Oxygenation of Phenols to Catechols by A (μ-η^2:η^2-Peroxo)dicopper(II) Complex: Mechanistic Insight into the Phenolase Activity of Tyrosinase

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Tyrosinase is a copper monooxygenase that catalyzes oxygenation of phenols to catechols (phenolase activity) and the subsequent two-electron oxidation of catechols to the corresponding *o*-quinones (catecholase activity).[1] Chemical and spectroscopic studies have indicated that the enzyme has a dinuclear copper active site nearly identical to that found in hemocyanin,[1,2] where a side-on type (μ-η^2:η^2) peroxo species[3] is generated by the reaction of the reduced dicopper(I) form and O_2.[1] As a pioneering work by Karlin and co-workers in Cu/O_2 chemistry, aromatic ligand hydroxylation in a dinuclear Cu(I) complex by O_2 was first reported in early 1980s.[4] The mechanistic studies have indicated that the aromatic ligand hydroxylation reaction involves an *electrophilic attack* on the arene ring by a (μ-η^2:η^2-peroxo)-dicopper(II) intermediate.[5] After their finding, several examples of the aromatic ligand hydroxylation have been reported using similar type of *m*-xylyl dinucleating ligands.[6] With respect to the intermolecular reactions between phenols and the peroxo intermediate, however, most of the reactions so far reported afford a C−C coupling dimer as a major product.[7] Casella and co-workers have recently reported the first synthetic (μ-η^2:η^2-peroxo)dicopper-(II) complex which can react with an exogenous phenolate to yield the corresponding catechol.[8,9] Unfortunately, the low yield of the product (20% based on the dicopper complex) has precluded the kinetic and mechanistic investigation on the reaction between the peroxo intermediate and the phenolate.[8] As such, the mechanism for the catechol formation via intermolecular reactions between the peroxo intermediate and phenol derivatives has yet to be clarified.[10]

We report herein that efficient conversion of phenol derivatives to the corresponding catechols is achieved for the first time by intermolecular reactions of a (μ-η^2:η^2-peroxo)dicopper(II) complex, supported by tridentate ligand $\mathbf{L^{Py2Bz}}$ (*N*,*N*-bis[2-(2-pyridyl)-ethyl]-α,α-dideuteriobenzylamine),[11] with lithium salts of phenols. The mechanistic studies on the catechol formation have been performed to provide valuable mechanistic insight into the phenolase activity of the enzyme.

Treatment of the copper(I) complex, $[Cu^I(\mathbf{L^{Py2Bz}})](PF_6)$, with dioxygen in anhydrous acetone at −94 °C afforded a brown color solution which exhibited a strong absorption band at 364 nm (ϵ = 26400 M^{-1} cm^{-1}) together with a small one at 530 nm (1500 M^{-1} cm^{-1}) and a resonance Raman band at 737 cm^{-1} that shifted to 697 cm^{-1} upon ^{18}O-substitution.[12,13] The frozen acetone solution of the intermediate was ESR silent at 77 K, and a $Cu:O_2$ = 2:1 stoichiometry was obtained for formation of the intermediate by manometry. These results unambiguously indicate that the oxygenated intermediate is a (μ-η^2:η^2-peroxo)dicopper(II) complex as suggested previously by Karlin et al.[11] This compound is quite stable (no self-decomposition) at the low-temperature enabling us to examine the reaction with external substrates.

Addition of lithium salts of *p*-substituted phenols (*p*-X-C_6H_4-OLi; X = Cl, Me, and CO_2Me) to the solution of the peroxo complex resulted in a spectral change shown in Figure 1A, where the absorption bands due to the peroxo species decreased obeying pseudo-first-order kinetics (see the inset of Figure 1A).[14] From the final reaction mixture in a preparative-scale was obtained the catechol derivatives in fairly good isolated yields (90, 69, and 60% based on the peroxo intermediate for X = Cl, Me, and CO_2-Me, respectively), but neither the corresponding *o*-quinone

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(12) When excited at 406.7 nm, the Raman band at 581 cm^{-1} (553 cm^{-1} upon ^{18}O-substitution) due to a bis(μ-oxo)dicopper(III) complex was also detected in addition to the Raman band of the peroxo intermediate [ν_{O-O} ($^{16}O_2$) = 737 cm^{-1}, ν_{O-O} ($^{18}O_2$) = 697 cm^{-1}]. Those Raman bands due to the bis(μ-oxo)dicopper(III) complex became negligibly smaller when the solution was excited at 514.5 nm. According to the Solomon's paper on a similar tridentate ligand system (Pidcock, E.; DeBeer, S.; Obias, H. V.; Hedman, B.; Hodgson, K. O.; Karlin, K. D.; Solomon, E. I. *J. Am. Chem. Soc.* **1999**, *121*, 1870−1878), the content of the bis(μ-oxo) species in solution may be less than a few percent.
(13) See Supporting Information.
(14) To prevent side reactions (autoxidation of the products), excess O_2 was completely removed by bubbling Ar gas into the solution of the (μ-η^2:η^2-peroxo)dicopper(II) complex for 15 min before addition of the phenolate substrates. During the bubbling of Ar gas, no spectral change of the peroxo complex was observed at −94 °C.

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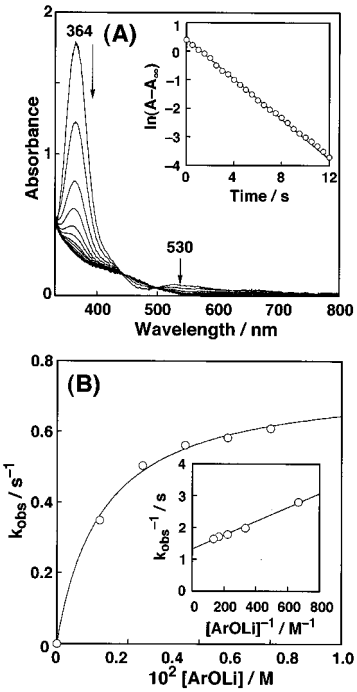
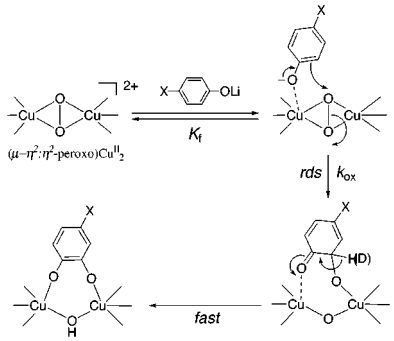


Figure 1. (A) Spectral change for the reaction of $[Cu^{II}_2(\mu\text{-}O_2)(\mathbf{L}^{\mathbf{Py2Bz}})_2]^{2+}$ (7.5×10^{-5} M) in acetone at −94 °C with lithium 4-chlorophenolate (1.5×10^{-3} M). Interval: 1.5 s. Inset: first-order plot based on the absorption change at 364 nm. (B) Plot of k_{obs} vs [*p*-Cl-C_6H_4OLi]. Inset: plot of k_{obs}^{-1} vs [*p*-Cl-C_6H_4OLi]$^{-1}$.

derivative nor the C−C or C−O coupling dimer[9f] was obtained from the final reaction mixture.[13,14] Thus, the catechols are formed as a solely isolable product nearly quantitatively by intermolecular reactions between the peroxo intermediates and the phenolates. Isotope labeling experiment using $^{18}O_2$ has confirmed that the origin of one of the two oxygen atoms of the catechol product is molecular oxygen.[13] In contrast to such an efficient catechol formation by the peroxo complex, no catechol was formed when a bis(μ-oxo)dicopper(III) complex supported by a bidentate ligand $\mathbf{L}^{\mathbf{Py1Bz}}$ (*N*-ethyl-*N*-[2-(2-pyridyl)ethyl]-α,α-dideuteriobenzylamine) was employed under the same experimental conditions.[15]

The pseudo-first-order rate constant k_{obs} was then plotted against the substrate concentration to afford a Michaelis−Menten type saturation curve as shown in Figure 1B. This indicates a complex formation between the substrate and the peroxo intermediate in the course of the reaction. Such a complex formation prior to the oxygenation together with the absence of the C−C coupling product rules out an electron-transfer pathway from the substrate to the peroxo intermediate to produce a free radical species.[16] From the double reciprocal plot according to the equation of $1/k_{obs} = 1/k_{ox} + (1/K_f k_{ox})(1/[\text{ArOLi}])$ (Lineweaver−Burk type plot) was obtained the formation constant K_f and the rate constant k_{ox} for the oxidation process as follows: for X = Cl, $K_f = 570$ M^{-1}, $k_{ox} = 0.76$ s^{-1}; for X = CO_2Me, $K_f = 940$ M^{-1}, $k_{ox} = 0.083$ s^{-1}.[17] The observed rate constant for *p*-Me-C_6H_4OLi (X = Me) was, however, too fast to be determined accurately. Thus, the reactivity of the substrate increases drastically with increasing the electron-donating ability of the *p*-substituent, whereas the K_f values are rather insensitive to the electronic effects of the *p*-substituents. In addition, no kinetic deuterium isotope effect has been observed as in the case of the enzymatic reaction,[1] when *p*-Cl-C_6D_4OLi was used instead of *p*-Cl-C_6H_4OLi as the substrate ($k_{ox}^H/k_{ox}^D = 1 \pm 0.1$).

Scheme 1



So far, an *electrophilic aromatic substitution* reaction by the side-on type peroxo intermediate has been suggested as a possible reaction mechanism for the phenolase activity of tyrosinase.[1,18] Our present results are consistent with such an ionic mechanism involving a rate-determining *electrophilic attack* of the (μ-η^2:η^2-peroxo)dicopper(II) intermediate to the phenolate ring in a binary complex as illustrated in Scheme 1. The electrophilic reactivity of the peroxo complex shows a sharp contrast with the well-established radical character (hydrogen atom abstraction) of the bis(μ-oxo)dicopper(III) complex.[19] Furthermore, it is obvious that deprotonation of the substrate prior to the reaction is essential, since only the C−C coupling dimer was obtained when the phenol itself was used instead of the phenolate as the exogenous substrate.[16] This suggests that deprotonation of the phenol substrates may also occur in the substrate-binding process of the enzymatic system.

In conclusion, the (μ-η^2:η^2-peroxo)dicopper(II) complex supported by $\mathbf{L}^{\mathbf{Py2Bz}}$ exhibits tyrosinase-like activity, that is, nearly quantitative conversion of exogenous phenolates to the corresponding catechols via an *electrophilic aromatic substitution mechanism*, where the oxygenation of the substrate (C−O bond formation) occurs along with the O−O bond cleavage of the peroxo intermediate (Scheme 1).[1,20] Further studies are now in progress to obtain more information about the mechanistic details.

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Supporting Information Available: Experimental details including synthetic procedures, resonance Raman measurements, product analysis, manometry, and kinetic measurements (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Details about the formation and characterization of the bis(μ-oxo)-dicopper(III) complex supported by $\mathbf{L}^{\mathbf{Py1Bz}}$ will be reported elsewhere; Taki, M.; Itoh, S.; Fukuzumi, S.

(16) The C−C coupling dimer became the major product when the phenol itself was used instead of the phenolate as in the case of other peroxo systems.[7] In such a case, hydrogen atom abstraction from the phenol occurs to produce the corresponding phenoxyl radical leading to the C−C coupling reaction.

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