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A Thiolate-Bridged, Fully Delocalized Mixed-Valence Dicopper(I,II) Complex That Models the Cu_A Biological Electron-Transfer Site

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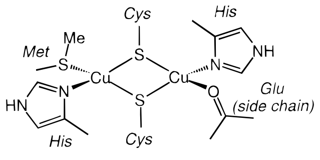
Nitrous oxide reductase (N_2OR) and cytochrome *c* oxidase (C*c*O) catalyze the reductions of N_2O to N_2 during microbial denitrification and of O_2 to H_2O during cellular respiration, respectively.[1,2] These disparate metalloenzymes have in common an unusual copper site, Cu_A, that functions analogously to the ubiquitous type 1 monocopper centers[3] to transfer electrons during enzyme turnover. Controversy surrounding the structure of Cu_A appears to have been resolved by recent X-ray crystallographic results[4] and spectroscopic and biochemical studies of N_2OR, C*c*O, and engineered Cu_A sites.[5] The combined data support a novel dithiolate-bridged, delocalized mixed-valence ($Cu^{1.5}Cu^{1.5}$) resting-state formulation with four-coordinate, distorted tetrahedral copper ions in close proximity (∼2.6 Å apart). A key spectroscopic signature that supports the delocalized nature of the site is an EPR signal with seven-line hyperfine coupling patterns in its low-field components arising from spin interactions with both $I = {}^3/_2$ copper ions.[5h] The structure represents a fundamentally new type of electron-transfer site in biology and is a novel entity from a purely inorganic chemical point of view as well. Although several complexes with bis(μ-thiolato)dicopper(I,I) cores are known,[6] fully delocalized "class III"[7] mixed-valence dicopper(I,II) complexes are extremely rare,[8,9] and no example of such a species bridged by thiolates has appeared in the literature. Herein we report the successful synthesis and characterization of a unique molecule with a $\{Cu_2(\mu\text{-SR})_2\}^+$ core that closely mimics the resting-state Cu_A geometry, oxidation level, and high degree of electron delocalization as reflected by EPR spectroscopy.



Cu_A

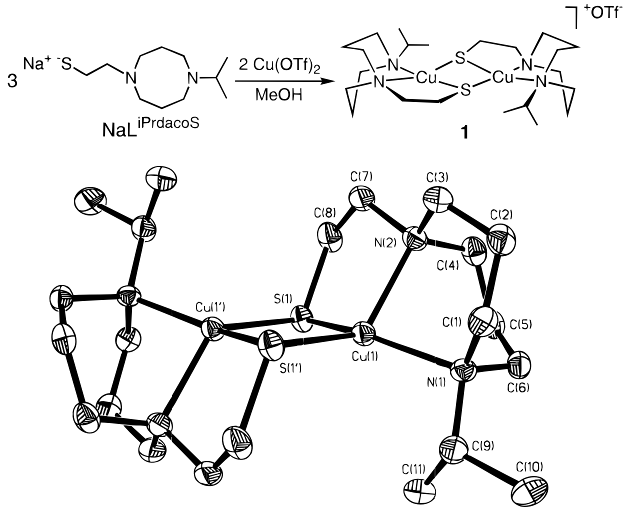


Figure 1. Synthesis of **1** and a representation of its X-ray crystal structure showing one of the two chemically similar binuclear cations generated from the two crystallographically independent half-dimers present in the unit cell (50% ellipsoids, hydrogen atoms omitted for clarity). Selected bond distances (Å) and angles (deg) for this cation: Cu1−S1, 2.250(1); Cu1−S1′, 2.292(1); Cu1−N1, 2.115(3); Cu1−N2, 2.125(3); Cu1···Cu1′, 2.9306(9); S1···S1′, 3.480(2); S1−Cu1−S1′, 99.64(3); S1−Cu1−N1, 149.23(9); S1−Cu1−N2, 90.25(9); S1′−Cu1−N1, 107.64(9); S1′−Cu1−N2, 122.20(9); N1−Cu1−N2, 87.2(1); Cu1-S1-Cu1′, 80.36(3). The similar parameters for the other cation are presented in the supporting information.

Our synthesis of the title complex hinged on the use of the new ligand $NaL^{iPrdacoS}$, which was prepared from 1,5-bis(*p*-toluenesulfonyl)diazacyclooctane by using precedented N-macrocycle deprotection and alkylation strategies.[10,11] Admixture of a 3:2 ratio of $NaL^{iPrdacoS}$ and $Cu(O_3SCF_3)_2$ in MeOH, followed by removal of solvent, extraction with CH_2Cl_2 to remove byproducts, and crystallization from MeOH/Et_2O, yielded $(L^{iPrdacoS}Cu)_2(O_3SCF_3)$ (**1**), analytically pure in 60% yield as deep blue crystals (Figure 1).[10] The 3:2 ligand−metal stoichiometry is critical for obtaining the pure product, as the

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(10) Cyclization of the disodium salt of *N,N*-bis(*p*-toluenesulfonyl)-propane-1,3-diamine with 1,3-bis(*p*-toluenesulfonyloxy)propane in DMF afforded 1,5-bis(*p*-toluenesulfonyl)diazacyclooctane.[10a] Single detosylation with 30% HBr/HOAc,[10b] alkylation with isopropyl bromide, and subsequent detosylation with H_2SO_4 afforded 1-isopropyl-1,5-diazacyclooctane. Functionalization with thiirane followed by deprotonation with NaH yielded $NaL^{iPrdacoS}$.[11] See supporting information for synthetic details and characterization data for all new compounds, including **1**. (a) Atkins, T. J.; Richman, J. E.; Oettle, W. F. *Organic Synthesis*; John Wiley & Sons: New York, 1988; Collect. Vol. 6, pp 652−662. (b) Sessler, J. L.; Sibert, J. W. *Tetrahedron* **1993**, *49*, 8727−8738.

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extra half equivalent of $\text{NaL}^{\text{iPrdacoS}}$ provides the needed reducing equivalent to afford the mixed-valence complex plus disulfide.

An X-ray crystal structure determination of **1** confirmed the presence of a single triflate counterion and a $\{Cu_2(\mu\text{-SR})_2\}^+$ core, as shown in Figure 1 for one of the dimers generated from one of the two crystallographically independent but chemically similar half-dimers in the unit cell.[12] The Cu_2S_2 unit is planar, with an average Cu···Cu distance of 2.92 Å that is slightly longer than that suggested for Cu_A (2.5 Å by EXAFS,[5e,f] 2.5−2.7 Å by X-ray crystallography).[4] The geometry of each of the metal ions is best described as distorted trigonal pyramidal, with N1 and the bridging thiolate sulfur atoms defining the trigonal plane, from which Cu1 is only slightly displaced (by 0.22 and 0.18 Å for the two crystallographically independent molecules). Principal distortions away from idealized trigonal pyramidal local symmetry involve displacement of N2 ~27° from the vector normal to the N1,S1,S1′ plane and angles in this plane that deviate from 120° (range 99−149°). The overall coordination geometry is analogous to those of "trigonal" type 1 copper protein sites and model complexes,[3,13,14] except the basal plane in **1** incorporates a NS_2 rather than a N_2S donor atom set.[15] Also similar to type 1 centers, the ligand arrangement in **1** can be construed to be intermediate between geometries favorable for Cu^I or Cu^{II} oxidation levels, presumably in order to facilitate redox reactions and, in this instance, to stabilize a $Cu^{1.5}Cu^{1.5}$ form.

The delocalized mixed-valence description implied by the X-ray structural data is supported by spectroscopic and physical information obtained for **1** in MeOH solution.[16] Most importantly, the X-band EPR spectrum of **1** in a MeOH/toluene glass at 4.2 K contains a nearly axial signal with clearly recognizable seven-line hyperfine splitting patterns in the two lower field components (Figure 2). Copper hyperfine coupling constants and *g* values were estimated via spectral simulation (assuming interaction of the unpaired spin with both metal ions) to be $g_1 = 2.010$, $g_2 = 2.046$, $g_3 = 2.204$, $A_2^{Cu} = 36.3$ G, and $A_3^{Cu} = 49.9$ G. Comparison of this signal to those reported for the Cu_A sites in C*c*O and N_2OR reveals striking similarities,[5h] notwithstanding differences in details such as A^{Cu} values for the synthetic model 12−15 G larger than for the proteins. We therefore assign analogous class III delocalized electronic structures to **1** and the biological sites.

The optical absorption spectrum of blue **1** in MeOH (Figure 3) contains features at $\lambda_{max} = 358$ (ϵ 2700 M^{-1} cm^{-1}), 602 (800), 786 (sh), and 1466 (1200) nm. The visible absorption bands are red-shifted and 2−3 times less intense relative to the purple Cu_A sites from various sources,[17] possibly due to differences in donor atom sets, metal−metal distances (shorter in Cu_A), or copper geometries (the metal ions in Cu_A have been suggested to be more akin to the tetrahedrally distorted class of type 1 copper sites than to the trigonal planar set better mimicked by those in **1**).[5i] The intriguing, intense band evident in the near-infrared region of the absorption spectrum of **1** has not yet been assigned. Finally, in its cyclic voltammogram, waves corresponding to one-electron reduction or oxidation of **1** in MeOH are apparent ($E_{pc} = -0.50$ V and $E_{1/2} = +0.35$ V vs SCE with 0.1 M $TBAPF_6$), but our finding that the former is completely irreversible and the latter becomes chemically reversible only at high scan rates (>1.0 V s^{-1}) attests to significant stabilization of the mixed-valence state.

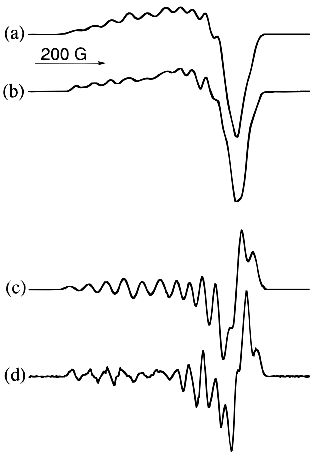

Figure 2. EPR data for **1** shown as (a) first-derivative simulated, (b) first-derivative experimental, (c) second-derivative simulated, and (d) second-derivative experimental spectra (9.213 GHz, 4.2 K, MeOH/toluene glass). Parameters used to obtain the simulated spectra are $g_1 = 2.010$, $g_2 = 2.046$, $g_3 = 2.204$, $A_2^{Cu} = 36.3$ G, and $A_3^{Cu} = 49.9$ G.

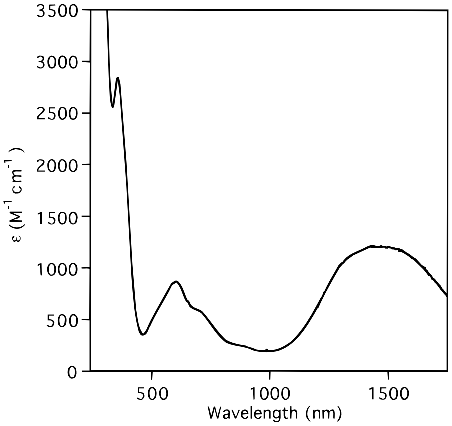

Figure 3. Optical absorption spectrum of **1** in MeOH (250−800 nm) or CD_3OD (800−1600 nm). See text for λ_{max} and ϵ values.

In sum, we have prepared a novel compound with a bis(μ-thiolato)dicopper(I,II) core that replicates essential elements of the structural and spectroscopic features of the Cu_A metalloprotein active site. The combination in **1** of a low coordination number (<5), thiolate bridges, and an unusual distorted trigonal pyramidal metal ion geometry that is supported by L^{iPrdacoS} contributes to the stabilization of the delocalized mixed-valence form of the $\{Cu_2(\mu\text{-SR})_2\}^+$ unit that heretofore was accessible only in dicopper(I,I)[6] or -(II,II)[11] oxidation states. By analogy, similar factors would appear to be responsible for the physicochemical properties of the biological Cu_A centers, for which numerous questions regarding correlation of structure and function remain unanswered.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds and full details of the X-ray crystal structure determination of **1** (17 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(12) Crystal data for $(L^{\text{iPrdacoS}}Cu)_2(O_3SF_3)$ (**1**): MW = 706.90, $C_{23}H_{46}Cu_2F_3N_4O_3S_3$, crystal dimensions 0.40 mm × 0.15 mm × 0.13 mm, monoclinic, space group $P2_1/n$, $a = 12.4726(3)$ Å, $b = 17.9093(5)$ Å, $c = 13.0768(3)$ Å, $\beta = 91.786(1)°$, $V = 2919.6(1)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.608$ g cm^{-3}, $2\theta_{max} = 50.14°$, Mo Kα radiation ($\lambda = 0.710\,73$ Å), $T = 173$ K. Data were collected using a Siemens SMART system, and the structure was solved via direct methods. Full-matrix least-squares refinement on F^2 using SHELXTL V5.0 converged with final $R1 = 0.0444$ and $wR2 = 0.0867$ for 5149 independent reflections with $I > 2\sigma(I)$ and 393 parameters.

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(16) Electrospray MS (MeOH): $[M - O_3SCF_3]^+ = 556$. Molar conductivity (MeOH): $\Lambda = 81$ cm^{-1} mol^{-1} Ω^{-1}. Magnetic moment (per complex, Evans method, CD_3OD, 298 K, 500 MHz): $\mu_{eff} = 1.7(1)$ μ_B.

(17) Visible absorption bands reported for Cu_A in N_2OR from *Achromobacter cycloclastes*:[5j] $\lambda_{max} = 350$ (sh), 481 (ϵ 5200 M^{-1} cm^{-1}), 534 (5300), 630 (sh), 780 (2900) nm. For the soluble Cu_A binding domain from *Pseudomonas denitrificans* C*c*O:[5d] $\lambda_{max} = 363$ (1200), 480 (3000), 532 (3000), 808 (1600) nm.