

Inhibitory Effects of a Palladium Complex on the Activity, Stability, and Structure of Tyrosinase Enzyme

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Background: Tyrosinase, as a copper-containing enzyme, is widely distributed in different levels of life span. It is also a key enzyme in melanin biosynthesis, which plays a crucial role in determining the color of mammalian skin and hair.

Objectives: The current study aimed to determine the effect of a palladium complex on cresolase and catecholase reactions of mushroom tyrosinase (MT).

Materials and Methods: The MT kinetics parameters were obtained from double reciprocal plots of Lineweaver-Burk and the inhibition constants (K_i) were determined by the secondary plots. Thermodynamic parameters were obtained from thermal and chemical denaturation of the tyrosinase with and without the presence of palladium complex. The tertiary and secondary structures of tyrosinase were detected by fluorescent and Circular Dichroism (CD) techniques.

Results: The inhibition modes of palladium complex were competitive in both activities of the enzyme with K_i values of 3.74 and 10.55 μM for cresolase and catecholase activities, respectively. In thermal denaturation, the melting points (T_m) of the enzyme were 59.4 °C and 51 °C for the sole enzyme and its treatment by palladium, respectively. In chemical denaturation, the magnitudes of half denaturant concentration (C_m) were 1 μM vs. 1.36 μM and the free energy of Gibbs ($\Delta G_{\text{H}_2\text{O}}$) were calculated 9.3 vs. 7.5 kJ/M for the sole enzyme and its treatment by palladium, respectively.

Conclusions: In overall the palladium complex acted as a good inhibitor of tyrosinase and induced the enzyme thermodynamic and conformational instability, therefore it can be considered in the hyper expression of tyrosinase in melanoma cancer.

Keywords: Mushroom; Tyrosinase; Palladium; Inhibition

1. Background

Tyrosinase (EC 1.14.18.1), as a bifunctional enzyme, catalyzes the o-hydroxylation of monophenols to the corresponding catechols (cresolase activity), and the oxidation of catechols to the corresponding o-quinones (catecholase activity). The enzyme active site can be found in three met, deoxy, and oxy forms (1). Structural models for the active site of these three enzyme forms are proposed by considering its binding with oxygen (2). The common mushroom tyrosinase (MT) from the species *Agaricus bisporus* is a copper containing enzyme with a molecular mass of 120 kD composed of two H subunits (43 kD) and two L subunits (13 kD) and has two active sites. The active sites include a pair of copper ions, each bound with three conserved histidine residues (3). After the two consecutive cresolase and catecholase activities as the enzymatic reactions and production of ortho-quinones, they can polymerize non-enzymatically to melanin as the most important natural biopolymer responsible for pigmentation and the color and patterns of mammalian skin. The production of abnormal melanin pigmentation (melasma, freckles, ephelide, senile lentigines, etc.) is a

serious esthetic problem in human beings (4). Tyrosinase pigments can be detected in the mammals' brains (5). Tyrosinase may play a role in neuromelanin formation in the human brain, -be central to dopamine neurotoxicity, and contribute to the neurodegeneration associated with the Parkinson (6). The enzyme can be utilized in pharmaceutical, cosmetic, and hygienic productions (7). Many tyrosinase inhibitors that suppress melanogenesis are actively studied to develop hyper pigmentation treatment (8-10). The studies showed that palladium complexes could restrict multiplication of some groups of tumor cells sensitive to cisplatin (e. g. pam212, vero, Hela) as well as some others resistant to cisplatin (e. g. pam-ras) (11, 12). Besides, palladium complexes are less toxic in kidneys than - cisplatin. Considering the aforementioned reasons, palladium complexes can be widely used to treat different kinds of diseases (13, 14). It is reported that the failure of cisplatin to treat tumors of gastrointestinal region is mainly due to high concentration of chloride in this region (15). However, since palladium complexes, containing chelating ligands, do not interact with chlo-