Tyrosinase: Recent Studies of Structure and Mechanism

Christine Dickerson   Literature Seminar   October 1, 2002

Copper is present in trace amounts in living organisms[1,2] and its primary function is to facilitate electron transfer.   Copper proteins are divided into three types based on their spectroscopic properties. Tyrosinase, a type 3 copper protein, is found in bacteria, plants and animals.[4] It functions to hydroxylate phenol to the respective catechol and subsequently to oxidize the catechol to an o-quinone (Figure 1).[1-4] Quinones may autopolymerize to produce polyphenolic catechol melanins,[1,4,6] and mutations in tyrosinase are thought to contribute to albinism (lack of melanin) in animals. Tyrosinase is also a contributing factor in the brown coloration in plants.[5]
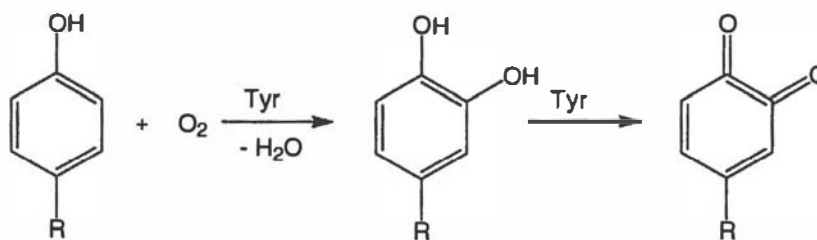

Figure 1. Reactions catalyzed by tyrosinase

There is no crystal structure of the active site of tyrosinase so reactivity studies and spectroscopic data have been used to elucidate the structure and the catalytic mechanism.[1-6] The structure of hemocyanin, also a type 3 copper protein, is known and hemocyanin has spectroscopic characteristics similar to those of tyrosinase.[6] Therefore, the active site of tyrosinase is assumed to be similar to that of hemocyanin, which provides a critical structural model, namely the presence of a μ-η^2:η^2-peroxodicopper (II) unit for comparative studies.

The mechanism by which an oxygen atom is transferred to the phenol substrate is proposed to begin with either a μ-η^2:η^2-peroxodicopper (II) intermediate or a bis-μ-oxodicopper (III) intermediate.[1,2,6,8] These intermediates have characteristic EPR and resonance Raman spectra. Synthetic studies provide models of both complexes and evidence for rapid equilibrium between the two forms. [8-12] However, the presence of the peroxo core is observed in the crystal structure of hemocyanin, whereas the bis-μ-oxo core has not been identified in a copper protein. Furthermore, density functional calculations (DFT) of model systems provide evidence that the peroxo complex is more stable then the bis-μ-oxodicopper unit.[3,9] Based on reactivity and DFT data, the peroxo complex is currently the most widely accepted species for the oxygen transfer intermediate.

Using the information known about the active site of hemocyanin, structural models can be synthesized that possess tyrosinase-like spectroscopic characteristics.[10-12] An example of a recent structural model is a mononuclear copper precursor which, when exposed to O_2, forms a binuclear compound: $[\{Cu(II)(MeAN)\}_2(O_2)]^{2+}$

5

(MeAN=N,N,N',N',N''-pentamethyldipropylenetriamine) (Figure 2).[9] The structure of the model was confirmed by resonance Raman spectroscopy and UV-Vis studies. The balance between the $\mu\text{-}\eta^2{:}\eta^2$-peroxodicopper (II) and the bis-μ-oxodicopper(III) species was shown to be sensitively dependent on both solvent and ligand substituents.

$\mu\text{-}\eta^2{:}\eta^2$-peroxodicopper (II)   R= H, Me   bis-μ-oxodicopper (III)
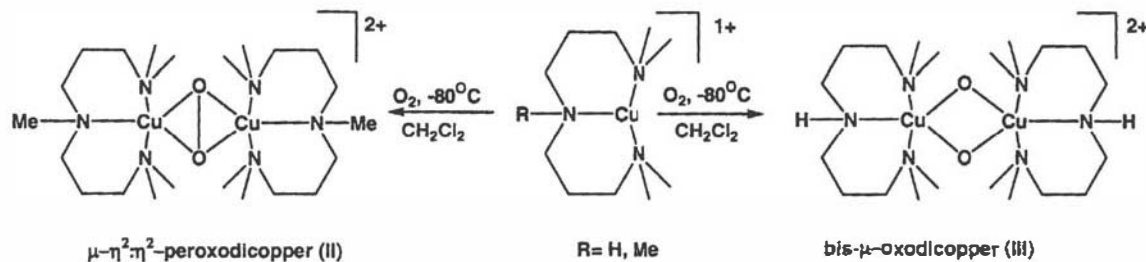
Figure 2. Structural proposals for $[\{Cu(II)(MeAN)\}_2(O_2)]^{2+}$ ($\mu\text{-}\eta^2{:}\eta^2$-peroxodicopper (II)) and of $[\{Cu(II)(AN)\}_2(O_2)]^{2+}$ (bis-μ-oxodicopper(III)).

Recent model systems display reactivity patterns similar to that of tyrosinase as monitored through UV-Vis, resonance Raman, kinetic, and XAS studies.[7,13] These model compounds catalyze either the hydroxylation of monophenols or the oxidation of catechols to *o*-quinones. The functional model $[\{Cu(II)(DBED)\}_2(\mu\text{-}\eta^2{:}\eta2\text{-}O_2)](CF_3SO_3)_2$, (DBED=di-*tert*-butylethylenediamine) reacts efficiently with phenolate to give a mixture of catechol and quinone (Figure 3). The product formation was characterized by NMR and GC-MS. Isotopic labeling ($^{18}O_2$) was used to show that transfer of an oxygen atom to the catechol and the quinone was from a bound O_2 molecule.
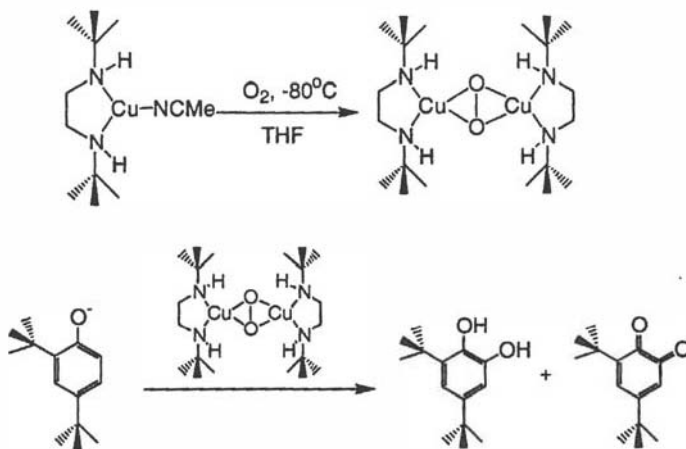
Figure 3. The reaction of $[\{Cu^{II}(DBED\}_2(O_2)]^{2+}$ with 3,5-di-*tert*-butylcatechol

Structural and functional model systems of tyrosinase provide a basis for chemists to learn about dioxygen activation by binuclear copper proteins. Model systems such as

$Cu(tacn)Cl_2$ which in the presence of oxygen forms a peroxodicopper(II) complex, are potential industrial applications of tyrosinase model complexes.[14]

References:

1.   Solomon, E.I.; Sundaram, U. M.; Machonkim, T. E. "Multicopper Oxidases and Oxygenases," *Chem. Rev.* **1996,** *96*, 2563-2605.

2.   Solomon, E. I.; Chen, P.; Metz, M.; Lee, S.-Kyu; Palmer, A. E. "Oxygen Binding, Activation, and Reduction to Water by Copper Proteins," *Angew. Chem. Int. Ed.* **2001,** *40*, 4570-4590.

3.   Holm, R. H.; Kennepohl, P.; Solomon, E. I. "Structural and Functional Aspects of Metal Sites in Biology," *Chem. Rev.* **1996,** *96*, 2239-2314.

4.   Lind, T.; Siegbahn, P. E. M.; Crabtree, R. H. "A Quantum Chemical Study of the Mechanism of Tyrosinase," *J. Phys. Chem. B* **1999,** *103*, 1193-1202.

5.   Decker, H.; Dillinger, R.; Tuczek, F. "How Does Tyrosinase Work? Recent Insights from Model Chemistry and Structural Biology," *Angew. Chem. Int. Ed.* **2000,** *39*, 1591-1595.

6.   Decker, H.; Tuczek, F. "Tyrosinase/Catecholoxidase Activity of Hemocyanins: Structural Basis and Molecular Mechanism," *TIBS* **2000,** *25*, 392-397.

7.   Mirica, L. M.; Vance, M.; Rudd, D. J.; Hedman, B.; Hodgson, K. O., Solomon, E. I., Stack, T.D. P. "A Stabilized μ-η^2:η^2-Peroxodicopper(II) Complex with a Secondary Diamine Ligand and Its Tyrosinase-like Reactivity," *J. Am. Chem. Soc.* **2002,** *124*, 9332-9333.

8.   Siegbahn, P. E. M.; Wirstam, M.   "Is the Bis-μ-Oxo Cu_2 (III, III) State an Intermediate in Tyrosinase?" *J. Am. Chem. Soc.* **2001,** *123*, 11819-11820.

9.   Liang, H.-C; Zhang, C. X.; Henson, M. J.; Sommer, R. D.; Hatwell, K. R.; Kaderli, S.; Zuberbühler, A. D.; Rheingold, A. L.; Solomon, E. I.; Karlin, K. D. "Contrasting Copper-Dioxygen Chemistry Arising from Alike Tridentate Alkyltriamine Copper (I) Complexes," *J. Am. Chem. Soc.* **2002,** *124*, 4170-4171.

10.   Holland, P. L.; Tolman, W. B. "Dioxygen Activation by Copper Sites: Relative Stability and Reactivity of (μ-η^2:η^2-peroxo) and Bis(μ-oxo)dicopper Cores," *Coord. Chem. Rev.* **1999,** 855-869.

11.   Mahadevan, V.; Henson, M. J.; Solomon, E. I.; Stack, T. D. P. "Differential Reactivity between Interconvertible Side-On Peroxo and Bis-μ-oxodicopper

Isomers Using Peralkylated Diamine Ligands," *J. Am. Chem. Soc.* **2000**, *122*, 10249-10250.

12.   Que, L.; Tolman, W.B. "Bis(μ-oxo)dimetal "Diamond" Cores in Copper and Iron Complexes Relevant to Biocatalysis," *Angew. Chem. Int. Ed.* **2002**, *41*, 1114-1137.

13.   Itoh, S.; Kumei, H.; Taki, M.; Nagatomo, S.; Kitagawa, T.; Fukuzumi, S. "Oxygenation of Phenols to Catechols by A (μ-η^2:η^2-Peroxo)dicopper(II) Complex: Mechanistic Insight into the Phenolase Activity of Tyrosinase," *J. Am. Chem. Soc.* **2001**, *123*, 6708-6709.

14.   Higashimura, H.; Fujisawa, K.; Moro-oka, Y.; Namekawa, S.; Kubota, M.; Shiga, A.; Uyama, H.; Kobayashi, S. "New Crystalline Polymers: poly(2,5-dialkyl-1,4-phenylene oxide)s," *Macromol. Rapid Commun.* **2000**, *21*, 1121-1124.