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Original Article

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.

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Yuniarti Falya, Deni Firmansyah, Nyi Mekar Saptarini, Yuli Andriani, Sri Adi Sumiwi, Jutti Levita. The active site of human Tyrosinase-related Protein: can it be inhibited by plants? J Adv Pharm Edu Res 2021;11(1):86-90.
Source of Support: Nil, Conflict of Interest: None declared.

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

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monooxygenase" [MeSH Terms] OR ("monophenol "[All Fields] AND "monooxygenase" [All Fields]) OR "monophenol 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 Nε 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 tyrosine 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].

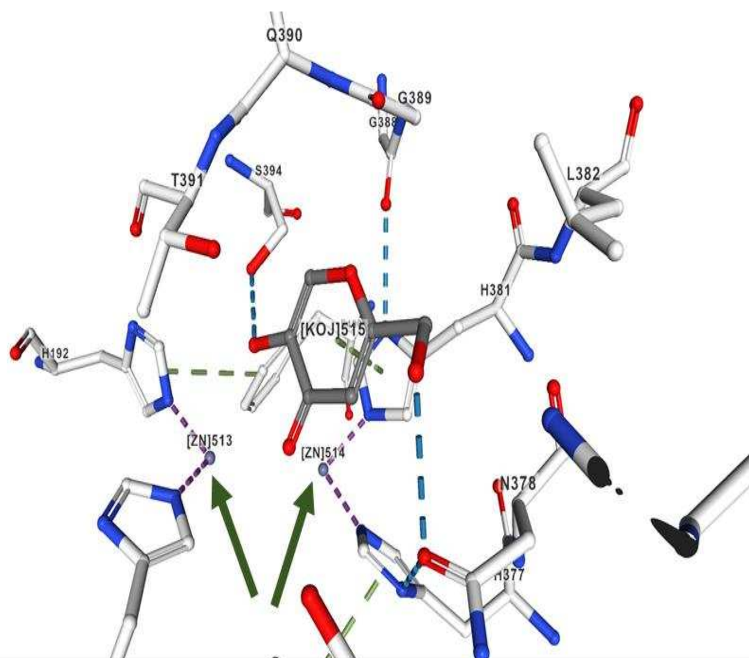

Figure 1. The active site of human TYRP-1 (PDB ID: 5M8M deposited by Lai et al., 2016) shows two zinc ions (shown by bold arrows) labeled as ZN513 and ZN514. Each zinc ion indicates two metal interactions (purple dashed lines) with histidine residues in the TYRP-1 active site. The TYRP-1 inhibitor, kojic 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).

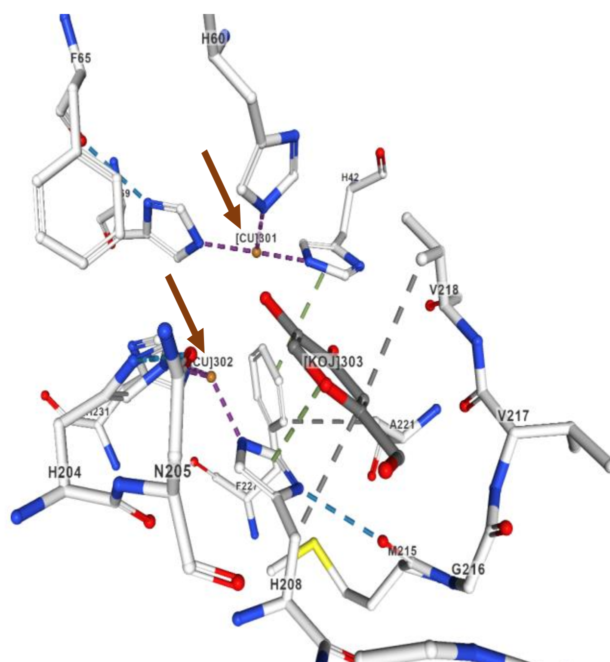

Figure 2. The active site of *Bacillus megaterium* tyrosinase (PDB ID: 5I38 deposited by Kanteev et al., 2016) shows two copper ions (shown by bold arrows) labeled as CU301 and CU302. Each copper ion indicates three metal interactions (purple dashed lines) with histidine residues in the tyrosinase active 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	*Artocarpus heterophyllus*	Artocarpanone is a promising compound for hyperpigmentation, skin lightening, and antioxidant therapy	[15]
Pyrano-cyclo-artobiloxanthone	*Artocarpus obtosus*	Pyrano-cyclo-artobiloxanthone 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	*Morus alba* twigs	Structure-activity relationships for flavonoids and glucosides, stylbena 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	*Dalbergia ecastaphyllum*	The phenolic compounds in the extract are mainly responsible for the inhibition of tyrosinase activity	[18]
N-(substituted-phenyl)-4-{(4-[(E)-3-phenyl-2-propenyl]-1-piperazinyl}	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 *Endarachne 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].

Conclusions and Future Perspectives

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.

Acknowledgment

The authors would like to thank the Rector of Universitas Padjadjaran for funding the publication fee of this article via the Directorate of Research and Community Engagement (DRPM).

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