^{II}(Me₂bpa)(NO₂)(ClO₄)] (2): The X-ray experiment was carried out on a MAC Science MXC18 diffractometer with graphite-monochromated Mo-K_α radiation (λ = 0.71069 Å). A blue (**1**) or green (**2**) crystal was mounted on a glass capillary. The reflection intensities were monitored by three standard reflections at every 150 measurements, and the decay of intensities was within 2%. The unit-cell parameters used for the refinement were determined by least-square calculations on the setting angles for 22 carefully centered reflections. An absorption correction was applied. Diffraction data were corrected for both Lorentz and polarization effects. The structure was solved by direct methods SIR92^[23] using the CRYSTAN-GM program system^[24] and refined anisotropically for non-hydrogen atoms by full-matrix least-squares calculations. Each refinement was continued until all shifts were smaller than one-third of the standard deviations of the parameters involved. Atomic scattering factors and anomalous dispersion terms were taken from the literature.^[25] All hydrogen atoms were located at their calculated positions. The fundamental crystal data and experimental parameters for structure determination are given in Table 3.

[Cu^I(Me₂bpa)(NO₂)₂·[(Ph₃P)₂NPF₆]] (4): The X-ray experiment was carried out at -70 °C on a Rigaku Mercury CCD area detector coupled with a Rigaku AFC-7R diffractometer with graphite-monochromated Mo-K_α radiation (λ = 0.71069 Å). The pale-yellow crystal was mounted on a glass capillary. The data were corrected for Lorentz and polarization effects, but not for secondary extinction. An empirical absorption correction was applied. The structure was solved by direct methods using the Crystal Structure crystallographic software package^[26] and refined anisotropically for non-hydrogen atoms by full-matrix least-squares calculations. Each refinement was continued until all shifts were smaller than one-third of the standard deviations of the parameters involved. Atomic scattering factors and anomalous dispersion terms were taken from the literature.^[25] All hydrogen atoms were located at their calculated positions. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.73 and -0.52 e Å⁻³, respectively. The fundamental crystal data and experimental parameters for structure determination are given in Table 3.

CCDC-251363 (for **1**), -251364, (for **2**) and -251365 (for **4**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Electronic-Structure Calculations: Electronic-structure calculations of the Cu^I and Cu^{II} complexes containing Me₂bpa and nitrite were carried out with the DFT method in the Gaussian 98 program.^[27] For all molecules, the geometries were fully optimized without any constraints. The three-parameter Becke–Lee–Yang–Parr (B3LYP) functional was adopted as the exchange–correlation functional.^[28] The core electrons of Cu were replaced with the effective core potential (ECP) of Hay and Wadt.^[29] The basis set for Cu was the split-valence set (LANL2DZ^[21]) for the ECP. The Huzinaga–Dunning (9s5p)/[4s2p] and (4s)/[2s] basis sets were used for the first row elements and hydrogen, respectively.^[30]

Supporting Information (see also footnote on the first page of this article): Further figures illustrating the ESR spectra of **1** and **2**, the GC chart, and the Arrhenius–Eyring plot for the reaction of **4** with proton are provided.

Table 3. Crystal data for **1**, **2**, and **4**

	1	2	4
Formula	C ₁₄ H ₁₉ Cl ₂ CuN ₃ O ₉	C ₁₄ H ₁₇ ClCuN ₄ O ₆	C ₆₄ H ₆₄ Cu ₂ F ₆ N ₉ O ₄ P ₃
Formula mass	507.80	436.30	1357.27
Color	blue	green	pale yellow
Crystal size [nm]	0.75 × 0.30 × 0.30	0.45 × 0.10 × 0.03	1.00 × 0.50 × 0.10
Crystal system	triclinic	triclinic	monoclinic
Space group	<i>P</i> 1	<i>P</i> 1̄	<i>C</i> 2/ <i>c</i>
<i>a</i> [Å]	8.558(5)	8.972(8)	30.72(1)
<i>b</i> [Å]	8.650(7)	12.395(9)	12.950(5)
<i>c</i> [Å]	7.820(5)	8.516(8)	15.596(6)
<i>α</i> [°]	113.18(5)	93.13(7)	–
<i>β</i> [°]	110.95(4)	109.33(7)	96.29(1)
<i>γ</i> [°]	71.86(5)	77.97(6)	–
<i>V</i> [Å ³]	487.1(5)	874.010010(1)	6166.7(3)
<i>Z</i>	1	2	4
<i>D</i> _{calcd.} [g cm ^{−3}]	1.731	1.657	1.462
Radiation	Mo- <i>K</i> _α (λ = 0.71073 Å)	Mo- <i>K</i> _α (λ = 0.71073 Å)	Mo- <i>K</i> _α (λ = 0.7107 Å)
μ [cm ^{−1}]	14.51	14.435	8.42
<i>F</i> ₀₀₀	259.00	446.00	2800.00
Scan method	θ–2θ	θ–2θ	ω–2θ
2θ _{max} [°]	52.94	52.86	53.46
No. reflections obsd.	2220	1566	6690
No. reflections used	2205	1454	4312
<i>R</i> ^[a]	0.069	0.069	0.064
<i>R</i> _w ^[b]	0.087	0.091	0.097

[a] $R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$. [b] $R_w = [\Sigma w(|F_o| - |F_c|)^2 / \Sigma w F_o^2]^{1/2}$; $w = 1/[\sigma^2(F_o) + 0.03 F_o^2]$ for **1** and **2**, $w = 1/[\sigma^2(F_o) + 0.005 F_o^2]$ for **4**.

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