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Enantio-differentiating catalytic oxidation by a biomimetic trinuclear copper complex containing L-histidine residues

Laura Santagostini,[a] Michele Gullotti,[a] Roberto Pagliarin,[b] Enrico Monzani[c] and Luigi Casella*[c]

[a] *Dipartimento di Chimica Inorganica, Metallorganica ed Analitica, Istituto ISTM, Università degli Studi di Milano, 20133 Milano, Italy*

[b] *Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, 20133 Milano, Italy*

[c] *Dipartimento di Chimica Generale, Università di Pavia, Via Taramelli 12, 27100 Pavia, Italy. E-mail: bioinorg@unipv.it; Fax: +39 0382 528544; Tel: +39 0382 507331*

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The trinuclear complex $[Cu_3PHI]^{6+}$, derived from a ligand containing two chiral L-histidine residues, performs the catalytic oxidation of L- and D-Dopa with remarkable enantio-differentiation; this depends on the anchoring effect provided by the copper center which is not participating in the catalytic reaction and recognizes the chirality of the substrate.

Bio-inspired catalytic oxidations by copper complexes mimicking enzyme active sites are intensively pursued in view of their potentially interesting synthetic applications.[1] Basically, all studies reported so far focused on catalytic systems consisting of mononuclear or dinuclear copper complexes which reproduce structural or functional features of enzyme centers of similar nuclearity (type 2 or type 3 Cu, respectively).[2–4] Trinuclear copper complexes have been little investigated,[5,6] in spite of the fact that clusters encompassing the type2–type 3 Cu assembly are contained in the well known group of multicopper oxidases, in association with a mononuclear type 1 Cu site.[7–10] The paucity of such studies reflects at least in part the difficulty of designing suitable octadentate nitrogen donor ligands to produce the necessary Cu_3N_8 core. We recently reported the synthesis of the chiral octadentate ligand PHI and the corresponding trinuclear complex $[Cu_3PHI]^{6+}$, where the metal ions are bound in two type 3-like A sites and in one type 2-like B site (Scheme 1).[11] The actual coordination number for the Cu(II) ions is five for sites A and four for site B,[11] with solvent molecules occupying the vacant sites, but the overall ligand donor atom distribution in this complex is similar to that present in the type 2–type 3 cluster of multicopper oxidases. Herein, we show that $[Cu_3PHI]^{6+}$ exhibits high stereoselectivity in the biomimetic catalytic oxidation of L- and D-dihydroxyphenylalanine (Dopa). The origin of the chiral recognition has been probed by binding studies of the complex with enantiomeric L-/D-amino acid couples. To our knowledge, this report represents the first example of stereoselective oxidation catalysis by biomimetic copper complexes.

The catalytic activity of $[Cu_3PHI]^{6+}$ in the oxidation of catechols was initially investigated using 3,5-di-*tert*-butylcatechol (Dtbc) in methanol/aqueous buffer at pH 5.1,† because the same conditions were employed previously in several biomimetic studies with Cu complexes.[6,12,13] The initial rates of quinone (Dtbq) formation were linearly dependent on the catalyst concentration and, when studied as a function of substrate concentration, exhibited saturation behavior. No dependence of the rates on molecular oxygen concentration was observed. These results are consistent with the preequilibrium formation of a precursor complex (characterized by the constant K_M) in the rate determining step (k_{cat}). Since no hydrogen peroxide was detected in the reaction medium through the lactoperoxidase-iodide assay,[12,13] the kinetic data can be interpreted according to the following mechanism (Cat is the copper catalyst):

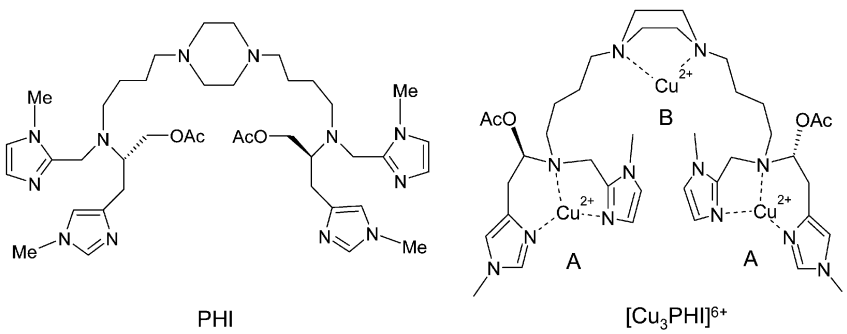
$$Cat_{ox} + Dtbc \rightarrow Cat_{red} + Dtbq + 2\ H^+ \text{ (slow)} \quad (1)$$

$$Cat_{red} + O_2 \rightarrow Cat_{red}\text{-}O_2 \text{ (fast)} \quad (2)$$

$$Cat_{red}\text{-}O_2 + Dtbc \rightleftharpoons [Cat_{red}\text{-}O_2\text{-}Dtbc] \rightarrow Cat_{ox} + Dtbq + 2\ H_2O \quad (3)$$

When reaction (1) is faster than reaction (3) the quinone band develops with biphasic behavior.[12,13] Since with $[Cu_3PHI]^{6+}$ this phenomenon is not observed, either the first step of the cycle is the rate-determining step or reactions (1) and (3) have similar rate constants. Catechol oxidation proceeds with the reduction of two of the three Cu(II) ions in the first step (eqn. (1)). Anaerobic experiments performed by reacting $[Cu_3PHI]^{6+}$ and Dtbc in 1 : 1 molar ratio showed that one type A and the type B Cu(II) center are involved in the two-electron redox process of step (1), since the CD and EPR features corresponding to one oxidized type A center are observed after completion of the reaction. Overall, the rate data collected in Table 1 indicate that the catalytic activity of $[Cu_3PHI]^{6+}$ in the catechol oxidation reaction is comparable to that of the dinuclear and trinuclear Cu(II) complexes studied previously by our group.[6,12,13]

The catalytic oxidation of L-/D-Dopa and their methyl esters (L-/D-DopaOMe) by $[Cu_3PHI]^{6+}$ was investigated in methanol/aqueous buffer at pH 8.6, because at lower pH the activity was very low.‡ To prevent further reactions of the Dopaquinone products, these reactions were studied in the presence of



Scheme 1 PHI and $[Cu_3PHI]^{6+}$; the copper binding sites are shown.

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Table 1 Rate constants for the catalytic oxidation of catechol derivatives by the complex $[Cu_3PHI]^{6+}$ in methanol/aqueous buffer at 20 °C. With Dtbc the reaction was studied at pH 5.1; with Dopa derivatives at pH 8.6

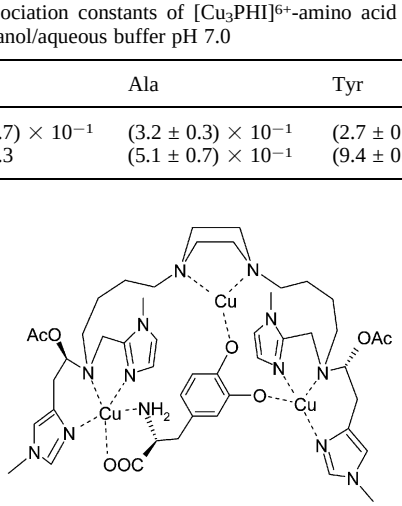
Substrate	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}s^{-1}$)
Dtbc	$(7.5 \pm 0.6) \times 10^{-1}$	$(2.6 \pm 0.05) \times 10^{-2}$	35
L-Dopa	$(4.1 \pm 0.8) \times 10^{-3}$	0.24 ± 0.01	58 000
D-Dopa	$(2.0 \pm 0.3) \times 10^{-2}$	0.28 ± 0.01	14 000
L-DopaOMe	$(1.4 \pm 0.2) \times 10^{-1}$	0.11 ± 0.01	770
D-DopaOMe	$(1.1 \pm 0.1) \times 10^{-1}$	0.08 ± 0.01	730

3-methyl-2-benzothiazoline hydrazone (Mbth), a quinone trapping reagent widely employed in the oxidations catalyzed by tyrosinase.[14] The kinetic data show that $[Cu_3PHI]^{6+}$ exhibits remarkable enantio-differentiation in the oxidation of L- and D-Dopa, but negligible enantio-differentiation toward their methyl esters (Table 1). The enantio-differentiating ability of the complex almost totally derives from the difference in K_M values for L- and D-Dopa, indicating that the binding interaction between the substrate and the catalyst active species is the critical parameter ruling the stereochemical preferences of the reaction and that both carboxylate and amino functions are required for chiral recognition of the substrate, since in this case binding to the metal involves formation of a chelate ring.

In order to provide a rationale for this interpretation, a series of binding experiments of $[Cu_3PHI]^{6+}$ with representative L-/D-amino acid couples were performed.§ Binding of ligands to the copper A site could be conveniently followed through the CD spectral changes undergone by the complex in the LF region upon formation of the adducts. From the binding isotherms, the equilibrium constants for 1 : 1 adducts reported in Table 2 were calculated. The data show that $[Cu_3PHI]^{6+}$ binds stronger to L- than D-amino acids and that the difference in binding strength is particularly large between L-Tyr and D-Tyr. In this case, the assistance by the second A site through the binding of the phenolic nucleus must be important. The binding experiments support the view that the observed stereoselectivity in the L-/D-Dopa oxidation by $[Cu_3PHI]^{6+}$ originates from the substrate anchoring effect provided by the metal center which does not participate in the catalytic reaction (Scheme 2). This hypothesis is consistent with the rate data reported in Table 1. In fact, the k_{cat} values are connected to the efficiency of the electron transfer from the bound catechol residue to the coppers, and are similar for the different Dopa derivatives because they have a similar redox potential and the catechol binds in a similar mode

Table 2 Dissociation constants of $[Cu_3PHI]^{6+}$-amino acid adducts (K_D, mM) in methanol/aqueous buffer pH 7.0

	His	Ala	Tyr
L	$(5.6 \pm 0.7) \times 10^{-1}$	$(3.2 \pm 0.3) \times 10^{-1}$	$(2.7 \pm 0.4) \times 10^{-3}$
D	1.5 ± 0.3	$(5.1 \pm 0.7) \times 10^{-1}$	$(9.4 \pm 0.9) \times 10^{-2}$



Scheme 2 Schematic view of the substrate anchoring effect provided by the A site Cu(II) center in the adduct between $[Cu_3PHI]^{6+}$ and L-Dopa.

to the two copper(II) ions. The K_M values reflect the overall strength of the substrate binding interaction and show a larger differentiation when chiral discrimination can occur. In conclusion, we have shown that chiral trinuclear complexes perform stereoselective catalysis through a new type of metal centered stereochemical assistance, which may find other useful applications in asymmetric catalysis.

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Notes and references

† Kinetic measurements of Dtbc oxidation were performed by using HP 8452A and HP8453 spectrophotometers equipped with a thermostated and magnetically stirred optical cell of 1-cm path length maintained at 20.0 ± 0.1 °C. A mixture of methanol/aqueous phosphate buffer (50 mM, pH 5.1) 30 : 1 (v/v) saturated with atmospheric oxygen was used as solvent. Formation of Dtbq was monitored through the development of its characteristic absorption band at 400 nm (ε_{400} = 1550 M^{-1} cm^{-1}). The dependence of the oxidation rate on Dtbc concentration was studied employing a 15 μM catalyst concentration, while the substrate concentration was varied between 0.07 and 8 mM. The initial rates of the reactions were obtained by fitting the plots of absorbance *vs*. time in the first few seconds of the reactions.

‡ Kinetics experiments on the catalytic oxidation of L-/D-Dopa, and L-/D-Dopa methyl ester were carried out similarly at 20.0 ± 0.1 °C, but employing a solvent mixture of methanol/aqueous phosphate buffer (50 mM, pH 8.6) 1 : 15 (v/v), and adding 1 mM Mbth to the solutions. The catalyst concentration was kept at 3 μM, while the substrate concentration was varied from 4 μM to 0.8 mM. The oxidations were monitored through the intense absorption band of the *o*-quinone-Mbth adduct at 500 nm (ε_{500} = 13 400 M^{-1} cm^{-1} for Dopaquinone-Mbth, and ε_{500} = 11 600 M^{-1} cm^{-1} for Dopaquinone methyl ester-Mbth).[14] The initial rates of the reactions were obtained as before.

§ Binding studies of amino acids to $[Cu_3PHI]^{6+}$ were performed by adding concentrate solutions of the amino acid in aqueous 0.1 M phosphate buffer pH 7.0 to solutions of the complex in methanol–water 1 : 18 (v/v). The titrations were followed by CD spectroscopy in the range 300–700 nm, and the data were analyzed at the λ_{max} of maximum CD change within the d–d envelope, as follows: 666 nm for L-Tyr, 617 nm for D-Tyr, 685 nm for L-His, 690 nm for D-His, 644 nm for L-Ala, and 631 nm for D-Ala. The equilibrium constants of adduct formation were calculated as described previously;[12] in all cases the adducts formed with a 1 : 1 stoichiometry.

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