International Journal of Biological Macromolecules 57 (2013) 151–155

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Antityrosinase and antimicrobial activities of furfuryl alcohol, furfural and furoic acid

Wei-Ming Chai [1], Xuan Liu [1], Yong-Hua Hu, Hui-Ling Feng, Yu-Long Jia, Yun-Ji Guo, Han-Tao Zhou *, Qing-Xi Chen **

School of Life Sciences, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361005, China

ARTICLE INFO

Article history:
Received 24 December 2012
Received in revised form 22 February 2013
Accepted 25 February 2013
Available online 4 March 2013

Keywords:
Antityrosinase activities
Inhibitory kinetics
Antimicrobial activities
Furfuryl alcohol
Furfural
Furoic acid

ABSTRACT

The inhibitory kinetics of furfuryl alcohol, furfural and furoic acid on mushroom tyrosinase have been investigated. The results showed that these furan compounds were reversible inhibitors of the enzyme. Furthermore, furfuryl alcohol and furfural were found to be mixed-type inhibitors while furoic acid is uncompetitive inhibitor. The inhibition constants have been confirmed and the order of the inhibiting ability was furfural > furoic acid > furfuryl alcohol. They indicate that the functional groups on the furan ring play a crucial role in the inhibition on the enzyme. In addition, it was also found that these furan compounds could inhibit the proliferation of *Salmonella bacteria* and *Bacillus subtilis* to different extents. The minimum inhibitory concentration (MIC) values of furfuryl alcohol, furfural and furoic acid against *B. subtilis* and *S. bacteria* were 0.115, 0.027, 0.015 and 0.115, 0.029, 0.009 μM, respectively. The minimum bactericidal concentration (MBC) values of that were 0.115, 0.027, 0.015 and 0.231, 0.121, 0.030 μM, respectively.

1. Introduction

Tyrosinase (EC 1.14.18.1), which is also known as polyphenol oxidase (PPO), is a copper-containing multifunction oxidase widely existed in microorganisms, animals, and plants [1]. It is a key enzyme associated with both melanin biosynthesis and sclerotization in insects. This enzyme plays an important role in normal developmental processes, such as cuticular tanning, scleration, wound healing, production of opsonins, encapsulation, and nodule formation for defense against foreign pathogens in insects [2]. The inhibition of activity on tyrosinase could lead to abrogation of insect defense mechanisms or abnormal body softening, both of which could lead to pest control [3]. Tyrosinase is essential for the formation of melanin [4]. In the bacterium, melanin can protect the bacterial cells and spores against UV radiation [5,6]. It can also bind heavy metals that are toxic to the cells, which is important to bacterial protection mechanisms [7]. Therefore the inhibition of activity of tyrosinase could lead to pest and bacterium control. In our previous research, a great many tyrosinase inhibitors [3,8–10] were screened and these compounds showed inhibition effects on tyrosinase. And many of the inhibitors consist of compounds which structurally analogous to phenolic substrate.

In the present study, furfuryl alcohol, furfural and furoic acid were used as source of tyrosinase inhibitor. Furfuryl alcohol, is one of the most important furan derivatives. Polymeric furfuryl alcohol has been used for preparing corrosion-protective materials, adhesives, pastes, electrode materials, and acid-resistant materials [11]. Furfural, colorless liquid, is a cheap chemical which is industrially obtained in a large amount from the treatment of lignocellulosic materials, derived from agricultural or forestry wastes [12]. Its derivatives have broad usages as braking pad resin, solvents, nematode control agent, and rocket fuel [13]. Furoic acid has been used as a pharmaceutical intermediate, fungicide, preservative hypolipidimic and anti-inflammatory agents [14]. Furan derivatives have been widely used as antimicrobial agents such as furazolidone, nifuroxazide, nitrofurantoin and furoxone [15]. However, there was no report about antimicrobial activities and inhibitory kinetics on the enzyme activity of mushroom tyrosinase with furfuryl alcohol, furfural and furoic acid. This study therefore aims to make a kinetic study about the inhibition on the enzyme activity of tyrosinase with furfuryl alcohol, furfural and furoic acid. In addition, their antimicrobial activities were also studied. This study will serve as the basis for the understanding of that these furan compound may be regarded as potential candidates for novel bioinsecticides and antimicrobials.

* Corresponding author.
** Corresponding author. Tel.: +86 592 2185487; fax: +86 592 2185487.
E-mail addresses: htzhou@xmu.edu.cn (H.-T. Zhou), chenqx@xmu.edu.cn (Q.-X. Chen).
[1] These authors contributed equally to this work.

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.02.019

2. Materials and methods

2.1. Reagents

Mushroom tyrosinase (with specific activity 6680 U/mg), dimethyl sulfoxide (DMSO) and L-3, 4-dihydroxyphenyl-alanine (L-DOPA) were purchased from Sigma (St. Louis, MO, USA). Furfuryl alcohol, furfural and furoic acid were obtained from Sinopharm (Shanghai, China). *Bacillus subtilis* and *salmonella bacteria* were collected from a colony preserved at −80 °C in Fujian academy of agricultural sciences (Fujian, China). Water used in this experiment was purified on a Millipore Milli-Q Apparatus (TGI Pure Water Systems, USA).

2.2. Enzyme assay

2.2.1. Inhibitory effects assay

The assay of the enzyme activity was performed as described by Qiu et al. [16]. In this investigation, L-DOPA was used as substrate for the enzyme activity assay. The reaction medium (3 mL) contained 0.1 mL inhibitor and DMSO, 0.3 mL L-DOPA, 0.75 mL sodium phosphate buffer (pH 6.8), 1.8 mL H_2O, and 0.05 mL enzyme. Then they were mixed and the absorbance values of solutions were measured by a Beckman UD-800 spectrophotometer immediately. The final concentration of mushroom tyrosinase was 6.67 μg/mL. The inhibitor was first dissolved in DMSO. The final concentration of DMSO in the test solution was 3.3% (v/v). The enzyme activity was monitored by dopachrome formation at 475 nm [17] accompanying the oxidation of the substrate. The extent of inhibition by the addition of the sample was expressed as the percentage necessary for 50% inhibition (IC_{50}). Controls, without inhibitor but containing 3.3% DMSO, were routinely carried out. All measurements were carried out at 30 °C.

2.2.2. Inhibitory mechanism assay

Inhibitory mechanism assay was assayed by changing the concentration of the enzyme in reaction medium (3 mL). The plots of the remaining enzyme activity versus the concentrations of enzyme in the presence of different concentrations of inhibitors gave a family of straight lines. If all straight lines pass through the origin, the inhibition is reversible; and if they are parallel lines, the inhibition is irreversible.

2.2.3. Inhibition type and inhibition constants assay

The inhibition type was assayed by the Lineweaver–Burk plot, and the inhibition constant was determined by the second plots of the apparent K_m/V_m or $1/V_m$ versus the concentration of the inhibitor. The mathematical equation between $1/v$ and $1/[S]$ was: $1/v = K_m/V_m \times (1 + [I]/K_I) \times (1/[S]) + 1/V_m \times (1 + [I]/K_{IS})$. Where K_m is Michaelis–Menten constant, V_m is maximum reaction velocity. [S] represents substrate concentration. K_I and K_{IS} are inhibitor constant. [I] represents inhibitor concentration. And v is the rate of reaction.

2.3. Antimicrobial assay

The antimicrobial assay was determined using the agar well diffusion method. Briefly, culture medium was mixed with the given microorganism, and then spread the bacterial inoculums in the media. Wells (7 mm diameter) were punched in the agar and filled with compounds in the concentrations of 0.087, 0.175, 0.35, 0.7 and 1.4 μM, respectively. In this experiment, the antimicrobial assay was carried out on tryptone beef extract agar, at pH 7.2, with an inoculum of 1×10^5 cells/mL. Control wells, containing neat DMSO (negative control) and standard antibiotic streptomycin sulfate (1000 U/mL) for the tested bacteria, were also run parallel in the same plate. Bacteria were incubated at 37 °C for 24 h. Antimicrobial activity was assessed by measuring the diameter of the zone of inhibition for the respective compound. The MIC and the MBC were tested by broth macrodilution methods according to Kubo et al. [18]. Briefly, serial 2-fold dilutions of the test compounds were prepared in DMSO, and 30 μL of each dilution was added to 3 mL of broth. Thirty microliters of the exponentially growing bacterial cells of *S. bacteria* and *B. subtilis* (final 1×10^5 cells/mL) were inoculated into the broth. And then they were incubated at 37 °C for 24 h. The MIC was determined as the lowest concentration of the test compound that demonstrated no visible growth. The MBC was determined as follows. After the determination of the MIC, 100-fold dilutions with compound-free broth from each tube showing no turbidity were incubated at 37 °C for 48 h. The MBC was the lowest concentration of the test compound that showed no visible growth in the compound-free cultivation.

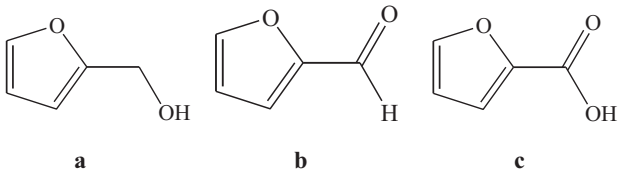

Fig. 1. Chemical structures of furfuryl alcohol (a), furfural (b) and furoic acid (c).

3. Results and discussion

3.1. Inhibitory effects of furfuryl alcohol, furfural and furoic acid on tyrosinase activity

Furfuryl alcohol, furfural and furoic acid (see Fig. 1 for structures) were first tested for the inhibitory effect on the oxidation of L-DOPA catalyzed by mushroom tyrosinase. The activity of tyrosinase decreased with increasing concentrations of the compounds as shown in Fig. 2. The values of IC_{50} for furfuryl alcohol (a), furfural (b) and furoic acid (c) were estimated to be 17.75, 3.38 and 10.00 mM, respectively.

Tyrosinase has two distinct kinds of catalysis functions: the hydroxylation of monophenols and the oxidation of *o*-diphenols to the corresponding *o*-quinone [19]. In present study, the inhibitory kinetics of furfuryl alcohol, furfural and furoic acid on the diphenolase activity of mushroom tyrosinase for the oxidation of L-DOPA

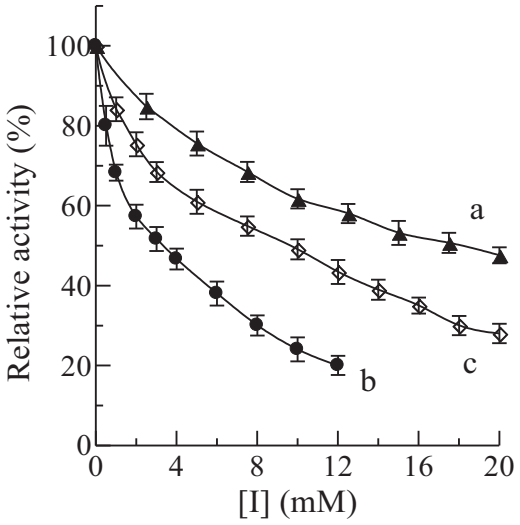

Fig. 2. Inhibitory effects of furfuryl alcohol (a), furfural (b) and furoic acid (c) on the diphenolase activity of mushroom tyrosinase.