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Tyrosinase-Like Reactivity in a $Cu^{III}_2(\mu\text{-}O)_2$ Species

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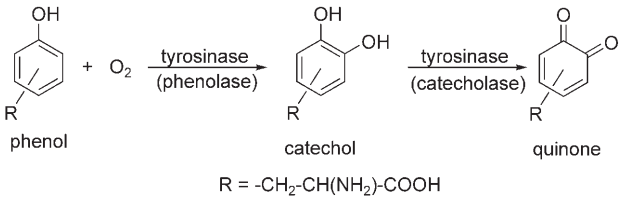
Understanding the mechanisms by which the oxidizing potential of the O_2 molecule is used in the oxidation of organic substrates is a formidable task with fundamental interest in technology and biochemistry.[1] Nature usually uses transition-metal ions to overcome kinetic barriers associated with spin multiplicity of the O_2 molecule.[2] For example, Type 3 copper proteins use a bimetallic site to bind and/or activate O_2. Among the enzymes that contain this type of active site structure, tyrosinase is a particularly interesting enzyme that catalyzes the *ortho*-hydroxylation of phenols to catechols and further oxidation to the corresponding quinones (Scheme 1).[3]

Recent X-ray crystallographic studies of this enzyme have shown the presence of a rather flexible active site that accommodates large changes in the Cu···Cu distance during its catalytic cycle,[4] and they confirm previous spectroscopic studies implicating a side-on (μ-η^2:η^2-peroxo)dicopper(II) species preceding the hydroxylation of the phenol moiety.[5] Extensive work has been done to unravel the reaction mechanism of this enzyme.[6] The generally accepted mechanism



Scheme 1. Biological function of tyrosinase towards the final synthesis of melanins.

arising from these studies proposes that a (μ-η^2:η^2-peroxo)dicopper(II) species is responsible for the hydroxylation step via an electrophilic attack over the aromatic ring. That is, O–O cleavage occurs after or along with C–O bond formation.[3,7] Nevertheless, this proposal has been challenged by the observation that the (μ-η^2:η^2-peroxo)dicopper(II) core is usually in nearly degenerate equilibrium with its bis(μ-oxo)dicopper(III) isomer,[8] leading to the proposal that this species may be the actual executor of the arene hydroxylation step. Along these lines, Tolman and co-workers have shown that bis(μ-oxo)dicopper(III) species can effect intramolecular arene hydroxylation.[9] In addition, more recently, Stack and co-workers have reported that $[Cu^{II}_2(\mu\text{-}\eta^2\text{:}\eta^2\text{-}O_2)(DBED)]^{2+}$ (DBED = *N,N′*-di-*tert*-butylethylenediamine) undergoes an intermolecular reaction at −120 °C with phenolates that proceeds by initial phenolate binding and subsequent O–O bond breakage to form $[Cu^{III}_2(\mu\text{-}O)_2(\text{phenolate})(DBED)]^{+}$, which further decays by electrophilic *ortho*-hydroxylation of the phenolate to give the corresponding catechol.[10] These observations firmly establish that the bis(μ-oxo)dicopper(III) core is capable of eliciting tyrosinase-like reactivity when an aromatic ring is placed in close proximity to it. These results contrast with the radical-type reactivity exhibited by most bis(μ-oxo)dicopper(III) species when they are allowed to react with an external phenolate.[6b,11] A logical question arising from these studies is whether O–O bond breakage necessarily occurs only after phenolate binding, thus suppressing the radical-type reactivity until the aromatic ring is in close proximity to the core. Here we report

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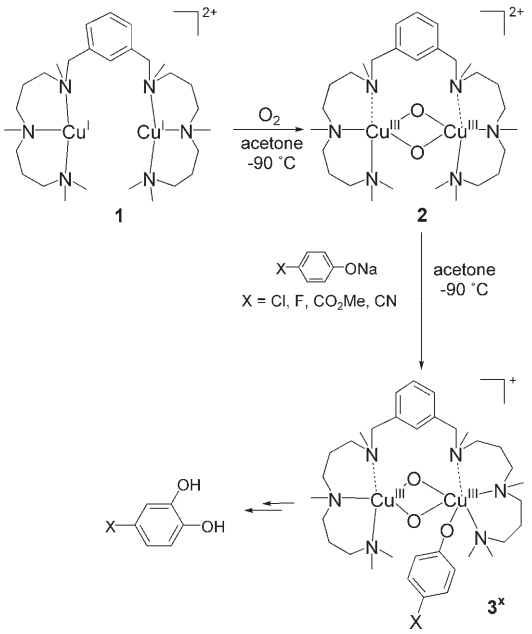
the first example of a bis(μ-oxo)dicopper(III) species that binds and then *ortho*-hydroxylates phenolates, thus mimicking the reactivity of tyrosinase. We have trapped and spectroscopically characterized a metastable species resulting from the low-temperature reaction of sodium *p*-chlorophenolate (*p*-Cl-C_6H_4ONa) with a bis(μ-oxo)dicopper(III) species preceding phenolate hydroxylation. Finally, kinetic analysis of the hydroxylation reaction has been performed to obtain activation parameters that can be compared with those found for tyrosinase.[12]

We recently found that the reaction of $[Cu^I_2(m\text{-}XYL^{MeAN})](SbF_6)_2$, **1**-$SbF_6$ (Scheme 2), with O_2 at −80 °C in



Scheme 2. The reactions described in this work.

THF resulted in the fast formation of a deep yellow species **2** that is characterized by two intense UV/Vis features at $\lambda_{max} = 308$ nm ($20\,000\ M^{-1}cm^{-1}$) and $\lambda_{max} = 413$ nm ($28\,000\ M^{-1}cm^{-1}$).[13] Resonance Raman experiments of frozen acetone solutions using laser excitation at 413 nm revealed a characteristic Cu_2O_2 breathing vibration peak at 600 cm^{-1} that showed a downshift of 23 cm^{-1} when $^{18}O_2$ was used. These are common spectral features for a $Cu^{III}_2(\mu\text{-}O)_2$ core[11,14] that led us to formulate **2** as $[Cu^{III}_2(\mu\text{-}O)_2(m\text{-}XYL^{MeAN})]^{2+}$.[13] Use of several solvents (THF, diethyl ether, CH_2Cl_2, or acetone) and counterions (ClO_4^-, $CF_3SO_3^-$, $BArF^-$, and SbF_6^-) did not change the nature of **2**, and no experimental evidence for the isomeric (μ-η^2:η^2-peroxo)dicopper(II) species was observed. This was further substantiated by DFT calculations at the B3LYP level, which indicated that the peroxo form was 35.5 kJ mol^{-1} higher in energy.[13] Extraction and analysis of the ligand after thermal decay of **2** did not show any evidence for ligand modification, indicating that intramolecular aromatic hydroxylation does not take place in this *meta*-xylyl-based system.

Nevertheless, addition of 10 equivalents of the sodium salt of *p*-chlorophenol at −90 °C caused immediate bleaching of the spectral features associated with **2**. Acidic work-up and HPLC analysis revealed that 4-chlorocatechol was formed in 67% yield with respect to the initial dicopper complex. The identity of the product was further confirmed by isolation with preparative HPLC and ^1H NMR analysis. Neither quinone nor C–C or C–O coupling products were obtained. Similar reactions with *p*-carbomethoxyphenolate and *p*-cyanophenolate also show the formation of the corresponding catechol as the sole oxidation product. Therefore, **2** constitutes a rare example of a bis(μ-oxo)dicopper(III) species capable of performing the phenolate hydroxylation to form a catechol, thus mimicking the activity exhibited by tyrosinase.

Insight into the reaction mechanism was obtained by trapping at very low temperature (−90 °C) a metastable reaction intermediate formed after reaction of **2** with sodium *p*-chlorophenolate (*p*-Cl-C_6H_4ONa). UV/Vis monitoring of the reaction in acetone showed that the initial features corresponding to the bis(μ-oxo) species (**2**) immediately disappear after phenolate addition (Figure 1, top). Concomitantly, new spectral features appear at 390 and 563 nm corresponding to a new species $\mathbf{3^{Cl}}$. The latter is thermally very sensitive and rapidly decomposes ($t_{1/2} \approx 20$ s) at −90 °C. Nevertheless, resonance Raman experiments (Figure 1, bottom) of frozen solutions of $\mathbf{3^{Cl}}$ with laser excitation at 407 nm show a resonance enhanced feature at 597 cm^{-1} that experiences a −26 cm^{-1} shift when $^{18}O_2$ is used in the generation of **2**. This feature is not enhanced when 568 nm laser excitation is used in the experiment. Moreover, no isotope-sensitive features that could be assigned to a (μ-η^2:η^2-peroxo)dicopper(II) species were observed in the 700–770 cm^{-1} region.[6a] On the other hand, laser excitation at 568 nm shows intense peaks at 1264, 1409, and 1642 cm^{-1}, characteristic of phenolate vibration modes.[15] These vibrational features are not affected by the use of $^{18}O_2$, and they are not enhanced with laser excitation at 407 nm. The Raman data thus provide direct evidence for phenolate binding to the Cu_2O_2 core in $\mathbf{3^{Cl}}$.

The accumulated data can be interpreted with two different scenarios. The first is that $\mathbf{3^{Cl}}$ is actually a mixture of residual bis(μ-oxo)dicopper(III) (**2**) and some type of copper–phenolate species. Alternatively, $\mathbf{3^{Cl}}$ may be formulated as $[Cu^{III}_2(\mu\text{-}O)_2(p\text{-}Cl\text{-}C_6H_4O)(m\text{-}XYL^{MeAN})]^+$, where bis(μ-oxo) and phenolate vibrations are uncoupled. We favor the latter hypothesis on the basis of the following observations. Kinetic analysis (vide infra) indicates that reaction of *p*-Cl-C_6H_4ONa is fast even for stopped-flow methodology and no residual **2** should be present under the experimental conditions used to prepare the resonance Raman sample. Also, the UV/Vis spectrum of $\mathbf{3^{Cl}}$ does not change upon varying the concentration of phenolate. Furthermore, the features associated with the bis(μ-oxo) core (390 nm) and with the phenolate (563 nm), decay with the same kinetic behavior (as monitored by UV/Vis spectroscopy). In addition, we have observed negligible perturbations in the energy of the Cu_2O_2 breathing mode in the resonance Raman spectra of

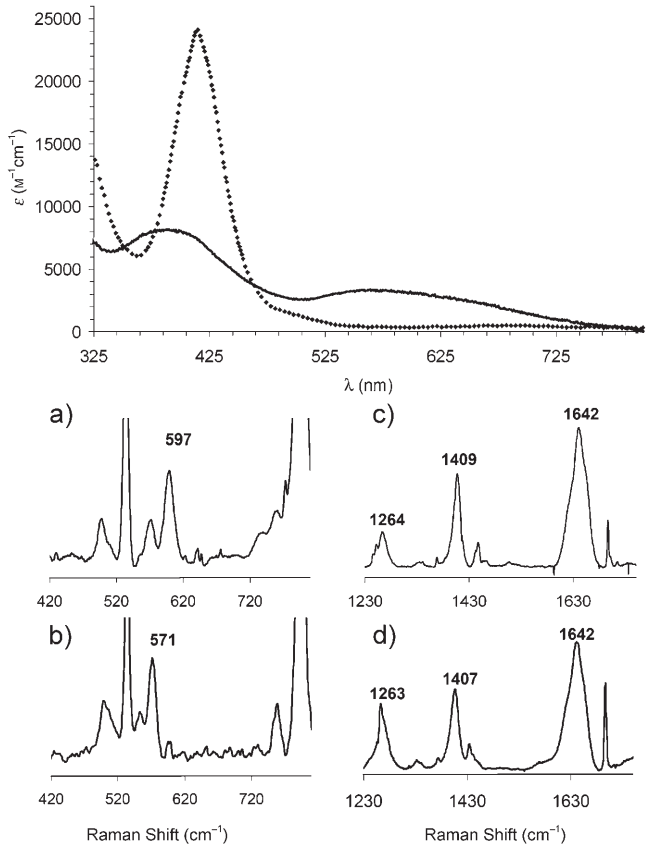


Figure 1. Top: UV/Vis spectra of **2** (dashed line) and $\mathbf{3^{Cl}}$ (solid line) in acetone at −90 °C. Experimental conditions: Complex $\mathbf{3^{Cl}}$ was generated by reaction of a 0.2 mM solution of **2** with 1.5 equivalents of *p*-Cl-C_6H_4ONa in acetone at −90 °C. Bottom: Resonance Raman spectra of $\mathbf{3^{Cl}}$ generated with $^{16}O_2$ (a) and $^{18}O_2$ (b) with laser excitation at 407 nm. Spectra of $\mathbf{3^{Cl}}$ generated with $^{16}O_2$ (c) and $^{18}O_2$ (d) with laser excitation at 568 nm.

the bis(μ-oxo) core in a related system upon coordination to a $CF_3SO_3^-$ group in acetone.[16] This observation can explain the similarity between the resonance Raman enhanced vibrations of the bis(μ-oxo) core in **2** and $\mathbf{3^{Cl}}$. Finally, the spectral features associated to $\mathbf{3^{Cl}}$ are reminiscent of those reported for the $[Cu^{III}_2(\mu\text{-}O)_2(\text{phenolate})(\text{DBED})]^+$ species recently described by Stack and co-workers.[10] Therefore, we conclude that $\mathbf{3^{Cl}}$ is best described as the phenolate adduct of the bis(μ-oxo) species **2** (Scheme 2).

Formation and decay of $\mathbf{3^{Cl}}$ were studied by UV/Vis stopped-flow methods. The reaction between **2** and the phenolate to form $\mathbf{3^{Cl}}$ is very fast ($k > 10^6\,M^{-1}s^{-1}$), too fast even for stopped-flow techniques at very low temperatures (−88 °C), and neither precise reaction rates nor activation parameters could be obtained for this process. On the other hand, kinetic analysis indicates that the decay of $\mathbf{3^{Cl}}$ is a first-order process. The analogous species $\mathbf{3^X}$ (X = F, CO_2Me and CN) were generated by addition of 1.5 equivalents of *p*-X-C_6H_4ONa to **2** at −80 °C in acetone (see Supporting Information for UV/Vis spectral features), and their corresponding decay rates were studied by UV/Vis and fitted to a single exponential function by nonlinear regression methods. Plotting the rate of decay of $\mathbf{3^X}$ against the corresponding Hammett substituent constants (σ^+) affords a linear correlation ($R^2 = 0.99$) that gives a ρ value of −1.9 for the hydroxylation step (Figure 2), indicative of an electrophilic oxidizing

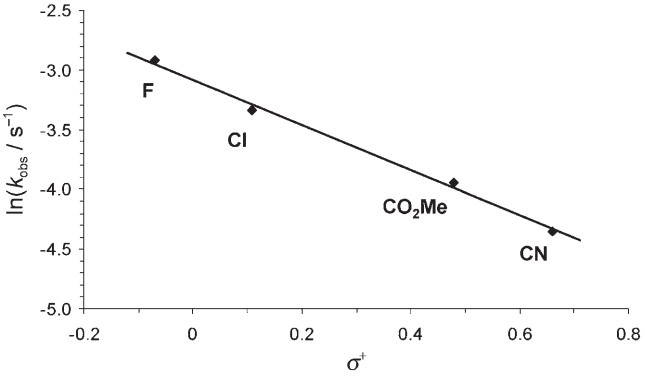


Figure 2. Hammett plot for the thermal decay of $\mathbf{3^X}$ at −80 °C in acetone (X = F, Cl, CO_2Me and CN). [**2**] = 0.050 mM, [*p*-X-C_6H_4ONa] = 0.075 mM.

species that attacks the aromatic ring. This value is in close proximity to the value reported for $[Cu^{III}_2(\mu\text{-}O)_2(\text{phenolate})(\text{DBED})]^+$ ($\rho = -2.2$),[10] selected model compounds with (μ-η^2:η^2-peroxo)dicopper(II) cores ($\rho = -1.8$ to -2.1),[11b,17] and to that found in tyrosinase ($\rho = -2.4$) (Table 1).[12]

Finally, kinetic analysis of the thermal decay of $\mathbf{3^{Cl}}$ in the −88.5 °C to −60 °C temperature range affords activation parameters for the hydroxylation step. Activation parameters for the reaction are characterized by a negative activation entropy and a relatively small activation enthalpy. Interestingly, the $\Delta S^{\neq}$ value is significantly smaller than that reported for intermolecular phenol hydroxylation by $[Cu^{II}_2(\text{MeL66})(O_2)]^{2+}$, but more closely related to the intramolecular arene hydroxylation exhibited by (μ-η^2:η^2-peroxo)dicopper(II) complexes supported by *m*-xylyl-bridged bis(2-pyridylethyl)amine $[Cu^{II}_2(\text{R-XYL})(O_2)]^{2+}$[19] and $^{i\text{Pr}}$TACN $[Cu^{II}_2(m\text{-}XYL^{i\text{Pr}4})(O_2)]^{2+}$ chelates.[20] Presumably, phenolate binding and hydroxylation are not completely independent reactions in the kinetic study of $[Cu^{II}_2(\text{MeL66})(O_2)]^{2+}$ and thus kinetic parameters for the hydroxylation are likely to

Table 1. Kinetic parameters for the hydroxylation of phenols by tyrosinase and selected model compounds.

Compound[a]	ρ	$\Delta H^{\neq}$ [kJ mol^{-1}]	$\Delta S^{\neq}$ [J K^{-1} mol^{-1}]	Ref.
$\mathbf{3^{Cl}}$	−1.9	37.1 ± 0.5	−55 ± 3	–
tyrosinase	−2.4	61 ± 9	−24/+21 ± 11	[12]
$[Cu^{II}_2(\text{DBED})_2(O_2)]^{2+}$	−2.2	[b]	[b]	[10]
$[Cu^{II}_2(\text{MeL66})(O_2)]^{2+}$	−1.8	29.1 ± 3.0	−115 ± 15	[17,18]
$[Cu^{II}_2(L^{Py2Bz})_2(O_2)]^{2+}$	−1.8	n.d.[c]	n.d.[c]	[12a]
$[Cu^{II}_2(\text{R-XYL})(O_2)]^{2+}$	−2.1	50 ± 1[d]	−35 ± 2[d]	[19]
$[Cu^{II}_2(m\text{-}XYL^{i\text{Pr}4})(O_2)]^{2+}$	–	50.1 ± 0.2	−50.4 ± 0.9	[20]

[a] See Supporting Information for a structural diagram of the complexes. [b] An Arhenius plot affords $E_a = 42.7$ kJ mol^{-1}, $A = 9 \times 10^{11}$. [c] Not determined. [d] R = H.

be influenced by bimolecular substrate binding. In addition, although the $\Delta S^{\neq}$ term is expected to be large and negative due to immobilization of the copper-bound phenolate in the transition state, the different structures of the Cu_2O_2 complexes may involve differences in phenolate binding and in the degree of immobilization. For instance, the four benzimidazole rings in complex $[Cu^{II}_2(MeL66)(O_2)]^{2+}$ are likely to participate in stacking interactions with the phenolate aromatic ring. In our case, phenolate binds rapidly and irreversibly in the first step, and first-order intramolecular decomposition of **3^{Cl}** occurs in the second step (for which the activation parameters were determined). On the other hand, the $\Delta H^{\neq}$ term determined for **3^{Cl}** is remarkably similar to that obtained for $[Cu^{III}_2(\mu\text{-}O)_2(phenolate)(DBED)]^+$ ($\Delta H^{\neq} = 40.3\ kJ\,mol^{-1}$, derived from $E_a = 10.2\ kcal\,mol^{-1}$).[10] Overall, the values obtained for **3^{Cl}** are in close proximity to those reported for aromatic hydroxylations by (μ-η^2:η^2-peroxo)dicopper(II) species and also $[Cu^{III}_2(\mu\text{-}O)_2(phenolate)(DBED)]^+$ (Table 1), which may suggest a coincident transition state in all cases. The activation parameters for the monophenolase reaction catalyzed by tyrosinase are different (Table 1).[12] In this case, a large $\Delta H^{\neq}$ value, and the analogy with the data for the diphenolase reaction, suggest that O–O cleavage is the main contributor to the enthalpic barrier, whereas small and substrate-dependent $\Delta S^{\neq}$ values indicate strong preorganization and complementarity of the active site with the transition state configuration of the reactants.[12]

In conclusion, we demonstrate for the first time that a bis(μ-oxo)dicopper(III) species is competent for binding and hydroxylating phenolates, and thus mimicking tyrosinase. Kinetic parameters establish a close similarity between our system and the (μ-η^2:η^2-peroxo)dicopper(II) species capable of performing aromatic hydroxylation. Complex **2** differs from any previously reported system in the fact that exclusive formation of bis(μ-oxo)dicopper(III) species is observed, before and after phenolate binding to the Cu_2O_2 site. This work further substantiates the notion that the bis(μ-oxo) core is competent for performing tyrosinase-like activity.

Experimental Section

Full experimental details for the preparation of the complexes, experimental procedures for the phenolate oxidation reactions, resonance Raman analysis, and kinetic analyses are included as Supporting Information.

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