Journal of Bioscience and Bioengineering
VOL. 121 No. 6, 607–613, 2016

www.elsevier.com/locate/jbiosc

New tyrosinase inhibitory decapeptide: Molecular insights into the role of tyrosine residues

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Received 7 July 2015; accepted 14 October 2015
Available online 14 November 2015

Tyrosinase, a rate-limiting enzyme in melanin biosynthesis, catalyzes the hydroxylation of L-tyrosine to 3,4-dihydroxy-L-phenylalanine (L-dopa) (monophenolase reaction) and the subsequent oxidation of L-dopa to L-dopaquinone (diphenolase reaction). Thus, tyrosinase inhibitors have been proposed as skin-lightening agents; however, many of the existing inhibitors cannot be widely used in the cosmetic industry due to their high cytotoxicity and instability. On the other hand, some tyrosinase inhibitory peptides have been reported as safe. In this study, we found that the peptide TH10, which has a similar sequence to the characterized inhibitory peptide P4, strongly inhibits the monophenolase reaction with a half-maximal inhibitory concentration of 102 μM. Seven of the ten amino acid residues in TH10 were identical to P4; however, TH10 possesses one N-terminal tyrosine, whereas P4 contains three tyrosine residues located at its N-terminus, center, and C-terminus. Subsequent analysis using sequence-shuffled variants indicated that the tyrosine residues located at the N-terminus and center of P4 have little to no contribution to its inhibitory activity. Furthermore, docking simulation analysis of these peptides with mushroom tyrosinase demonstrated that the active tyrosine residue was positioned close to copper ions, suggesting that TH10 and P4 bind to tyrosinase as a substrate analogue.

[**Key words:** Melanogenesis; Skin-lightening agents; Tyrosinase inhibitor; Peptide; Action mechanism]

Human skin is repeatedly exposed to environmental DNA-damaging agents such as ultraviolet radiation (UVR) (1,2), and therefore requires numerous endogenous systems for protection from this damage (3,4). Skin pigmentation caused by melanin deposition is one of these protection systems and functions as a physical barrier to scatter UVR and as an absorbent filter to limit the penetration of UVR through the epidermis. Thus, skin pigmentation is an indispensable system for human health (4,5). However, the excess deposition of melanin can lead to skin disorders and diseases, such as freckles and melanoderma, respectively (6). Therefore, methods to control melanin production are strongly desired in the cosmetic field.

Tyrosinase (EC 1.14.18.1), a rate-limiting enzyme in melanin biosynthesis, catalyzes the hydroxylation of L-tyrosine to 3,4-dihydroxy-L-phenylalanine (L-dopa) and the subsequent oxidation of L-dopa to L-dopaquinone (Fig. 1A) (7,8). The former and latter steps are defined as monophenolase and diphenolase reactions, respectively. Two copper ions in the active pocket of tyrosinase are individually bound by three histidine residues and are directly involved in the activity of each catalytic reaction via oxy-, deoxy-, and met-forms (Fig. 1B) (9). L-Dopaquinone generated by the diphenolase reaction is subsequently converted to melanin through a series of non-enzymatic processes. Due to the importance of tyrosinase activity in melanin biosynthesis, several tyrosinase inhibitors have been proposed as therapeutic agents for melanin-related skin disorders (10,11). Kojic acid, arbutin, and hydroquinone are well-known inhibitory agents currently used in the cosmetic field. While these agents produce strong therapeutic effects, they also have serious side effects. Kojic acid and hydroquinone have been active ingredients in commercially available skin-lightening products since the 1960s, but are known to cause genotoxicity and carcinogenesis (12–14). Arbutin is a glycosylated hydroquinone extracted from wheat, pear skins, and the leaves of blueberries, and is easily converted on the skin's surface to harmful hydroquinone (15). Therefore, safe and stable agents are urgently needed.

Several tyrosinase inhibitory peptides have been reported over the past ten years (16–19). P4 is the best-known model peptide with high inhibitory potency against mushroom tyrosinase (half-maximal inhibitory concentration [IC_{50}] = 40 μM) (17,20), and is currently used as a therapeutic agent for skin disorders (21). Due to the absence of inherent toxicity, tyrosinase inhibitory peptides have been emphasized as safe agents. Approximately half of the already-known tyrosinase inhibitory peptides include tyrosine in their sequences, suggesting that this residue plays an important role in the inhibitory function of these peptides.

Rice (*Oryza sativa*) is a well-known staple food worldwide, and global rice production was estimated at 645 million tons (22).

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1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2015.10.010

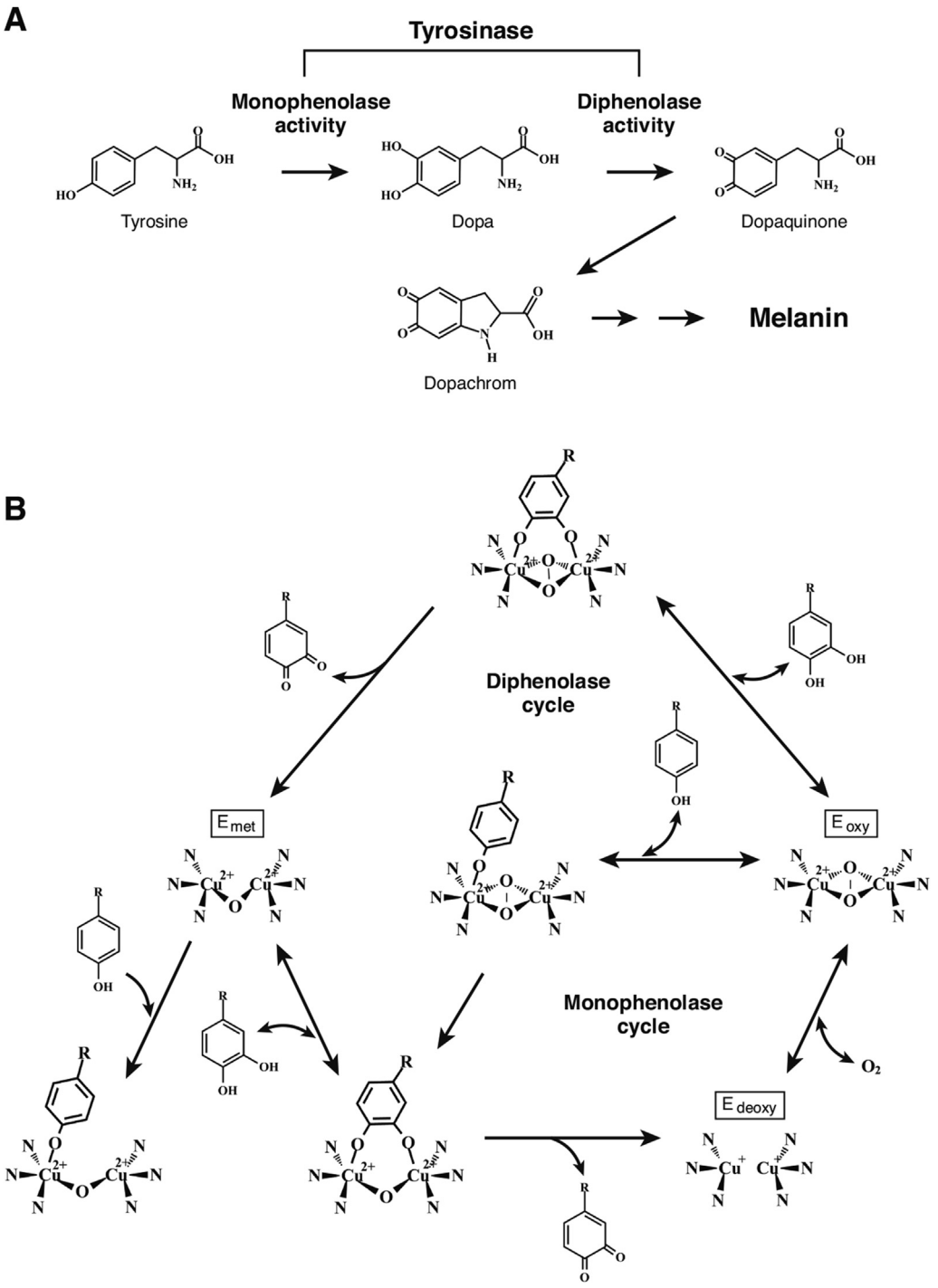

FIG. 1. Role of tyrosinase in melanin synthesis. (A) Two reactions of tyrosinase in melanin synthesis. (B) Detailed catalytic cycles of the hydroxylation of monophenol and oxidation of *o*-diphenol to *o*-quinone by tyrosinase.

Rice bran, the brown layer of rice kernel, is produced as a byproduct of the rice milling process and contains several nutrients, minerals, and physiologically active phytochemicals such as γ-oryzanol, ferulic acid, and tocopherols (22). However, most of these are ultimately discarded as waste (22). Therefore, we recently explored the usage of rice-derived unused biomass, including bran. During this process, we conducted a homology search and found a peptide sequence homologous to peptide P4 in the translated genomic DNA database of rice. This peptide, designated as TH10, is a partial ten-amino acid sequence of ED40-like domain-containing protein (GenBank ID: AK061891) and can be cut out by a specific proteinase, thermolysin. Chemically synthesized TH10 showed strong inhibition against mushroom tyrosinase. Seven of the ten residues completely matched with P4. TH10 possesses one N-terminal tyrosine residue, whereas P4 contains three tyrosine residues located at its N-terminus, center, and C-terminus (Table 1). In this study, a new tyrosinase inhibitory peptide was identified, and subsequent analysis using variants of TH10 and P4 revealed the functional role of tyrosine at various positions in their sequences.