Kinetic Evaluation of Dioxygen Activation Mechanism by Dicopper Enzymes

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Tyrosinase is a copper monooxygenase with a coupled dinuclear copper active site (type-3 copper), catalyzing phenol-oxygenation (phenolase activity) as well as catechol-dehydrogenation (catecholase activity) using O_2 as the oxidant.[1] In this study, a very simple tyrosinase reaction system has been developed using borate ion as a trapping agent of catechols and hydroxylamine as an external reductant in order to evaluate the phenolase activity without the interference of catecholase activity.[2] Reactivities of variously *p*-substituted phenols in this system were compared directly to those of the phenols in the model reactions, demonstrating that the enzymatic oxygenation reaction of phenols proceeds via the same mechanism as the model reaction, that is, electrophilic aromatic substitution mechanism. Catalase activity and peroxygenase activity of mushroom tyrosinase have also been examined kinetically by using amperometric O_2- and H_2O_2-sensors.[3] It has been found that the catalase activity of mushroom tyrosinase is three-order of magnitude greater than that of octopus hemocyanin. The higher catalase activity of tyrosinase could be attributed to easier accessibility of H_2O_2 to the dinuclear copper site of tyrosinase. Mushroom tyrosinase has also been demonstrated for the first time to catalyze oxygenation reaction of phenols with H_2O_2 (peroxygenase activity). The reaction has been investigated kinetically in 0.5 M borate buffer (pH 7.0) under aerobic conditions. Similarity of the substituent effects of a series of *p*-substituted phenols in the peroxygenase reaction with H_2O_2 to those in the native phenolase reaction by O_2 as well as no kinetic deuterium isotope effect with a perdeuterated substrate (*p*-Cl-C_6D_4OH vs. *p*-Cl-C_6H_4OH) clearly demonstrated that the oxygenation mechanism of both reactions are the same, that is the electrophilic aromatic substitution reaction by a (μ-η^2:η^2-peroxo)dicopper(II) intermediate of oxy-tyrosinase.

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[2] Yamazaki, S.; Itoh, S. *J. Am. Chem. Soc*. **2003**, *125*, 13034–13035.
[3] Yamazaki, S.; Morioka, C.; Itoh, S. *Biochemistry* **2004**, *43*, 11546–11553.