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Molecular inhibitory mechanism of tricin on tyrosinase

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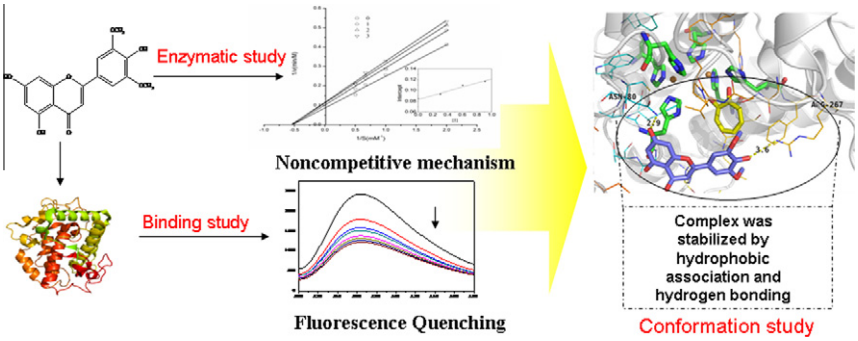
HIGHLIGHTS

- Tricin effectively inhibits tyrosinase in a non-compatitive manner.
- Tricin quenches tyrosinase fluorescence by forming complex with tyrosinase.
- The tricin-enzyme complex is stabilized by hydrogen bonds and hydrophobic forces.
- A simulated model for the molecular interaction of tricin and tyrosinase.

GRAPHICAL ABSTRACT



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ABSTRACT

Tricin was evaluated as a type of tyrosinase inhibitor with good efficacy compared to arbutin. Tricin functioned as a non-competitive inhibitor of tyrosinase, with an equilibrium constant of 2.30 mmol/L. The molecular mechanisms underlying the inhibition of tyrosinase by tricin were investigated by means of circular dichroism spectra, fluorescence quenching and molecular docking. These assays demonstrated that the interactions between tricin and tyrosinase did not change the secondary structure. The interaction of tricin with residues in the hydrophobic pocket of tyrosinase was revealed by fluorescence quenching; the complex was stabilized by hydrophobic associations and hydrogen bonding (with residues Asn80 and Arg267). Docking results implied that the possible inhibitory mechanisms may be attributed to the stereospecific blockade effects of tricin on substrates or products and flexible conformation alterations in the tyrosinase active center caused by weak interactions between tyrosinase and tricin. The application of this type of flavonoid as a tyrosinase inhibitor will lead to significant advances in the field of depigmentation.

Introduction

Melanin synthesis is initiated from tyrosine by tyrosinase (EC 1.14.18.1), which is considered to be the rate-limiting enzyme in melanin biosynthesis [1,2]. Tyrosinase plays a key role in processes such as pigmentation in vertebrates and unfavorable browning in food products [3]. Melanin overproduction may cause hyperpigmentation diseases, such as flecks in mammals [4]. In the food industry, browning can cause deleterious changes in the organoleptic properties of food products, which results in the loss of quality in fruits and vegetables [5]; thus, preventing this unfavorable browning reaction has always been a challenge in food science [4]. Tyrosinase is uniquely associated with the insect molting process and wound healing [4,6], and tyrosinase inhibitors have played important roles in the fields of medicine, agriculture, food sciences, and cosmetics. Accordingly, there is a developing need to screen for tyrosinase inhibitors with higher bioactivity and lower toxicity to explore the inhibitory mechanisms. Many plant-derived compounds, such as arbutin and some types of flavones, can inhibit tyrosinase activity [7,8].

Tricin is a type of methylated flavone that accumulates in most cereal crop plants [9,10]. Tricin has long been recognized for its

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beneficial health effects, such as antioxidant [11], antiviral [12] and antitumor activities [13]. Therefore, the pharmacological and physiological activities of tricin and its application are currently attracting great interest.

Tyrosinase belongs to the oxidoreductase family. For many tyrosinase inhibitors, one of their inhibitory mechanisms is that they can antagonize tyrosinase activation by oxygen as antioxidants [14]. As a result, tyrosinase cannot switch from the deoxy form (E_{deoxy}) to the catalytic form (E_{oxy}). Thus, as a well-known antioxidant, tricin may have potential tyrosinase inhibition activity.

In the present study, the inhibitory efficacy of tricin on tyrosinase was evaluated, and the molecular mechanism underlying this interaction was extensively explored. These findings will be useful for the exploration of tyrosinase inhibitors with higher bioactivity and lower toxicity and provide fundamental data for the pharmacological and physiological activities of tricin.

Materials and methods

Materials

HPLC-grade tricin was produced in our laboratory. L-tyrosine and mushroom tyrosinase (EC 1.14.18.1) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade, and distilled and deionized water was used.

Tyrosinase activity assay

The enzyme assay was performed as previously described [15,16] with the following modifications. Tricin was dissolved in dimethyl sulphoxide at 1 mg/mL and diluted for dose dependence in vitro experiments. Briefly, 70 μL of inhibitor was combined with 30 μL of tyrosinase (333 U/mL in phosphate buffer) in triplicate reactions in a 96-well plate. After incubation at 25 °C for 5 min, 110 μL of substrate (1.0 mM L-tyrosine or 2.0 mM L-dopa in 50 mM K_2HPO_4–KH_2PO_4 buffer, pH 6.8) was added to each well and incubated for 30 min at 25 °C. The optical densities of the samples in each well were then determined at 492 nm with a plate reader (Sunrise, TECAN, Switzerland). Arbutin was used as a positive control. All of the doses mentioned in the study were the final concentrations. The inhibition of tyrosinase activity was calculated as follows:

$$\%\text{inhibition} = [1 - (B - C)/A] \times 100\%, \quad (1)$$

where A is the absorbance at 492 nm without the test sample, B is the absorbance at 492 nm with the test sample, and C is the absorbance at 492 nm without tyrosinase.

Circular dichroism spectroscopy

Circular dichroism (CD) spectra were recorded with an MOS 450 Circular Dichroism spectrometer (BioLogic Inc., France) at 25 °C. The CD measurements were made in the range of 190–250 nm using a 2 mm quartz cuvette at a scan speed of 60 nm/min; three scans were averaged for each CD spectra. The cuvette contained a 1.0 mL mixture composed of 0.9 mL of tyrosinase (0.1 mg, dissolved in water) and 0.1 mL of tricin (dissolved in 10% methanol aqueous solution). The molar concentration ratio of tricin to tyrosinase varied (0:1, 1:1 and 4:1) [17,18].

Fluorescence quenching

Fluorescence spectra were recorded using an F-7000 spectrofluorophotometer (Hitachi Inc., Japan) equipped with a 150 W xenon lamp and a thermostat bath. Fluorescence emission spectra were recorded at wavelengths of 295–480 nm upon excitation at 280 nm. The excitation and emission bandwidths were both 5 nm [19,20].

Model building and molecular docking

The crystal structure of tyrosinase from Agaricus bisporus (AbTYR; PDB code, 2Y9X) [37] was chosen as the protein model for the present study. The docking algorithm was based on ROSETTALIGAND (http://www.rosettacommons.org) as previously described [21,22]. The Cu^{2+} in 2Y9X were replaced by Zn^{2+} and the ligands in 2Y9X were removed before the docking computation was performed, for there is no definition of Cu^{2+} in the database of ROSETTA and Zn^{2+} has the similar size to Cu^{2+}. The best docking result was selected according to the lowest total score. The figures were produced with the PyMOL molecular graphics system (http://www.pymol.org).

Results and discussion

Inhibitory effects of tricin on tyrosinase

Fig. 1 shows the inhibitory effects of tricin and arbutin at different doses on tyrosinase activity. To compare the inhibitory potencies, arbutin was employed as a positive control. A commercially available tyrosinase inhibitor, arbutin is a glycosylated hydroquinone and may carry similar cancer risks [23]. The results showed that the flavonoid obtained in this study had a dose-dependent inhibitory effect on tyrosinase activity, which indicated that tricin was a potent tyrosinase inhibitor. Moreover, the inhibitory efficacy of tricin on tyrosinase activity was better than arbutin, with absolute safety. The inhibitor concentration at which half of the original tyrosinase activity remained (IC50) was 269.46 μg/mL for tricin; this concentration was determined from the slope of the regression curves of the inhibition rate versus the concentration of tricin.

Kinetics analysis

Effects of tricin on the monophenolase activity of mushroom tyrosinase

The inhibition kinetics of mushroom tyrosinase by tricin were studied. Fig. 2 shows the kinetic progression of L-tyrosine oxidation by tyrosinase in the presence of different concentrations of tricin. A marked lag time, a characteristic of monophenolase activity, was simultaneously found with the appearance of the product [24], as shown in Fig. 2a. The lag time was prolonged with the increase in tricin concentration, from 5 min without tricin to approximately 15 min. After the lag time, the system reached a steady state, and

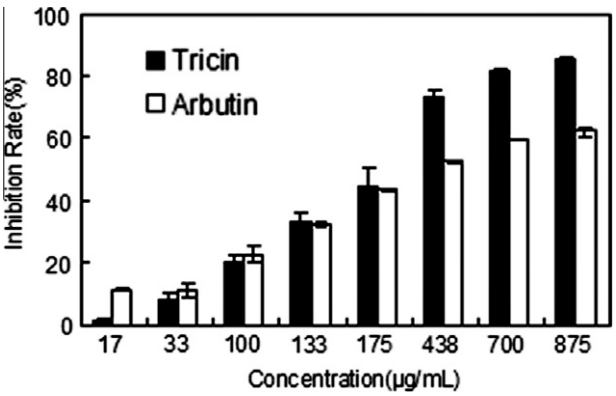

Fig. 1. Inhibitory effect of tricin on tyrosinase (arbutin as a control).