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Inhibition of Tyrosinase Activity on Dopamine Hydrochloride by Kojic Acid

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The inhibition effect of kojic acid on tyrosinase activity was studied and the rate of enzymatic reaction in presence and absence of the inhibitor was calculated. It was shown that increasing the concentration of inhibitors reduces the enzymatic reaction rate linearly. The kinetics of inhibition by kojic acid was studied and the Michaelis constants calculated. It was found that kojic acid could non-competitively inhibit the tyrosinase action on its substrate, dopamine hydrochlodride under specified conditions.

Key Words: Tyrosinase, Enzyme kinetics, Enzyme inhibition, Polyphenol oxidase, Kojic acid.

INTRODUCTION

Polyphenol oxidases (PPO) (EC 1.14.18.1) are oxido-reductases that catalyze the hydroxylation of monophenols and the subsequent oxidation of *o*-diphenols to *o*-quinones[1]. The enzymatic products, *o*-quinones, are susceptible to oxidation, leading to polymerization and the formation of brown, red or black pigments[2, 3]. The most important endogenous phenolic substrates for PPO in apple and potato sources are chlorogenic acid, catechol, caffeic acid, L-3,4-dihydroxyphenylalanine (L-DOPA), 4-methyl catechol, *p*-hydroxyphenyl acetic acid (DHPAA), 4-hydroxyphenyl pyruvic acid, *p*-coumaric acid and *m*- and *p*-cresol[4].

Polyphenol oxidase (PPO) mediated browning in raw fruits and vegetables is a major cause of quality deterioration in fruits and vegetables and their derived food products[5]. Enzymatic browning affects the appearance, organoleptic properties and nutritional quality of food products. The control of enzymatic browning is, therefore, a challenge to the food industry[6, 7]. Sulfur amino acids such as L-cysteine and tripeptide glutathione (reduced form) have been reported as effective inhibitors of browning in fruit juices[8]. The use of natural substances such as honey has also been reported as potential natural inhibitor of PPO[9].

In man, pigment formation increases with age and the inhibition of tyrosinase by some drugs will reduce the rate of their formation. Therefore, many researchers in the field of biochemistry have focused their attention on the inhibitors of

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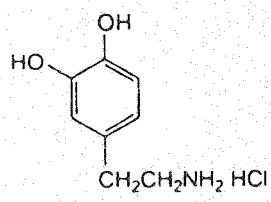
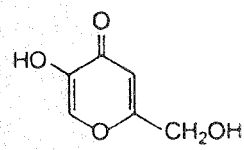
tyrosinase. Kojic acid is an aromatic organic acid (Fig. 1b) with antibiotic activity that can reduce the rate of tyrosinase reactions.



(a) Dopamine hydrochloride



(b) Kojic acid

Fig. 1. The chemical structures of (a) tyrosinase substrate and (b) its non-competitive inhibitor.

In this work, the kinetics of mushroom tyrosinase activity on one of its substrates, dopamine hydrochloride, was studied in the presence of different concentrations of kojic acid as inhibitor. The enzymatic reaction was followed using 3-methyl-2-benzothiazolinone hydrazone (MBTH) as the colour reagent. The product of tyrosinase activity on dopamine hydrochloride reacted with the amino group in MBTH to produce a deep pink coloured complex with a maximum absorption at 503 nm. A solution of dimethyl formamide (DMF) was added to the reaction mixture in order to keep the resulting coloured complex in solution state during the course of our investigations.

EXPERIMENTAL

Mushroom tyrosinase, dopamine hydrochloride and MBTH were purchased from Sigma Aldrich Chemical Company. Sodium mono- and di-phosphates, DMF and kojic acid were purchased from Merck representative in Iran.

Preparation of the enzyme and substrate solution

(a) Enzyme solution: Pure mushroom tyrosinase (1 mg/mL) was used without further purification and diluted to 1/160th of its original concentration.

(b) Substrate solution: Dopamine hydrochloride (44 mM) was freshly prepared in phosphate buffer (pH 6.8) containing 2% (v/v) DMF and 5 mM MBTH. This solution was stored in dark, as the direct light changed its colour.

Enzyme assay

The enzymatic reaction was initiated by addition of a known amount of the enzyme to the substrate solution containing known amounts of DMF and MBTH. The progress of the reaction was followed by measuring the intensity of the resulting pink colour at 503 nm. A typical reaction mixture with a total volume of 1.0 mL contained 100 μL enzyme solution (a) 500 μL substrate solution (b) and 400 μL phosphate buffer (pH 6.8). To study the inhibitory effect of kojic acid, 400 mL of the inhibitor (5.0–100 μM) replaced the phosphate buffer. The temperature was kept constant (20°C) during the course of the reaction and the UV-Vis spectrophotometer was equipped with a circulator and a thermostated cell holder.

The effect of temperature on the rate of enzymatic reaction was also studied and the Arhenius plot obtained. The results of this part of the work will be published soon.

RESULTS AND DISCUSSION

It has been reported that generally two classes of PPO inhibitors affect tryrosinase activity[1]. The first class interacts with copper site in the enzyme and act as competitive inhibitors, while the second class interferes with the site for the phenolic substrate and their inhibition is of non-competitive type.

Although the inhibitory effects of some compounds have been studied on mushroom tyrosinase, the kinetics of inhibition depends highly on the type of substrate. A number of organic compounds including aromatic carboxylic acids and halide salts (sulfites) have been identified as effective inhibitors of PPO. However, the use of these compounds has been restricted due to their potential hazards. It has been shown that a combination (1 : 1) of G-cyclodextrin (g-CD) and L-ascorbate-2-triphosphate (L-AATP) could reduce the rate of quinone formation in apple juice. The kinetic studies have shown that they are non-competitive inhibitors of PPO[10]. The inhibition kinetics on the diphenolase activity of mushroom tyrosinase by some alkyl benzaldehydes has been investigated. It has been shown that the alkyl benzaldehydes cause a reversible inhibition; *o*-tolualdehyde and *m*-tolualdehyde are mixed-type inhibitors whereas *p*-alkyl benzaldehydes are uncompetitive inhibitors[11].

Dopamine hydrochloride was used to study the effect of kojic acid as an inhibitor on mushroom tyrosinase activity. The rate of tyrosinase reaction on dopamine hydrochloride was obtained at 20°C (Fig. 2). The hyperbolic dependence of the rate on substrate concentrations confirmed non-linear regression to the Michaelis equation.

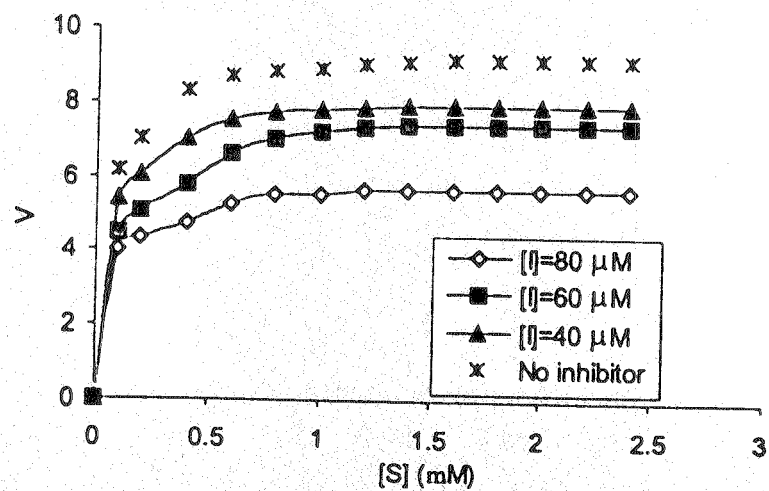


Fig. 2. Dependence of rate (µM/min) on dopamine hydrochloride concentration in absence and presence of various concentrations of kojic acid

To study the nature of inhibition, a set of different concentrations of kojic acid (40–80 mM) were used and compared with the kinetic behaviour of a control set

(without addition of the inhibitor). The double reciprocal Lineweaver-Burk plot obtained for the effect of kojic acid on mushroom tyrosinase from this study (Fig. 3) showed that the nature of inhibition was non-competitive. A non-competitive inhibitor binds to the enzyme or enzyme substrate complex and increasing the substrate concentration cannot overcome its effect. The K_m value of 0.11 mM calculated from this plot remained relatively constant in the presence of different concentrations of kojic acid, while the value of V_{max} reduced (1/V increased). The value of K_m indicates the affinity of an enzyme towards its substrates; the greater the value of K_m, the less is the affinity[12–15]. Kojic acid, therefore, does not change the affinity of tyrosinase towards dopamine hydrochloride, but it reduces the rate of enzymatic reaction (V_{max}).

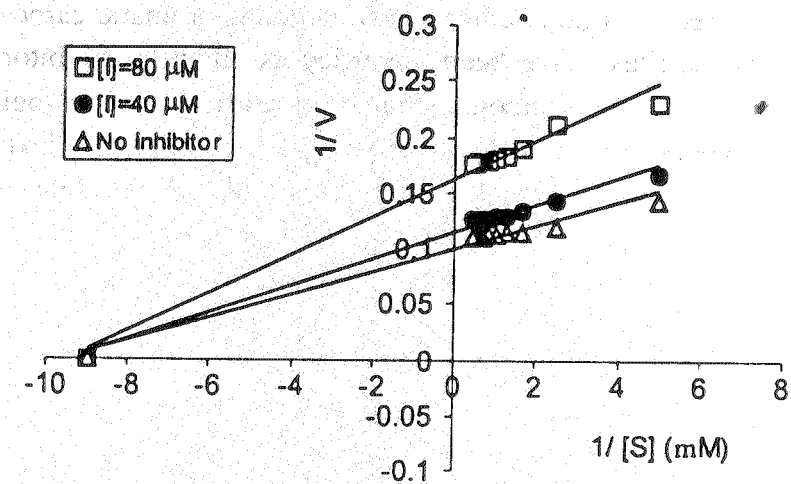


Fig. 3. Double reciprocal Lineweaver-Burk plot (1/V in µM/min *vs.* 1/[S] in µM) of inhibition of mushroom tyrosinase by two different concentrations of kojic acid (I) in phosphate buffer (pH 6.8) containing 2% (v/v) DMF and 5 mM MBTH to a total volume of 1.0 mL. According to the plot, Km = 0.111 (mM)

Conclusions

It is clear from the results obtained in the present study that kojic acid is a non-competitive inhibitor for the reaction of tyrosinase on dopamine hydrochloride, its most common substrate. The double reciprocal Lineweaver-Burk plot obtained for the effect of kojic acid on muashroom tyrosinase indicated that the nature of inhibition was non-competitive. The kinetic studies showed that the value of 0.11 mM for K_m remained relatively constant in the presence of different concentrations of kojic acid, while the value of V_{max} reduced (1/V increased). As the value of K_m indicates the affinity of an enzyme towards its substrates, kojic acid does not change the affinity of tyrosinase towards dopamine hydrochloride, but it reduces the rate of enzymatic reaction (V_{max}).

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