# Sensing Tyrosinase Activity

Subjects: Chemistry, Medicinal

Contributor: Ping Wang

Tyrosinase (TYR, E.C. 1.14.18.1), a critical enzyme participating in melanogenesis, catalyzes the first two steps in melanin biosynthesis including the ortho-hydroxylation of L-tyrosine and the oxidation of L-DOPA. Previous pharmacological investigations have revealed that an abnormal level of TYR is tightly associated with various dermatoses, including albinism, age spots, and malignant melanoma.

Keywords: tyrosinase (TYR) ; enzymatic activity ; optical substrates ; TYR inhibitors ; high-throughput screening

## 1. Introduction

Tyrosinase (TYR, E.C. 1.14.18.1), a type-3 binuclear copper-containing oxidoreductase, efficiently catalyzes *o*-hydroxylation of monophenols to diphenols (monophenolase activity) and the oxidation of diphenols to quinones (diphenolase activity), without any additional cofactors (**Figure 1**) [1][2]. It is ubiquitously distributed in organisms ranging from bacteria to eukaryotes and plays a pivotal role in the enzymatic browning of fruit or fungi, as well as mammalian melanin synthesis [3][4]. In mammals, melanin is exclusively synthesized in melanosomes via complex biochemical reactions (**Figure 2**), and this endogenous substance is primarily responsible for the pigmentation of retina and skin [5][6]. TYR catalyzes the first two steps in melanin biosynthesis: the *o*-hydroxylation of L-tyrosine and the oxidation of L-DOPA. Since the remainder of the reaction sequence can proceed spontaneously at physiological pH, the conversion of L-tyrosine to dopaquinone (DQ) has been implicated as a crucial rate-limiting procedure in melanogenesis [7][8]. DQ could spontaneously convert into dopachrome, which gradually decomposes into 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) through a succession of redox reactions [5][9]. Ultimately, these dihydroxyindoles are oxidized to eumelanin. Alongside, in the presence of cysteine or glutathione, DQ is converted to 5-*S*-cysteinyl dopa or glutothionyl dopa, finally yielding pheomelanin [10][11]. The types and relative amounts of these melanin constitute color-based ethnic diversification. Three tyrosinase-like enzymes co-regulate melanogenesis, including TYR and TYR-related proteins 1 (TRP-1) and 2 (TRP-2). TRP-1 shows DHICA oxidase and low tyrosine hydroxylase activity when zinc is replaced by copper. TRP-2 contains two zinc ions at the active site and isomerizes dopachrome to DHICA. They are metal-containing glycoproteins and share ~40% amino acid sequence identity and ~70% similarity [1]. Despite TYR and TYR-related proteins 1 (TRP-1) and 2 (TRP-2) being necessary for melanogenesis, TYR is the most critical rate-limiting enzyme [10].

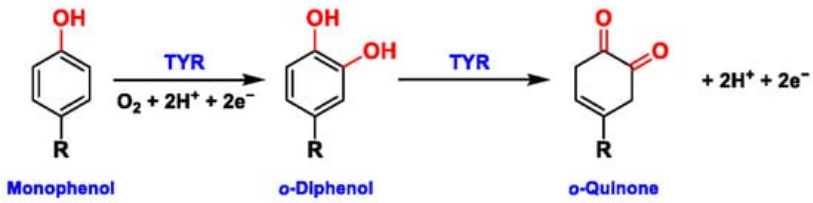


**Figure 1.** The slow *ortho*-hydroxylation of monophenol and the fast oxidation of catechol catalyzed by TYR.

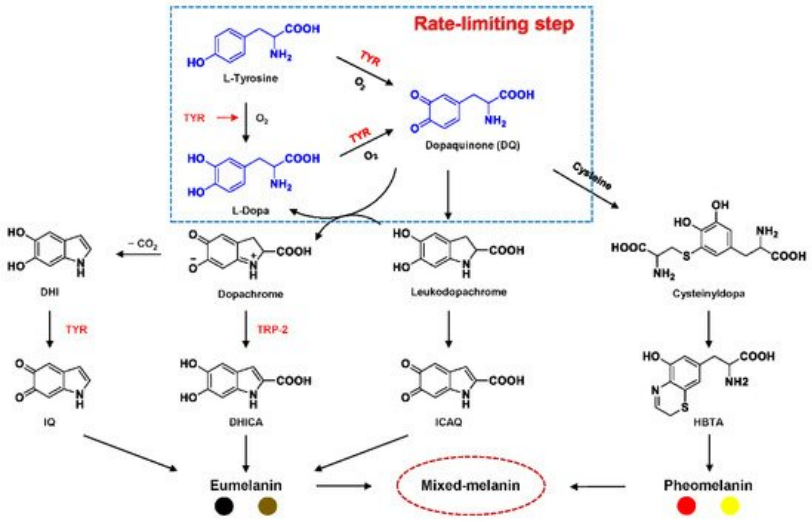


**Figure 2.** Biosynthetic pathway of melanin [7][8][10]. DQ: dopaquinone; L-Dopa: L-3,4-dihydroxyphenylalanine; DHICA: 5,6-dihydroxyindole-2 carboxylic acid; DHI: 5,6-dihydroxyindole; ICAQ: indole-2-carboxylic acid-5,6-quinone; IQ: indole-5,6-quinone; HBTA: 5-hydroxy-1,4-benzothiazinylalanine.

Recent pharmacological investigations have revealed that the abnormal metabolism of melanocytes and imbalance in TYR activity are indirectly or directly responsible for various dermatoses [1][12][13]. For example, functional mutations in the gene encoding TYR (TYR, 11q14-21, MIM 606933) would inactivate TYR and cause a deficiency in melanin, thereby resulting in oculocutaneous albinism type 1 (OCA1, MIM 203100), an autosomal recessive disorder characterized by the absence of pigment in hair, skin, and eyes [14]. On the contrary, excess melanin accumulation or abnormal distribution would give rise to hyperpigmentation disorders, including age spots, post-inflammatory hyperpigmentation, and even malignant melanoma [13][15]. In particular, the overexpression of TYR and TRP 1 is significantly associated with the risk of melanoma, a fatal skin carcinoma [16][17][18]. To this end, it has been viewed as a relatively specific biomarker and therapeutic target for melanoma lesions. Additionally, the abnormal level of TYR induces dopamine neurotoxicity and neurodegeneration, which is related to Parkinson's disease (PD) [19][20][21]. Monitoring TYR activity in complex biosystems undoubtedly remains critical and challenging for biomedical research and drug high-throughput screening (HTS).

In recent decades, various analytical techniques, including immunochemical analysis, mass spectrometry-based proteomics, and substrate-based biochemical assays, have flourished for the quantification of TYR. However, only the substrate-based biochemical assay could rapidly and sensitively determine the real activity of TYR in complex biological systems, leading to its common use in drug discovery and clinical studies [22].

## 2. Biochemical Characteristics of TYR

### 2.1. Structural Feature and Catalytic Mechanism of TYR

Human TYR is a glycoprotein (13% carbohydrate) predominantly located in the melanosome membrane of melanocytes [23][24]. The presence of the transmembrane domain and glycans renders it difficult to isolate homogeneous TYR from melanocytes, which impedes crystallographic studies. Fortunately, the most characteristic TYR can be acquired from *Streptomyces glausescens*, the fungi *Neurospore crassa*, or *Agaricus bisporus* [7][25][26]. To compare the conservation of catalytic cavity, the crystal structures of TYR from bacteria (*Streptomyces castaneoglobisporus* [25] and *Bacillus megaterium* [27]), fungi *Agaricus bisporus* [28], and walnut leaves [29] were retrieved from Protein Date Bank. A salient feature of TYR from various sources (**Figure 3**) is the presence of strictly conserved binuclear copper atoms at the active site, each of copper atoms is coordinated with three conserved histidines, respectively [3]. Moreover, the normal redox state of copper atoms is exceedingly significant for enzyme activity. Due to the relatively high similarity and homology with mammalian TYR, mushroom TYR from *Agaricus bisporus* acts as a model for enzyme kinetics and inhibitor screening [28][30][31]. Although extensive studies are devoted to TYR, its catalytic mechanism remains controversial. According to the presence/absence of oxygen and the oxidation state of copper ions [Cu (Ⅱ)/Cu (Ⅰ)], three enzymatic forms ($E_{oxy}$, $E_{met}$, and $E_{deoxy}$) participate in the catalytic cycle (**Figure 4**) [3][32]. The resting form of TYR is found to be a mixture of 85% met and 15% oxy forms, while only the latter could act on the monophenol [7][33][34]. During the monophenolase cycle, to form $E_{oxy}$-monophenol complex ($E_{oxy}M$), the oxygen atom on the deprotonated monophenol is coordinated with the coppers of $E_{oxy}$. Then, the phenol is *o*-hydroxylated to generate $E_{met}$-diphenol (D) complex ($E_{met}D$) [26][35]. Reducing agents could well draw the $E_{met}$ into the $E_{deoxy}$, with concomitant oxidation to the corresponding *o*-quinone [36]. Since the deoxy form is the only one capable of reacting with oxygen to regenerate $E_{oxy}$ and continue the catalytic action, monophenolase activity

usually manifests as a characteristic lag time until a sufficient amount of catechol helps $E_{met}$ to become $E_{deoxy}$ [36]. Remarkably, this period depends on several factors, including enzyme concentration, monophenol concentration and the presence of reducing agents, especially *o*-diphenol derivatives (such as L-DOPA) that could shorten and even abolish the lag time [30][33][37]. In the diphenolase cycle, $E_{oxy}$ continues to bind *o*-diphenol to originate the $E_{oxy}D$ complex, while both $E_{oxy}$ and $E_{met}$ are capable of oxidizing the diphenol to the *o*-quinone. After this, $E_{met}$ is regenerated to complete the catalytic cycle continuously [30][34].

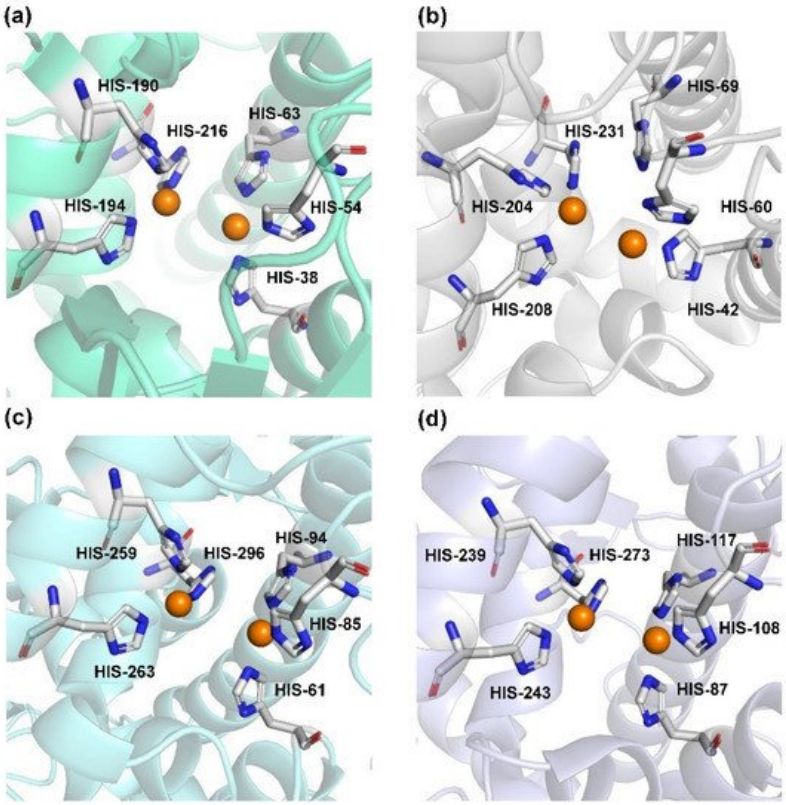


**Figure 3.** The conserved cavity of TYR from different sources. (**a**) The crystal of TYR from *Streptomyces castaneoglobisporus* (PDB ID: 2ZMX). (**b**) The crystal of TYR from *Bacillus megaterium* (PDB ID: 3NQ1). (**c**) The Crystal of TYR from fungus (PDB ID: 2Y9W, *Agaricus bisporus*). (**d**) The Crystal of TYR from plant (PDB ID: 5CE9, *Juglans regia*). Two copper ions (orange) are coordinated with three histidine residues, respectively.

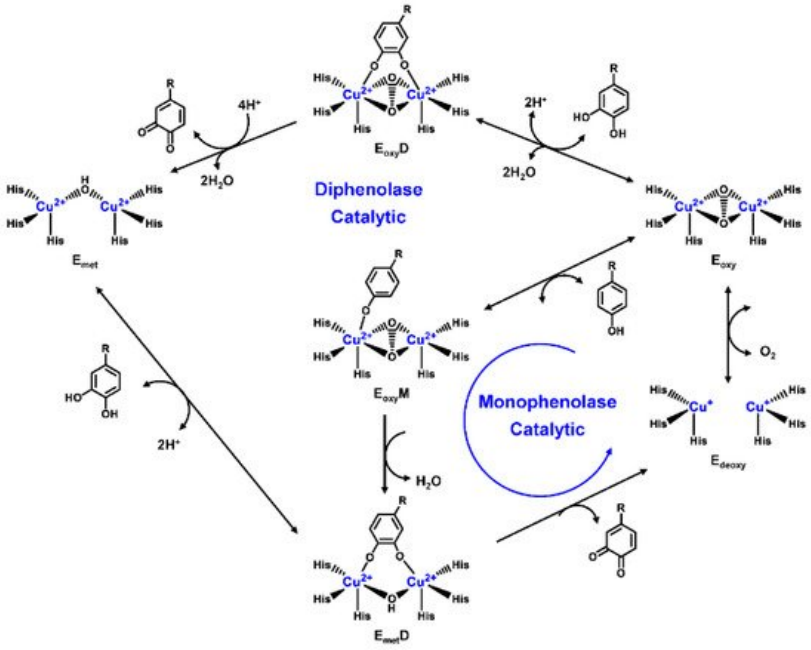


**Figure 4.** Catalytic cycle of TYR.

### 2.2. Substrate Specificity of TYR

Based on the broad substrate spectrum, in principle, any simple monophenol or corresponding catechol appears to be its substrate [38]. Besides, TYR also oxidizes various aromatic amines, *o*-aminophenols, and aromatic *o*-diamines (**Figure 5**),

despite the reaction rates being orders of magnitude smaller than the corresponding phenols or catechol [39][40]. In terms of phenols, mammalian TYR tends to be relatively specific for its physiological substrate (L-tyrosine and L-DOPA) and has a higher affinity for the L-isomers [41]. A prevalent characteristic in monophenol substrates is without substituents in the *ortho*-position of the phenolic hydroxyl group. Understandably, large side-chain substituents increase the difficulty of substrate interaction with the key catalytic residues; this is unpropitious for the recognition and catalytic process between the enzyme and ligand [36]. A kinetic study [42] quantitatively discussed the effects of substituents in the 1-position of the aromatic ring on the rate of hydroxylation catalyzed by TYR. The results revealed that monophenols with a high electron donor tend to be oxidized faster [42]. In sharp contrast, the oxidation rate of catechol is positively correlated with the electron-withdrawing capacity of the *para*-substituents [36]. As such, the steric hindrance, stereochemical characteristics, and electronic effects of substituents have a distinct influence on the rate of TYR-mediated catalysis.

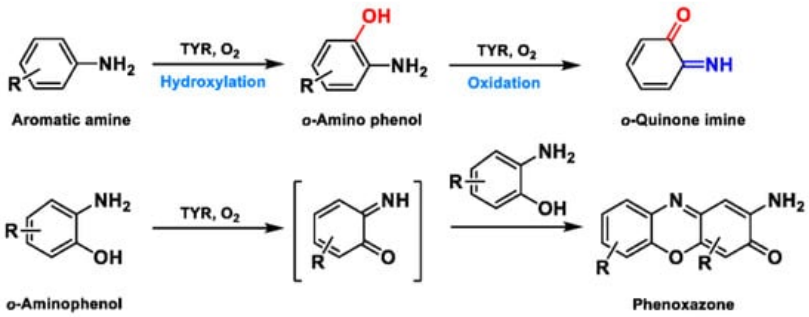


**Figure 5.** The catalytic reaction of TYR-mediated aromatic amine and *o*-aminophenol. Adapted with permission from ref. [43]. 1987, American Chemical Society.

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