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The effect of validamycin A on tyrosinase: Inhibition kinetics and computational simulation

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ABSTRACT

In this study, we investigated validamycin A as a tyrosinase inhibitor based on its structural properties. We found that the reversible inhibition of tyrosinase by validamycin A occurred in a mixed-type manner with $K_i = 5.893 \pm 0.038$ mM, as determined by integrating kinetics studies and computational simulations. Time-interval tyrosinase studies showed that the inhibition followed first-order kinetics with two phases. Fluorescence measurements of ANS binding showed that validamycin A induced changes in the tertiary protein structure of tyrosinase. To obtain further insight, computational docking and molecular dynamics were applied, and the results indicated that HIS85, HIS244, GLU256, HIS259, and ASN260 of tyrosinase interacted with validamycin A. This strategy of predicting tyrosinase inhibition based on hydroxyl group numbers might be useful in the design and screening of potential tyrosinase inhibitors.

1. Introduction

Tyrosinase (EC 1.14.18.1) which belongs to the type 3 copper protein family [1–3] is well known for its important role in the pathway of melanin biosynthesis. Because it catalyzes the first two reactions of the melanogenesis process namely: the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone as Scheme 1 shows [4,5]. As the reason of this function, tyrosinase is very important for insects to produce melanin, harden and stabilize the exoskeleton, and activate the immune response [6–9]. Thus, finding a good inhibitor for tyrosinase, would facilitate the control of insect growth.

Although numerous tyrosinase inhibitors have been reported, only a few are used today because many of them have side effects such as dermatitis and skin irritation, post-inflammatory pigmentation, ochronosis and skin cancer [10–13]. Therefore, new candidates that show effective tyrosinase inhibition without negative side effects need to be identified. In recent years, the mechanism of tyrosinase inhibition has been reported by our laboratory [14–18]. We found that the hydroxyl groups in the molecular structure of a tyrosinase inhibitor are important for its inhibitory action. Based on these findings, validamycin A was investigated as a tyrosinase inhibitor because of its relatively low toxicity to humans and because its molecular structure contains many hydroxyl groups (Scheme 2).

Validamycin A is produced by *Streptomyces hygroscopicus* fermentation and has been widely used as an aminoglycoside agricultural antibiotic against the rice sheath blight caused by the phytopathogenic fungus *Rhizoctonia solani* [19–21]. In recent years, validamycin A was also reported to be a potential glucosidase and trehalase inhibitor [22–25]. However, the inhibitory effect of validamycin A on tyrosinase is not known; thus, identifying validamycin A as a tyrosinase inhibitor would provide a theoretical basis for its application as an insect growth regulator.

In this study, the inhibitory function of validamycin A, its kinetics and its interaction with tyrosinase were investigated by computational simulation. Our results showed that validamycin A binds directly to several residues in the active site of tyrosinase, including HIS85, HIS244, GLU256, HIS259 and ASN260. The kinetics studies and the computational simulations indicated that

Abbreviations: DOPA, 3,4-dihydroxyphenylalanine; ANS, 1-anilinonaphthalene-8-sulfonate.

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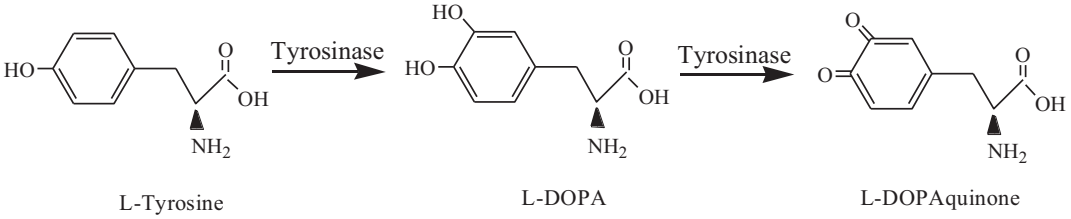
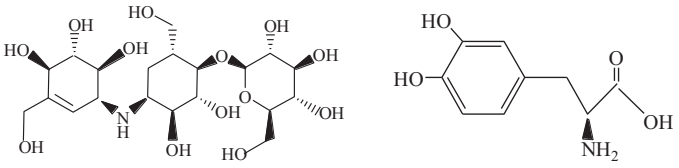


Scheme 1.



Scheme 2.

validamycin A is an effective tyrosinase inhibitor and provided useful information about the inhibition mechanism of aminoglycosides on tyrosinase.

2. Materials and methods

2.1. Materials

Tyrosinase (M.W. 128 kDa), L-DOPA and ANS were purchased from Sigma–Aldrich, Shanghai, China. Validamycin A was provided by Qianjiang Biochem Co. Ltd., China. When L-DOPA was used as a substrate in our experiments, the tyrosinase had a K_m of 0.393 ± 0.062 mM ($V_{max} = 0.2083 \pm 0.009$ mM min^{-1}), which was calculated using a Lineweaver–Burk plot.

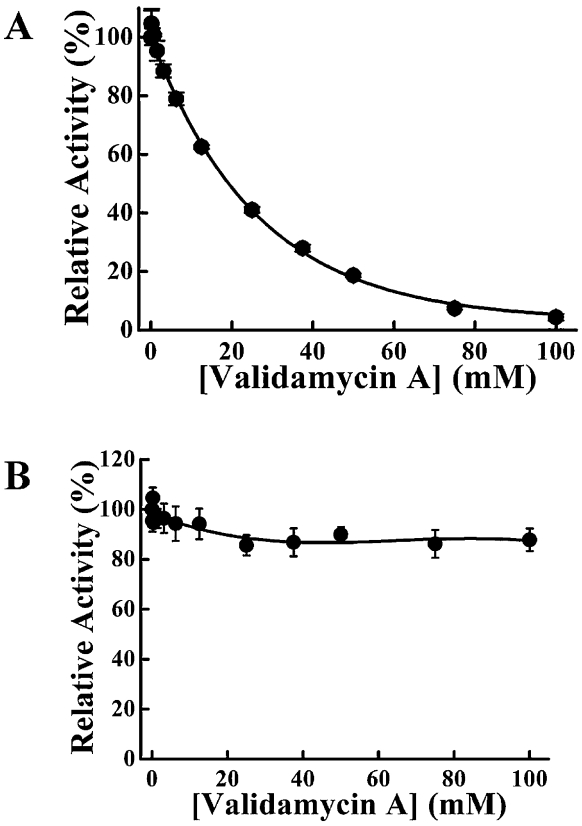


Fig. 1. Inhibitory effect of validamycin A on tyrosinase. Tyrosinase was incubated with different validamycin A concentrations for 3 h at 25 °C, pH 7.0, respectively, and then added to the assay system containing 2 mM L-DOPA with the corresponding validamycin A concentrations (A) or with the absence of validamycin A (B). The final concentrations of L-DOPA and tyrosinase were 2 mM and 1.0 μg/mL, respectively.

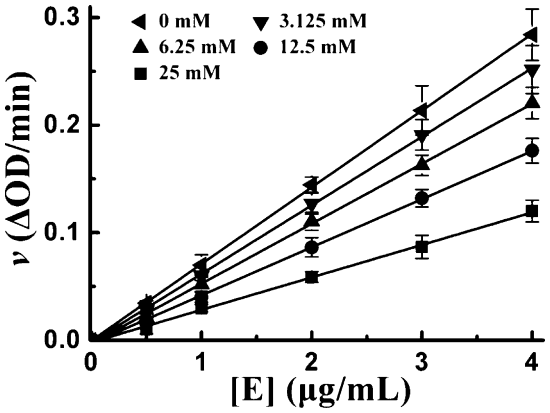


Fig. 2. Further determination of the effect of validamycin A on tyrosinase. The ν value indicates the change in absorbance at 475 nm/min at validamycin A concentrations of 0 mM, 3.125 mM, 6.25 mM, 12.5 mM and 25 mM under the condition of 25 °C and pH 7.0. The final L-DOPA concentration was 2 mM.

2.2. Tyrosinase assay

A spectrophotometric tyrosinase assay was performed as previously described [26,27]. To begin the assay, a 10 μL sample of enzyme solution was added to 1 mL of reaction mix containing 2 mM L-DOPA and 50 mM sodium phosphate buffer. Tyrosinase activity (ν) was recorded as the change in absorbance at 475 nm/min using a SHIMADZU UV-1800 spectrophotometer under the temperature of 25 °C and pH 7.0.

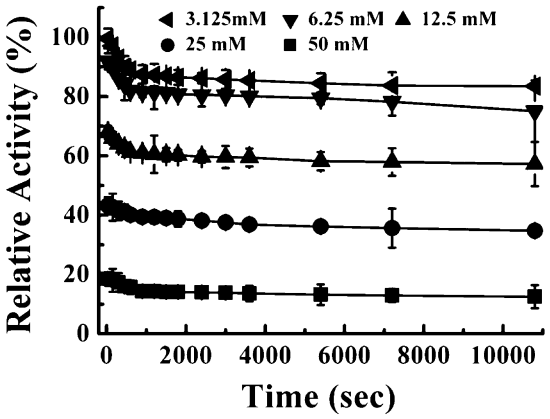


Fig. 3. Time course of tyrosinase inhibition in the presence of validamycin A. The enzyme solution was mixed with validamycin A at 3.125 mM, 6.25 mM, 12.5 mM, 25 mM and 50 mM, and aliquots were collected for assaying at the indicated time intervals under the condition of 25 °C and pH 7.0. The final concentrations of L-DOPA and tyrosinase were 2 mM and 1.0 μg/mL, respectively.