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Catecholase Activity of a Series of Dicopper(II) Complexes with Variable Cu–OH(phenol) Moieties

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The catecholase activity of a series of dicopper(II) complexes containing different numbers of phenol groups coordinated to the metal centers was studied to identify functional as well as structural models for the type III copper enzymes tyrosinase and catechol oxidase. The syntheses and characterization of complexes $[Cu_2(H_2$-bbppnol$)(\mu$-OAc$)(H_2O)_2]Cl_2\cdot 2H_2O$ (**1**) and $[Cu_2($Hbtppnol$)(\mu$-OAc$)](ClO_4)_2$ (**2**) were previously reported by us (*Inorg. Chim. Acta* **1998**, *281*, 111–115; *Inorg. Chem. Commun.* **1999**, *2*, 334–337), and complex $[Cu_2(P1–O^-)(OAc^-)](ClO_4)_2$ (**3**) was previously reported by Karlin et al. (*J. Am. Chem. Soc.* **1997**, *119*, 2156–2162). The catalytic activity of the complexes **1**–**3** on the oxidation of 3,5-di-*tert*-butylcatechol was determined spectrophotometrically by monitoring the increase of the 3,5-di-*tert*-butyl-*o*-benzoquinone characteristic absorption band at about 400 nm over time in methanol saturated with O_2/aqueous buffer pH 8 solutions at 25 °C. The complexes were able to oxidize 3,5-di-*tert*-butylcatechol to the corresponding *o*-quinone with distinct catalytic activity. A kinetic treatment of the data based on the Michaelis–Mentèn approach was applied. The $[Cu_2(H_2$bbppnol$)(\mu$-OAc$)(H_2O)_2]Cl_2\cdot 2H_2O$ complex showed the highest catalytic activity of the three complexes as a result of a high turnover rate ($k_{cat} = 28\ h^{-1}$) combined with a moderate substrate–catalyst binding constant ($K_{ass} = 1.3 \times 10^3\ M^{-1}$). A mechanism for the oxidation reaction is proposed, and reactivity differences, k_{cat}/K_M of the complexes, were found to be dependent on $(\Delta E)_{1,2}$, the difference in the driving force for the reduction reactions $Cu^{II}{}_2/Cu^{II}Cu^{I}$ and $Cu^{II}Cu^{I}/Cu^{I}{}_2$.

Introduction

Copper has been known as a bioessential element for a long time,[1] but its biological relevance was only fully recognized in the last 20−30 years due to the development of its bioinorganic chemistry and successful interaction between the chemistry of model complexes and metalloprotein biochemistry.[2,3]

Copper-containing proteins are involved in distinct processes in living systems. Hemocyanin (O_2 transport), tyrosinase (hydroxylation of monophenols and oxidation of catechols), and catechol oxidase (oxidation of catechols) are classified as type III copper proteins and have coupled binuclear copper centers in their active sites. Recently, structures of the oxidized and the reduced forms of catechol oxidase from sweet potato were determined by X-ray crystallography.[4] They consist of a binuclear copper center

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each coordinated by three histidine nitrogen atoms. In the oxidized catechol oxidase structure, the two copper(II) centers contain a hydroxide bridging group completing the four-coordinated trigonal pyramidal coordination sphere, and the Cu(II)−Cu(II) distance was determined as 2.9 Å. In the reduced form, the Cu(I)−Cu(I) separation increases to 4.4 Å and a water molecule coordinates to one copper (CuA) which assumes a distorted trigonal pyramidal geometry, whereas the coordination of the other copper (CuB) can be described as square planar with one missing coordination site. The dinuclear copper site in tyrosinase and catechol oxidase is highly accessible to exogenous ligands compared to hemocyanin.[4] Tyrosinase catalyses the oxidation of monophenols to *o*-diphenols (cresolase activity) and the further oxidation of the resulting catechols to *o*-quinones (catecholase activity). This enzyme participates in the biosynthesis of melanin pigments and many other polyphenolic natural products. Solomon et al.[5−7] suggest a mechanism for the tyrosinase activity based on associative ligand substitution at the tyrosinase site. A phenol substrate could bind to oxytyrosinase (Cu(I)−O_2−Cu(I)) in an axial fashion, and rearrangement in the ternary Cu_2/O_2/substrate complex could be accompanied by *ortho*-hydroxylation, followed by loss of water and coordination of the diphenol product. Intramolecular electron transfer would result in the release of *o*-quinone, and the dicopper(I) produced could react with O_2 again to produce oxytyrosinase. Catechol oxidase catalyses the oxidation of a broad range of catechols to the corresponding *o*-quinones through the four-electron reduction of molecular oxygen to water, without acting on tyrosine. The resulting highly reactive quinones autopolymerize to form brown polyphenolic catechol melanins, a process thought to protect the damaged plant from pathogens or insects.[4] In the proposed catalytic pathway a simultaneous binding of the catechol substrate (monodentate) and oxygen (peroxide in a bridging side-on μ-η^2:η^2) to the reduced enzyme is suggested. The copper center in which the substrate binds would be six-coordinated with the substrate in axial position in a distorted octahedral coordination. In the ternary catechol oxidase/O_2^{2-}/substrate complex two electrons could be transferred from the substrate to the peroxide followed by protonation of the peroxide group and cleavage of the O−O bond, accompanied by the loss of water and oxidation of the catechol (release of *o*-quinone). Protonation of the bridging group by solvent brings the active site into the resting hydroxide-bridged dicupric state, which could react with other molecule of catechol reducing the Cu(II)−OH−Cu(II) state back to the dicuprous form and repeating the catalytic cycle.

It has been of great interest to investigate the catecholase activity of copper complexes using 3,5-di-*tert*-butylcatechol as a convenient model substrate for the identification of functional models for the metalloenzymes, in a biomimetic approach, or for new catalysts for oxidation reactions.[8−15] On the basis of the proposed catalytic mechanisms and the X-ray information available for catechol oxidase and tyrosinase, we have planned the synthesis of model compounds having as a main requirement the presence of two copper centers in close proximity to permit the binding of the catechol substrate as a bridging ligand, prior to the electron-transfer reaction. In recent notes[16,17] we reported the syntheses, characterization, and crystal structure of complexes $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(H_2O)_2]Cl_2\cdot 2H_2O$ (**1**) and $[Cu_2(Hbtppnol)(\mu\text{-}OAc)](ClO_4)_2$ (**2**), which contain uncommon phenol groups coordinated axially to two and one copper(II) center(s), respectively, as relevant models for the intermediate enzyme−phenolic substrate in the catalytic cycle of tyrosinase. To identify these complexes also as functional models for tyrosinase and catechol oxidase we investigated their abilities to catalyze the oxidation of catechol to quinone (catecholase activity) by employing 3,5-di-*tert*-butylcatechol (3,5-D*t*BC), a well-known model substrate. We also investigated the catecholase activity of a related complex $[Cu_2(P1\text{-}O^-)(OAc^-)](ClO_4)_2$ (**3**) previously reported by Karlin et al.[18] with the ligand 1,3-bis[bis(2-pyridylmethyl)amino]-propanolate for comparison. The ligands H_3bbppnol, H_2-btppnol, and P1-OH (Chart 1) belong to a class of organic ligands with the same 1,3-diamine-2-propanol central skeleton with different arms containing nitrogen donor atoms

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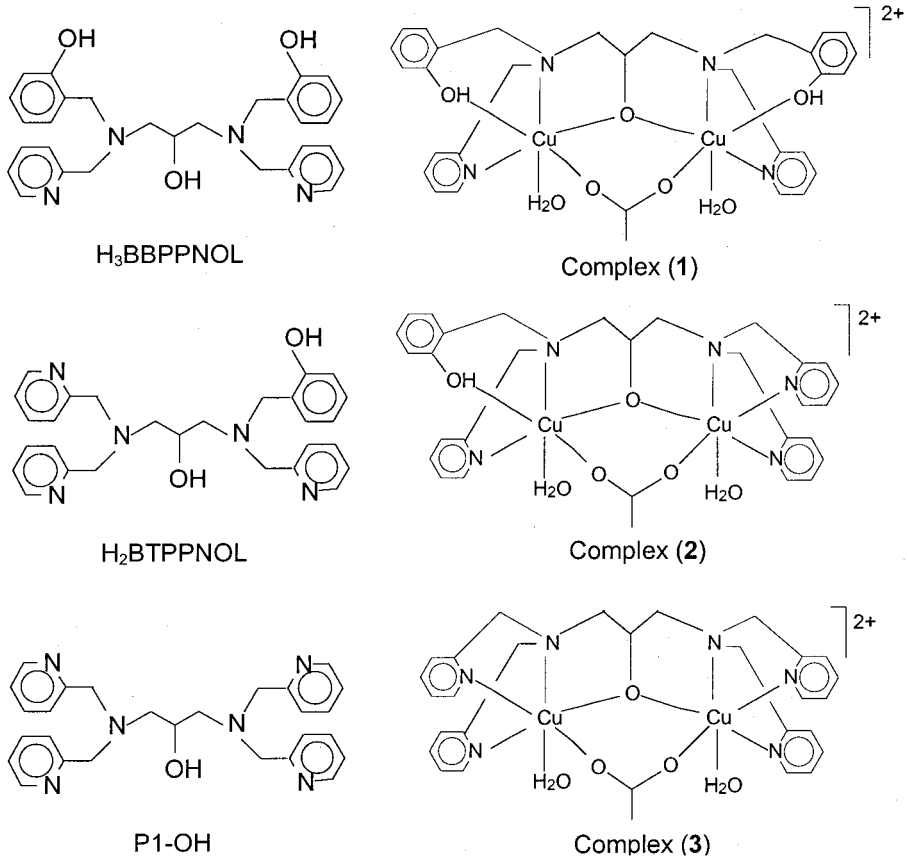
Chart 1



from pyridine and two, one, and no oxygen donor atoms from phenols, respectively.

Experimental Section

Abbreviations: H_3bbppnol, *N*,*N*′-bis(2-hydroxybenzyl)-*N*,*N*′-bis-(pyridylmethyl)]-2-hydroxy-1,3-propanediamine; H_2btppnol, *N*-(2-hydroxybenzyl)-*N*,*N*′,*N*′-tris(2-pyridylmethyl)-1,3-diaminopropan-2-ol; P1-OH, 1,3-bis[bis(2-pyridylmethyl)amino]propanolate; 3,5-D*t*BC, 3,5-di-*tert*-butylcatechol; 3,5-D*t*BQ, 3,5-di-*tert*-butylquinone; MES, 2-morpholinoethanesulfonic acid; TRIS, tris(hydroxymethyl)-aminomethane.

Materials. All reagents and solvents used in the syntheses were reagent grade and used without further purification. In complexes characterization and kinetic experiments spectroscopic grade solvents from Merck dried with molecular sieves were used.

Syntheses. $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(H_2O)_2]Cl_2\cdot 2H_2O$ (**1**),[16] $[Cu_2(Hbtppnol)(\mu\text{-}OAc)](ClO_4)_2$ (**2**),[17] and $[Cu_2(P1\text{-}O^-)(OAc^-)](ClO_4)_2$ (**3**)[18] were prepared by following previously reported procedures.

Safety Note. Perchlorate salts are potentially explosive and should be handled in small quantities with care! No problems were encountered with the complexes reported in this study.

Potentiometric Equilibrium Determinations. The potentiometric studies were performed with a Micronal B375 pH meter fitted with blue-glass and calomel reference electrodes calibrated to read $-\log[H^+]$ directly, designated as pH. Bidistilled water in the presence of $KMnO_4$ was used to prepare the water solutions. The electrode was calibrated using the data obtained from a potentiometric titration of a known volume of a standard 0.0100 M HCl solution with a standard 0.100 M KOH. The ionic strength of the HCl solution was maintained at 0.100 M by addition of KCl. The temperature was 25.00 ± 0.05 °C, and the experimental solutions, adjusted to 0.100 M in ionic strength by addition of KCl, were titrated with 0.100 M standard CO_2-free KOH. Equilibrium measurements were made on solutions containing 0.05 mmol of complex. The experiments were carried out on 50.00 mL of experimental solution in a thermostated cell, purged with argon cleaned by two 0.100 M KOH solutions. Computations were all carried out with the BEST7 program, and species diagrams were obtained with SPE and SPEPLOT programs.[19]

Cyclic Voltammetry. Electrochemical measurements were carried out using a Princeton Applied Research (PARC) model 273 potentiostat/galvanostat. Cyclic voltammograms were obtained at room temperature for acetonitrile solutions containing 10^{-3} M complex and 0.1 M [TBA][PF_6] as the supporting electrolyte under an argon atmosphere. The electrochemical cell employed was of a standard three-electrode configuration: platinum working electrode; platinum wire counter electrode; SCE reference electrode. The Fc^+/Fc couple of ferrocene ($E° = 0.400$ V vs NHE) was used as an internal standard.[20]

Kinetics of 3,5-Di-*tert*-butylcatechol Oxidation. Kinetic experiments for the oxidation of 3,5-di-*tert*-butylcatechol were monitored spectrophotometrically on a HP-8452A diode-array spectrophotometer by following the increase of the 3,5-di- *tert*-butyl-*o*-benzoquinone characteristic absorption band at about 400 nm over time. In a typical experiment, 100 μL of a complex solution in methanol ($[C]_o = 2.4 \times 10^{-5}$ M) was added to a 1-cm path-length cell containing 3 mL of methanol saturated with O_2 and 100 μL of aqueous buffer TRIS pH 8.0 at 25 °C. The reaction was initiated

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by the addition of 100 μL of catechol solution ([3,5-D*t*BC]$_o$ = 3.0 $\times$ 10^{-3} to 9.0 $\times$ 10^{-3} M). In a separate set of experiments, the kinetic determinations were performed without the catalyst. The reaction rate was obtained from the slope of the $A \times t$ plot over the first 800 s of reaction. The conversion of the reaction rate units was done using ϵ = 1900 M^{-1} cm^{-1} for 3,5-D*t*BQ in methanol.[21] A kinetic treatment on the basis of the Michaelis−Mentèn approach was applied, and the results were evaluated from Lineweaver−Burk double-reciprocal plots.

Detection of Hydrogen Peroxide in the Oxidation Reaction. To detect the formation of hydrogen peroxide during the catalytic reaction we employed a modification of the iodometric method.[22] Reaction mixtures were prepared as in the kinetic experiments. After 1 h of reaction an equal volume of water was added and the quinone formed was extracted three times with dichloromethane. The aqueous layer was acidified with H_2SO_4 to pH $\cong$ 2 to stop further oxidation, and 1 mL of a 10% solution of KI and three drops of 3% solution of ammonium molybdate were added. In the presence of hydrogen peroxide occurs the reaction $H_2O_2 + 2I^- + 2H^+ \rightarrow 2H_2O + I_2$, and with an excess of iodide ions, the triiodide ion is formed according to the reaction $I_2(aq) + I^- \rightleftharpoons I_3^-$. The reaction rate is slow but increases with increasing concentrations of acid, and the addition of an ammonium molybdate solution renders the reaction almost instantaneous. The formation of I_3^- could be monitored spectrophotometrically due to the development of the characteristic I_3^- band (λ = 353 nm, ϵ = 26 000 M^{-1} cm^{-1}).[13]

Results and Discussion

Acid−Base Equilibrium Determination. The potentiometric titration of complexes **1**−**3** showed the neutralization of 2 mol of KOH/mol of complex. Initially, this consumption of base would be attributed to the deprotonation of the phenols or of the water molecules coordinated axially to the Cu^{II} centers in complex **1**. But after the analysis of the entire data available for complexes **1**−**3** (Chart 1), where complex **3** is coordinated only by nitrogen donor groups, the consumption of 2 mol of base/mol of complex was attributed to the deprotonation of two metal-bound water molecules. In this complex each Cu^{II} center has a square pyramidal arrangement[18] and each water molecule is expected to be at the sixth position in the coordination sphere of the metal ions in solution, as was identified also in the X-ray crystal structure of complex **1**.[16] In complexes **2** and **3**, the water molecules were not identified in the crystal structures probably due to the shorter and consequently more polarized Cu−N bonds in the axial positions (Cu−N = 2.211[17] and 2.157[23] Å in complexes **2** and **3**, respectively) compared with the Cu−OH(phenol) in complex **1**. UV−vis measurements of water solutions of complexes **1**−**3** did not show any substantial spectral changes with pH in the 400−450 nm range. The deprotonation of the metal-bound phenol groups in complexes **1** and **2** should be accompanied by the appearance of charge transfer transitions of the type Cu(II) to phenolate (axial) MLCT or phenolate (equatorial) to Cu(II) LMCT at about 400 nm, as recently discussed in the literature.[24] Studies of the protonation of phenolate groups in copper(II) complexes with remarkable change in the electronic spectra have been reported.[25] However, complexes **1**−**3** show significant ligand field spectral shifts within the pH range of interest for kinetic studies. For complex **3** the wavelength maximum of the d−d band shifts from [λ_{max}, nm (ϵ, M^{-1} cm^{-1})] 645 (170) at pH 4.9 to 905 (300) at pH 9.0 (Figure 1), while for **1** and **2** the maxima change respectively from 663 (215) to 726 (254) and 685 (172), 874 (142) to 720 (200), 842 (229) under the same pH conditions as those for complex **3**. We propose that these pH-driven interconversion processes are associated with the change from H_2O ligands to OH^- ligands in agreement with the potentiometric titration data.

The protonation constants of the two hydroxide ions in the dinuclear copper(II) complexes **1**−**3** represented by eq 1 were determined by potentiometric pH titration and are shown in Table 1.

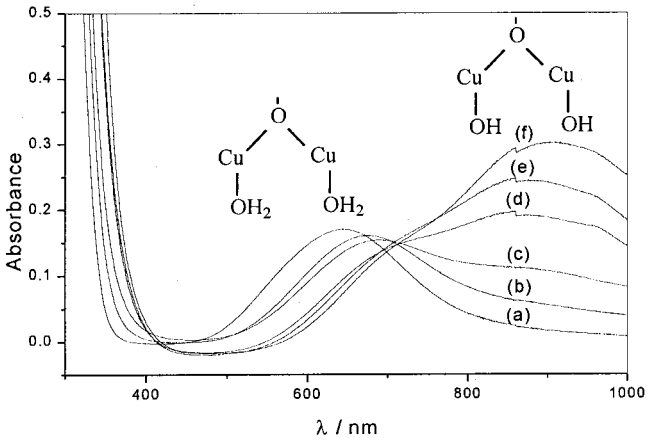

Figure 1. UV−visible spectra of 1 $\times$ 10^{-3} M aqueous solutions of [Cu_2(P1-O^-)(OAc^-)](ClO_4)$_2$ (**3**) as a function of pH: (a) pH = 4.9 (buffer acetate); (b) pH = 6.3 (buffer MES); (c) pH = 7.1 (buffer TRIS); (d) pH = 7.5 (buffer TRIS); (e) pH = 8.1 (buffer TRIS) and pH = 9.0 (buffer TRIS).

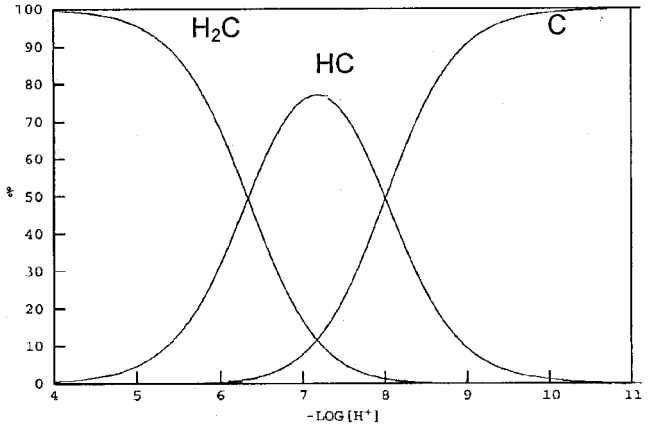

Figure 2. Species distribution curves of protonated species of the binuclear copper(II) complex **2**. H_2C is the dinuclear copper(II) complex with two water molecule coordinated to the metal center, HC is the monohydroxo species, and C is the dihydroxo one.

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Table 1. log Values of Protonation Constants for Complexes **1**−**3**[a]

K	complex **1**	complex **2**	complex **3**
[HC]/[C][H$^+$]	7.42	7.96	8.15
[H$_2$C]/[HC][H$^+$]	5.70	6.32	6.82

[a] T = 25.0 °C and μ = 0.100 (KCl).

H_2C represents the binuclear copper(II) complex with two water molecules coordinated one on each Cu^{2+} ion, HC is the complex with a water molecule coordinated to one Cu^{2+} ion and a hydroxide ion coordinated to the other Cu^{2+} ion, and C represents the complex with two hydroxide ions coordinated to the dinuclear metal centers. For complex **1**, the H_2C species predominates at pH values below 5.70. The dissociation of a metal-bound water molecule gives the HC species reaching a maximum of 78.3% formed at pH 6.60. The concentration of this species decreases at higher pH values giving rise to a corresponding increase in the dihydroxo species C that predominates at pH values above 7.42. For complex **2**, the diaquo complex predominates at pH values lower than 6.3. At neutral pH, the monohydroxo species predominates, reaching a maximum of 77% formed at pH 7.10. The dihydroxo is the major species at pH values above 8.00. For complex **3**, the diaquo complex predominates at pH values lower than 6.80, the monohydroxo species reaches a maximum of 70% at pH 7.50, and the dihydroxo species is the major species at pH values above 8.15. The distribution curves of the species present for complex **2** are shown in Figure 2. Finally, the absence of titratable protons in the 4.0−5.0 pH range for **1**−**3** guarantees that the μ-acetate group remains coordinated in these complexes under these experimental conditions.

$$H_{n-1}C + H^+ \rightleftharpoons H_nC \qquad (1)$$

Electrochemistry. The electrochemistry of the complexes was investigated, as the redox potential is an important parameter in such electron-transfer reactions we are studying. The redox potential should be such as to permit the reoxidation of the reduced copper centers by molecular oxygen to maintain the catalytic cycle. The reduction potentials for complexes **1**−**3** were measured by cyclic voltammetry (CV) at a working platinum electrode in acetonitrile solution containing 0.1 M [TBA][PF_6] as the supporting electrolyte in the range −1.6 to + 1.6 V vs SCE. The cyclic voltammograms of all complexes show irreversible cathodic reduction peaks, and the electrochemical data are summarized in Table 2. The reduction peaks are tentatively assigned to the one-electron processes $Cu^{II}Cu^{II} \rightarrow Cu^{II}Cu^{I}$ and $Cu^{II}Cu^{I} \rightarrow Cu^{I}Cu^{I}$. All the complexes exhibit an additional sharp anodic oxidation peak in the range from −0.21 to −0.12 V vs NHE, probably corresponding to a process involving Cu^0 at the electrode surface. All complexes have negative reduction potentials which do not compare with the reported value of +0.36 V vs SCE for the enzyme tyrosinase isolated from mushroom.[26]

Kinetic Studies for Catecholase Activity. The catecholase activity of tyrosinase and catechol oxidase is carried out by the oxy form (Cu(II)−O_2^{2-}−Cu(II)) and by the met form (Cu(II)−Cu(II)) of the enzymes through a two electron-transfer reaction. The catecholase activity of Cu(II) complexes has been determined by the catalytic oxidation of 3,5-di-*tert*-butylcatechol.[8−15] The kinetic studies of the oxidation of 3,5-D*t*BC by complexes **1**−**3** were carried out by the method of initial rates by monitoring the increase in the characteristic quinone (3,5-D*t*BQ) absorption band at 400 nm over time. It was found that under anaerobic conditions little product was formed, so the solvent was saturated with O_2 before the kinetic experiments. Oxygen must participate directly in the catalytic cycle of the oxidation reaction acting as a thermodynamic driving force by reoxidizing any generated copper(I) species back to the active copper(II) species. The dependence of the oxidation reaction catalyzed by complexes **1**−**3** on the pH was studied by adding a small amount of aqueous buffer to the oxygen saturated methanol solution. To minimize the pH effect on the spontaneous reaction, we used the same solution without adding the catalyst as an internal reference. All complexes exhibit the same behavior with a sudden increase in the reaction rates at pH around the second pK_a value. For complex **1**, there is almost no catalytic activity until pH 7.0 is reached, and the reaction rate suddenly increases at pH higher than 7.5, as shown in Figure 3. For complexes **2** and **3**, there is almost no catalytic activity until pH 8.0 is reached. The data were fitted using a Boltzman model resulting in pK_a values of 7.8 ± 0.1 for complex **1**, 8.1 ± 0.1 for complex **2**, and 8.2 ± 0.1 for complex **3**. The pK_a values obtained from the kinetic experiments are consistent with the second dissociation constant of the complexes determined by potentiometric titrations (Table 1), indicating that the most active species for the oxidation reaction is the totally deprotonated form. On the other hand, the catechol substrate may not be dissociated under these pH conditions since the pK_a values of 10.354 and 14.7 are reported.[27] The pH dependence may be due to the fact that in alkaline media the water molecules coordinated to the copper(II) centers in the complex are dissociated and probably the metal-coordinated hydroxide groups are able to interact with catechol promoting the deprotonation of the substrate and coordination to the metal centers. Because the oxidation reaction of 3,5-D*t*BC was found to be pH dependent, complete kinetic studies were performed in a pH range where there are higher reaction

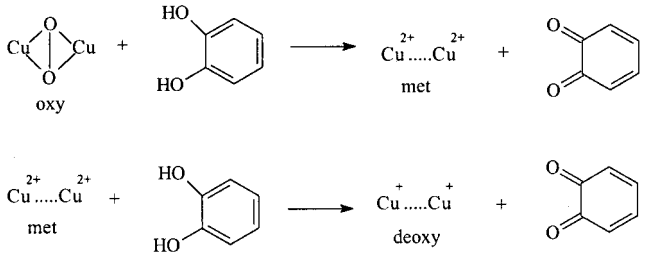

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Table 2. Electrochemical Data for Dicopper(II) Complexes **1**−**3**

complex	$E(\text{red})_1$[a] (V vs NHE)[c]	$E(\text{red})_2$[b] (V vs NHE)[c]	$(\Delta E)_{1,2}$ (V vs NHE)
$[Cu_2(H_2bbppnol)(\mu\text{-OAc})(H_2O)_2]Cl_2\cdot 2H_2O$ (**1**)	−0.527	−0.713	0.186
$[Cu_2(Hbtppnol)(\mu\text{-OAc})](ClO_4)_2$ (**2**)[17]	−0.890	−1.11	0.220
$[Cu_2(P1\text{-}O^-)(OAc)](ClO_4)_2$ (**3**)	−0.615	−0.917	0.302

[a] Reduction potential attributed to the process Cu(II)−Cu(II) → Cu(II)−Cu(I). [b] Reduction potential attributed to the process Cu(II)−Cu(I) → Cu(I)−Cu(I). [c] Potentials were referred in V vs NHE by adding 0.400 V to the potentials corrected in V vs Fc^+/Fc.[20]

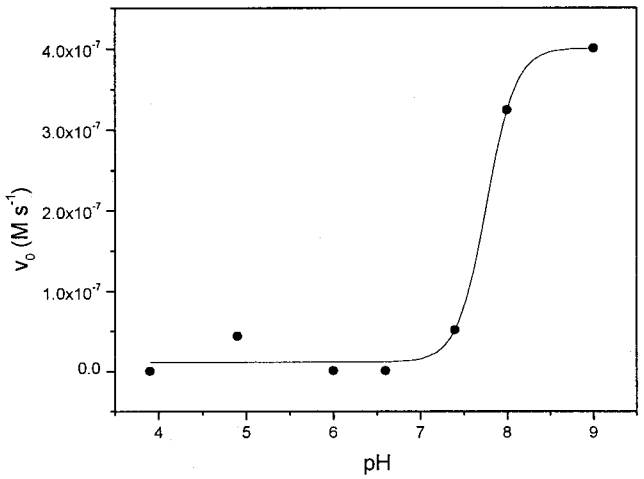

Figure 3. Dependence of the reaction rates on pH for the oxidation of 3,5-D*t*BC catalyzed by complex **1**. The reactions were performed in methanol saturated with O_2/aqueous buffer (30:1) (acetate pH 3.9 and 4.9, MES pH 6.0 and 6.6, and TRIS pH 7.4, 8.0, and 9.0). [c] = 2.4×10^{-5} M and [3,5-D*t*BC] = 5×10^{-3} M at 25 °C.

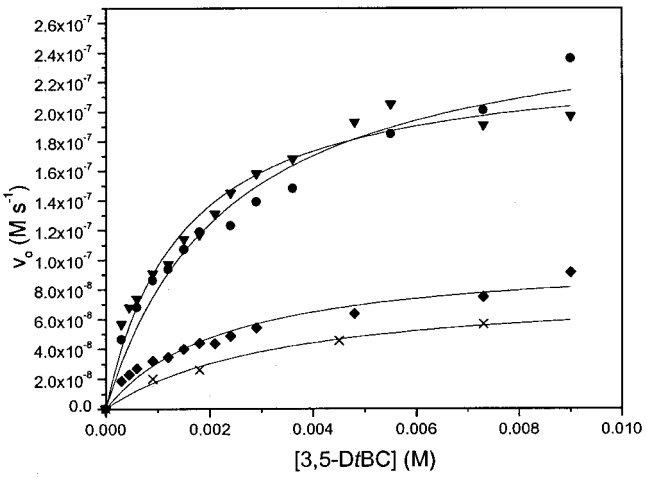

Figure 4. Dependence of the reaction rates on the 3,5-D*t*BC concentrations for the oxidation reaction catalyzed by dicopper(II) complexes: (▼) **1**; (●) **2**; (◆) **3**; (×) blank. The reactions were performed in methanol saturated with O_2/aqueous buffer TRIS pH 8.0 (30:1), with [c] = 2.4×10^{-5} M and [3,5-D*t*BC] = 3.0×10^{-4}−9.0×10^{-3} M at 25 °C.

rates and less influence from the spontaneous reaction, to obtain information concerning the reaction mechanism. The formation of hydrogen peroxide during the catalytic reaction was determined by iodometry.[22]

Saturation kinetics were found for the initial rates versus the 3,5-D*t*BC concentration for all complexes (Figure 4). An analysis of the data on the basis of the Michaelis−Mentèn model, originally developed for enzyme kinetics, was applied. The results evaluated from Lineweaver−Burk plots are shown in Table 3. Complexes **1** and **2** show the same turnover rate (ca. 28 h^{-1}) and little differences in the K_M values which results in some differences in the second-order rate constant k_2 ($=k_{cat}/K_M$). Complex **3** shows the lowest catalytic activity of the series as a result of a low turnover rate (10 h^{-1}) combined with a comparable substrate−catalyst binding constant (K_{ass}). In fact, several factors need to be considered in assessing the difference in the catalytic activities of complexes **1**−**3**, such as electrochemical properties, exogenous donors, and steric match. We found a very good correlation between the electrochemical parameter $(\Delta E)_{1,2}$ ($=E(\text{red})_1 - E(\text{red})_2$) and the kinetic parameter k_2 ($=k_{cat}/K_M$) (Figure 5) although there is no correlation between k_{cat}/K_M and the individual $E(\text{red})_1$ and $E(\text{red})_2$ of the complexes. $(\Delta E)_{1,2}$ is an important parameter since it represents how readily the metal centers accept the electron pair from the substrate in the oxidation reaction. In addition, the correlation between k_{cat}/K_M and $(\Delta E)_{1,2}$ supports the mechanistic idea that a one-electron redox intermediate is disfavored in this two-electron reaction.

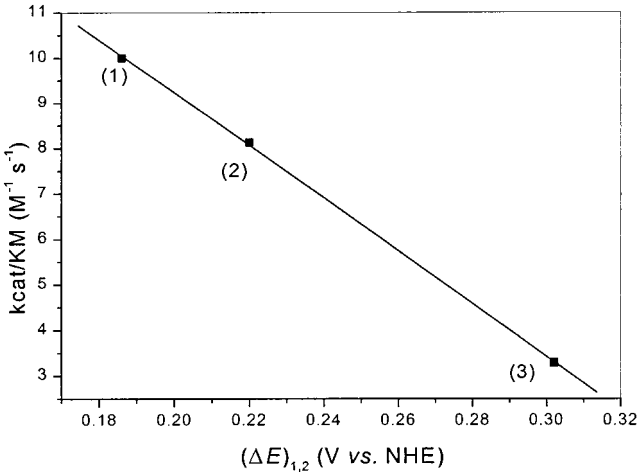

Figure 5. Correlation between electrochemical ($(\Delta E)_{1,2}$) and kinetic (k_{cat}/K_M) parameters for the series of dicopper(II) complexes **1**−**3**.

Table 3. Kinetic Parameters for the Oxidation of 3,5-D*t*BC Catalyzed by Dicopper(II) Complexes

complex	V_{max} ($M\ s^{-1}$)	K_M (M)	K_{ass} (M^{-1})	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}\ s^{-1}$)
1	1.9×10^{-7}	7.9×10^{-4}	1.3×10^3	0.0079	10
2	1.9×10^{-7}	9.5×10^{-4}	1.0×10^3	0.0078	8.1
3	6.7×10^{-8}	8.6×10^{-4}	1.2×10^3	0.0028	3.3

We have also investigated the dependence of the catalytic reaction rate on the concentration of acetate close to saturation of D*t*BC in the presence of variable amounts of sodium acetate. For complex **1** the rate was found to decrease from 1.9×10^{-7} M s^{-1} at $[OAc^-] = 0.0$ M to 6.5×10^{-8} M s^{-1} at $[OAc^-] = 5 \times 10^{-3}$ M. In the studied complexes **1**−**3** the fifth coordination site of the Cu(II) centers is occupied by the bridging acetate and thus some inhibition should be

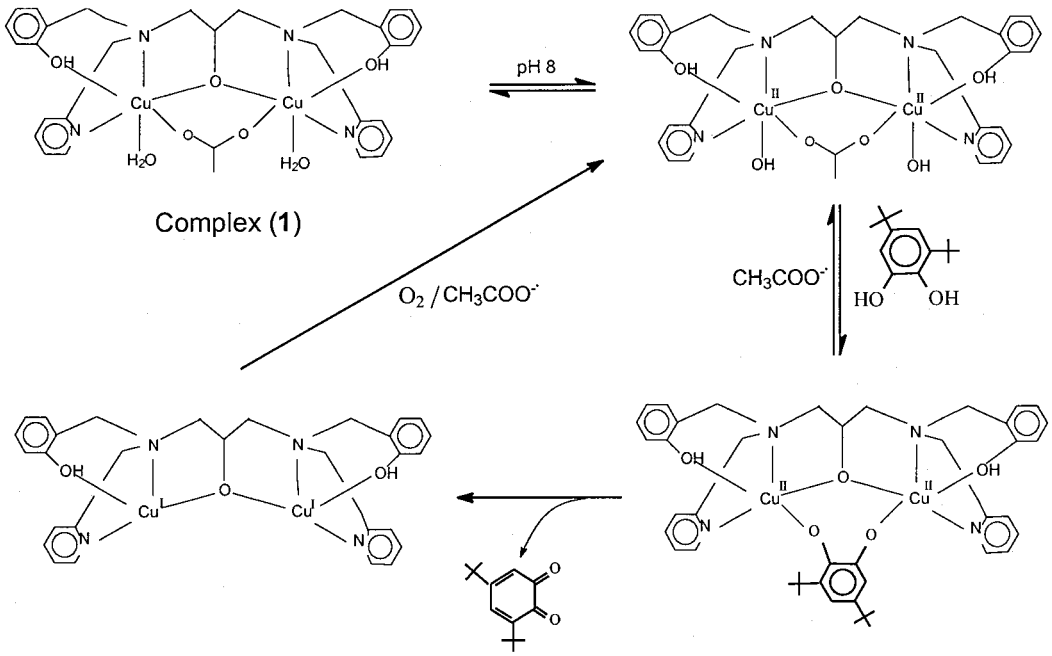

Figure 6. Mechanism for the 3,5-D*t*BC oxidation catalyzed by dicopper(II) complexes proposed on the basis of the structure of complex **1**.

expected if acetate is replaced by the substrate. Further, the Cu^{II}---Cu^{II} distances (~3.40 Å) as observed in the X-ray structures of **1**−**3**[16−18] allow a bridging catechol coordination compatible with the distance between two *o*-diphenol oxygen atoms (a Cu^{II}---Cu^{II} distance of 3.25 Å has been observed for the unique *o*-catecholate-bridged dicopper complex described in the literature).[3e]

The experimental data permit the postulation of the following mechanism shown in Figure 6 on the basis of the structure of complex **1**. First, we propose a preequilibrium between the complex and its deprotonated form since we found the oxidation reaction to be pH dependent. The coordination of diphenol as a bridging ligand prior to the intramolecular electron-transfer reaction is supported by the dependence of the reaction rate on substrate concentration which is in agreement with the Michaelis−Mentèn mechanism. Moreover, the incoming catecholate is a stronger ligand than acetate and so replacement should be occur. The electron-transfer reaction, as the rate-determining step, results in the oxidation of the catechol substrate to the corresponding *o*-quinone and reduction of the copper centers to Cu(I). The Cu(I)−Cu(I) complex in which the Cu(I)'s are 4-coordinate, in the presence of dioxygen, is immediately oxidized back to the original form completing the catalytic cycle. It is important to note that the accumulation of hydrogen peroxide in the catechol oxidation was found to be rather small when compared with blank experiments and that H_2O_2 is not decomposed by the complexes when O_2 is used as the oxidant. Thus, we propose that the generated H_2O_2 is consumed in a fast reoxidation reaction of the dicopper(I) complex or alternatively the catalytic reaction occurs through the catechol oxidase cycle. Finally, despite the lack on information concerning the stabilization of the Cu^{II}−O_2^{2-}−Cu^{II}, we believe that this simple model is sufficient to describe our kinetic results, although a much more complicated mechanism may be involved in the catalytic cycle.

Summary and Perspectives

We have synthesized and characterized three Cu^{II}_2 complexes as analogues for the tyrosinase active site, which show catecholase activity through a mechanism similar to that proposed for tyrosinase[5−7] and catechol oxidase[4] enzymes. This system of structurally very similar dinuclear complexes represents a strong piece of experimental evidence that the difference of the driving force for the reduction of the Cu^{II} centers plays an important role in the simultaneous two electron oxidation of the model substrate 3,5-di-*tert*-butylcatechol. However, unfortunately we lack information regarding the stabilization of the peroxide-bridging complex in our synthetic models. This information is necessary to ascertain whether these complexes can be considered functional models for the catecholase activity of the met form of the enzymes mentioned above. Therefore, we are currently investigating the synthesis and characterization of copper(I) complexes with the same ligands employed in this work.

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