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Rifampicin as a novel tyrosinase inhibitor: Inhibitory activity and mechanism

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ABSTRACT

In this study, the inhibitory effect and mechanism of rifampicin on the activity of tyrosinase were investigated for developing a novel tyrosinase inhibitor. It was found to have a significant inhibition on the activity of tyrosinase (IC_{50} = 90 ± 0.6 μM). From the kinetics analysis, it was proved to be a reversible and noncompetitive type inhibitor of the enzyme with the K_I value of 94 ± 3.5 μM. The results obtained from intrinsic fluorescence quenching indicated that rifampicin could interact with tyrosinase. In particular, the drastic decrease of fluorescence intensity was due to the formation of a rifampicin-enzyme complex in a static procedure which was mainly driven by hydrophobic forces and hydrogen bonding. Moreover, the ANS-binding fluorescence analysis suggested that rifampicin binding to tyrosinase changed the polarity of the hydrophobic regions. Molecular docking analysis further revealed that the hydrogen bonds were generated between rifampicin and amino residues Leu7, Ser52, and Glu107 in the B chain of the enzyme. And the hydrophobic forces produced through the interaction of rifampicin with B chain residues Pro9, Pro14, and Trp106. This work identified a novel tyrosinase inhibitor and potentially contributed to the usage of rifampicin as a potential hyperpigmentation drug.

1. Introduction

Tyrosinase (EC 1.14.18.1) is a multifunctional oxidase widely distributed in the animal, plant and fungi kingdom [1,2]. It is an important enzyme for melanin biosynthesis of human skin and hair, and in normal retinal development [3–5]. The enzyme could catalysis the oxidation of 3,4-dihydroxyphenylalanine (L-DOPA) to the dopamine quinone in the melanin synthesis [6]. However, the melanin accumulation may cause serious skin diseases such as melasma, freckles, senile lentigines, age spots, and sites of actinic damage in human beings [7]. Therefore, many tyrosinase inhibitors find their application in cosmetics and pharmaceutical products for the prevention of overproduction of melanin in the epidermis.

In recent years, a large number of naturally occurring and synthetic tyrosinase inhibitors have already been reported [8–11], but the safety regulations for drug uses limit their applications in vivo. Rifampicin (see Fig. 1 for structure), one of the most potent and broad semisynthetic antibiotics against bacterial pathogens and is a key component of anti-tuberculosis therapy [12]. In previous study, rifampicin has showed inhibitory activity against amyloid β protein aggregation [13] and *Taq* RNA polymerase (RNAP) [14]. However, there have been no reports on the tyrosinase inhibitory activity of rifampicin. Hence, in this research, we investigated the inhibitory effect of rifampicin on tyrosinase activity. Kinetics analysis, fluorescence quenching, and molecular docking analysis were studied to expound the inhibition mechanism of rifampicin on tyrosinase. The results of this study potentially provided scientific evidence in the developing of hyperpigmentation drugs.

2. Experimental

2.1. Reagents

Mushroom tyrosinase (EC 1.14.18.1) from mushroom (with specific activity 6680U/mg), rifampicin, 3,4-Dihydroxyphenylalanine (L-DOPA) and dimethyl sulfoxide (DMSO) were products of Sigma-Aldrich (St. Louis, MO, USA). 1-Anilino-8-naphthalene sulfonate (ANS) was purchased from Solarbio (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). The water used was redistilled and ion-free.

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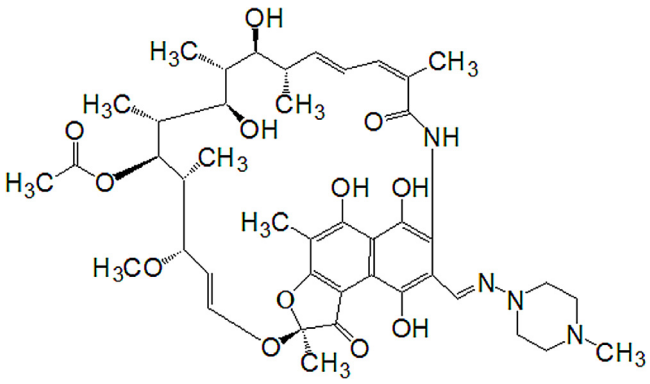

Fig. 1. Chemical structure of rifampicin.

2.2. Enzyme assay

Enzyme assay was performed as previously reported [15–17] with slight modifications. In this investigation, L-DOPA was selected as substrate for the enzyme activity assay. Firstly, 300 μL of a 0.5 mM-DOPA solution was mixed with 750 μL of 50 mM sodium phosphate buffer (pH 6.8) and incubated at 30 °C for 5 min. Then, 100 μL of rifampicin with different concentrations, 1800 μL H_2O, and 50 μL of 0.2 mg/mL the enzyme were added in this order to the mixture and the final volume of the reaction system was kept at 3 mL. The enzyme activity was measured immediately by following the increasing absorbance at 475 nm every 5 s accompanying the oxidation of the substrate with a molar absorption coefficient of 3700 (M^{-1} cm^{-1}) using a Beckman DU-730 (USA) spectrophotometer [18]. The initial slope of the kinetic curve (reaction in initial 30 s) was used as an indicator of tyrosinase activity.

The inhibitory effect of rifampicin on the tyrosinase was expressed as the concentration that inhibited 50% of the enzyme activity (IC_{50}). All the assay were performed as three separate replicates. An inhibitory mechanism assay was carried out by changing the enzyme concentration in the reaction medium. The inhibition type was obtained by the Line-weaver Burk plot, and the inhibition constant was determined by the secondary plots of the apparent K_m/V_m or $1/V_m$ versus the concentration of the rifampicin [19].

2.3. Intrinsic and ANS-binding fluorescence quenching analysis

The fluorescence assay of tyrosinase in the presence of rifampicin was measured according to reference of Kim et al. [20] using a Varian Cary Eclipse fluorescence spectrophotometer (Varian Scientific Instruments, Mulgrave, Australia). The excitation wavelength was set at 290 nm and the range of emission wavelength was set from 300 to 500 nm. Briefly, the reaction medium (1 mL) contained 100 μL of tyrosinase solution (0.2 mg/mL), 800 μL of 50 mM sodium phosphate buffer (pH 6.8), and 100 μL of rifampicin solution (dissolved in DMSO) with different concentrations. Before the measurement, the mixture of tyrosinase and inhibitor were incubated at room temperature for 30 s.

The fluorescence quenching was described by the Stern-Volmer equation [21]:

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

In the equation, F_0 and F are the fluorescence intensities in the absence and presence of rifampicin, respectively. K_q is the biomolecule quenching rate constant. τ_0 is the average lifetime of the fluorophore in the absence of the quencher (the value is 10^{-8} s). [Q] represents the concentration of rifampicin and K_{SV} is the Stern-Volmer quenching constant, which could be calculated by linear regression of a plot of F_0/F against [Q].

Fluorescence quenching might result from a variety of processes, including dynamic quenching, static quenching and a combination of them [22]. When the type was static, the apparent binding constant (K_A) and the number of binding sites (n) could be estimated by the following equation [23]:

$$\log[(F_0 - F)/F] = \log K_A + n\log[Q] \quad (2)$$

There were four main types of noncovalent interactions between the ligand and macromolecule: electrostatic interactions, multiple hydrogen bonds, van der Waals interactions and hydrophobic forces [24]. If the temperature did not vary drastically, the enthalpy change (ΔH°) could be regarded as a constant, and then its value and the value of entropy change (ΔS°) could be calculated from the Van't Hoff equation [24]:

$$\log K_A = -\frac{\Delta H^\circ}{2.303RT} + \frac{\Delta S^\circ}{2.303R} \quad (3)$$

The free energy change (ΔG°) could be determined from the following equation:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (4)$$

Where T represents the absolute temperature used in the experiment. K_A is the binding constant at the corresponding temperature. R is the gas constant (the value is 8.314 J mol^{-1} K^{-1}).

What's more, the ANS-binding fluorescence quenching analysis of tyrosinase was also studied using a Varian Cary Eclipse fluorescence spectrophotometer with a range of emission wavelength from 400 to 600 nm and an excitation wavelength of 350 nm. Tyrosinase was labeled with 100 μL of 100 μM ANS for 5 min prior to the measurements, and the other measurements were the same with the intrinsic fluorescence experiments [25,26].

2.4. Molecular docking

The X-ray structure of tyrosinase from *Agaricus bisporus* (pdb entry 2Y9W) [27] was used as the initial model for molecular docking. Before docking, the water molecules in the enzyme were removed, afterwards Gasteiger charges and polar hydrogen atoms were added to the enzyme molecule by using the AutoDock tools. The 3D structure of rifampicin was produced by Chem Bio Draw Ultra 8.0. Docking process and calculations were carried out according to the default parameters. The docked results obtained from conformation with lowest free energy [28].

3. Results and discussion

3.1. Inhibitory effect of rifampicin on tyrosinase activity

Rifampicin was first investigated for its inhibitory effect on the oxidation of L-DOPA catalyzed by tyrosinase. The result showed that the enzyme activity reduced obviously with the increase of rifampicin concentration, but it was not completely suppressed (see Fig. 2A). The IC_{50} value was estimated to be 90 ± 0.6 μM (Fig. 2A). Compared with arbutin [29] (IC_{50} = 180 μM) and amoxicillin [30] (IC_{50} = 900 μM), rifampicin in this study was better inhibitor of tyrosinase.

3.2. Inhibitory mechanism of rifampicin on tyrosinase activity

Furthermore, the inhibitory mechanism by rifampicin against the tyrosinase activity was tested. The result revealed that the relationship between the enzyme activity and the concentration of rifampicin gave a family of straight lines, which all passed through the origin (Fig. 2B). Therefore, the inhibition of the enzyme by