A Structural Model of the Type 1 Copper Protein Active Site: N_2S(thiolate)S(thioether) Ligation in a Cu(II) Complex

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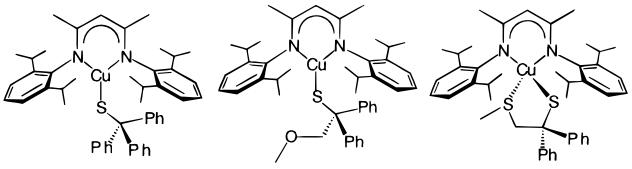
The ubiquitous type 1 copper biological electron-transfer sites have a single Cu ion bonded to two histidine imidazoles and a cysteine thiolate (Cu−S ∼2.15 Å) in an approximately trigonal geometry, usually with a fourth donor (typically a methionine thioether) present at a long yet bonding distance (2.6−2.9 Å).[1] Recent work has shown that this classic motif is in fact variable, and there is great interest in understanding how the subtle changes in the Cu coordination sphere in the various type 1 sites relate to differences in their spectral and redox properties.[1,2] Studies of synthetic copper complexes are helpful for understanding such structure/property relationships.[3] Despite attempts over several decades to prepare inorganic complexes that reproduce the type 1 coordination geometry, however, no Cu(II) compound with sole N_2S(thiolate)S(thioether) ligation has been characterized definitively.[4,5] We describe herein the successful attainment of this longstanding goal.

We recently reported[6] the synthesis of the first three-coordinate Cu(II) complexes, including thiolate **1** that models the trigonal type 1 site in fungal laccase,[7] by using a highly hindered β-diketiminate as a supporting ligand (L).[8] In an extension of this approach, we treated solutions of LCuCl in THF with NaSC-$(Ph)_2CH_2ECH_3$ (E = O or S).[9] For the case of E = O, product **2** was isolated as a stable blue-purple crystalline solid. The pendant ether is not coordinated to the Cu(II) ion in the complex and it adopts a solution geometry similar to that of **1**, as shown by



1 **2** **3**

spectral data (Figures 1 and S1). Thus, the UV−vis and EPR spectral features of **1** and **2** are nearly identical, key features being the analogous S → Cu(II) LMCT band energies and intensities

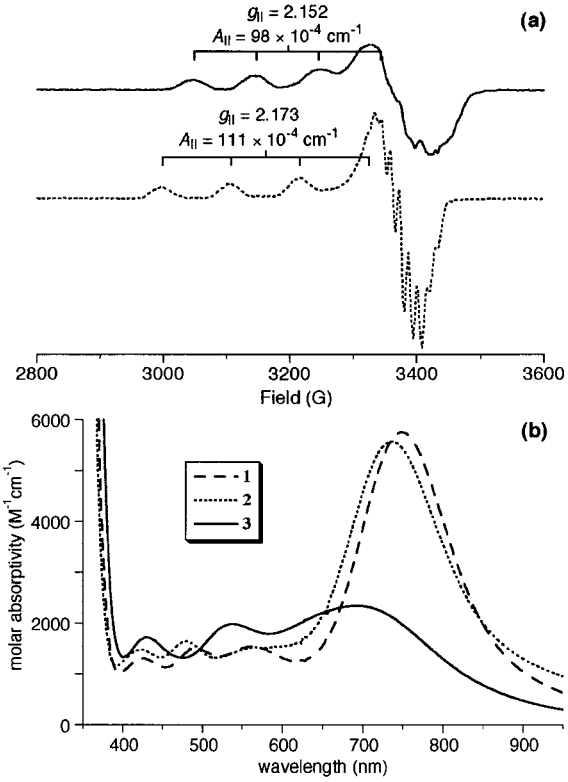


Figure 1. (a) X-band EPR spectra of toluene solutions of **2** (dashed line) and **3** (solid line), T = 20 K. (b) UV−visible spectra of solutions in heptane (**1**), pentane (**2**), or toluene (**3**) at ambient temperature.

as well as axial EPR signal parameters.[10] The X-ray crystal structure of **2** (Figure 2a) also is similar to that of **1**. The Cu geometry is trigonal planar [the Cu atom lies 0.125(2) Å from the N_2S plane], and the metrical parameters mimic the coordination sphere of the three-coordinate type 1 Cu(II) site in fungal laccase in much the same way as described earlier for **1**.[6]

In contrast, using a ligand with a thioether functionality (E = S) in the place of the ether resulted in the formation of a product (**3**) with significantly perturbed spectroscopic and structural features. Importantly, the X-ray crystal structure of **3** (Figure 2b) reveals coordination of the thioether group to the metal ion [Cu−S(2) = 2.403(1) Å] to yield the first example of a structurally defined Cu(II) complex with the same donor set as the classical type 1 biological site. As a result of strong thioether binding, the remainder of the Cu coordination sphere in **3** differs significantly from those of **1** and **2**. The metal−ligand distances in **3** are lengthened, consistent with its higher metal coordination number. In addition, in **3** the β-diketiminate ligand is "folded" along the

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(9) For synthetic procedures and complete characterization data, see the Supporting Information.

(10) Data for **2** (yield 54%): UV/vis (pentane) [λ_{ex}, nm (ϵ, mM^{-1} cm^{-1})] 343 (∼35), 423 (1.4), 480 (1.6), 566 (1.4), 738 (5.6); EPR (9.61 GHz, toluene, 20 K): $g_{||}$ = 2.17, $A_{||}^{Cu}$ = 111 × 10^{-4} cm^{-1}, $g_{\perp}$ = 2.04, $A_{\perp}^{N}$ = 13 × 10^{-4} cm^{-1}; cyclic voltammogram (0.2 M NBu_4PF_6/THF electrolyte, referenced to internal ferrocene): $E_{1/2}$ = −0.12 V vs NHE, $E_{pa} - E_{pc}$ = 0.12 V, $i_a \approx i_c$. Anal. Calcd for $C_{44}H_{56}CuN_2OS$: C, 72.94; H, 7.79; N, 3.87. Found: C, 72.78; H, 7.89; N, 3.84. Data for **3** (yield 54%): UV/vis (toluene) [λ_{ex}, nm (ϵ, mM^{-1} cm^{-1})] 354 (∼15), 430 (1.9), 538 (2.1), 691 (2.3); EPR (9.61 GHz, toluene, 20 K): $g_{||}$ = 2.15, $A_{||}^{Cu}$ = 98 × 10^{-4} cm^{-1}, g_x = 2.01, g_y = 2.06, $A_x^N = A_y^N$ = 10 × 10^{-4} cm^{-1}; cyclic voltammogram (0.2 M NBu_4PF_6/THF electrolyte, referenced to internal ferrocene): −0.20 V vs NHE, $E_{pa} - E_{pc}$ = 0.11 V, $i_a \approx i_c$. Anal. Calcd for $C_{44}H_{56}CuN_2S_2$: C, 71.36; H, 7.62; N, 3.78. Found: C, 71.92; H, 8.23; N, 3.48. Selected data for **1** (ref 6): λ_{max} = 749 nm, ϵ = 5.8 mM^{-1} cm^{-1}; : $g_{||}$ = 2.17, $A_{||}^{Cu}$ = 111 × 10^{-4} cm^{-1}, $g_{\perp}$ = 2.04, $A_{\perp}^{N}$ = 13 × 10^{-4} cm^{-1}; $E_{1/2}$ = −0.18 V vs NHE.

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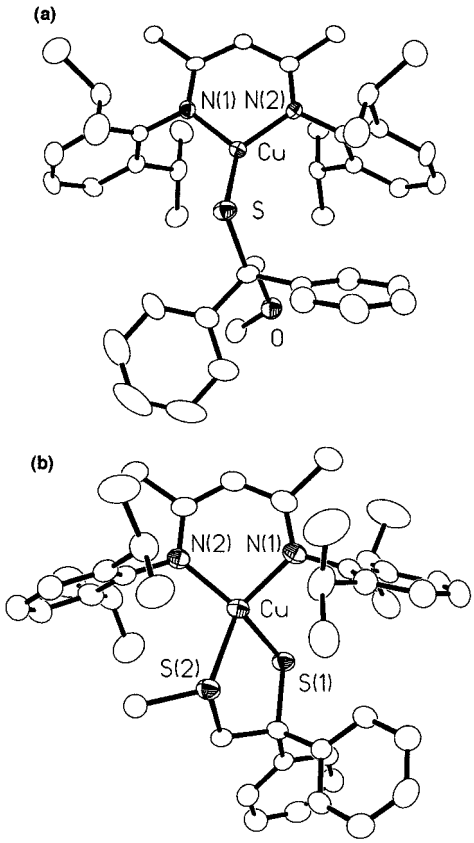

Figure 2. Thermal-ellipsoid plots (50% probability) of (a) **2** and (b) **3**, with hydrogen atoms omitted for clarity. Selected bond distances and angles: **2**, Cu−S = 2.119(1) Å; Cu−N = 1.900(4), 1.908(4) Å; Cu−S−C = 112.3(1)°. **3**, Cu−S(1) = 2.242(1) Å, Cu−S(2) = 2.403(1) Å, Cu−N = 1.987(3), 1.952(3) Å, Cu−S(1)−C = 109.4(1)°.

N−N vector [fold angle = 14.6(2)°] and the metal adopts a flattened tetrahedral geometry [55.10(8)° twist angle between the N_2Cu and S_2Cu planes] with the copper atom residing 0.484(2) Å above the N_2S(thiolate) plane (Figure S4). In contrast to the trigonal ligand disposition in **1** and **2** which results in very similar S(thiolate)−Cu−N angles (average 130.5°; range 126−136°), these angles in **3** differ considerably [98.29(9) and 146.53(9)°]. Because of this distortion, the overall geometry of **3** does not accurately replicate that of the "classic" type 1 sites (e.g. plastocyanin and azurin)[1] despite the identity of the donor atoms. The structure of **3** does, however, resemble the "Type 1.5" sites (e.g. nitrite reductase and several azurin mutants containing Gln, His, or Glu in place of the Met ligand)[11] that are characterized by stronger axial ligand interactions and a tetragonal distortion relative to the $\sim C_{3v}$ geometry of the type 1 centers.[1c] This resemblance is illustrated by a comparison of the core of **3** to the relevant copper coordination spheres in the crystallographically determined structures of nitrite reductase and of M121H azurin (Figure 3).

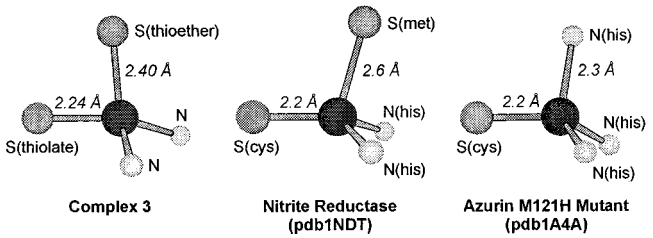

Figure 3. Comparison of the coordination sphere of **3** to those of structurally characterized "perturbed" type 1 copper cores.

The spectral characteristics of **3** suggest that the differences from **2** seen in the solid state are retained in solution (Figure 1). In the EPR spectrum of **3**, the $A_{\|}^{Cu}$ and $g_{\|}$ values (98×10^{-4} cm^{-1} and 2.15, respectively) are smaller than those for **2** (111×10^{-4} cm^{-1} and 2.17). In addition, the signal for **3** is rhombic: our best simulation has the $g_{\perp}$ region split into $g_x = 2.01$ and $g_y = 2.06$. The EPR spectrum is quite different from an independently prepared tetragonal copper(II) complex of L.[12] The differences between the EPR data for **2** and **3** parallel those between the type 1 and 1.5 protein sites.[13] Compared to **1** and **2**, the electronic absorption spectrum of **3** shows a substantial shift of the characteristic low-energy S → Cu LMCT band to 691 nm, along with a decrease in its intensity ($\epsilon = 2300$ M^{-1} cm^{-1}). These data are also consistent with a four-coordinate solution structure, and show a striking resemblance to the changes observed upon deprotonation of Glu in the Met121Glu mutant of azurin.[11b] These changes may be attributed to a longer Cu−S(thiolate) bond.[13,14] Interestingly, cyclic voltammograms of **1**−**3** contain pseudoreversible waves with redox potentials that fall within a narrow range ($E_{1/2}$ −0.12 to −0.20 V vs NHE).[10]

In conclusion, by examining complexes that differ by only a single donor atom (E = O vs S), we have been able to address directly in a model system the structural and spectroscopic effects of thioether coordination to the type 1 N_2S(thiolate)Cu core. The thioether ligand in complex **3** is strongly bound, leading to a flattened tetrahedral structure distinct from the geometries of the three-coordinate molecules **1** and **2**. This geometric difference is reflected in divergent spectral features. Structurally, complex **3** most closely matches the perturbed "type 1.5" biological sites, with similarities and differences in spectroscopic and redox properties that warrant further study.

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Supporting Information Available: Experimental details for all new compounds, EPR simulations, resonance Raman spectra, cyclic voltammograms (PDF) and X-ray crystallographic files (CIF) for **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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