International Journal of Biological Macromolecules 50 (2012) 694–700

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Kinetic, structural and molecular docking studies on the inhibition of tyrosinase induced by arabinose

Wei-Jiang Hu[a], Li Yan[a], Daeui Park[b], Hyoung Oh Jeong[b], Hae Young Chung[b], Jun-Mo Yang[c], Zhuo Ming Ye[e,*], Guo-Ying Qian[d,**]

[a] Zhejiang Provincial Key Laboratory of Applied Enzymology, Yangtze Delta Region Institute of Tsinghua University, Jiaxing 314006, PR China
[b] Molecular Inflammation Research Center for Aging Intervention (MRCA), College of Pharmacy, Pusan National University, Busan 609-735, Republic of Korea
[c] Department of Dermatology, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul 135-710, Republic of Korea
[d] College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, PR China
[e] Department of Environmental Health, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, PR China

ARTICLE INFO

Article history:
Received 19 October 2011
Received in revised form
26 November 2011
Accepted 29 December 2011
Available online 8 January 2012

Keywords:
Tyrosinase
Arabinose
Inhibition kinetics
Folding
Docking simulation

ABSTRACT

Tyrosinase plays a central role in biological pigment formation, and hence knowledge of tyrosinase catalytic mechanisms and regulation may have medical, cosmetic, and agricultural applications. We found in this study that arabinose significantly inhibited tyrosinase, and this was accompanied by conformational changes in enzyme structure. Kinetic analysis showed that arabinose-mediated inactivation followed first-order kinetics, and single and multiple classes of rate constants were measured. Arabinose displayed a mixed-type inhibitory mechanism with $K_i = 0.22 \pm 0.07$ mM. Measurements of intrinsic and ANS-binding fluorescence showed that arabinose induced tyrosinase to unfold and expose inner hydrophobic regions. We simulated the docking between tyrosinase and arabinose (binding energies were −26.28 kcal/mol for Dock6.3 and −2.02 kcal/mol for AutoDock4.2) and results suggested that arabinose interacts mostly with His61, Asn260, and Met280. The present strategy of predicting tyrosinase inhibition by simulation of docking by hydroxyl groups may prove useful in screening for potential tyrosinase inhibitors, as shown here for arabinose.

1. Introduction

Tyrosinase (EC 1.14.18.1) is a ubiquitous enzyme with diverse physiological roles related to pigment production. Tyrosinase catalyzes the pigmentation of skin [1,2], the browning of fruits and vegetables [3,4], wound healing [5,6], and cuticle formation in insects [7,8]. Tyrosinase inhibitors have potential applications in medicine, cosmetics (e.g., as whitening agents), and agriculture (as bio-insecticides). Structurally, tyrosinase belongs to the type 3 copper protein family [9,10], with two copper ions each coordinately bonded with a distinct set of three histidine residues within the active site [11]. The tyrosinase mechanism is complex, in that this enzyme can catalyze multiple reactions. The copper atoms participate directly in hydroxylation of monophenols to *o*-diphenols (cresolase activity) and in the oxidation of *o*-diphenols to *o*-quinones (catechol oxidase activity) [12]. The overall 3D structures and architecture of the active site in tyrosinase from various sources are gradually emerging [11,13,14]. Studies of this enzyme mechanism demand a variety of kinetic and computational methods to derive key structure–function relationships, e.g., between substrates and ligands of the enzyme [15–19].

Arabinose is a natural five-carbon sugar component of vegetables and fruits, an aldopentose having both sugar and aldehyde groups in its structure [20]. The inhibitory effects of some compounds with sugar backbones on tyrosinase may be of interest in studies of the enzyme mechanism [21–25]. The D-arabinose was used to immobilize mushroom tyrosinase on a re-usable glass bead preparation [26]. In this context, the binding affinities of arabinose for tyrosinase and copper ions at the tyrosinase active site identified arabinose as a potential tyrosinase inhibitor.

In this study, we investigated the mechanism of tyrosinase inhibition and unfolding induced by arabinose using kinetic analysis and computational simulation. We hypothesized that the aldehyde (CHO) functional group of arabinose participates in blocking catalysis and inducing unfolding by binding to tyrosinase. Previous findings show the importance of aldehyde groups in tyrosinase inhibition [27–29] in terms of molecular position, number, and specific interactions with the enzyme; these findings further support

Abbreviations: DOPA, 3,4-dihydroxyphenylalanine; ANS, 1-anilinonaphthalene-8-sulfonate; MD, molecular dynamics; ns, nanosecond.

* Corresponding author. Tel.: +86 20 61648329; fax: +86 20 61648334.
** Corresponding author. Tel.: +86 574 88222298; fax: +86 574 88222298.
E-mail addresses: yzhuom@fimmu.com (Z.M. Ye), qianguoying_wanli@hotmail.com (G.-Y. Qian).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2011.12.035

our hypothesis. Experimentally, arabinose exerted a mixed-type of inhibition on tyrosinase. Kinetic parameters suggested that arabinose binds to the active site, while measurements of intrinsic and ANS-binding fluorescence revealed significant changes in tertiary structure of tyrosinase. To further explore the docking action of arabinose on tyrosinase, we used computational simulations. Our findings point to new applications for arabinose as a tyrosinase inhibitor.

2. Materials and methods

2.1. Materials

Tyrosinase (M.W. 128 kDa), L-DOPA, ANS and arabinose were purchased from Sigma–Aldrich.

2.2. Tyrosinase assay

A spectrophotometric tyrosinase assay was performed as previously described [30,31] in 50 mM sodium phosphate buffer (pH 6.8). To begin the assay, a 10-μl sample of enzyme solution was added to 1 ml of reaction mix. Tyrosinase activity (v) was recorded as the change in absorbance per min at 492 nm using a Perkin Elmer Lambda Bio U/V spectrophotometer.

2.3. Kinetic analysis for the mixed-type inhibition

To describe the mixed-type inhibition mechanism, the Lineweaver–Burk equation in double-reciprocal form can be written as:

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{\alpha K_i}\right) \quad (1)$$

Secondary plots can be constructed from

$$Slope = \frac{K_m}{V_{max}} + \frac{K_m[I]}{V_{max}K_i} \quad (2)$$

$$\text{and} \quad Y\text{-}intercept = \frac{1}{V_{max}^{app}} = \frac{1}{V_{max}} + \frac{1}{\alpha K_i V_{max}}[I] \quad (3)$$

Values for α, K_i, K_m, and V_{max} can be derived from the equations above. The secondary replot of *Slope* or *Y-intercept* vs. [I] is linearly fitted, assuming a single inhibition site or a single class of inhibition site.

2.4. Rate constants calculations

$$\Delta A = A_\infty - A_t \quad (4)$$

where A_∞ is the enzyme activity at the end of the reaction reaching the equilibrium state and A_t is the enzyme activity at time t during inactivation. The experimental data was fitted to first-order expressions as:

$$\Delta A = P_1 \exp\left(\frac{-k_1}{t}\right) + P_2 \exp\left(\frac{-k_2}{t}\right) \quad (5)$$

where P_1 and P_2 indicate the fractions reacting with the rate constants k_1 and k_2, respectively, for a biphasic reaction.

2.5. Intrinsic and ANS-binding fluorescence measurements

Fluorescence emission spectra were measured with a Jasco FP750 spectrofluorometer using a cuvette with a 1-cm path length. Tryptophan fluorescence was measured following excitation at 280 nm, and the emission wavelength ranged between 300 and 410 nm. Changes in the ANS-binding fluorescence of tyrosinase were measured following excitation at 390 nm, and the emission wavelength ranged from 400 to 520 nm. The tyrosinase was labeled with 40 μM ANS for 30 min prior to measurements.

2.6. Molecular docking of tyrosinase and arabinose

Among the many tools available for protein–ligand docking studies, DOCK6.3 and AutoDock4.2 were applied for their automation capability. To model the tyrosinase structure, we used the crystal structure of *Agaricus bisporus* tyrosinase (PDB ID: 2Y9X) [11]. Because the docking programs use a set of predefined 3D grids of the target protein with a systematic search technique [32], we selected the previously defined 3D grids on tyrosinase which are interacted with tropolone as the binding pocket. The scores for docking simulation are approximate binding energies. The docking score for the ligand with receptor is represented by various energy terms such as electrostatic energy, van der Waals energy, and solvation energy. For ligand–protein docking, the

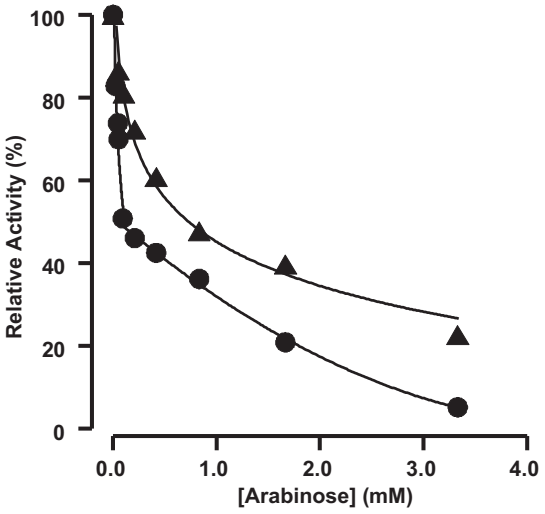

Fig. 1. Inhibitory effect of arabinose on tyrosinase. Tyrosinase was incubated with arabinose at various concentrations for 3 h at 25 °C and then added to the assay system at the corresponding arabinose concentrations (●) or in the absence of arabinose (▲). Data are presented as means (n = 3). The final concentrations of L-DOPA and tyrosinase were 2 mM and 2.9 μg/ml, respectively.

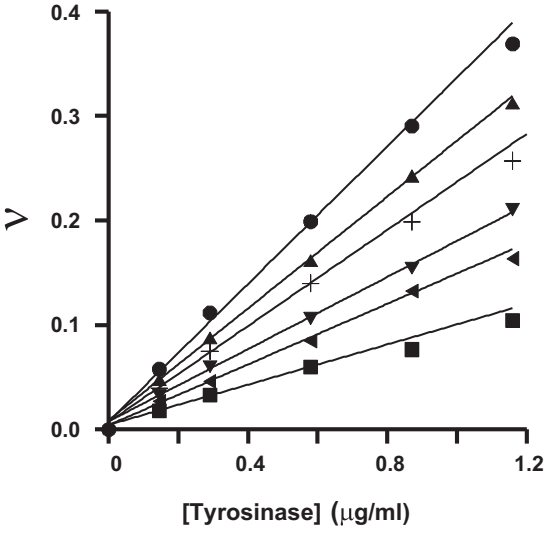

Fig. 2. Plots of v vs. [Tyrosinase]. The v value indicates the change in absorbance at 492 nm per min at arabinose concentrations of 0 (●), 0.208 (▲), 0.416 (+), 0.832 (▼), 1.665 (◄) and 3.33 (■) mM. The final L-DOPA concentration was 2 mM.