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Paramagnetic Active Site Models for the Molybdenum−Copper Carbon Monoxide Dehydrogenase

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A unique heterobimetallic Mo/Cu active site is present in the carbon monoxide dehydrogenase (CODH) from *Oligotropha carboxidovorans*.[1,2] This enzyme catalyzes the oxidation of CO to CO_2, thereby providing carbon and energy to the organism and maintaining sub-toxic levels of CO in the troposphere.[1,2] The presence of a square pyramidal (Mo) oxidized active site, i.e., $\{(\mathrm{MCD})\mathrm{Mo^{VI}OX}(\mu\text{-S})\mathrm{Cu^{I}}(\mathrm{S{-}Cys})\}^{n-}$ (MCD = molybdopterin cytosine dinucleotide; X = OH[3] or O[4] (Figure 1)), has been confirmed, but the enzyme's mechanism and the role of the μ-sulfido ligand in catalysis are still subjects of debate. Some mechanisms invoke the direct participation of the sulfido ligand in the formation of thiocarbonate intermediates,[3,5] while others preclude a direct role for this ligand.[5,6] Paramagnetic forms of the enzyme have been partially characterized, and the reconstitution of inactive enzyme has been examined.[3,7] Detailed studies of well-defined $\mathrm{MoO}(\mu\text{-S})\mathrm{Cu}$ complexes are essential to a comprehensive description of the electronic structure and mechanism of CODH.

We report here the synthesis and characterization of the first $\mathrm{Mo^{V}O}(\mu\text{-S})\mathrm{Cu^{I}}$ complexes that model the core unit of the paramagnetic form of active CODH, along with evidence for extensive, mechanistically critical electronic delocalization in the heterobimetallic $\mathrm{Mo}(\mu\text{-S})\mathrm{Cu}$ moiety.

Red-brown, air-sensitive $\mathrm{Tp}^{i\mathrm{Pr}}\mathrm{MoO(OAr)}(\mu\text{-S})\mathrm{Cu(Me_3tcn)}$ ($\mathrm{Tp}^{i\mathrm{Pr}}$ = hydrotris(3-isopropylpyrazol-1-yl)borate; OAr = $\mathrm{OC_6H_3{}^{t}Bu_2}$-3,5 (**1**) and $\mathrm{OC_6H_4Ph}$-4 (**2**); $\mathrm{Me_3tcn}$ = 1,4,7-trimethyl-1,4,7-triazacyclononane) were prepared by reacting $\mathrm{CoCp_2[Tp^{\mathit{i}Pr}MoOS(OAr)]}$ with $\mathrm{[Cu(NCMe)(Me_3tcn)]BF_4}$ in NCMe or by adding $\mathrm{CoCp_2}$ to mixtures of $\mathrm{Tp^{\mathit{i}Pr}MoOS(OAr)}$ and $\mathrm{[Cu(NCMe)(Me_3tcn)]BF_4}$ in the same solvent. The generality of such cupration reactions is currently under investigation. Interestingly, reconstitution of inactive CODH by sulfide and Cu(I) also requires reducing conditions.[7]

Microanalytical, mass spectrometric, and spectroscopic data are consistent with the proposed formulations. The isotropic X-band EPR spectra of **1** and **2** (Figure 2) reveal striking quartet resonances centered at g ca. 1.94, due to coupling of the Mo(V) unpaired electron to a single $^{63,65}\mathrm{Cu}$ center (A_{Cu} ca. $53 \times 10^{-4}\ \mathrm{cm^{-1}}$). Hyperfine coupling to $^{95,97}\mathrm{Mo}$ (A_{Mo} ca. $40 \times 10^{-4}\ \mathrm{cm^{-1}}$) was also observed. The frozen-glass EPR spectra (Figure S1) are highly rhombic, with strong hyperfine coupling to the $^{63,65}\mathrm{Cu}$ center. Importantly, the anisotropic EPR spectra of **1** and **2** display the same distinctive $^{63,65}\mathrm{Cu}$ hyperfine pattern observed for the paramagnetic form of active CODH.[4] Electronic structure calculations (vide infra) verify extensive electronic delocalization in the SOMO of the $\mathrm{Mo}(\mu\text{-S})\mathrm{Cu}$ unit as the origin of the unique EPR spectra of **1**, **2**, and CODH and support strongly directional Mo−S π-bonding in the $\mathrm{Mo}(\mu\text{-S})\mathrm{Cu}$ moiety.

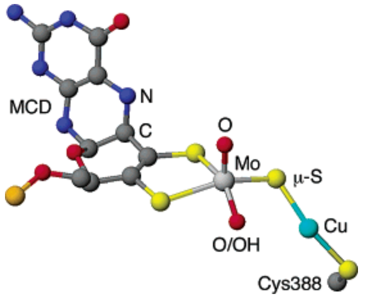

Figure 1. Proposed active site of CODH. Only the pterin−dithiolene portion of MCD is shown.[3,4]

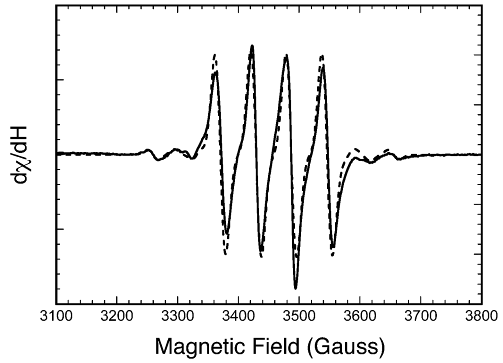

Figure 2. EPR spectrum of **2** at room temperature in acetonitrile (solid line) and a spectral simulation thereof (dashed line).

The structure of **1**, determined by X-ray diffraction, is shown in Figure 3. The complex contains a six-coordinate, distorted octahedral Mo center linked via a single, bent μ-sulfido bridge to a four-coordinate Cu center. The Mo−S and Cu−S distances are shorter than expected for M−S single bonds to, for example, thiolate and μ-sulfido ligands ($\mathrm{Mo{-}S_{av}} = 2.317$ Å[8]) or μ_3-sulfido ligands ($\mathrm{Cu{-}S_{av}} = 2.163$ Å[8]). The Mo coordination sphere is completed by terminal oxo, phenolate, and *fac*-$\mathrm{Tp}^{i\mathrm{Pr}}$ ligands, the Cu coordination sphere by a tridentate $\mathrm{Me_3tcn}$ ligand. The separation between the metal centers is 3.806 Å, and the distances and angles compare favorably with those of CODH, where the respective d(Mo−S), d(Cu−S) and ∠(Mo−S−Cu) parameters are 2.27 Å, 2.21 Å, and 113° for the $\mathrm{Mo^{VI}}$ form, and 2.32 Å, 2.18 Å, and 122° for the $\mathrm{Mo^{IV}}$ form.[3,4]

The reactions of **1** and **2** with excess $\mathrm{NEt_4CN}$ in dichloromethane mimic those involved in the cyanolysis of CODH; that is, decupration and desulfurization are observed.[3] The first step in the reactions is MoS−Cu bond scission, with formation of $[\mathrm{Tp}^{i\mathrm{Pr}}\mathrm{MoOS(OAr)}]^-$. Subsequently, cyanide attacks the Mo(V) anion to produce $\mathrm{Tp}^{i\mathrm{Pr}}\mathrm{MoOCl(OAr)}$ and $\mathrm{SCN^-}$, the chloro ligand being derived from the solvent. By analogy, we predict cyanide deactivation of the enzyme

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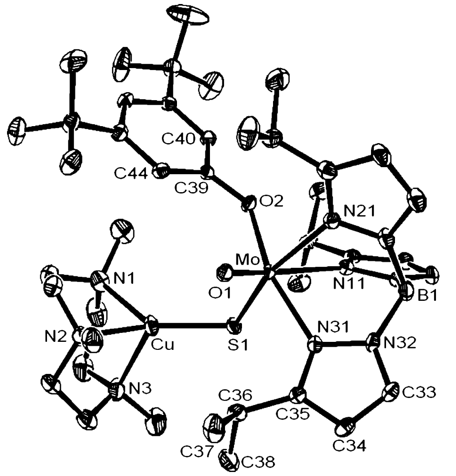

Figure 3. ORTEP projection of **1** drawn at the 30% probability level. Selected bond distances (Å) and angles (deg) include: Mo−O(1) 1.695(3), Mo−O(2) 1.975(2), Mo−S(1) 2.2844(11), Mo−N(11) 2.378(3), Mo−N(21) 2.293(3), Mo−N(31) 2.222(3), Cu−S(1) 2.1348(11), Cu−$N_{av.}$ 2.168, Mo···Cu 3.806, Mo−S(1)−Cu 118.90(5), O═Mo−S−Cu dihedral angle 38.7(1).

center to be a multistep process, resulting first in the loss of copper and then the sulfido ligand from the active site.

Key to the catalytic mechanism of the CODH site is the electronic communication between the Mo and Cu atoms. The observation of unusually large ^{63,65}Cu hyperfine coupling constants in frozen-glass EPR spectra of the title complexes ($A = 88 \times 10^{-4}$ cm^{-1} for **2**; see Figure S1) and CODH ($A =$ ca. 60×10^{-4} cm^{-1}) indicates a very high degree of delocalization in the Mo(μ-S)Cu moiety. The magnitude of the ^{63,65}Cu hyperfine splitting is remarkable in light of the Cu(I) formulation. For example, blue copper proteins display ^{63,65}Cu hyperfine of comparable magnitude ($A_{\|} = 63 \times 10^{-4}$ cm^{-1}) when the spin primarily resides *on* the Cu(II) center.[9] Furthermore, the large isotropic component in the Mo(μ-S)Cu systems strongly suggests a significant Fermi contact term originating from Cu s-orbital character in the SOMO wave function. Thus, the large isotropic contribution to the ^{63,65}Cu hyperfine tensor reveals the nature of the dominant Cu d-orbital contribution to the SOMO, as only the d_{z^2} orbital on Cu in a pseudo-C_{3v} (**1** and **2**) or linear (CODH) environment possesses the appropriate symmetry to mix with the metal s-orbitals on Cu. The d−s orbital mixing is supported by bonding calculations performed on $[(NH_3)_3MoO(OPh)(\mu\text{-}S)Cu(tcn)]^+$ that show extensive delocalization of the SOMO over the Mo (Mo d_{xy}, 44%) and S (S p, 25%) and onto the Cu site (21%, primarily Cu d_{xz} and d_{z^2} with some s character) via a pseudo-σ^* Cu−S orbital interaction (Figure 4). Extensive delocalization of the SOMO over the Mo(μ-S)Cu unit of CODH would provide a conduit for electrons to reduce Mo upon substrate oxidation at Cu, without having to invoke $C_{substrate}-S_{sulfido}$ bond formation during the course of catalysis. To the extent that electron delocalization in the catalytically competent Mo(VI)−S−Cu(I) oxidation state contributes significantly to the rate of the reductive half-reaction, CO oxidation could occur directly at the Cu(I) site, with two-electron transfer to Mo occurring via a highly efficient superexchange pathway mediated by the bridging sulfido ligand.

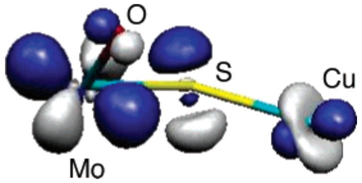

Figure 4. SOMO wave function for computational model displaying Mo−S π^* and S−Cu pseudo-σ^* interactions. Only the atoms of the MoO(μ-S)Cu core are shown (see Figure S2 for views of the SOMO on the complete model). Geometry optimization of the computational model yielded an O═Mo−S−Cu dihedral angle of 46°, in good agreement with the value in Figure 3.

In summary, we have described the first synthetic models for the paramagnetic form of CODH. Moreover, extensive electronic delocalization over the Mo(μ-S)Cu moiety has been verified as the origin of the enzyme's unusual EPR signal. Extension of the work to other relevant oxidation states and related dithiolene and thiolate complexes is under investigation. Studies probing the correlation of spectral properties with core geometry and electronic structure, across a range of complexes, will allow detailed insights into how electron delocalization across the Mo(μ-S)Cu unit contributes to catalysis.

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Supporting Information Available: Synthetic procedures, routine characterization and frozen-glass EPR data, crystallographic data (in CIF format), computational method and Cartesian coordinates of the optimized model complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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