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Review

# Small-molecule models of tyrosinase: From ligand hydroxylation to catalytic monooxygenation of external substrates

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ABSTRACT

The reactivities of tyrosinase and catechol oxidase have been of continued interest during the last decades. To gain mechanistic insights into the conversion of mono- and diphenols by these type 3 copper enzymes, a large range of model systems has been developed. This review describes our newest results in this field. Our approach involves with the synthesis of mono- and dinucleating ligands based on imine and/or different heterocyclic groups. The influence of the ligand framework on the catalytic conversion of external substrates is investigated. Besides catalytic systems we also investigated new dicopper complexes exhibiting ligand hydroxylation reactions. The implications of these results on the mechanism of tyrosinase and catechol oxidase are discussed.



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## 1. Introduction

The ubiquitous biopolymer melanin is found in plants, animals, fungi and bacteria. It is involved in all kinds of biological pigmentation processes [1–3] such as the coloring of hair, eyes and skin [4,5] and the browning of fruits and vegetables [6]. Moreover, it protects the skin from damage caused by ultraviolet (UV) or ionizing radiation [2,7,8], removes reactive oxygen species [2,9], and plays an important role in wound healing and the immune response [10–13]. The color of melanin varies from yellow to black.

*Abbreviations:* BIMZ, bis(1-methylimidazol-2-yl)methane; BPM, 1,1′-methylenebis-1*H*-pyrazole; CO, catechol oxidase; CT, charge transfer; dmBPM, 1,1′-methylenebis(3,5-di-methyl-1*H*-pyrazole; DFT, density functional theory; L-DOPA, 3,4-dihydroxy-L-phenylalanine; 2,4-DTBP-H, 2,4-di-*tert*-butyl-phenol; 3,5-DTBC-$H_2$, 3,5-di-*tert*-butylcatechol; 3,5-DTBSQ, 3,5-di-*tert*-butyl-semiquinone; 3,5-DTBQ, 3,5-di-*tert*-butyl-*ortho*-quinone; Hb, hemoglobin; Hc, hemocyanin; mBPM, 1,1′-methylenebis(3-methyl-1*H*-pyrazole; MeBA-OH, 4-hydroxybenzoic acid methyl ester; 4-MeP-H, 4-methylphenol; NATEE, *N*-acetyl-L-tyrosine ethyl ester; 4-OMeP-H, 4-methoxyphenol; P-H, phenol; PPO, polyphenol oxidase; rRaman, resonance Raman; 3-TBP-H, 3-*tert*-butyl-phenol; TOF, turnover frequency; TON, turnover number; Ty, Tyrosinase.

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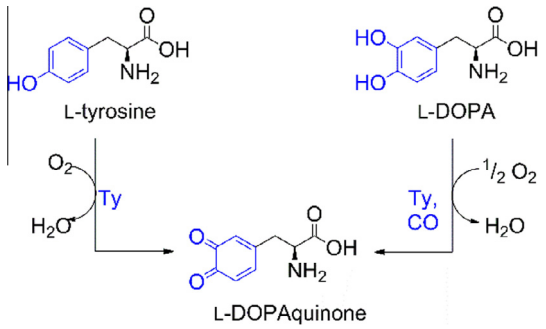


**Scheme 1.** Tyrosinases catalyze the *ortho*-hydroxylation of the amino acid L-tyrosine and the subsequently two-electron oxidation to L-DOPAquinone. The related enzymes catechol oxidases only catalyze the oxidation step.

Correspondingly, it is classified into two forms, (i) eumelanin and (ii) sulphurous pheomelanin [1,2].

### 1.1. Type 3 copper enzymes

The type 3 copper enzyme tyrosinase (Ty) catalyzes the initial step of melanogenesis corresponding to the conversion of the amino acid L-tyrosine to L-DOPAquinone. This reaction involves both hydroxylation and two-electron oxidation of the substrate, accompanied by the formation of water (monophenolase activity; Scheme 1 left). The subsequent spontaneous polymerization of *ortho*-quinone leads to the biopolymer melanin [3,13–18]. An overproduction of the enzyme causes miscellaneous dermatological disorders such as melanosis and age spots which are related to hyperpigmentation [18,19]. Mutations in tyrosinase are also associated with the formation of malignant melanoma [2,8]. Absence or damage of tyrosinase, on the other hand, lead to oculocutaneous albinism type 1 [5].

The related type 3 copper enzyme catechol oxidase (CO) catalyzes the two-electron oxidation of L-DOPA to L-DOPAquinone or, more generally, the two-electron oxidation of catechols to the corresponding *ortho*-quinones (diphenolase activity; Scheme 1 right) [3,14,15]. In contrast to tyrosinase [3,15–17,20,21], catechol oxidase cannot mediate the hydroxylation step involved in monophenolase activity.

Tyrosinases and catechol oxidases are both referred to as polyphenol oxidases (PPOs) [3,13,15]. The third class of type 3 copper proteins comprises hemocyanin (Hc) which functions as oxygen carrier protein in molluscs and arthropods [22]. The electronic absorption bands of *oxy*-Hc cause the characteristic blue color of the hemolymph of molluscs and arthropods [3].

### 1.2. Active site of type 3 copper enzymes

The active site of the type 3 copper enzymes Ty, CO and Hc contains two copper centers, $Cu_A$ and $Cu_B$, both of which are coordinated by three histidines. During the monophenolase and diphenolase catalytic cycles, the copper ions pass through three different states, *deoxy* (two $Cu^I$ ions/$[Cu^I_2]^{2+}$), *oxy* ($[Cu^{II}_2O_2]^{2+}$), and *met* (two $Cu^{II}$ ions bridged by a hydroxide ligand/$[Cu^{II}_2(OH)]^{3+}$). In case of deactivation by a phenolic oxidation mechanism (suicide-inactivation), *deact*-Ty (one copper $Cu^0$ and the second $Cu^{II}$) is formed [3,14,18,23–25].

Both enzymatic cycles start with the binding of dioxygen. Thereby, the two copper ions of the active site are oxidized from $Cu^I$ (*deoxy*) to $Cu^{II}$ (*oxy*) [3,16,26], and dioxygen is coordinated as peroxide in a characteristic side-on bridging ($\mu$-$\eta^2$:$\eta^2$) coordination. In the *oxy* form, the copper centers are strongly antiferromagnetically coupled. Two spectral features appear in the UV/Vis absorption spectrum, an intense band at ~345 nm and a second less intense absorption band at ~580 nm, both of which related to the peroxo → $Cu^{II}$ charge transfer (CT) transitions. The O—O stretching vibration of the $Cu_2O_2$ species is observed at ~750 $cm^{-1}$ in the resonance Raman spectrum [3,16].

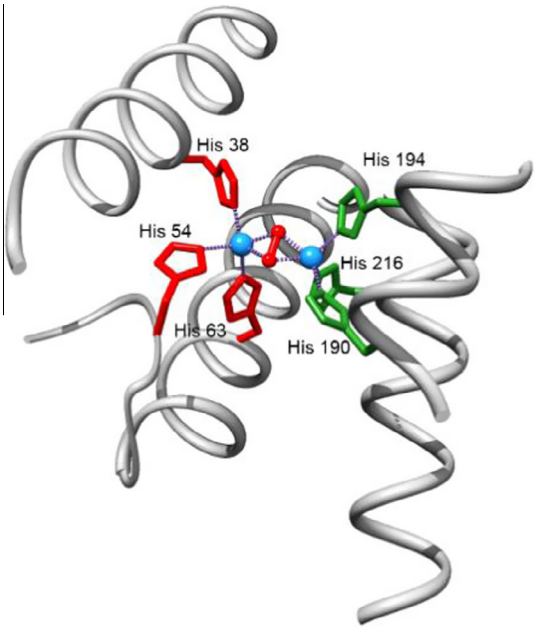


**Fig. 1.** Crystal structure of the active site of tyrosinase (*oxy*-form) isolated from the bacterium *S. castaneoglobisporus* [27]. Each copper ion is coordinated by three histidine residues provided by α-helices.

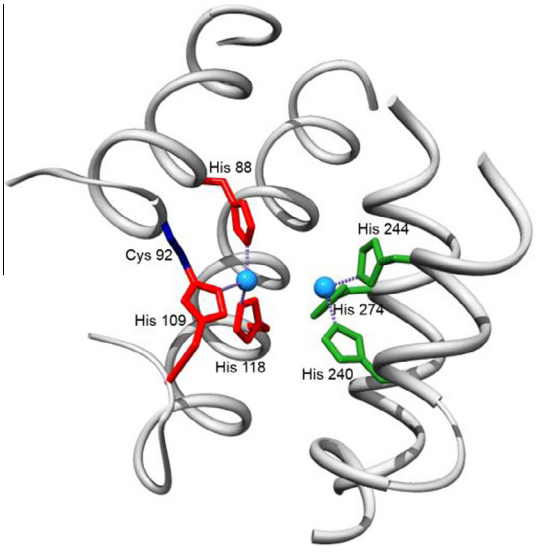


**Fig. 2.** Active site of catechol oxidase isolated from *Ipomoea batatas* [20].

In 2006, Matoba and coworkers obtained the first X-ray crystal structure of tyrosinase from the bacterium *Streptomyces castaneoglobisporus* (Fig. 1) [27]. During the last years, more crystal structures of PPOs were solved; e.g., from the insect *Manduca sexta* [28], the bacterium *Bacillus megaterium* [29] and the fungi *Agaricus bisporus* [30] and *Aspergillus oryzae* [31]. Very recently the first plant tyrosinase (*Juglans regia*) was crystallographically characterized [25,32].

In 1937, the first successful isolation of catechol oxidases (from potatoes) was achieved by Kubowitz [33,34]. The first crystal

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