A Synthetic Analogue of the Iron/Copper Bridged Assembly in Cytochrome *c* Oxidase

Sonny C. Lee[1] and R. H. Holm*

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

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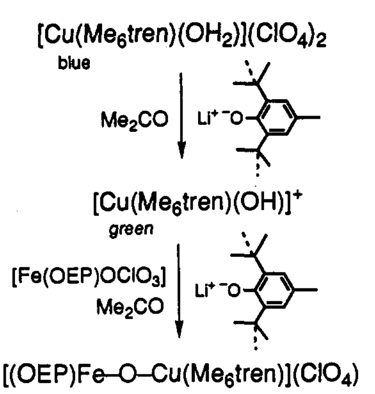
Cytochrome *c* oxidases (CcO) and the related prokaryotic terminal oxidases are indispensable components in the biochemical machinery of aerobic life on Earth, exergonically coupling the reduction of atmospheric dioxygen to the oxidation of molecular foodstuffs.[2] In specific function, these membrane-bound enzymes catalyze the reduction $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$, with concomitant proton translocation against a transmembrane concentration gradient. Structurally, they possess several metal centers, of which a binuclear iron–copper assembly is the site of dioxygen binding and reduction. The copper (Cu_B) coordination sphere is believed to be minimally defined by at least three histidine imidazoles, while the iron occurs as a heme (heme a_3) with imidazole ligation on the face distal to Cu_B; the oxygen chemistry occurs in the space intervening the two metals.

Although current evidence remains inconclusive for bimetallic bridging interactions in the catalytic cycle,[3] there is general agreement on an intimately bridged heme a_3/Cu_B dimer in purified preparations of the oxidized enzyme (the "as-isolated" state). This bridged site remains tentatively defined by spectroscopic deduction despite extensive investigation; studies of the binuclear site are complicated by the presence of other, spectroscopically interfering metal centers in the enzyme and the existence of multiple conformations of the "as-isolated" state. Support for the bridged model depends largely on magnetic susceptibility data[4] and the absence of odd-spin EPR signals[5] of the oxidized site, both of which suggest a strongly antiferromagnetically coupled interaction (estimated $J \geq 200$ cm^{-1}) between the Fe(III) and Cu(II) sites to yield an $S = 2$ system spin state. Site probes with certain exogenous ligands have also produced results indicative of bridged interactions implicating these ligands.[6]

We are currently pursuing the directed syntheses of asymmetric, bridged metal assemblies of biological relevance. Previously, all synthetic compounds considered related to the CcO binuclear site have failed the necessary criterion of strong electronic coupling between metal centers.[7] To promote this condition, we have chosen an oxo-bridged heme–copper moiety as a plausible synthetic target.[8] Our approach has been implemented in Scheme I.

Scheme I



A blue solution of $[Cu(Me_6tren)(OH_2)](ClO_4)_2$[9] (1 equiv) was treated with lithium 2,6-di-*tert*-butyl-4-methylphenoxide (2 equiv) in acetone to give a dark green solution.[10] Addition of $Fe(OEP)(OClO_3)$[11] (1 equiv) afforded a dark purple-red solution.[12] Introduction of ether produced a microcrystalline precipitate which was isolated, redissolved in THF, and filtered to remove insoluble copper residues. Volume reduction followed by ether addition afforded $[(OEP)Fe\text{-}O\text{-}Cu(Me_6tren)](ClO_4)$ (**1**)[12,13] as violet microcrystals (50%).

The structure[14] of **1** establishes the unprecedented Fe–O–Cu unit linking a square-pyramidal iron porphyrin to trigonal-bipyramidal copper (Figure 1). Two independent molecules crystallize in the asymmetric unit with essentially identical dimensions. The Fe–N and Fe–O (mean 1.747(6) Å) bond distances are consistent with those of five-coordinate high-spin and μ_2-oxo Fe(III) porphyrins.[15] The Cu–N distances are also normal, with the axial distance slightly shorter than the equatorial distances, as well precedented.[16] While no structurally proven examples exist of μ_2-oxo Cu(II) bridges, Cu–O distances do compare favorably with like distances in nonbridging copper alkoxide moieties (1.90(3) Å).[17] These distances, taken together with the charge of the complex (1+) and the Cu–O–Fe angle (mean, 176.0°), allow the unambiguous assignment of the atom as a bridging oxo.[18] The (Me_6tren) ligand exhibits a large degree

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[1] National Science Foundation Predoctoral Fellow, 1987–1990.

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[4] (a) Tweedle, M. F.; Wilson, L. J.; Garcia-Iñiguez, L.; Babcock, G. T.; Palmer, G. *J. Biol. Chem.* **1978,** *253,* 8065. (b) Moss, T. H.; Shapiro, E.; King, T. E.; Beinert, H.; Hartzell, C. *J. Biol. Chem.* **1978,** *253,* 8072.

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[6] Li, W.; Palmer, G. *Biochemistry* **1992,** *32,* 1833 and references therein.

[7] (a) Nanthakumar, A.; Nasir, M. S.; Karlin, K. D.; Ravi, N.; Huynh, B. H. *J. Am. Chem. Soc.* **1992,** *114,* 6564 and references therein. (b) Serr, B. R.; Headford, C. E. L.; Anderson, O. P.; Elliott, C. M.; Spartalian, K.; Fainzilberg, V. E.; Hatfield, W. E.; Rohrs, B. R.; Eaton, S. S.; Eaton, G. R. *Inorg. Chem.* **1992,** *31,* 5450.

[8] EXAFS studies suggest sulfur or chlorine as the bridge in the "as-isolated" state.[2d] Different workers, however, disagree on bond distances and the copper coordination sphere. Given the difficulties associated with the study of CcO as mentioned in the text, we believe the identity of the bridging moiety remains an open question.

[9] This compound was originally formulated as $[Cu(Me_6tren)OClO_3]ClO_4$: Ciampolini, M.; Nardi, N. *Inorg. Chem.* **1966,** *5,* 41. We have established the identity of the cation as the aquo complex $[Cu(Me_6tren)(OH_2)]^{2+}$ (Cu–O, 1.979(6) Å) by an X-ray structure determination. ((Me_6tren) = tris(*N,N*-dimethylethyl)amine.

[10] The solution at this point presumably contains a terminal $[CuOH]^+$ species. We are unaware of the previous existence of any terminal copper hydroxide. We have structurally characterized the compound $[Cu(Me_6tren)(OH)]ClO_4 \cdot H_2O$, (Cu–O, 1.875(4) Å), confirming that (Me_6tren) can support terminal hydroxide ligation. A recently published structure of a purported terminal copper hydroxide is incorrect: Mathews, I. I.; Sudhakara Rao, S. P.; Nethaji, M. *Polyhedron* **1992,** *11,* 1397. Bond distances support the assignment of aquo for the alleged hydroxide, with a ligating hydroxymethyl group deprotonated.

[11] Dolphin, D. H.; Sams, J. R.; Tsin, T. B. *Inorg. Chem.* **1977,** *16,* 711. (OEP = octaethylporphyrinate(2−)).

[12] ^{1}H NMR studies of this reaction indicate a high-spin heme coproduct (*ca.* 30%), which we currently believe to be the enolate complex $Fe(OEP)(OC(Me){=}CH_2)$. The use of lithium 2,6-di-*tert*-butyl-4-methylphenoxide as the base allows clean separation of the reaction products on the basis of solubility differences.

[13] The Fe:Cu:Cl atom ratio is 1:1.0:1.1 by microprobe analysis. Absorption spectrum (CH_2Cl_2): λ_{max} (ϵ_M) 286 (18 300), 342 (sh, 35 200), 371 (43 700), 427 (74 700), 499 (sh, 4000), 541 (11 600), 574 (12 700), 634 (1500). ^{1}H NMR (acetone-d_6, 292 K): δ 20.0 and 17.4 (CH_2), 3.63 (CH_3), −16.3 (br, CH).

[14] Compound **1** was recrystallized from acetonitrile/ether. X-ray data were collected at 173 K on a Nicolet P3F diffractometer with Mo Kα radiation. Structure solutions were achieved by standard Patterson and difference Fourier methods. Crystallographic data are given as a, b, c (Å); α, β, γ; space group, Z, $2\theta_{min/max}$, number of unique data, R/R_w (%). **1**: 17.847(3), 25.085(4), 25.356(4); 90, 108.95(1), 90; $P2_1/n$, 8, 3.0°/45.0°, 8907 ($F_o^2 \geq 1.5\sigma(F_o^2)$), 9.33/10.25. **2**: 12.673(3), 13.832(4), 16.901(5); 93.69(2), 105.64(2), 92.91(2); $P\bar{1}$, 2, 3.0°/50.0°, 5208 ($F_o^2 \geq 3\sigma(F_o^2)$), 4.84/4.87.

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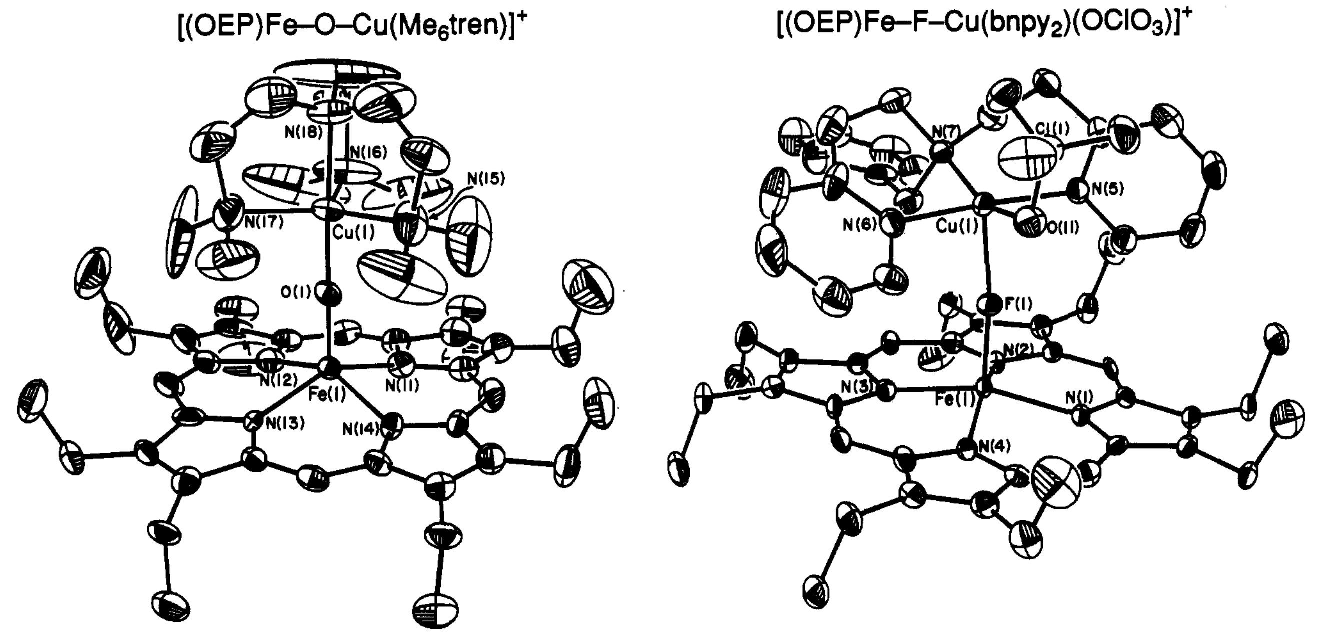

Figure 1. The structures of [OEP)Fe-O-Cu(Me$_6$tren)]$^+$ (**1**, left) and [(OEP)Fe-F-Cu(bnpy$_2$)(OClO$_3$)]$^+$ (**2**, right) showing 50% thermal ellipsoids and selected atom-labeling scheme. Averaged distances (Å) and angles (deg) for two independent molecules of **1**: Fe(1)···Cu(1), 3.571(2); Fe–O, 1.747(6); Fe–N$_{porph}$, 2.109(7); Ct′···Fe, 0.67; Cu–O, 1.827(6), Cu–N$_{ax}$, 2.050(8); Cu–N$_{eq}$, 2.14(2); Fe–O–Cu, 176.0(7). Distances (Å) and angles (deg) for **2**: Fe(1)···Cu(1), 3.956(1), Fe(1)–F(1), 1.865(3); Fe(1)–N$_{porph}$, 2.049(4), Ct′···Fe(1), 0.36; Cu(1)–F(1), 2.101(3); Cu(1)–O(11), 2.139(3), Cu(1)–N(5), 1.972(4); Cu(1)–N(6), 1.985(4); Cu(1)–N(7), 2.041(4); Fe(1)–F(1)–Cu(1), 171.9(1). Ct′ is the centroid of the 24-atom (C,N) porphyrin core; N$_{eq}$ and N$_{ax}$ represent equatorial and axial positions, respectively.

of thermal motion; ellipsoids are elongated in the trigonal plane, and the N–C–C–N chelate rings are nearly planar instead of puckered. We attribute the disorder to the close interligand contact enforced by the oxo-bridge. Given the present iron–copper separation, calculation of distances between the porphyrin plane and an idealized (Me$_6$tren) ligand (using the puckered arm conformation generally found for this ligand) shows contact between the methyl groups and the porphyrin ring at slightly less than the van der Waals minimum. We take the nearly linear Fe–O–Cu angle as further indication of the steric demands of the ligand set. This relatively strong interligand repulsion probably prevents the flexible (Me$_6$tren) from adopting one clear lowest energy conformation; instead, the ligand can only assume a range (dynamic and/or static) of higher energy orientations.

The magnetic susceptibility behavior of **1** is well-described by Curie–Weiss law, with only a minor deviation to higher susceptibility above 200 K; the curvature is sufficiently slight that the complete temperature range (4–300 K) can be accurately fitted in its entirety by $\chi^M = 3.15/(T + 2.03)$. *This firmly establishes compound 1 as a tightly coupled, $S = 2$ system.*[19] The EPR spectrum of **1**, silent at 125 K, is also consistent with this assignment. Further indication of the strong electronic coupling is evidenced by the paramagnetically shifted ^{1}H NMR signals[13] of the OEP ligand which occur in regions previously unencountered for Fe(III) porphyrins. This, along with the presence of diastereotopic methylene protons (split by 2.6 ppm), is strong evidence for retention of the bridge structure in solution. The zero-field spectrum of **1** at 4.2 K consists of a quadrupole doublet with $\delta = 0.48$ mm/s and $\Delta E_q = 1.20$ mm/s, supporting the structural formulation of high-spin ($S = {}^5/_2$) heme[20] and implying antiferromagnetic coupling with Cu(II) ($S = {}^1/_2$) to achieve the $S = 2$ state. The absorption spectrum exhibits a strongly red-shifted Soret band (427 nm, as compared to 385 nm for [Fe(OEP)]$_2$O). An increase in negative charge on the axial ligand has been correlated with a decrease in Soret transition energy;[21] this interpretation is consistent with our expectation of a weaker Cu(II) affinity for oxo as compared to Fe(III).

Other Cu(II)–Fe(III) bridged species are accessible by similar means. Thus, reaction of Fe(OEP)(OClO$_3$) (2 equiv) with [Cu$_2$F$_2$(bnpy$_2$)$_2$](PF$_6$)$_2$[22] (1 equiv) in acetone initially gives a black-brown solution; diffusion of Et$_2$O yields black crystals of [(OEP)Fe-F-Cu(bnpy$_2$)(OClO$_3$)](PF$_6$) (**2**). Structural characterization[14] reveals an asymmetric fluoride bridge (Cu–F, 2.101(3) Å; Fe–F, 1.865(3) Å) linking five-coordinate high-spin Fe(III) to square-pyramidal Cu(II) (Figure 1). The bridging ability of unsupported fluoride appears poor in this environment; in essence, the fluoride has been nearly completely abstracted by Fe(III) from Cu(II) inasmuch as the Fe–F distance in Fe(OEP)F is 1.834(2) Å.[23] While the fluoride ligates strongly to the heme, a weaker axial interaction is observed for Cu(II); unexpectedly, the poorly ligating perchlorate occupies the vacant strong-field equatorial position, a situation unique to our knowledge. Related behavior is noted for CcO, where added fluoride ion uncouples the EPR-silent bridged assembly of CcO by binding to heme a$_3$.[24]

The synthesis of **1** allows the first detailed spectroscopic comparisons between the spin-coupled enzyme site and a structurally defined, electronically congruent synthetic analogue. Moreover, the reaction chemistry has proven viable for the formation of bridges both strong and weak. The physical characterization of assemblies **1** and **2** and the construction of other relevant bridges are the focus of our continuing studies.

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Supplementary Material Available: Positional and isotropic thermal parameters for compounds **1** and **2** (5 pages). Ordering information is given on any current masthead page.

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(19) For $S = 2$, the Curie constant $C = 3.00$ emu K/mol assuming $g_e = 2$.

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(22) A compound prepared in a similar manner has been described as [Cu$_2$(OH)$_2$(bnpy$_2$)$_2$](PF$_6$)$_2$: (a) Karlin, K. D.; Gultneh, Y.; Hayes, J. C.; Zubieta, J. *Inorg. Chem.* **1984**, *23*, 519. (b) Sanyal, I.; Mahroof-Tahir, M.; Nasir, M. S.; Ghosh, P.; Cohen, B. I.; Gultneh, Y.; Cruse, R. W.; Farooq, A.; Karlin, K. D.; Liu, S.; Zubieta, J. *Inorg. Chem.* **1992**, *31*, 4322. Our characterization (including an X-ray structure determination) indicates that it is actually the (μ_2-F)$_2$ complex with unsymmetrical bridges (Cu–F, 1.918(2), 2.232(2)).[23] (bnpy$_2$ = *N*,*N*-bis(2-pyridylethyl)benzylamine.)

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