

The active site of human Tyrosinase-related Protein: can it be inhibited by plants?

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ABSTRACT

Tyrosinase and tyrosinase-related proteins (TYRPs) play an important role in melanogenesis. TYRPs present in the membrane of melanosome, are known to function in the activation and stabilization of tyrosinase, melanosome synthesis, increase eumelanin/pheomelanin ratio, and in reducing oxidative stress due to its peroxidase effect. Besides, the tyrosinase enzyme is the main enzyme that catalyzes the rate of melanin synthesis, whereas the downregulation of tyrosinase is the most prominent way of developing the inhibitors of melanogenesis. The active site of the tyrosinase-like subdomain of TYRP-1 contains two zinc ions, while that of tyrosinase contains two copper ions. This article focuses on the role of an isoform of TYRP, namely TYRP-1, in melanogenesis, the character of its active site, and the mechanism of action of plant-based lightening agents.

Keywords: Flavonoids, Melanin, Tyrosine, Melanogenesis, Tyrosinase-related protein

Introduction

Skin color is influenced by the amount of melanin synthesized by melanosomes and the pattern of melanosome distribution in melanocytes. Melanin in keratinocytes acts as a photoprotector through body staining and scavenging reactive oxygen species such as singlet oxygen and superoxide anions [1]. When the skin is exposed to UV light, melanogenesis, catalyzed by tyrosinase, occurs [1, 2]. Melanogenesis is a physiological process that produces the synthesis of a complex dark-pigmented biopolymer defined as melanin. Melanin is synthesized by melanosome in melanocytes whose function is to protect the skin from direct exposure to UV rays, drugs, and chemicals. In the process of

melanogenesis, three main enzymes play important roles, namely tyrosinase, tyrosinase-related protein-1 (TYRP-1), and tyrosinase-related protein-2 (TYRP-2) [3]. Melanogenesis is initiated by tyrosine oxidation by the tyrosinase enzyme to form dopaquinone. This quinone product, a reactive precursor of melanin synthesis, is then converted to dopa and dopachrome through the process of auto-oxidation, and finally, produces melanin [4]. These reactions occur repeatedly with the help of the TYRPs. This article is devoted to reviewing the characteristic of the TYRP-1 binding site and the mechanism of TYRP-1 in melanogenesis to be able to discover plant-based lightening agents.

Materials and Methods

This review was obtained from several databases: (1) the Google Scholar database using the keywords "tyrosinase" AND "hydroquinone" AND "whitening agent" AND "kojic acid" AND "melanogenesis"; (2) PubMed using the keywords "whitening [All Fields] AND agent [All Fields] AND ("monophenol monooxygenase" [MeSH Terms] OR ("monophenol" [All Fields] AND "monooxygenase" [All Fields]) OR "monophenol

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monooxygenase" [[MeSH] All Fields OR "tyrosinase" [All Fields] OR "catechol oxidase" [MeSH Terms] OR ("catechol" [All Fields] AND "oxidase" [All Fields]) OR "catechol oxidase" [All Fields]) AND melanogenesis [All Fields]; (3) Science Direct using the keywords "melanogenesis" AND "Acute dermal" AND "tyrosinase" AND "inhibitors". The exclusion criteria were as follows: articles <2000, articles not in English, and clinical studies.

Tyrosinase-related proteins and tyrosinase

TYRP-1, expressed in human melanocytes, is one of the three tyrosinase-like glycoenzymes. This transmembrane glycoprotein is produced within the endoplasmic reticulum and transported through the Golgi to melanosomes. TYRP-1, also known as gp75 glycoprotein (TYRP-1/gp75) due to its molecular weight (75 kDa), is important to the production of melanin, a compound that is responsible for the pigmentation of skin, eye, and hair [5]. The active site of the tyrosinase-like subdomain of TYRP-1 has been reported to contain two zinc ions (**Figure 1**), which is different from that of tyrosinase that contains copper ions (**Figure 2**). This difference explains why TYRP-1 exhibits no tyrosinase redox activity [6].

Tyrosinase catalyzes 2 reactions on its phenolic substrates, e.g. (1) the hydroxylation of the monophenols to *o*-diphenols (catalyzed by the monophenolase activity of tyrosinase) and (2)

the oxidation of the *o*-diphenols to *o*-quinones (catalyzed by the diphenolase activity of tyrosinase) [7]. The cytoplasmic domain of tyrosinase contributes to its translocation from the nucleus to the melanosomes [8]. The internal domain contains the catalytic site with histidine residues, where the Cu ions bind (**Figure 2**) [9], through the N ϵ nitrogen atoms [4].

When the copper in the catalytic site is oxidized by its reaction with the phenolic substrates, tyrosinase will be inactive. A mechanism in which the enzymatic reaction produces an oxy-tyrosinase is responsible for inactivation. The chemical structure of the substrates, at least two -OH groups in ortho-position in the benzene ring, is very essential in the suicide inactivation process. The rate constant of this reaction is directly related to the nucleophilic property of the -OH attached to C-1. The suicide inactivation may happen if the -OH attached C-2 transferred its proton to the protonated peroxide. In addition, the coplanarity among the ring, the oxygen of the C-1, and the Cu atom would only allow the reduction/oxidation reaction, thus inactivates the enzyme [7]. Deactivated tyrosinase is formed from oxy-tyrosine during the suicide inactivation. Concisely, this inactivation takes place when the diphenolic substrates are processed as monophenols, and one of the Cu ions is reduced in this route [10]. However, this enzyme can be reactivated by electron donor molecules, e.g. nitric oxide, superoxide anion, ascorbic acid, and L-DOPA (NO) [8].

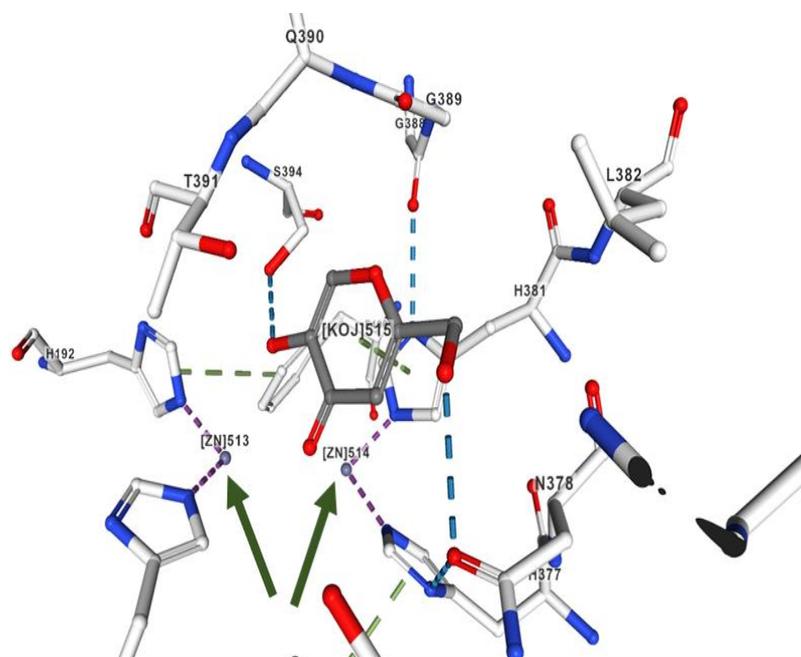


Figure 1. The active site of human TYRP-1 (PDB ID: 5M8M deposited by Lai *et al.*, 2016) shows two zinc ions (shown by bold arrows) labeled as ZN513 and ZN514. Each zinc ion indicates two metal interactions (purple dashed lines) with histidine residues in the TYRP-1 active site. The TYRP-1 inhibitor, kojic acid, labeled as KOJ515 (grey-red structure), builds two hydrogen bonds (blue dashed lines) with Ser394 and Asn378 and one pi-pi interaction with His381 (green dashed line) (downloaded from <http://www.rcsb.org/3d-view/5M8M/1> and viewed by using 3D NGL-WebGL powered by MMTF).

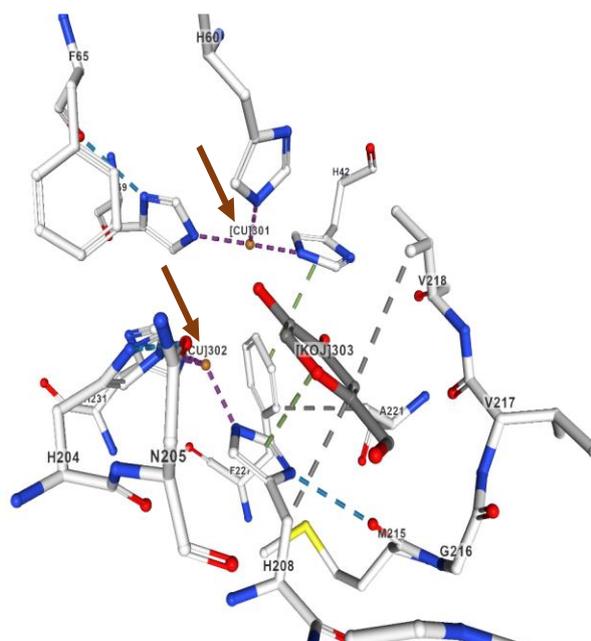


Figure 2. The active site of *Bacillus megaterium* tyrosinase (PDB ID: 5I38 deposited by Kanteev *et al.*, 2016) shows two copper ions (shown by bold arrows) labeled as CU301 and CU302. Each copper ion indicates three metal interactions (purple dashed lines) with histidine residues in the tyrosinase active site. The tyrosinase inhibitor, kojic acid, labeled as KOJ303 (grey-red structure), builds two hydrophobic contacts (grey dashed lines) with Val218 and His208, and one pi-pi interaction with His208 (downloaded from <http://www.rcsb.org/3d-view/5I38/1> and viewed by using 3D NGL-WebGL powered by MMTF).

It was proposed that TYRP-1 might be important in stabilizing the tyrosinase. Tyrosinase and TYRP-1 complex may prevent the premature death of melanocytes by attenuating tyrosinase-mediated cytotoxicity [5].

Tyrosinase inhibitors as skin lightening agents

Skin lightening agents, contained in cosmetics, are used to brighten or eliminate unwanted skin tones [11, 12]. The basic mechanism of the skin lightening agents is by inhibiting the catalytic activity of tyrosinase [13]. Various chemicals have been popularly used as skin lightening agents in cosmetics.

Hydroquinone

Hydroquinone is a phenolic compound known chemically as 1,4-dihydroxybenzene. This compound inhibits the enzymatic oxidation of tyrosine, as well as reducing melanin production by inhibiting the sulfhydryl group and acting as a tyrosinase substrate. It is covalently bound to histidine and interacts with copper in the active site of tyrosinase. This activity does not 'whiten the skin' but gradually suppresses the melanin

production. Hydroquinone interacts with *Bacillus megaterium* tyrosinase by building two hydrogen bonds with Asn205 and Pro219, two hydrophobic contacts with Ala221 and Val218, and one pi-pi interaction with His208. Hydroquinone can act as both *Bacillus megaterium* tyrosinase substrate and an inhibitor [9].

Kojic acid

Kojic acid, a hydrophilic compound, is widely used as a skin whitening agent. This compound inhibits both TYRP-1 and tyrosinase [14]. Kojic acid interacts with human TYRP-1 by building two hydrogen bonds (Ser394 and Asn378) and one pi-pi interaction (His381) in the TYRP-1 binding site (<http://www.rcsb.org/3d-view/5M8M/1>), whereas, with *Bacillus megaterium* tyrosinase, kojic acid builds two hydrophobic contacts with Val218 and His208, and one pi-pi interaction with His208 (<http://www.rcsb.org/3d-view/5I38/1>).

Plants as inhibitors of tyrosinase

Many studies reported the tyrosinase inhibitory activity of plants (Table 1):

Table 1. Plants that Inhibit Tyrosinase Activity

Compound/ Extract	Source	Description	Reference
Artocarpanone	<i>Artocarpus heterophyllus</i>	Artocarpanone is a promising compound for hyperpigmentation, skin lightening, and antioxidant therapy	[15]
Pyrano-cyclo-artobioxanthone	<i>Artocarpus obtusus</i>	Pyrano-cyclo-artobioxanthone is a strong inhibitor of the fungus tyrosinase. The activity is supported by the structure-activity relationship of phenolics related to the presence of C-3 substituents and 4-substituted resorcinol.	[16]

Moracin	<i>Morus alba</i> twigs	Structure-activity relationships for flavonoids and glucosides, stilbena and glucoside, a 2-arylbenzofuran derivative, unsubstituted resorcinol groups in 2'- and 4'- OH in the B-ring flavonoids play an important role in tyrosinase inhibitory activity	[17]
Hydro-ethanol extracts	<i>Dalbergia ecastaphyllum</i>	The phenolic compounds in the extract are mainly responsible for the inhibition of tyrosinase activity	[18]
N-(substituted-phenyl)-4-[(E)-3-phenyl-2-propenyl]-1-piperaziny]	Butanamids	The presence of two methyl groups in 1 and 4 positions in the N-aryl group greatly influences tyrosinase inhibition.	[19]
2-hydroxytyrosol	Metarhizium sp.	Inhibits melanin pigmentation of B16 melanoma cells	[20]

Flavonoids contained in the rhizomes of Zingiberaceae plants have been proven could inhibit the catalytic function of tyrosinase thus reduces melanogenesis [21, 22], e.g. 4-hydroxypanduratin-A and isopanduratin-A [23]. A phenolic compound contained in ginger, 6-shogaol, effectively inhibits the activity of tyrosinase better than arbutin [24, 25].

Moreover, algae (green and brown), due to their flavonoids, phlorotannins, and carotenoids content, have been reported in possessing inhibitory activity towards tyrosinase [26-28]. *Sargassum silquastrum*, *Ecklonia cava*, *Schizymenia dubyi*, and *Enderachne binghamiae* have been proven to have tyrosinase inhibitory effect similar to that of kojic acid [29]. *Digenea simplex*, *Laurencia papillosa*, and *Laurencia paniculata* indicated a significant inhibitory effect on monophenolase and diphenolase (of mushroom tyrosinase) in zebrafish model compared to kojic acid [30].

Conclusion

TYRP-1, also known as TYRP-1/gp75, plays a role in the melanosome synthesis, stabilization and activation of tyrosinase, increases eumelanin/pheomelanin ratio and reduces oxidative stress due to its peroxidase effect. The active site of the TYRP-1 has been reported to contain two zinc ions, which is different from that of tyrosinase that contains copper ions. Kojic acid, a well-known skin lightening agent, interacts with human TYRP-1 by building two hydrogen bonds (Ser394 and Asn378) and one pi-pi interaction (His381) in the TYRP-1 binding site. Many plants and marine algae, particularly those containing flavonoids, have been proven to inhibit mushroom or bacteria tyrosinase. However, further explorations on human TYRP-1 are still needed for developing prospective cosmeceuticals.

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References

- Tada M, Kohno M, Niwano Y. Alleviation effect of arbutin on oxidative stress generated through tyrosinase reaction with L-tyrosine and L-DOPA. *BMC Biochem.* 2014;15(1):1-7. doi:10.1186/1471-2091-15-23.
- Gillbro JM, Olsson MJ. The melanogenesis and mechanisms of skin-lightening agents - Existing and new approaches. *Int J Cosmet Sci.* 2011;33(3):210-21. doi:10.1111/j.1468-2494.2010.00616.x.
- Akaberi M, Emami SA, Vatani M, Tayarani-Najaran Z. Evaluation of Antioxidant and Anti-Melanogenic Activity of Different Extracts of Aerial Parts of *N. Sinenisii* in Murine Melanoma B16F10 Cells. *Iran J Pharm Res.* 2018;17(1):225-35.
- Matoba Y, Kihara S, Bando N, Yoshitsu H, Sakaguchi M, Kayama K, et al. Catalytic mechanism of the tyrosinase reaction toward the Tyr98 residue in the caddie protein. *PLoS Biol.* 2018;16(12):e3000077. doi:10.1371/journal.pbio.3000077.
- Ghanem G, Fabrice J. Tyrosinase related protein 1 (TYRP1/gp75) in human cutaneous melanoma. *Mol Oncol.* 2011;5(2):150-5. doi:10.1016/j.molonc.2011.01.006.
- Lai X, Wichers HJ, Soler-Lopez M, Dijkstra BW. Structure of Human Tyrosinase Related Protein 1 Reveals a Binuclear Zinc Active Site Important for Melanogenesis. *Angew Chem Int Ed.* 2017;56(33):9812-5.
- Muñoz-Muñoz JL, Garcia-Molina F, Varon R, Garcia-Ruiz PA, Tudela J, Garcia-Cánovas F, et al. Suicide inactivation of the diphenolase and monophenolase activities of tyrosinase. *IUBMB Life.* 2010;62(7):539-47. doi:10.1002/iub.00348
- Videria IFS, Moura DFL, Magina S. Mechanisms regulating melanogenesis. *An Bras Dermatol.* 2013;88(1):76-83.
- Deri B, Kanteev M, Goldfeder M, Lecina D, Guallar V, Adir N, et al. The unravelling of the complex pattern of tyrosinase inhibition. *Sci Rep.* 2016;6(1):34993.
- Cieńska M, Labus K, Lewańczuk M, Koźlecki T, Liesiene J, Bryjak J. Effective L-Tyrosine Hydroxylation by Native and Immobilized Tyrosinase. *PLoS ONE* 2016;11(10):e0164213. doi:10.1371/journal.pone.0164213

11. Pillaiyar T, Manickam M, Jung SH. Recent development of signaling pathways inhibitors of melanogenesis. *Cell Signal*. 2017;40:99-115. doi:10.1016/j.cellsig.2017.09.004.
12. Aljunaiyeh H, Naif A, Kadhim K. Non-prescription Skin Lightening Preparations; Their Use & Hazards in Iraq. *Int J Pharm Res Allied Sci*. 2018;7(2):209-17.
13. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem Photobiol*. 2008;84(3):539-49. doi:10.1111/j.1751-1097.2007.00226.x
14. Ahn SJ, Koketsu M, Ishihara H, Lee SM, Ha SK, Lee KH, et al. Regulation of melanin synthesis by selenium-containing carbohydrates. *Chem Pharm Bull (Tokyo)*. 2006;54(3):281-6. doi:10.1248/cpb.54.281.
15. Arung ET, Shimizu K, Kondo R. Inhibitory effect of artocarpone from *Artocarpus heterophyllus* on melanin biosynthesis. *Biol Pharm Bull*. 2006;29(9):1966-9. doi:10.1248/bpb.29.1966.
16. Hashim NM, Rahmani M, Lian GC, Sukari MA, Yahayu M, Amin MAM, et al. Antioxidant, antimicrobial and tyrosinase inhibitory activities of xanthenes isolated from *Artocarpus obtusus* F.M. Jarrett. *Molecules* 2012;17(5):6071-82. doi:10.3390/molecules17056071.
17. Zhang L, Tao G, Chen J, Zheng ZP. Characterization of a New Flavone and Tyrosinase Inhibition Constituents from the Twigs of *Morus alba* L. *Molecules*. 2016;21(9):1130. doi:10.3390/molecules21091130.
18. Morais DVd, Costa MAPdC, Santa Bárbara MF, Silva FdL, Moreira MM, Delerue-Mato C, et al. Antioxidant, photoprotective and inhibitory activity of tyrosinase in extracts of *Dalbergia ecastaphyllum*. *PLoS ONE* 2018;13(11):e0207510. doi:10.1371/journal.pone.0207510.
19. Raza H, Abbasi MA, Siddiqui SZ, Hassan M, Abbas Q, Hong H, et al. 'Synthesis, molecular docking, dynamic simulations, kinetic mechanism, cytotoxicity evaluation of N-(substituted-phenyl)-4-{(4-[(E)-3-phenyl-2-propenyl]-1-piperazinyl} butanamides as tyrosinase and melanin inhibitors: In vitro, in vivo and in silico approach. *Bioorganic Chem*. 2017;94:103445. doi:10.1016/j.bioorg.2019.103445.
20. Uchida R, Ishikawa S, Tomoda H. Inhibition of tyrosinase activity and melanin pigmentation by 2-hydroxytyrosol. *Acta Pharm Sinica Bull*. 2014;4(2):141-5. doi:10.1016/j.apsb.2013.12.008
21. Solano F, Briganti S, Picardo M, Ghanem G. Hypopigmenting agents: an update review on biological, chemical and clinical aspects. *Pigment Cell Res*. 2006;19(6):550-71.
22. Sangi SM, Bawadekji A, Alotaibi NM, Aljlaud NA. Preventive and Curative Effects of Metformin, *Nigella sativa*, *Punica granatum* and *Zingiber officinale* on Male Reproductive Dysfunction in Diabetic Rats. *Int J Pharm Res Allied Sci*. 2019;8(2):48-57.
23. Yoon JH, Shim JS, Cho Y, Baek NI, Lee CW, Kim HS, et al. Depigmentation of melanocytes by isopanduratin-A and 4-hydroxypanduratin-A isolated from *Kaempferia pandurata* RoxB. *Biol Pharm Bull*. 2007;30(11):2141-5.
24. Huang HC, Chang SJ, Wu CY, Ke HJ, Chang TM. 6-Shogaol Inhibits α -MSH-Induced Melanogenesis through the Acceleration of ERK and PI3K/Akt-Mediated MITF Degradation. *BioMed Res Int*. 2014;2014. doi:10.1155/2014/842569
25. Moghadam ZK, Najfabady MT, Abedi P, Haghighizadeh MH. Investigating the Effect of Ginger Pill on the Treatment of Nausea and Vomiting of Pregnancy (NVP) in Pregnancy Women. *Int J Pharm Phytopharmacol Res*. 2019;9(3):9-15.
26. Pereira L. Seaweeds as Source of Bioactive Substances and Skin Care Therapy—Cosmeceuticals, Algototherapy, and Thalassotherapy. *Cosmetics* 2018;5(4):68. doi:10.3390/cosmetics5040068
27. Alseroury FA. Simultaneous Decolorization of Dye Contaminated Wastewater and Energy Production Using Algae. *J Biochem Technol*. 2018;9(4):15-21.
28. Amasha RH, Aly MM. Removal of Dangerous Heavy Metal and Some Human Pathogens by Dried Green Algae Collected from Jeddah Coast. *Pharmacophores*. 2019;10(3):5-13.
29. Cha SH, Ko SC, Kim D, Jeon YJ. Screening of marine algae for potential tyrosinase inhibitor: Those inhibitors reduced tyrosinase activity and melanin synthesis in zebrafish. *J Dermatol*. 2011;38(4):354-63. doi:10.1111/j.1346-8138.2010.00983.x
30. Namjoyan F, Farasat M, Alishahi M, Jahangiri A, Mousavie H. The Anti-melanogenesis Activities of Some Selected Red Macroalgae from Northern Coasts of the Persian Gulf. *Iran J Pharm Res*. 2019;18(1):383-90.