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Cyanide Poisoning: An Analogue to the Binuclear Site of Oxidized Cyanide-Inhibited Cytochrome *c* Oxidase

Sonny C. Lee,[1a,b] Michael J. Scott,[1a] Karl Kauffmann,[1c]
E. Münck,*[1c] and R. H. Holm*[1a]

Departments of Chemistry
Harvard University
Cambridge, Massachusetts 02138
Carnegie Mellon University
Pittsburgh, Pennsylvania 15213

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In our continuing study of biologically relevant, bridged metal assemblies, the binuclear heme a_3-Cu$_B$ site of cytochrome *c* oxidase[2] (CcO), known to exist in several bridged states when fully oxidized,[2,3] has proven amenable to the synthetic analogue approach. The complexes [(OEP)Fe–F–Cu(bnpy$_2$)]$^{2+}$ [4] and [(OEP)Fe–O–Cu(Me$_6$tren)]$^+$,[5] together with the achievement of a related Fe^{III}–O–Cu^{II} species by Nanthakumar *et al.*,[6] demonstrate the formation of heteronuclear bridges and imply that other Fe^{III}–X–Cu^{II} linkages may also be stable. The complex with the bridge X = CN$^-$ is of potential interest in relation to cyanide toxicity. Although cyanide can interfere with a variety of protein sites, its lethal toxicity has been traced to rapid and irreversible binding to CcO at the binuclear site, at which point it terminates the reduction $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ catalyzed by the enzyme.[2,7] MCD spectra[8] of the beef heart enzyme and Mössbauer studies[9] on this enzyme and that from *Thermus thermophilus* have shown that the binuclear site of CcO–CN has integer electron spin and that the two lowest spin levels are nearly degenerate ($\Delta < 0.3$ cm^{-1}). While previous research relevant to this unit has provided interesting spectroscopic data,[10] it did not yield a proof of structure. We report here our initial approach to this problem.

Treatment of 0.84 mmol of [Fe(OEP)]$_2$O with 10% excess Me$_3$SiCN in 50 mL of pyridine/dichloromethane (1:9 v/v, 18 h) followed by solvent removal and recrystallization of the residue from dichloromethane/hexane[11] afforded violet microcrystalline [Fe(OEP)(CN)(py)][12,13a] (**1**, 92%). Reaction of [Cu(Me$_6$tren)(OH$_2$)](ClO$_4$)$_2$[4] (**2**) with equimolar KCN in ethanol gave blue [Cu(Me$_6$tren)(CN)](ClO$_4$)[13b] (**3**) in high yield. Complexes **1**–**3** are precursors to the desired bridged assembly, and **1** and **3** provide structural comparisons. Equimolar quantities of **1** (0.14 mmol) and **2** were dissolved in a minimal volume (*ca.* 3 mL) of acetone. Diffusion of several volume equivalents of ether in the solution over 48–72 h gave, after standard workup, violet [(py)(OEP)Fe–CN–Cu(Me$_6$tren)](ClO$_4$)$_2$[13c] (**4a**, 87%). For structural characterization, ether was slowly diffused into a concentrated acetone solution of **4a** and excess NaSbF$_6$, resulting in the formation of large black needlelike crystals of [(py)(OEP)Fe–CN–Cu(Me$_6$tren)](SbF$_6$)$_2$·Me$_2$CO[13d] (**4b**).

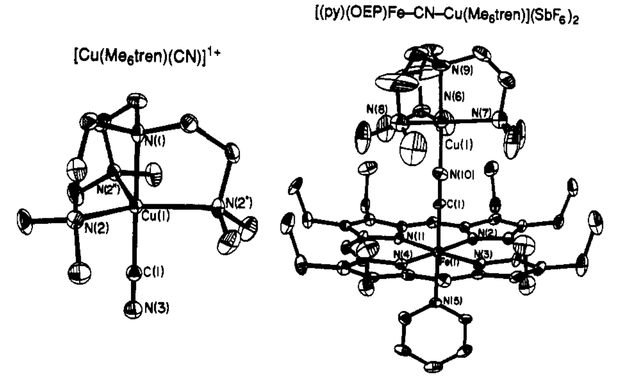

Figure 1. Structures of the cations of **3** (left) and the bridged assembly **4b** (right) showing 50% and 30% probability ellipsoids, respectively, and atom labeling schemes. Primed and unprimed atoms of **3** are related by an imposed C_3 axis. Selected bond distances (Å) and angles (deg): **3**, Cu–N(1) 2.01(1), Cu–N(2) 2.184(4), Cu–C(1) 1.96(1), C(1)–N(3) 1.13(1), N(1)–Cu–C(1) = Cu–C(1)–N(3) 180; **4b**, CuN(9) 1.99(1), Cu–N(6–8) 2.14(1), Cu(1)–N(10) 1.88(1), C(1)–N(10) 1.14(2), Fe–C(1) 1.92(2), Fe–N(1–4) 2.00(2), Fe–N(5) 2.04(1), Fe···Cu 4.94, Cu–N(10)–C(1) 174(1), Fe–C(1)–N(10) 179(1), C(1)–Fe–N(5) 180.0(5). The data for **4b** refer to one of the two cations in the asymmetric unit.

The structure of **3** (Figure 1) is trigonal bipyramidal with an imposed C_3 axis N(1)–N(3) and normal distances and angles. There are two essentially isostructural cations in the asymmetric unit of **4b**. The binuclear assembly (Figure 1) features the virtually linear bridge unit Fe^{III}–C(1)–N(10)–Cu^{II}, a nearly planar porphyrin ring with a slight saddle-shape distortion, and a small displacement of the Fe atom (0.15 Å) toward N(5) of the pyridine ligand. The [Fe(OEP)(CN)(py)] and [Cu(Me$_6$tren)]$^{2+}$ fragments of **4b** are essentially congruent with **1**[12b] and the corresponding portion of **3**, respectively. The principal difference is the longer Fe–N(py) bond in **1** (2.087 Å) and a slight displacement (0.053 Å) from the porphyrin plane toward the cyanide ligand.[12b] These structural similarities insure that **4b** contains low-spin Fe(III). Structure refinement favors the Fe–C–N–Cu bridge over the linkage isomer Fe–N–C–Cu. The latter was sought by reaction of equimolar **3** and [Fe(OEP)(OClO$_3$)] in the presence of excess pyridine in acetone solution.[11] The ^{1}H NMR spectrum of the reaction product was identical to that of the bridged species derived from **1** and **2**,[13c] which is most readily recognized by the characteristic diastereotopic OEP methylene signals at 8.14 and 11.57 ppm. These results support the Fe–C–N–Cu formulation.

Mössbauer spectra of **4a** are shown in Figures 2A–C. The zero-field spectrum at 4.2 K consists of a doublet with ΔE_Q = 1.23(2) mm/s (1.07 mm/s at 200 K) and δ_{Fe} = 0.22(2) mm/s;

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[1] (a) Harvard University. (b) National Science Foundation Predoctoral Fellow, 1987–1990. (c) Carnegie Mellon University.

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[11] Experimental details: all reactions were performed under a pure dinitrogen atmosphere at room temperature. Diffraction data were collected on a Nicolet P3F diffractometer with Mo Kα radiation. Crystal structures were solved by direct methods and refined using SHELXTLPLUS or SHELXL-93. IR spectra were recorded as hydrocarbon mulls.

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[13] (a) **1**: absorption spectrum (acetone) λ_{max} (ϵ_M) 346 (29 700), 406 (81 900), 541 (7200) nm; ν_{CN} 2129 cm^{-1}. (b) **3**: a = 10.003(3) Å, c = 32.699(13) Å, $R3c$, Z = 6, 895 unique data ($F_o \geq 6\sigma F_o$), $R(R_w)$ = 4.19(4.58); ν_{CN} 2136 cm^{-1}. (c) **4a**: absorption spectrum (acetone) λ_{max} (ϵ_M) 348 (27 400), 404 (89 400), 527 (8100), 553 (sh, 6300) nm; ^{1}H NMR (Me$_2$CO) δ 11.57, 8.14 (CH$_2$), 1.25 (CH$_3$), 11.75, 8.49, –11.14 (py-H), 3.70 (meso-H). (d) **4b**·Me$_2$CO: ν_{CN} 2177 cm^{-1}; a = 13.833(3) Å, b = 16.552(4) Å, c = 31.532(6) Å, α = 94.90(2)°, β = 94.66(2)°, γ = 112.52(2)°, $P\bar{1}$, Z = 4, 11 231 unique data ($F_o \geq 4\sigma F_o$), $R(wR^2)$ = 7.62(16.66). Compounds **4a** and **4b** have identical IR (ν_{CN}) and isotropically shifted ^{1}H NMR spectra.

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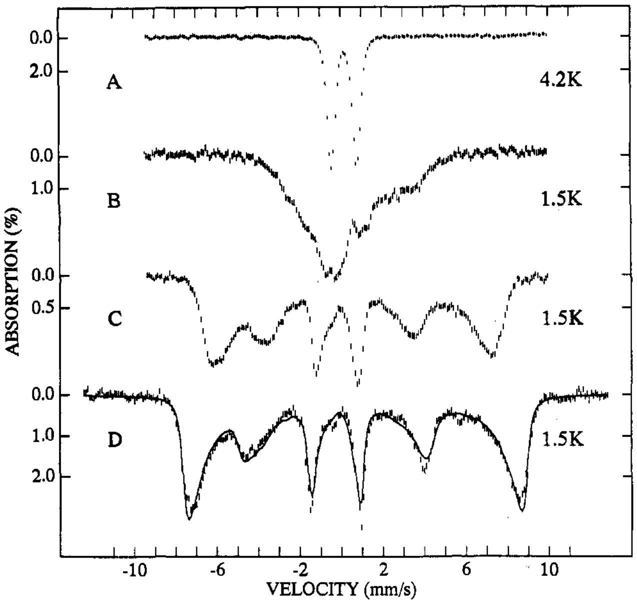

Figure 2. Mössbauer spectra of polycrystalline **4a** (A–C) and **1** (D) recorded in zero-field (A) and in parallel applied fields of 1.0 (B) and 8.0 T (C,D). The solid line is a spectral simulation with an $S = {}^1/_2$ Hamiltonian for the low-spin Fe(III) site of **1** using $g_x = 0.8$, $g_y = 1.0$, $g_z = 3.6$, $A_x = -20$ MHz, $A_y = +50$ MHz, and $A_z = +118$ MHz, $\delta = 0.23$ mm/s, $\Delta E_Q = -1.33$ mm/s, and $\eta = -1.6$.

these values are similar to those of precursor **1** (1.33 mm/s, 0.23 mm/s). Complex **1** exhibits Mössbauer spectral features (Figure 2D) and a broad X-band EPR signal at $g = 3.6$ similar to those of low-spin ($S = {}^1/_2$) $\mathrm{Fe^{III}}$–heme–CN complexes.[14,15] With a standard X-band cavity, **4a** in toluene is EPR-silent down to 5 K. Figure 2D shows a fit of the Mössbauer spectrum of **1** to an $S = {}^1/_2$ Hamiltonian with parameters similar to those of hemin(py)(CN).[14]

The shapes of the applied field Mössbauer spectra of **4a**, its EPR silence, and the observation of a quadrupole doublet in applied fields <0.1 T for frozen solution as well as polycrystalline samples show that **4a** has *integer* electronic spin, suggesting that the Fe(III) and Cu(II) sites are exchange-coupled. For Cu–Fe complexes containing high-spin Fe(III), it suffices to consider only isotropic exchange ($H = JS_1{\cdot}S_2$). Complexes involving low-spin Fe(III), on the other hand, require consideration of anisotropic and antisymmetric exchange.[16] If isotropic exchange dominates, the coupled system will consist of singlet and triplet levels well separated in energy. However, if all exchange terms have comparable magnitudes, the four spin levels mix and the triplet/singlet distinction becomes meaningless. Although we have been able to fit the spectra of Figure 2A–C to an exchange-coupled system, the multiparameter problem requires data from complementary techniques to resolve ambiguities; zero-field splittings of the spin triplet up to 10 cm^{-1} are compatible with the spectra of Figure 2A–C. Our preliminary analysis suggests ferromagnetic coupling. This is compatible with the structure of **4b** which suggests that the unpaired electrons are centered on (d_{xz},d_{yz}) of Fe(III) and d_{z^2} of Cu(II). Finally, the similarities between the 8.0-T spectra of **4a** and **1** suggest similar electronic structures; however, the z-component of the ^{57}Fe A-tensor is 10% smaller for **4b**.

Low-spin Fe(III) hemes generally have $\Delta E_Q > 2.0$ mm/s;[17] in contrast, heme–CN complexes exhibit quadrupole splittings in the range 1.2–1.9 mm/s.[14,17] Thus, a comparison of ΔE_Q and δ values for CcO–CN from *T. thermophilus* (1.25 mm/s, 0.28 mm/s)[9a] and from beef heart (1.13 mm/s, 0.26 mm/s)[9b] with those found for **1** and **4a** suggests that these cyanide-treated oxidases and the bridged assembly [(py)(OEP)Fe–CN–Cu(Me$_6$-tren)]$^{2+}$ approach, by a Mössbauer criterion, an electronic convergence. If isotropic exchange dominates for the oxidases, MCD[8] and Mössbauer[9] observations imply ferromagnetic coupling and a negative zero-field splitting of the $S = 1$ multiplet. Our Mössbauer studies of **4a** suggest that the two lowest spin levels are split by $\Delta \approx 2$–4 cm^{-1}.[18] Given the electronic similarities between **4a** and fully oxidized CcO–CN, the formation of the Fe–CN–Cu bridge is a manifestation of the terminal inhibition of the enzyme by cyanide.

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Supplementary Material Available: Positional and isotropic thermal parameters for compounds **3** and **4b** (33 pages); observed and calculated structure factors for **3** and **4b** (47 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(18) The exact nature of the lowest two spin levels has not yet been established for either **4a** or CcO–CN. The larger splitting for **4a** compared to CcO–CN does not neutralize the former as a meaningful analogue of the enzyme site inasmuch as Δ will be a sensitive function of ligation and the precise coordination geometry.