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Catalytic Promiscuity in Biomimetic Systems: Catecholase-like Activity, Phosphatase-like Activity, and Hydrolytic DNA Cleavage Promoted by a New Dicopper(II) Hydroxo-Bridged Complex

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Presented in this Communication are the structure, physicochemical properties, and catalytic promiscuity of a new dinuclear $Cu^{II}(\mu$-OH)Cu^{II} complex containing a novel N,O-donor symmetric dinucleating ligand.

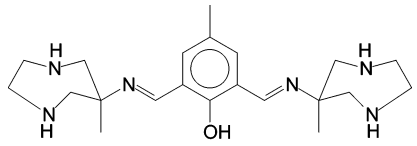
Catalytic promiscuity, defined as the ability of a single active site to catalyze more than one chemical transformation, constitutes a very important property of many enzymes, having a natural role in evolution and, occasionally, in the biosynthesis of secondary metabolites.[1] An illustrative example of this interesting feature is chymotrypsin, which catalyzes the hydrolysis of several chemically different substrates, such as amides, thiol esters, acid chlorides, and anhydrides. Thus, chymotrypsin exhibits catalytic promiscuity by catalyzing both amidase and phosphotriesterase reactions at its active site.[2]

Catechol oxidases are ubiquitous plant enzymes belonging to the oxidoreductase class. They contain a dinuclear Cu center in their active site, which catalyzes the two-electron oxidation of a broad range of *o*-diphenols (catechols) to the corresponding *o*-quinones, coupled with the reduction of molecular oxygen to water. The structure of the sweet potato catechol oxidase (ibCO) has been previously described and possesses, in its resting $Cu^{II}Cu^{II}$ (*met*) state, two cupric ions separated by a very short distance (2.9 Å), bridged by an exogenous hydroxo ligand. Each of the Cu^{II} centers completes its four-coordinate trigonal-pyramidal coordination with three N atoms from histidine residues.[3]

The development of synthetic analogues for the active sites of metalloenzymes containing dinuclear Cu centers has become an attractive approach to obtaining information concerning the mechanisms involved in their catalytic cycles.

In this Communication, we report the synthesis and structural characterization of a new dicopper(II) μ-hydroxo complex (**1**) containing a symmetric Schiff base derived from the recently described facial tridentate ligand AAZ,[4,5] as a model for the *met* form of the active site of catechol oxidase. We also show that, in addition to the expected catecholase-like activity, this system is able to catalyze the hydrolysis reaction of the phosphate diester 2,4-bis(dinitrophenyl)-phosphate (BDNPP) and to cause hydrolytic damage in plasmid DNA, exhibiting, in this way, a broad spectrum of catalytic activities.

The precursor 2,6-diformyl-4-methylphenol was obtained by modifying the method published by Gagné et al.[6] The heterocyclic compound AAZ was synthesized as described in the literature.[4] The Schiff base ligand HL was prepared in situ by reacting 2,6-diformyl-4-methylphenol (170 mg, 1 mmol) and AAZ (260 mg, 2 mmol) in MeOH under reflux.



HL

The molecular structure of the dinuclear cation in complex **1**[7,8] (Figure 1) reveals two pentacoordinated cupric ions, which are bridged by the phenolate oxygen O1 of the ligand HL and by an exogenous hydroxo ion. Bridging atoms

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(1) Kazlauskas, R. J. *Curr. Opin. Chem. Biol.* **2005**, *9*, 195−201.
(2) O'Brien, P. J.; Herschlag, D. *Chem. Biol.* **1999**, *6*, R91−R105.
(3) Klabunde, T.; Eicken, C.; Sacchettini, J. C.; Krebs, B. *Nat. Struct. Biol.* **1998**, *5*, 1084−1090.
(4) Aime, S.; Calabi, L.; Cavallotti, C.; Gianolio, E.; Giovenzana, G. B.; Losi, P.; Maiocchi, A.; Palmisano, G.; Sisti, M. *Inorg. Chem.* **2004**, *43*, 7588−7590.
(5) Peralta, R. A.; Neves, A.; Bortoluzzi, A. J.; Casellato, A.; dos Anjos, A.; Greatti, A.; Xavier, F. R.; Szpoganiccz, B. *Inorg. Chem.* **2005**, *44*, 7690−7692.
(6) Gagné, R. R.; Spiro, C. L.; Smith, T. J.; Hamann, C. A.; Thies, W. R.; Shiemke, A. K. *J. Am. Chem. Soc.* **1981**, *103*, 4073−4081.

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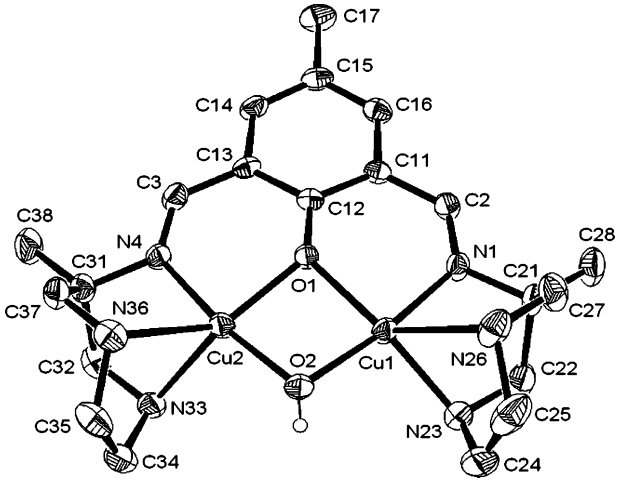

Figure 1. ORTEP plot of the cation $[Cu_2(\mu\text{-OH})(C_{21}H_{33}ON_6)]^{2+}$. Ellipsoid at the 40% probability level. Selected bond lengths and angles (Å and deg): Cu1−Cu2, 2.896(1); Cu1−O2, 1.920(5); Cu1−N1, 1.928(6); Cu1−O1, 1.954(4); Cu1−N23, 2.041(7); Cu1−N26, 2.524(7); Cu2−N4, 1.912(6); Cu2−O2, 1.914(5); Cu2−O1, 1.963(4); Cu2−N33, 2.092(6); Cu2−N36, 2.372(6); N1−C2, 1.256(9); C3−N4, 1.258(9); Cu1−O1−Cu2, 95.3(2); Cu2−O2−Cu1, 98.1(2); O2−Cu1−N1, 172.2(2); O2−Cu1−O1, 81.3(2); N1−Cu1−O1, 90.9(2); O2−Cu1−N23, 100.6(2); N1−Cu1−N23, 86.8(3); O1−Cu1−N23, 160.8(3); O2−Cu1−N26, 108.4(2); N1−Cu1−N26, 76.3(2); O1−Cu1−N26, 127.7(2); N23−Cu1−N26, 70.2(3); N4−Cu2−O2, 172.1(2); N4−Cu2−O1, 91.1(2); O2−Cu2−O1, 81.2(2); N4−Cu2−N33, 85.3(2); O2−Cu2−N33, 102.1(2); O1−Cu2−N33, 151.7(2); N4−Cu2−N36, 80.5(2); O2−Cu2−N36, 104.1(2); O1−Cu2−N36, 134.9(2); N33−Cu2−N36, 72.1(2).

occupy equatorial positions in the coordination sphere of the metals. The geometry around each Cu^{II} is distorted square-pyramidal, with values for the Addison[9] parameter τ of 0.19 for Cu1 and of 0.34 for Cu2. Cu1 completes its equatorial coordination plane with two N atoms (N1 and N23) from the AAZ pendent arm. The average Cu1−equatorial ligand bond length is 1.96 Å. The apical position is occupied by N26, the other AAZ nitrogen atom [Cu1−N26 = 2.524(7) Å]. On the other hand, the equatorial coordination plane of Cu2 is completed by the second AAZ unit N4 and N33 atoms. The average Cu2−equatorial ligand bond length is 1.97 Å. The N36 atom, from AAZ, occupies the apical position, completing thus the Cu2 coordination sphere [Cu2−N36 = 2.372(6) Å]. One of the outstanding features of this complex is the short intermetallic distance of 2.896(1) Å, which is, in fact, among the shortest $Cu^{II}\cdots Cu^{II}$ distances reported in the literature for hydroxo-bridged dicopper compounds.[10] It should be emphasized that this value is very similar to that determined for the *met* form of ibCO. In view of the crystallographic data discussed above, complex **1** can be considered to be one of the best structural models for the *met* form of the active site of catechol oxidases reported to date.

The reflectance spectrum of **1** (Figure S1 in the Supporting Information) shows a symmetrical absorption centered at 368 nm, assigned to a pair of different overlapped ligand-to-metal charge-transfer bands, namely, $Cu^{II} \leftarrow OH^-$ and $Cu^{II} \leftarrow {}^-OPh$, and an asymmetrical absorption at 608 nm, related to d−d transitions. The same feature is observed in MeOH [364 nm (ϵ = 12 100 mol L^{-1} cm^{-1}); 606 nm (145)] and MeCN [366 (7700); 607 (165)] solutions, suggesting that the molecular structure of the complex is maintained under these experimental conditions.

The cyclic voltammogram of **1** (Figure S2 in the Supporting Information) was measured in a MeCN solution and is characterized by two electrochemical signals. The first of them is a *quasi*-reversible wave at $E_{1/2} = -0.70$ V vs NHE, which can be assigned to the redox couple $Cu^{II}Cu^{II}/Cu^{II}Cu^{I}$. When the scan rate is increased, a second process, evidenced by a cathodic peak at −1.08 V vs NHE, appears. This can be attributed to the formation of the $Cu^{I}Cu^{I}$ species. The fact that this process is completely irreversible suggests a fast decomposition of the $Cu^{I}Cu^{I}$ species, as evidenced by Torelli et al.[10c] for a Cu_2 compound of the symmetric ligand H−BPMP. These results, confirmed by square-wave voltammetry (Figure S3 in the Supporting Information) and chronoamperommetric experiments, are in agreement with those obtained by other authors.[10c,11]

Complex **1** is able to catalyze the oxidation of the model substrate 3,5-di-*tert*-butylcatechol (3,5-dtbc) at 25.0 °C in a methanolic solution saturated with O_2. The reaction was monitored by means of UV−vis spectroscopy (400 nm) and shows a saturation kinetics pattern. To take into account the spontaneous oxidation of the substrate, correction was carried out using a reference cell under identical conditions but without addition of the catalyst. Initially, a pH-dependence study was carried out to determine the pH value at which catecholase activity is at a maximum (Figure S4 in the Supporting Information). A kinetic pK_a of 8.36 ± 0.06, probably related to the formation of a deprotonated form of **1**, was found, and therefore the dependence of the reaction on the substrate concentration was investigated under optimum activity conditions, i.e., at pH 9.0. For comparison, the activity of catechol oxidases[12] has been observed between

(7) HL readily reacts with copper(II) perchlorate hexahydrate (740 mg, 2 mmol) and sodium hydroxide (2 mmol) to give a dark-green solution, which was allowed to stand at room temperature for 30 min prior to being filtered off to eliminate any undesirable precipitate. After a few hours, green crystals suitable for X-ray analysis were formed. They were separated by vacuum filtration, washed with small amounts of cold methanol and diethyl ether, and dried in vacuo. Yield: 330 mg (44%). Found: C, 33.37; H, 4.73; N, 10.94. Calcd for $[Cu_2(\mu\text{-OH})(C_{21}H_{33}ON_6)](ClO_4)_2\cdot H_2O$: C, 33.79; H, 4.86; N, 11.26. Selected IR data (KBr): 3404, 3272, 1643, 1550, 1458, 1445, 1329, 1147, 1117, 1082, 888, 627 cm^{-1}. ***Caution!*** *Perchlorate salts of metal complexes with organic ligands are potentially explosive and should be handled in small quantities and with care.*

(8) X-ray analysis: $C_{21}H_{36}Cl_2Cu_2N_6O_{11}$, fw 746.54, monoclinic, $P2_1/c$, a = 14.452(1) Å, b = 13.254(1) Å, c = 16.386(3) Å, β = 109.158(9)°, V = 2964.9(6) $Å^3$, Z = 4, μ = 1.680 mm^{-1}, unique 5265 [R(int) = 0.0315], parameters 373, GOF(F^2) = 1.048, R1 [$I > 2\sigma(I)$] = 0.0598, wR2 (all data) = 0.1880.

(9) Addison, A. W.; Rao, T. N.; Reedijk, J.; van Rijn, J.;Verschoor, G. C. *J. Chem. Soc., Dalton Trans.* **1984**, 1349−1356.

(10) (a) Belle, C.; Beguin, C.; Gautier-Luneau, I.; Hamman, S.; Philouze, C.; Pierre, J. L.; Thomas, F.; Torelli, S. *Inorg. Chem.* **2002**, *41*, 479−491. (b) Dapporto, P.; Formica, M.; Fusi, V.; Micheloni, M.; Paoli, P.; Pontellini, R.; Rossi, P. *Inorg. Chem.* **2000**, *39*, 4663−4665. (c) Torelli, S.; Belle, C.; Gautier-Luneau, I.; Pierre, J. L.; Saint-Aman, E.; Latour, J. M.; Le Pape, L.; Luneau, D. *Inorg. Chem.* **2000**, *39*, 3526−3536 and references cited therein.

(11) Kannappan, R.; Mahalakshmy, R.; Rajendiran, T. M.; Venkatesan, R.; Sambasiva Rao, P. *Proc. Indian Acad. Sci. (Chem. Sci.)* **2003**, *115*, 1−14.

(12) Rompel, A.; Fischer, H.; Meiwes, D.; Buldt-Karentzopoulos, K.; Dillinger, R.; Tuczek, F.; Witzel, H.; Krebs, B. *J. Biol. Inorg. Chem.* **1999**, *4*, 56−63.

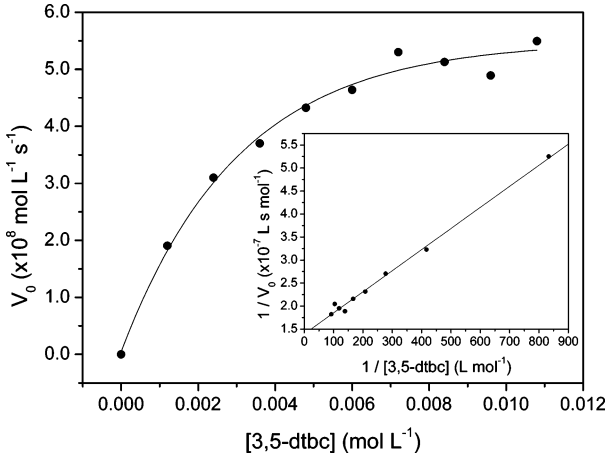

Figure 2. Oxidation of 3,5-dtbc catalyzed by **1**: Dependence of the reaction rates on [3,5-dtbc] at pH 9.0, in a methanol/water (32:1, v/v) mixture solution. Inset: double reciprocal plot. Conditions: $[\mathbf{1}]_{final} = 2.4 \times 10^{-5}$ mol L^{-1}; $[3,5\text{-dtbc}]_{final} = 1.2 \times 10^{-3}-1.1 \times 10^{-2}$ mol L^{-1}; at 25.0 °C.

pH 5 and 8, with maximum activity at pH 8. The initial rates obtained for the range of 3,5-dtbc concentrations used were fitted to the Michaelis−Menten equation (Figure 2) and linearized by means of the Lineweaver−Burk method (Figure 2, inset) to give the kinetic parameters $K_M = 4.0 \times 10^{-3}$ mol L^{-1}, $V_{max} = 8.3 \times 10^{-8}$ mol L^{-1} s^{-1}, the catalytic constant $k_{cat} = 3.46 \times 10^{-3}$ s^{-1}, and $k_{cat}/K_M = 0.88$ L mol^{-1} s^{-1}. These values are in the range of those commonly observed for other dinuclear $Cu^{II}Cu^{II}$ complexes.[10a,13] Thus, although complex **1** is not particularly active in the oxidation of 3,5-dtbc, it can be considered as a functional model for the active site of catechol oxidases. The reason for the low activity of **1** may be related to the geometry of the Cu centers. In a square-pyramidal arrangement, the steric bulk around the Cu atoms is increased, thus preventing the catechols from approaching.[10c] In this particular case, the intermetallic distance seems not to be a preponderant factor for activity.

Because complex **1** possesses in its structure a potential nucleophile constituted by the metal-bridging hydroxyl group, its ability to promote the hydrolysis of the activated phosphate diester BDNPP was investigated. In many cases, activated phosphate esters with good leaving groups are used as models of biologically relevant, unactivated phosphate esters, such as DNA. This study was performed in a 50% water/acetonitrile medium at 50.0 °C. We monitored the hydrolysis of BDNPP spectrophotometrically by following the absorption increase at 400 nm due to the formation of 2,4-dinitrophenolate over time. The pH-dependence plot for this reaction indicates optimum activity conditions around pH 7 (Figure S5 in the Supporting Information). A kinetic pK_a of 6.0 ± 0.2 was obtained from the acidic part of the curve, which is related to the formation of the μ-hydroxo species (**1**). At lower pH values, the diaqua $(H_2O)Cu^{II}(\mu\text{-phenoxo})Cu^{II}(OH_2)$ complex should predominate.[10c] The hydrolysis dependence on the complex concentration was studied at pH 6.0, and a second-order rate constant of 2.1×10^{-2} L mol^{-1} s^{-1} was found, which is comparable to the constants published by Young et al.[14] for the hydrolytic cleavage of BDNPP by two mononuclear Cu^{II} compounds.

Encouraged by the results obtained in the hydrolysis of BDNPP, we decided to evaluate the effect of **1** toward nucleic acid degradation. In fact, **1** is active as a DNA cleavage agent. Several Cu complexes were demonstrated to cleave DNA[15] and, in the vast majority of cases, an oxidative reaction pathway was suggested. Complex **1** should mediate DNA phosphodiester cleavage through a hydrolytic mechanism because a classical radical scavenger, such as dimethyl sulfoxide (DMSO), was not capable of inhibiting the activity (Figure S6 in the Supporting Information).

In summary, complex **1** shows both oxidoreductase and hydrolase activities. However, the catalytically active species are not the same: in the presence of catechols and a weakly basic medium, a deprotonated form of **1** acts by means of an oxidative mechanism. On the other hand, when the phosphate diester BDNPP is the substrate, complex **1** itself (which predominates in the solution from pH 6.0 to 7.5) breaks the P−O linkage following a hydrolytic pathway rather than an oxidative one. This is of great interest because the catalytic promiscuity of the system can be modulated by controlling the pH of the medium. Additional work is in progress to identify unequivocally the catecholase-like active species and to elucidate the mechanisms involved. The results obtained will be the subject of a full paper.

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Supporting Information Available: X-ray crystallographic details in CIF format and Figures S1−S6 showing a solid-state UV−vis spectrum, cyclic voltammograms, a square-wave voltammogram, pH-dependence plots for the oxidation of 3,5-dtbc and the hydrolysis of BDNPP, and a plasmid DNA cleavage experiment in the presence of DMSO, respectively (PDF). This material is available free of charge via the Internet at http://pubs.acs.org. CIF data can also be obtained free of charge as CCDC 613432 from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif or 12 Union Road, Cambridge CB2 1EZ, U.K. (tel +44 1223 336408, fax +44 1223 336033).

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(13) Peralta, R. A.; Neves, A.; Bortoluzzi, A. J.; dos Anjos, A.; Xavier, F. R.; Szpoganicz, B.; Terenzi, H.; Oliveira, M. C. B.; Castellano, E.; Friedermann, G. R.; Mangrich, A. S.; Novak, M. A. *J. Inorg. Biochem.* **2006**, *100*, 992−1004.

(14) Young, M. J.; Wahnon, D.; Hynes, R. C.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 9441−9447.

(15) (a) Oliveira, M. C. B.; Couto, M. S. R.; Severino, P. C.; Foppa, T.; Martins, G. T. S.; Szpoganicz, B.; Peralta, R. A.; Neves, A.; Terenzi, H. *Polyhedron* **2005**, *24*, 495−499. (b) Rossi, L. M.; Neves, A.; Bortoluzzi, A. J.; Hörner, R.; Szpoganicz, B.; Terenzi, H.; Mangrich, A. S.; Pereira-Maia, E. C.; Castellano, E. E.; Haase, W. *Inorg. Chim. Acta* **2005**, *358*, 1807−1822. (c) Liu, C.; Wang, M.; Zhang, T.; Sun, H. *Coord. Chem. Rev.* **2004**, *248*, 147−168. (d) Mitić, N.; Smith, S. J.; Neves, A.; Guddat, L. W.; Gahan, L. R.; Schenk, G. *Chem. Rev.* **2006**, *106*, 3338−3363.