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A Tris(μ-hydroxy)tricopper(II) Complex as a Model of the Native Intermediate in Laccase and Its Relationship to a Binuclear Analogue

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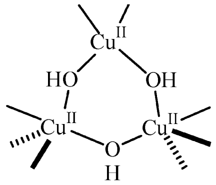
The reaction of a copper(I) complex with a sterically demanding secondary diamine ligand and O_2 yields a tris(μ-hydroxy)tricopper(II) complex. This complex is a structural model of the proposed native intermediate in multicopper oxidases, with interesting structural, magnetic, and solution properties.

Multinuclear copper clusters in metalloenzymes have evolved to facilitate multielectron redox processes. Both binuclear copper enzymes (e.g., hemocyanin, tyrosinase, catechol oxidase) and multicopper oxidases (e.g., laccase, ascorbate oxidase, cerulloplasmin) are engaged in dioxygen (O_2) activation and subsequent substrate oxidation.[1] Detailed mechanistic postulates from studies of biological systems with their inherent ligation and geometric restrictions provide key insights for the development of bio-inspired catalysts.[2] Finding synthetic ligand environments that stabilize complexes related to the postulated intermediates in the catalytic cycle is an important step in this endeavor.[3] Whereas binuclear copper complexes dominate the area of modeling O_2-activating copper enzymes, the characterization of magnetically coupled, triangular trinuclear copper(II) clusters[4−6] has significantly lagged behind their binuclear analogues.

Chart 1



Multicopper oxidases[7] reduce O_2 to H_2O at a trinuclear copper(I) cluster through two sequential, two-electron steps that involve a peroxide and native intermediate, respectively.[1,8] A tris-hydroxy-bridged trinuclear copper(II) cluster (Chart 1) is currently postulated as the catalytically competent native intermediate found in these enzymes.[9] We report here a structurally analogous tris(μ-hydroxy)tricopper(II) complex, along with its intriguing magnetic properties and solution behavior. The spectroscopic and physical characteristics of this complex will provide valuable comparative benchmarks for characterization of the native intermediate of multicopper oxidases.[10]

The copper(I) complex of *N*,*N′*-di-*tert*-butyl-ethylenediamine (DBED), [(DBED)Cu(MeCN)](X) ($X = ClO_4^-$, $CF_3SO_3^-$, BF_4^-), reacts with O_2 in THF to form a variety of complexes. At −80 °C, a binuclear μ-η^2:η^2-peroxodicopper(II) complex is formed that is a spectroscopic and functional model of tyrosinase.[11] By contrast, the reaction of [(DBED)-

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(1) Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. *Chem. Rev.* **1996**, *96*, 2563.

(2) (a) Fenton, D. E.; Okawa, H. *J. Chem Soc., Dalton Trans.* **1993**, 1349. (b) Kaim, W.; Rall, J. *Angew. Chem.* **1996**, *108*, 47; *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 43.

(3) Mirica, L. M.; Ottenwaelder, X.; Stack, T. D. P. *Chem. Rev.* **2004**, *104*, 1013.

(4) (a) Comarmond, J.; Dietrich, B.; Lehn, J.; Louis, R. *J. Chem. Soc., Chem. Commun.* **1985**, 74. (b) Cole, A. P.; Root, D. E.; Mukherjee, P.; Solomon, E. I.; Stack, T. D. P. *Science* **1996**, *273*, 1848. (c) Root, D. E.; Henson, M. J.; Machonkin, T.; Mukherjee, P.; Stack, T. D. P.; Solomon, E. I. *J. Am. Chem. Soc.* **1998**, *120*, 4982. (d) Machonkin, T. E.; Mukherjee, P.; Henson, M. J.; Stack, T. D. P.; Solomon, E. I. *Inorg. Chim. Acta* **2002**, *341*, 39. (e) Suh, M. P.; Han, M. Y.; Lee, J. H.; Min, K. S.; Hyeon, C. *J. Am. Chem. Soc.* **1998**, *120*, 3819. (f) Ferrer, S.; Haasnoot, J. G.; Reedijk, J.; Muller, E.; Cingi, M. B.; Lanfranchi, M.; Lanfredi, A. M. M.; Ribas, J. *Inorg. Chem.* **2000**, *39*, 1859. (g) Kodera, M.; Tachi, Y.; Kita, T.; Kobushi, H.; Sumi, Y.; Kano, K.; Shiro, M.; Koikawa, M.; Tokii, T.; Ohba, M.; Okawa, H. *Inorg. Chem.* **2000**, *39*, 226. (h) Lopez-Sandoval, H.; Contreras, R.; Escuer, A.; Vicente, R.; Bernes, S.; Noth, H.; Leigh, G. J.; Barba-Behrens, N. *J. Chem. Soc., Dalton Trans.* **2002**, 2648. (i) Angaridis, P. A.; Baran, P.; Boca, R.; Cervantes-Lee, F.; Haase, W.; Mezei, G.; Raptis, R. G.; Werner, R. *Inorg. Chem.* **2002**, *41*, 2219. (j) Clerac, R.; Cotton, F. A.; Dunbar, K. R.; Hillard, E. A.; Petrukhina, M. A.; Smucker, B. W. *C. R. Acad. Sci. Paris, Chim./Chem.* **2001**, *4*, 315. (k) Cage, B.; Cotton, F. A.; Dalal, N. S.; Hillard, E. A.; Rakvin, B.; Ramsey, C. M. *J. Am. Chem. Soc.* **2003**, *125*, 5270.

(5) (a) Ferrer, S.; Lloret, F.; Bertomeu, I.; Alzuet, G.; Borras, J.; Garcia-Granda, S.; Liu-Gonzalez, M.; Haasnoot, J. G. *Inorg. Chem.* **2002**, *41*, 5821. (b) Liu, X. M.; De Miranda, M. P.; Mcinnes, E. J. L.; Kilner, C. A.; Halcrow, M. A. *Dalton Trans.* **2004**, 59.

(6) Kozlowski, M. C.; Li, X.; Carroll, P. J.; Xu, Z. *Organometallics* **2002**, *21*, 4513.

(7) (a) Messerschmidt, A.; Rossi, A.; Ladenstein, R.; Huber, R.; Bolognesi, M.; Gatti, G.; Marchesini, A.; Petruzzelli, R.; Finazzi-Agro, A. *J. Mol. Biol.* **1989**, *206*, 513. (b) Messerschmidt, A.; Ladenstein, R.; Huber, R.; Bolognesi, M.; Avigliano, L.; Petruzzelli, R.; Rossi, A.; Finazzi-Agro, A. *J. Mol. Biol.* **1992**, *224*, 179. (c) Messerschmidt, A.; Luecke, H.; Huber, R. *J. Mol. Biol.* **1993**, *230*, 997. (d) Zaitseva, I.; Zaitsev, V.; Card, G.; Moshov, K.; Bax, B.; Ralph, A.; Lindley, P. *J. Biol. Inorg. Chem.* **1996**, *1*, 15.

(8) Solomon, E. I.; Chen, P.; Metz, M.; Lee, S.-K.; Palmer, A. E. *Angew. Chem.* **2001**, *113*, 4702; *Angew. Chem., Int. Ed.* **2001**, *40*, 4570.

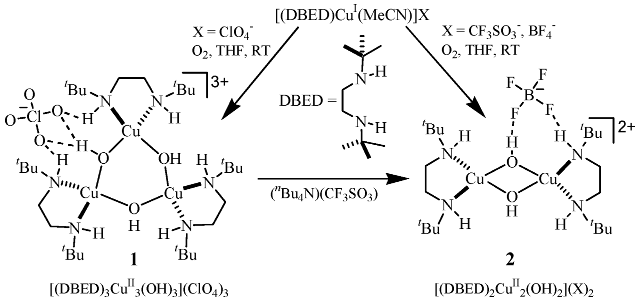
(9) (a) Palmer, A. E.; Lee, S. K.; Solomon, E. I. *J. Am. Chem. Soc.* **2001**, *123*, 6591. (b) Lee, S. K.; George, S. D.; Antholine, W. E.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **2002**, *124*, 6180.

(10) Yoon, J.; Mirica, L. M.; Stack, T. D. P.; Solomon, E. I. *J. Am. Chem. Soc.* **2004**, *126*, 12586.

(11) Mirica, L. M.; Vance, M.; Rudd, D. J.; Hedman, B.; Hodgson, K. O.; Solomon, E. I.; Stack, T. D. P. *J. Am. Chem. Soc.* **2002**, *124*, 9332.

10.1021/ic048182b CCC: $30.25

Scheme 1. Synthesis of Complexes **1** and **2**[a]



[a] One of the three ClO_4^- counteranions that forms hydrogen bonds with the N−H and O−H groups is shown for **1**·$(ClO_4)_3$. The BF_4^- counteranion that forms hydrogen bonds with two N−H groups is shown for **2**·$(BF_4)_2$.

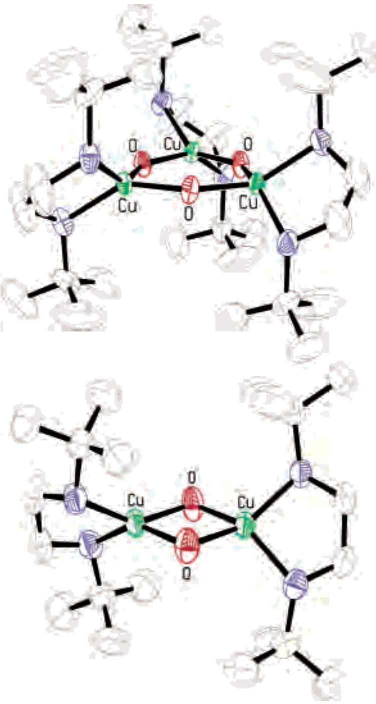


Figure 1. Structures of cations **1** (top) and **2** (bottom). Selected distances (Å) for **1** (**2**): Cu···Cu 3.643 (2.973), O···O 2.837 (2.392), Cu−O$_{avg}$ 1.914 (1.908), Cu−N$_{avg}$ 2.025 (2.009).

Cu(MeCN)](ClO_4) with O_2 at 25 °C yields a tris(μ-hydroxy)tricopper(II) complex $[(DBED)_3Cu_3(\mu\text{-}OH)_3](ClO_4)_3$ (**1**·$(ClO_4)_3$, Scheme 1). Alternatively, a change of the weakly coordinating counteranion of the copper(I) complex leads to the formation of the bis(μ-hydroxy)dicopper(II) complex $[(DBED)_2Cu_2(\mu\text{-}OH)_2]X_2$ (**2**·$(X)_2$, X = $CF_3SO_3^-$ or BF_4^-). The X-ray crystal structure of **1**·$(ClO_4)_3$ reveals a trinuclear copper cluster that has crystallographically imposed 3-fold symmetry (Figure 1, top).[12] The presence of three perchlorate anions per trinuclear cluster supports a composition of three

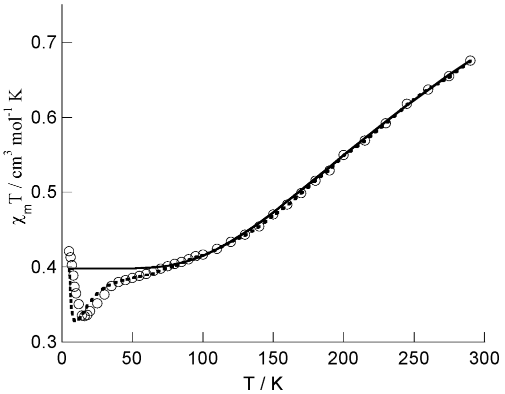


Figure 2. Thermal dependence of $\chi_M T$ for **1**·$(ClO_4)_3$. The solid line corresponds to the best theoretical fit for the data in the 80−290 K temperature range, and the dotted line to the fit for the 5−290 K temperature range, including antisymmetric exchange. See text for details.

Cu(II) centers bridged by three hydroxide groups. The $Cu_3(OH)_3$ moiety is planar, and each copper center has a distorted tetrahedral geometry with a twist angle of 46.5° between the N−Cu−N and O−Cu−O planes. The ligands are disposed in a helical arrangement that projects the *tert*-butyl groups above and below the $Cu_3(OH)_3$ plane to maximize their spatial distribution. The metrical parameters are typical for four-coordinate Cu(II) centers (Cu−O = 1.914 Å and Cu−N = 2.025 Å), with Cu···Cu and O···O distances of 3.643 and 2.837 Å, respectively. Two oxygen atoms of each ClO_4^- counteranion form hydrogen bonds with N−H groups of two ligands of the same molecule (O···N$_{avg}$ = 3.037 Å), as well as two bifurcated hydrogen bonds with the intercalating O−H group (O···N$_{avg}$ = 3.202 Å, Scheme 1).

Cyclic trinuclear copper(II) complexes such as **1** create a classic example of a *spin-frustrated* system with interesting magnetic properties.[13] For **1**·$(ClO_4)_3$, $\chi_M T$ at 300 K is ~0.69 cm^3 mol^{-1} K, much lower than the value expected for three uncoupled $S = {}^1/_2$ spins (~1.2 cm^3 mol^{-1} K), and it decreases to 0.4 cm^3 mol^{-1} K at 80 K, close to the value for a single uncoupled Cu(II) center (Figure 2).[14] Such behavior is consistent with a spin-frustrated doublet ground state.[5] A fit of the $\chi_M T$ data in the 80−290 K temperature range to an equation derived for an equilateral triangle distribution of the three $S = {}^1/_2$ Cu(II) ions[13] gives $J = -104.7$ cm^{-1} and $g = 2.06$ as best values. The large coupling constant between the doublet and quartet state is in close agreement with density functional theory calculations that also predict a doublet ground state ($J = -111$ cm^{-1}).[14] At lower temperatures, $\chi_M T$ decreases well below the value of 0.4 cm^3 mol^{-1} K. To interpret this behavior, it is necessary to go beyond the framework of the above isotropic exchange model and include an *antisymmetric exchange* interaction.[15] Such interaction is proposed to occur between two nearly degenerate $S = {}^1/_2$ states in triangular metal complexes, as observed initially by Tsukerblat[16] and recently by others.[5] The increase

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(12) Crystal data for **1**·$(ClO_4)_3$: $C_{34}H_{83}Cl_3Cu_3N_6O_{16}$, MW = 1129.03, T = 143 K, rhombohedral (hexagonal axes), space group $R\bar{3}c$ (No. 167), a = 14.585(1) Å, c = 45.609(4) Å, V = 8402.4(1) Å^3, Z = 6. Refinement of 590 unique reflections and 91 parameters gave R1 = 0.048 and wR2 = 0.136. Crystal data for **2**·$(BF_4)_2$: $C_{20}H_{50}B_2Cu_2F_8N_4O_2$, MW = 679.34, T = 184 K, monoclinic, space group $C2/c$ (No. 15), a = 29.989(2) Å, b = 11.697(1) Å, c = 18.921(1) Å, α = 90°, β = 104.968(1)°, γ = 90°, V = 6412.0(6) Å^3, Z = 8. Refinement of 6507 unique reflections and 441 parameters gave R1 = 0.0426 and wR2 = 0.1186. More crystallographic data are provided as Supporting Information.

(13) Kahn, O. *Molecular Magnetism*; VCH: New York, 1993.

(14) See the Supporting Information.

(15) Tsukerblat, B. S.; Belinskii, M. I.; Fainzil'berg, V. E. *Sov. Sci. Rev. B: Chem.* **1987**, *9*, 339.

(16) Tsukerblat, B. S.; Kuyavskaya, B. Y.; Belinskii, M. I.; Ablov, A. V.; Novotortsev, V. M.; Kalinnikov, V. T. *Theor. Chim. Acta* **1975**, *38*, 131.

of the $\chi_M T$ product below 16 K suggests the presence of weak ferromagnetic intermolecular interactions,[17] possibly due to the hydrogen bonding between neighboring cationic clusters and counteranions. A Hamiltonian including antisymmetric exchange and intermolecular interactions terms is sufficient to fit the experimental data for the entire temperature range,[18] yielding the same intramolecular coupling constant ($J = -105$ cm^{-1}) as found for the high-temperature range, an antisymmetric exchange constant $G = 35$ cm^{-1} and an effective intermolecular ferromagnetic interaction $J' = +1.1$ cm^{-1}.[19]

X-ray structure analysis of the binuclear bis(μ-hydroxy)dicopper(II) complex **2**$\cdot(BF_4)_2$ reveals a similar distorted tetrahedral geometry of each copper center (Figure 1), with an average twist angle of 46.4° between the N−Cu−N and O−Cu−O planes.[20] Such geometric distortions are uncommon for bis(μ-hydroxy)dicopper(II) complexes; only one other complex is known with such a large tetrahedral distortion.[21] The noncentrosymmetric disposition of the *tert*-butyl groups around the $Cu_2(OH)_2$ core maximizes their spatial distribution, and the metrical parameters of **2** are unexceptional for a bis(μ-hydroxy) dimer ($Cu-O_{avg} = 1.908$ Å, $Cu-N_{avg} = 2.009$ Å, Cu···Cu = 2.972 Å, O···O = 2.392 Å). The main structural differences between **2** and **1** are the O−Cu−O and Cu−O−Cu angles (77.8°, 101.6° for **2** and 95.7°, 144.2° for **1**), supporting a more compact structure for **2**. Solid-state magnetic measurements are consistent with two Cu(II) centers that are strongly antiferromagnetically coupled even at room temperature to give a singlet ground state.[13,22] In contrast to **1**, two F atoms of only one BF_4^- counteranion form hydrogen bonds with a N−H and an O−H group in **2**$\cdot(BF_4)_2$ (F···N/O ≈ 3.0 Å, Scheme 1).

The distinct solution properties of **1**$\cdot(ClO_4)_3$ and **2**$\cdot$**(X)**$_2$ (X = $CF_3SO_3^-$ or BF_4^-) excludes preferential crystallization as an explanation for the selective formation of each product. Examples do exist in the solid state in which weakly coordinating anions affect the nuclearity of metal complexes,[23] but we are unaware of any known examples in solution. Solution magnetic susceptibility measurements of **1**$\cdot(ClO_4)_3$ in THF at 298 K give a $\chi_M T$ value of ~0.7 cm^3 mol^{-1} K, consistent with more than one unpaired spin per trinuclear species and similar to that observed in the solid state.[24] This susceptibility is invariant whether the sample is prepared in situ or by dissolving crystalline material of **1**$\cdot(ClO_4)_3$. By contrast, the susceptibility measurements of **2**$\cdot(CF_3SO_3)_2$ under the same conditions indicate a diamagnetic complex. The visible absorption features of **1**$\cdot(ClO_4)_3$ and **2**$\cdot(CF_3SO_3)_2$, although similar,[14] are sufficiently different to assess a conversion from one species to the other. The addition of a 25-fold molar excess of (nBu$_4$N)(CF_3SO_3) to a THF solution of **1**$\cdot(ClO_4)_3$ at 25 °C leads to the conversion to **2** over 24 h as evidenced by the appropriate change in the visible spectrum and ES-MS.[25] Combined, these experiments suggest that the energy associated with the conversion of 2 equiv of trimer **1** to 3 equiv of dimer **2** is small and that the hydrogen-bonding and ion-pairing interactions with the weakly coordinating counteranions in THF determine what species is formed.

The generation of **1**$\cdot(ClO_4)_3$ in solution rather than the binuclear analogue was initially surprising as the latter is generally thermodynamically preferred. Yet, bis(μ-hydroxy)dicopper(II) complexes prefer to maintain a planar $N_4Cu_2(OH)_2$ core, unlike that found in the structure of **2**. Twisting of the N−Cu−N planes relative to the $Cu_2(OH)_2$ plane will electronically destabilize the dimer core, a distortion mandated by the severe steric demands of the DBED ligand. This suggests a reduction of the energetic cost of forming the less-compact, higher-nuclearity trinuclear complex. Interestingly, the two other structurally characterized Cu(II)[6] and Zn(II)[26] tris(μ-hydroxy) complexes carrying no additional bridging groups employ ligands with similar attributes to DBED. Thus, destabilizing an energetically preferred dimeric complex through increased ligand steric demands appears to be a viable strategy for creating higher-order species in solution, with the potential of studying their functional chemistry.

Modeling studies over the past decades have clearly demonstrated that almost all metal clusters in metalloproteins have intrisinic stability in homogeneous solution if appropriate solvents and sterically demanding ligands are selected. These restrictive conditions can be equated to the site-isolation and ligand geometrical constraints within a protein active site. The chemical plausibility of a tris(μ-hydroxy)tricopper(II) complex in a homogeneous solution as shown here and elsewhere[6] foreshadows its potential relevance in the catalytic cycle of multicopper oxidases.

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Supporting Information Available: Synthetic, spectroscopic, and analytical data for **1** and **2**; crystallographic information file (CIF); and additional tables of crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Bakalbassis, E.; Bergerat, P.; Kahn, O.; Jeannin, S.; Jeannin, Y.; Dromzee, Y.; Guillot, M. *Inorg. Chem.* **1992**, *31*, 625.

(18) To fit the experimental data, theoretical susceptibilities were calculated according to a generalized suscesptibility equation (ref 5b and Gerloch, M. *Magnetism and Ligand Field Analysis*; Cambridge University Press: Cambridge, U.K., 1983). Also see the Supporting Information.

(19) A similar value for the antisymmetric exchange constant was obtained by a different method in ref 10.

(20) DFT geometry optimization (B3LYP/6-31G*) converges to the distorted tetrahedral geometry with a twist angle of 43°.

(21) Funahashi, Y.; Nakaya, K.; Hirota, S.; Yamauchi, O. *Chem. Lett.* **2000**, 1172.

(22) $-2J > 600$ cm^{-1}, as observed in SQUID magnetic measurements ($\chi_M T = 0.09$ cm^3 mol^{-1} at 290 K).

(23) Patra, G. K.; Mostafa, G.; Tocher, D. A.; Datta, D. *Inorg. Chem. Commun.* **2000**, *3*, 56.

(24) (a) Evans, D. F. *J. Chem. Soc.* **1959**, 2003. (b) Sur, S. K.; *J. Magn. Reson.* **1989**, *82*, 169.

(25) The reverse experiment of adding (nBu$_4$N)(ClO_4) to a solution of **2**$\cdot(CF_3SO_3)_2$ does not lead to conversion to the trimer **1** within 24 h.

(26) Gorrell, I. B.; Looney, A.; Parkin, G.; Rheingold, A. L. *J. Am. Chem. Soc.* **1990**, *112*, 4068.