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Kinetics of inhibitory effect of isoferulic acid on mushroom tyrosinase

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Synopsis

A study on the kinetics of inhibitory effect of isoferulic acid on the monophenolase and diphenolase activity of mushroom tyrosinase was carried out using enzymological kinetic analysis method in a Na_2HPO_4–NaH_2PO_4 buffer solution (pH = 6.8) at 30°C. It was found that isoferulic acid efficiently inhibits both monophenolase and diphenolase activities of mushroom tyrosinase under experimental conditions. Concentrations of isoferulic acid leading to 50% rate inhibition (IC_{50}) on monophenolase and diphenolase activity were calculated to be 0.13 mmol/L and 0.39 mmol/L, respectively, which are much lower than that of arbutin (IC_{50} = 5.3 mmol/L for diphenolase activity). The presence of isoferulic acid also prolongs the lag period in the oxidation process of L-tyrosine via tyrosinase—a 4.3-min lagging was observed with the presence of 0.20 mmol/L isoferulic acid—compared to a 1.1-min lagging in the absence of isoferulic acid. The Lineweaver–Burk plot demonstrates a competitive behavior of isoferulic acid in the tyrosinase oxidation of L-3,4-dihydroxyphenylalanine, with maximum reaction rate (v_m) and inhibition constant (K_I) at 64.5 μM/min and 0.11 mmol/L, respectively.

INTRODUCTION

Tyrosinase, also known as polyphenol oxidase, is a copper-containing mixed-function oxidase widely distributed in microorganisms, animals, and plants (1,2). It is recognized as a pivotal enzyme in the process of melanin biosynthesis. Tyrosinase catalyzes two critical reactions in melanin synthesis: (i) hydroxylation of monophenol to *o*-diphenol (monophenolase activity) and (ii) conversion of *o*-diphenol to the corresponding *o*-quinone (diphenolase activity). The resulting quinone is subsequently subjected to a series of oxidation/polymerization processes to form dark pigments, also known as "melanine."

It is evident that tyrosinase is a key enzyme controlling the melanization process of skin, eye, inner ear, and hair, as well as the enzymatic browning process in fruits and vegetables (3–5). Meanwhile, the application of tyrosinase inhibitors has attracted more and more attention in the fields of cosmetic, food, and pharmaceutical industry, primarily because

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of their high efficacy in mitigating hyperpigmentation (6). Much effort has been made in searching for feasible and effective tyrosinase inhibitors. For instance, based on systematic studies on the inhibitory effect of quercetin, dodecyl gallate, and thymol on mushroom tyrosinase, Kubo *et al.* proposed a kinetic model of the inhibition process and pointed out some favorable features in molecular structure for a potential effective inhibitor (4,6,7). Nerya *et al.* analyzed a series of inhibitors extracted from the root of Licorice (8). Gong *et al.* has previously reported some potent tyrosinase inhibitors, such as ferulic acid and cinnamic acid (9,10).

Isoferulic acid is an active component found in *Rhizoma Cimicifugae*. Reported in this paper is a kinetic study on the inhibitory effect of isoferulic acid on mushroom tyrosinase. By investigating the *in vitro* inhibitory effect of isoferulic acid on both monophenolase and diphenolase activities of mushroom tyrosinase, a competitive inhibition model was established and the kinetic parameters were calculated. The current results provide experimental support for the potential application of isoferulic acid as a high-efficacy anti-pigment ingredient in industry.

EXPERIMENTAL

MATERIALS

Mushroom tyrosinase, L-tyrosine, and L-3,4-dihydroxyphenylalanine (L-DOPA) were purchased from Sigma (Shanghai, China). Isoferulic acid was obtained from the National Institute for the Control of Pharmaceutical and Biological Product (Guangdong, China). Dimethyl sulfoxide (DMSO) and other reagents were of analytical grade and obtained from commercial suppliers. Double distillated and de-ionized water was used unless stated otherwise.

METHODS

The diphenolase activity assay was performed as previously reported (9). The monophenolase activity assay was performed with L-tyrosine as substrate. Using a microsyringe, a tyrosinase solution (0.20 ml, 0.38 mmol/L) was added to a thermostatic solution (5.0 ml, 30°C) containing 50 mmol/L Na_2HPO_4–NaH_2PO_4 (pH = 6.8), 1.5 mmol/L L-tyrosine and different concentrations of isoferulic acid (predissolved in DMSO). The resulting mixture was mixed thoroughly using the syringe and immediately monitored by spectrophotometry at 475 nm for 10 min. The molar absorption coefficient of the oxidation product (*o*-quinone) from the substrate (L-tyrosine or L-DOPA) is calculated to be 3700 $(mmol/L \cdot cm)^{-1}$. Absorption data were recorded on an HP 6010 UV spectrophotometer by Agilent Technologies (Shanghai), China.

RESULTS AND DISCUSSION

EFFECT OF ISOFERULIC ACID CONCENTRATION ON MONOPHENOLASE ACTIVITY OF TYROSINASE

The inhibitory effects of different concentrations of isoferulic acid on the oxidation of L-tyrosine via tyrosinase were studied. The kinetic course of the oxidation of L-tyrosine in

the presence of isoferulic acid in different concentration levels (c_I) is shown in Figure 1. The lag period and inhibition rate against monophenolase activity of tyrosinase (I_M) increased accordingly with the increase in concentration of isoferulic acid, as shown in Figure 2. The lag period was estimated to be 1.1 min in the absence of inhibitor and almost quadrupled to 4.3 min in the presence of 0.20 mmol/L isoferulic acid. The inhibitor concentration leading to 50% monophenolase activity lost (IC_{50}) was estimated to be 0.13 mmol/L.

EFFECT OF ISOFERULIC ACID CONCENTRATION ON DIPHENOLASE ACTIVITY OF TYROSINASE

The inhibitory effect of isoferulic acid on the oxidation of L-DOPA via tyrosinase was also studied. As shown in Figure 3, the kinetic curves of the oxidation of L-DOPA in the presence of isoferulic acid in different concentration levels indicate that there is no lag period in L-DOPA oxidation. Increasing the concentration of isoferulic acid (c_I) resulted in a rapid increase of the inhibition rate against the diphenolase activity of tyrosinase (I_D), reaching a fairly high inhibition rate of 73.2% when c_I was 0.80 mmol/L, as shown in Figure 4. The inhibitor concentration leading to 50% diphenolase activity lost (IC_{50}) was estimated to be 0.39 mmol/L.

KINETIC PARAMETERS IN L-DOPA OXIDATION VIA TYROSINASE WITH ISOFERULIC ACID AS INHIBITOR

A steady-state analysis was performed to estimate the inhibition type and the kinetic parameters of the reaction system during the oxidation of L-DOPA. Lineweaver–Burk plot for the inhibitory effect of isoferulic acid against diphenolase activity of tyrosinase was shown in Figure 5.

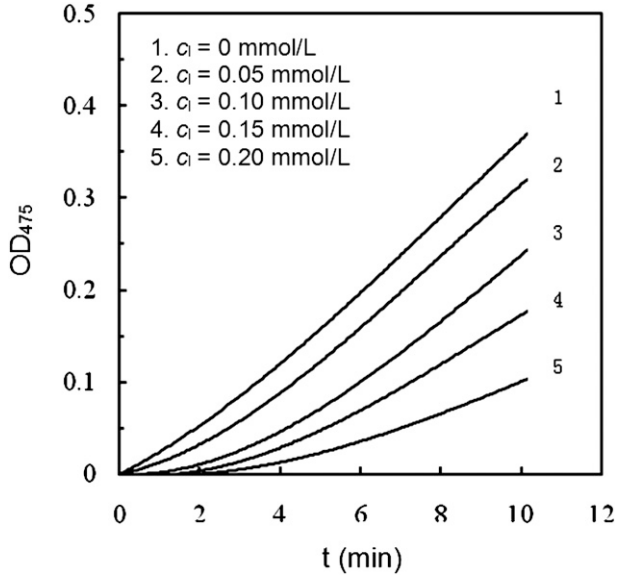

Figure 1. Progress curves of L-tyrosine oxidation via tyrosinase with isoferulic acid as inhibitor.

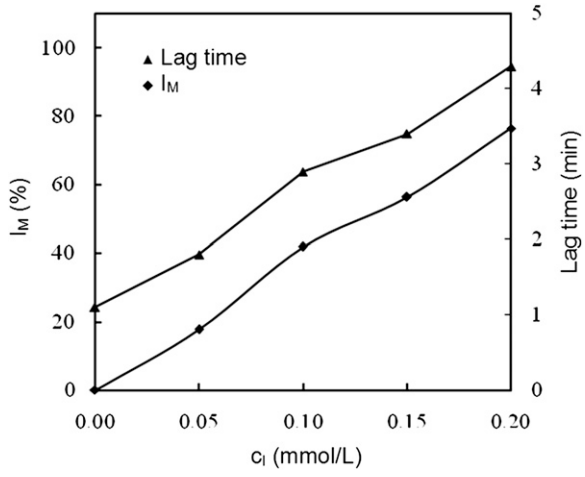

Figure 2. Effect of isoferulic acid concentration on monophenolase inhibition rate and lag time.

Under the conditions employed in the current investigation, the oxidation of L-DOPA by tyrosinase complies with a Michaelis–Menten mechanism. However, since the experiments were carried out in air-saturated aqueous solutions, the Michaelis constant (K_m) and the maximum reaction rate (v_m) deduced are only apparent. The effect of oxygen concentration on the kinetic parameters needs to be further investigated in subsequent studies.

The result illustrated in Figure 5 shows that isoferulic acid is a competitive inhibitor, as increasing the concentration of isoferulic acid resulted in a family of lines with different slopes but sharing a common intercept on the $1/v$ axis.

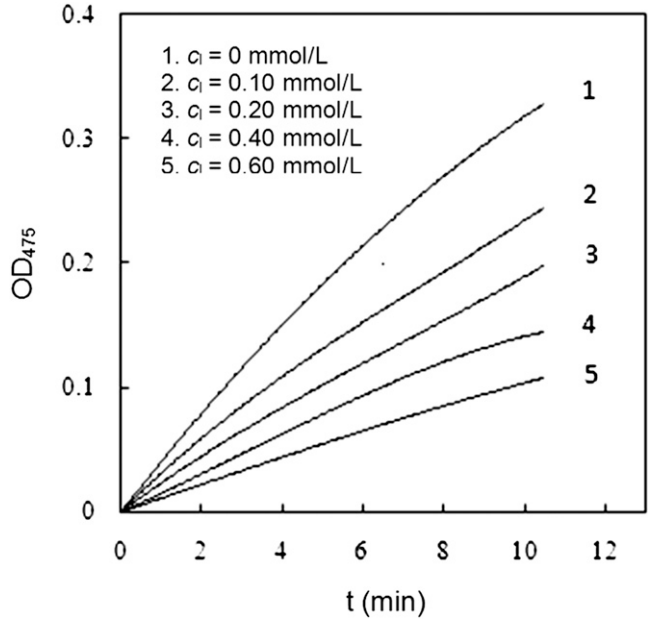

Figure 3. Progress curves of L-DOPA oxidation via tyrosinase with isoferulic acid as inhibitor.

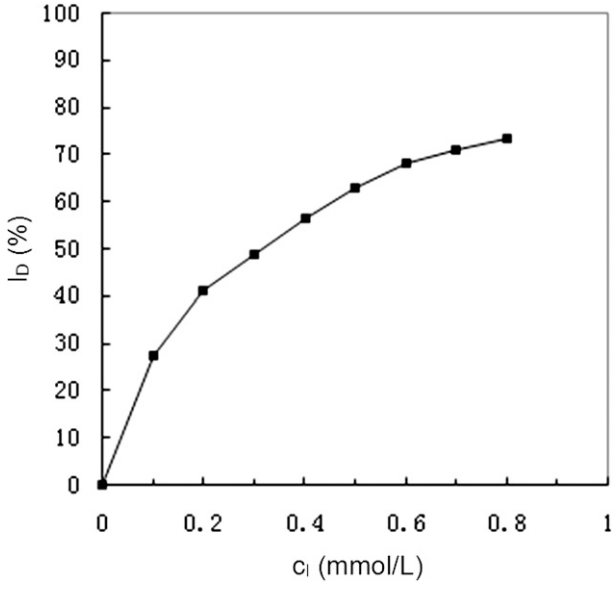

Figure 4. Effect of concentration of isoferulic acid on diphenolase inhibition rate.

The kinetic parameters of K_m and v_m for mushroom tyrosinase were calculated from the Lineweaver–Burk plot, using a method introduced by Huang *et al.* (5). The equilibrium constant K_I, in the binding and dissociation equilibrium of the inhibitor and the enzyme, was obtained from the linear plot of K_m versus c_I, as shown in Figure 6. The inhibition constant of isoferulic acid obtained from the experiment data is also listed in Table I.

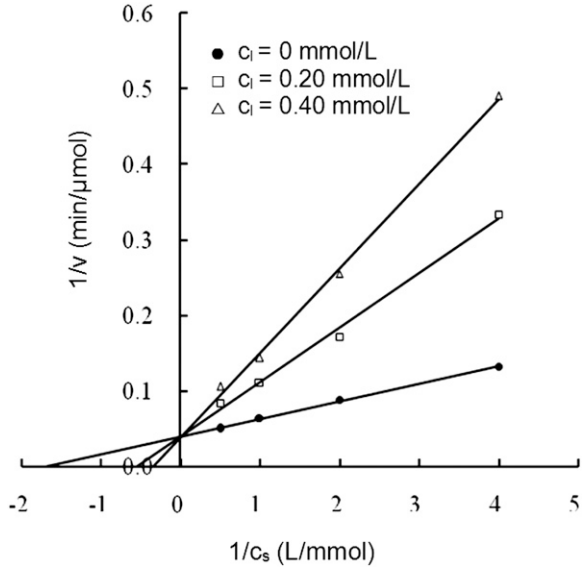

Figure 5. Lineweaver–Burk plot of isoferulic acid inhibitory effect on diphenolase activity of tyrosinase.

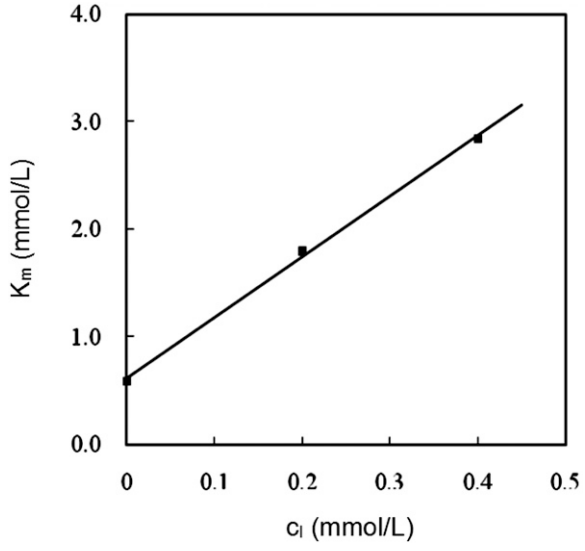

Figure 6. Effect of isoferulic acid concentration on Michaelis constant (K_m).

CONCLUSIONS

Described in this paper is a detailed investigation on the inhibition kinetics of isoferulic acid on both monophenolase and diphenolase activities of mushroom tyrosinase. The results showed that isoferulic acid is an effective inhibitor against both monophenolase and diphenolase activities of mushroom tyrosinase. Additionally, the presence of isoferulic acid prolongs the lag period in the inhibited monophenol oxidation. Furthermore, a competitive inhibition behavior was observed for isoferulic acid, when it was inhibiting the diphenolase activity of mushroom tyrosinase. Concentrations of isoferulic acid leading to 50% rate inhibition (IC_{50}) on monophenolase and diphenolase activity were calculated to be 0.13 mmol/L and 0.39 mmol/L, respectively, which are much lower than that of arbutin (IC_{50} = 5.3 mmol/L for diphenolase activity).

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Table I
Kinetic Parameters of the Isoferulic Acid Inhibitory Effect on Diphenolase Activity

c_I (mmol/L)	Michaelis–Menten equation	K_m (mmol/L)	v_m (μM/min)	K_I (mmol/L)
0	$1/v = 0.023/c_S + 0.039$	0.59		
0.20	$1/v = 0.071/c_S + 0.040$	1.79	64.5	0.11
0.40	$1/v = 0.111/c_S + 0.039$	2.84		

REFERENCES

(1) L.G. Fenoll, M.J. Peñalver, J.N. Rodríguez-López, R. Varón, F. García-Cánovas and J. Tudela, Tyrosinase kinetics: discrimination between two models to explain the oxidation mechanism of monophenol and diphenol substrates, *Int. J. Biochem. Cell Biol.*, **36**, 235-246 (2004).
(2) I. Kubo, Q.X.Chen and K.I.Nihei, Molecular design of antibrowning agents: antioxidative tyrosinase inhibitors, *Food Chem.*, **81**, 241-247 (2003).
(3) O. Zarivi, A. Bonfigli, P. Cesare, F. Amicarelli, G. Pacioni and M. Miranda, *FEMS Microbiol. Lett.*, Truffle thio-flavours reversibly inhibit truffle tyrosinase, **220,** 81-88, (2003).
(4) H. Huang, X.D. Liu and Q.X.Chen, (title in Chinese: 苯甲醛族化合物抑制蘑菇酪氨酸酶活力的研究), *J. of Xiamen University (Nature Science)*, **42**, 97-101 (2003).
(5) C. Tan, W.Y. Zhu and Y. Lu, Aloin, cinnamic acid and sophorcarpidine are potent inhibitors of tyrosinase, *Chin. Med. J.*, **115**, 1859-1862 (2002).
(6) I. Kubo and K.H. Ikuyo, 2-Hydroxy-4-methoxybenzaldehyde: A Potent Tyrosinase Inhibitor from African Medicinal Plants, *Planta Med.*, **65**, 19-22 (1999).
(7) H. Satooka and I. Kubo, Effects of Thymol on Mushroom Tyrosinase-Catalyzed Melanin Formation, *J. Agric. Food Chem.*, **59**, 8908-8914 (2011).
(8) O. Nerya, J. Vaya, R. Musa, S. Izrael, R. Ben-Arie, and S. Tamir, Glabrene and Isoliquiritigenin as Tyrosinase Inhibitors from Licorice Roots, *J. Agric. Food Chem.*, **51**, 1201-1207 (2003).
(9) S.Z. Gong, J. Cheng and Z.R. Yang, Inhibitory Effect of Ferulic Acid on Oxidation of L-DOPA Catalyzed by Mushroom Tyrosinase, *Chinese J. of Chem. Engineering*, **13**, 771-775 (2005).
(10) S.Z. Gong, Z.R. Yang and J. Cheng, (title in Chinese: 肉桂酸抑制酪氨酸酶催化反应的动力学研究), *J. of Chemical Engineering of Chinese University*, **21**, 345-349 (2007).