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Inhibition of Tyrosinase Activity on Dopamine Hydrochloride by Thiol Compounds

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This paper reports inhibits of tyrosinase activity by two thiol compounds, thioglycolic acid and 2-mercapto ethanol, using dopamine hydrochloride as substrate. Both of these —SH containing compounds inhibited tyrosinase activity in a noncompetitive way, with K_m values of 0.4050 and 0.2823 for thioglycolic acid and 2-mercaptoethanol, respectively. Other kinetic constants, V_{max}, K_{cat}, K_i were also obtained in each case.

Key Words: Tyrosinase, Inhibition, 2-Mercaptoethanol, Thioglycolic acid, Polyphenol oxidase.

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-dihydroxyphenyl alanine (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) medicated browning in raw fruits and vegetables is the major cause of quality deterioration in fruits and vegetables and their derived food products[5]. Enzymatic browning is catalyzed by two major groups of enzymes: PPO (EC 1.14.18.1) and peroxidase (EC 1.11.1.7). The relative contribution of these two groups is still unknown and may differ with plant source[6]. 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[7,8].

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 have been identified as effective inhibitors of PPO. However, the use of these compounds has been restricted due to there potential hazards. Thiol compounds have rarely been used as tyrosinase inhibitors in food industry, although cysteine and glutathione have been referred to as inhibitors of mammalian melanocyte tyrosinase[9].

In this study, the inhibition by some thiol containing compounds of dopamine hydrochloride oxidation by mushroom tyrosinase was investigated. The progress of

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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 maximun absorption at 503 nm.

EXPERIMENTAL

Mushroom tyrosinase, dopamine hydrochloride and MBTH were purchased from Sigma Aldrich Chemical Company. Sodium mono- and di-phosphates, DMF, thioglycolic acid and mercaptoethanol 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 1/160 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. To prevent its colour change by the action of direct light, this solution was stored in dark until use.

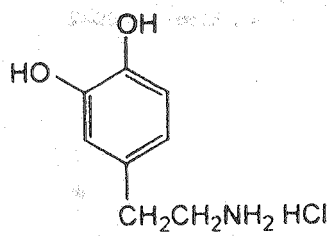
Enzyme assay: The enzymatic reaction was initiated by addition of a known amount of the enzyme to a solution of substrate containing dimethyl formamide DMF and MBTH. DMF was included in the reaction mixture in order to keep the resulting coloured complex in solution state during the course of investigations. 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, and (b) 400 μL phosphate buffer (pH 6.8). To study the inhibitory effect of thioglycolic acid and mercaptoethanol, 400 mL of each 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.

RESULTS AND DISCUSSION

The inhibitory effect of two thiol compounds, thioglycolic acid and mercaptoethanol on tyrosinase activity using dopamine hydrochloride as the substrate is investigated. Fig. 1 shows the chemical structures of the substrate and both inhibitors.

TABLE-1
INHIBITORY EFFECT OF —HS CONTAINING COMPOUNDS ON TYROSINASE ACTIVITY

Inhibitor	K_m (mM)	V_{max} (μM/min)	Type of inhibition	IC_{50} (μM)
Non	0.6356	1.250	—	—
Thioglycolic acid	0.4050	0.240	mixed	17
2-Mercaptoethanol	0.2823	0.550	mixed	69



Dopamine hydrochloride

HS—CH_2—CH_2OH

2-Mercaptoethanol

HS—CH_2—COOH

Thioglycolic acid

Fig. 1. The chemical structures of (a) tyrosinase substrate and (b) the inhibitors

The rate of tyrosinase reaction on dopamine hydrochloride was obtained at 20°C in the absence and presence of both inhibitors (Fig. 2). The hyperbolic dependence of the rate on substrate concentrations confirmed non-linear regressions to the Michaelis equation.

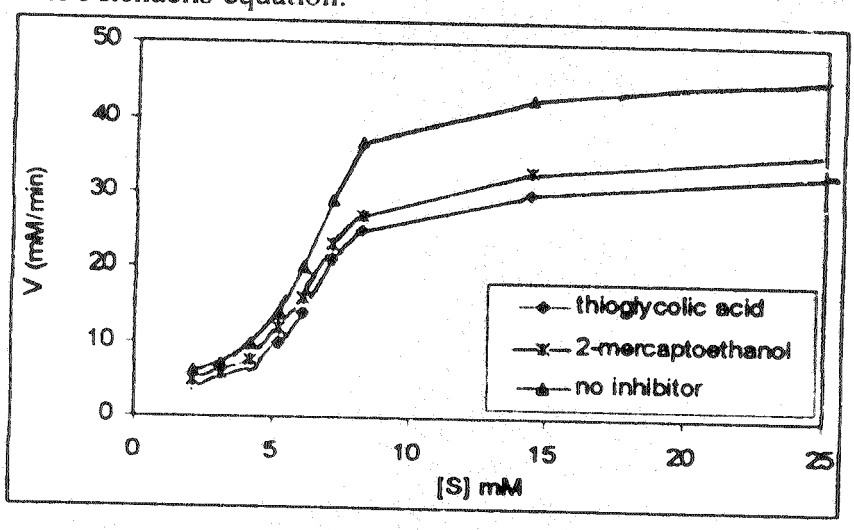


Fig. 2. Dependence of rate (μM/min) on dopamine hydrochloride concentration in absence and presence of inhibitors. The symbols used for legends are the same as in Fig. 1

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

The double reciprocal Lineweaver-Burk plot obtained for the effect of thioglycolic acid and mercaptoethanol on mushroom tyrosinase from the study (Fig. 3) showed that the nature of inhibition was non-competitive. It is known that a non-competitive inhibitor binds to the enzyme substrate complex and that increasing the substrate concentration cannot overcome its effect. The K_m value of 2.11 mM calculated from this plot remained relatively constant in the presence of different concentrations of thioglycolic acid and mercaptoethanol, 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[10-12]. Thioglycolic acid and mercaptoethanol, therefore, do not change the affinity of tyrosinase towards dopamine hydrochloride, but they reduce the rate of enzymatic reaction (V_{max}). The value of IC_{50} obtained for the two inhibitors (Table-1) indicates

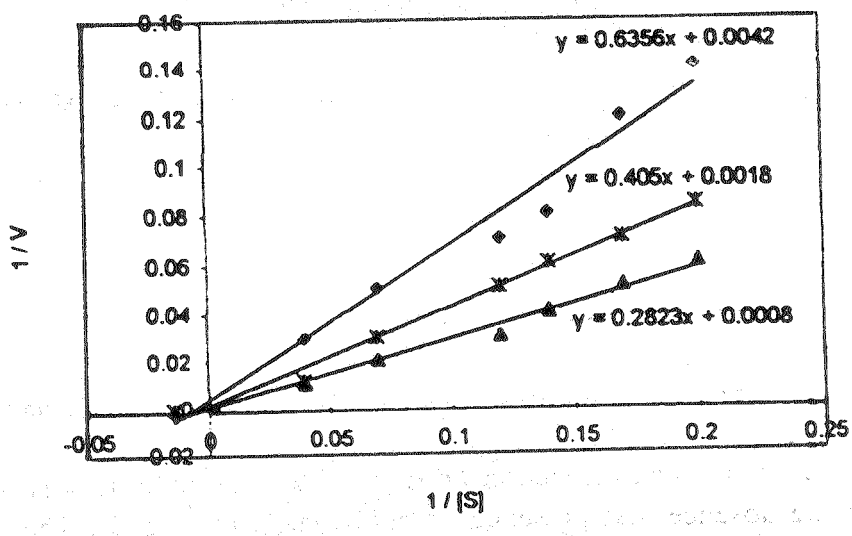


Fig. 3. Double reciprocal Lineweaver-Burk plot (1/V in μM/min *vs.* 1/[S] in μM) of inhibition of mushroom tyrosinase by thioglycolic acid and 2-mercaptoethanol in phosphate buffer (pH 6.8) containing 2% (v/v) DMF and 5 mM MBTH

that thioglycolic acid is a more potent inhibitor than 2-mercaptoethanol, showing that the acidic character of the former may also have an inhibitory effect on the enzyme activity.

Conclusions

The results obtained in the present study indicate that thioglycolic acid and mercaptoethanol are non-competitive inhibitors for the reaction of tyrosinase on dopamine hydrochloride, its most common substrate.

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