Journal of Enzyme Inhibition and Medicinal Chemistry, December 2006; 21(6): 711–717

informa
healthcare

The inhibitory effect of benzenethiol on the cresolase and catecholase activities of mushroom tyrosinase

A. A. SABOURY[1], S. ZOLGHADRI[1], K. HAGHBEEN[2], & A. A. MOOSAVI-MOVAHEDI[1]

[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, and* [2]*The National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran*

(Received 20 February 2006; in final form 12 April 2006)

Abstract
The inhibitory effect of benzenethiol on the cresolase and catecholase activities of mushroom tyrosinase (MT) have been investigated at two temperatures of 20 and 30°C in 10 mM phosphate buffer solution, pHs 5.3 and 6.8. The results show that benzenethiol can inhibit both activities of mushroom tyrosinase competitively. The inhibitory effect of benzenethiol on the cresolase activity is more than the catecholase activity of MT. The inhibition constant (K_i) value at pH 5.3 is smaller than that at pH 6.8 for both enzyme activities. However, the K_i value increases in cresolase activity and decreases in catecholase activity due to the increase of temperature from 20 to 30°C at both pHs. Moreover, the effect of temperature on K_i value is more at pH 6.8 for both cresolase and catecholase activities. The type of binding process is different in the two types of MT activities. The binding process for catecholase inhibition is only entropy driven, which means that the predominant interaction in the active site of the enzyme is hydrophobic, meanwhile the electrostatic interaction can be important for cresolase inhibition due to the enthalpy driven binding process. Fluorescence and circular studies also show a minor change in the tertiary structure, without any change in the secondary structure, of the enzyme due to the electrostatic interaction in cresolase inhibition by benzenethiol at acidic pH.

Keywords: *Mushroom tyrosinase, benzenethiol, competitive inhibition, inhibition constant, fluorescence, circular dichroism*

Introduction

Tyrosinase (Monophenol mono-oxygenase; polyphenol oxidase; catechol oxidase; and oxygen oxidoreductase; EC. 1.14.18.1) is a copper-containing enzyme, responsible for the formation of the pigments of skin, hair, and eye [1–6]. In the presence of molecular oxygen it catalyses the o-hydroxylation of monophenols to the corresponding catechols (monophenolase or cresolase activity), and successive oxidation of the catechols to the corresponding *o*-quinones (diphenolase or catecholase activity) [3–6]. *o*-Quinones are highly reactive substances, which polymerize spontaneously to macromolecules like melanin [7].

Tyrosinases are widely distributed among animals, plants and fungi [2,8]. They are responsible for many biologically essential functions, such as pigmentation, sclerotization, primary immune response and host defense [9–10]. In edible mushroom (*Agaricus bisporus*), as well as in fruits and vegetables, the enzyme is responsible for browning, a commercially undesirable phenomenon [8,11–12]. Mushroom tyrosinase (MT) with a molecular mass of 120 kD, is composed of two H subunits (43 kD) and two L subunits (13 kD) and contains two active site [13–14]. Its active site has a di-copper center, resembling that of the hemocyanins [10,15], but not identical [16]. Each copper ion in the active site is coordinated by three nitrogen atoms coming from three adjacent histidine residues and the enzyme can experience three forms of met, oxy and deoxy [17–18].

Tyrosinase may play a role in neuromelanin formation in the human brain and central to

Correspondence: A. A. Saboury, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. Tel.: 98 21 66956984, Fax: 98 21 66404680. E-mail: saboury@ut.ac.ir

ISSN 1475-6366 print/ISSN 1475-6374 online © 2006 Informa UK Ltd.
DOI: 10.1080/14756360600810787

dopamine neurotoxicity as well as contribute to the neurodegeneration associated with Parkinson's disease [19]. Therefore many tyrosinase inhibitors that suppress melanogenesis have been actively studied with the aim of developing preparations for the treatment of hyper pigmentation [20–28]. Tyrosinase inhibitors have attracted attention recently due to undesired browning in vegetables and fruits in post-harvest handling [29]. Besides, tyrosinase inhibitors may be clinically used for treatment of some skin disorders associated with melanin hyper-pigmentation and are also important in cosmetics for skin whitening effects [30–32]. Hence, the interest in tyrosinase inhibitors should have broad applications [33–34] and its inhibition is one of the major strategies in developing new whitening agents [35]. Although a large number of natural and synthetic inhibitors against catecholase, cresolase or both reactions of tyrosinase have been described in the literature, the search for new natural products and synthetic compounds with such activity still continues [36]. Synthetic tyrosinase inhibitors may be used as drugs and chemicals. In the case of clinical drugs, captopril, an antihypertensive drug, and methimazole act as tyrosinase inhibitors [37–38]. Polyphenols, aldehydes and their derivatives are the most important inhibitors from plant natural sources [39–43]. Thiol compounds such as cysteine, glutathione, methimazole, diethyldithiocarbamate, 2-mercaptobenzothiazole have been also used as inhibitors of tyrosinase from different sources [44–48].

We have attempted to obtain additional information about the structure, function and relationship of MT [49–53] to understand the mechanism of enzyme action and inhibition. Two new bi-pyridine synthetic compounds were recently introduced as potent uncompetitive MT inhibitors [54]. Also, the inhibitory effects of three synthetic n-alkyl dithiocarbamates, with different tails, were described [55]. Since, there is not any report on the inhibition of MT by benzenethiol (thiophenol), the aim of the present investigation is to carry out a kinetic study on the enzyme in the presence of this compound in two different pHs at two temperatures. Moreover, the structural change in the enzyme due to the binding with benzenethiol was followed by fluorescence and circular dichroism spectroscopic techniques.

Materials and methods

Materials

Mushroom tyrosinase (MT; EC 1.14.18.1), specific activity 3400 units/mg, was purchased from Sigma. Caffeic acid was an authentic samples. Analytical grade of benzenethiol was prepared from Sigma. Phosphate buffer (10 mM, pH 6.8 and 5.3) was used throughout this research and the corresponding salts were obtained from Merck. All experiments were carried out at two temperatures, 20 and 30°C.

Methods

Kinetic measurements. Kinetic assay of catecholase and cresolase activities were carried out through depletion of caffeic acid and coumaric acid, respectively, for 2 and 15 min, with an enzyme concentration of 12.51 μg/ml and 78.24 μg/ml, at 311 nm and 288 nm wavelengths using a Cary spectrophotometer, 100 Bio-model, with jacketed cell holders. Freshly prepared enzyme and the substrate, benzenethiol were used in this work. All the enzymatic reactions were run in phosphate buffer (10 mM) at pH 6.8 and 5.3 in a conventional quartz cell thermostated to maintain the temperature at 20 ± 0.1°C and 30 ± 0.1°C. Catecholase activity was carried out using seven different fixed concentrations of substrate (33.3, 50.0, 66.6, 100.0, 116.6, 133.3 and 166.6 μM) in different fixed concentrations of the inhibitor (0, 13.3, 20.0, 33.3 and 46.7 μM). Cresolase reactions were followed using five different concentrations of substrate (3.3, 6.6, 13.0, 20.0, and 26.0 μM at 20°C and 20, 26, 33, 40, 46 μm at 30°C) in different concentrations of the inhibitor (0, 0.099, 0.132, 0.162 and 0.400 μM at pH 5.3 and 0, 0.033, 0.066, 0.100 and 0.140 μM at pH 6.8). The selected conditions of solvent, buffer, pH, temperature, and enzyme concentration applied for assaying the oxidase activity of MT followed the method introduced by EI-Bayuomi and Frieden [55–56]. Substrate addition followed after incubation of the enzyme with different concentrations of benzenethiol. Addition of tyrosinase into a benzenethiol solution in phosphate buffer does not show any change in the spectrum which means that benzenethiol is not a substrate.

Circular dichroism spectroscopy. The far-UV CD region (190–260 nm), which corresponds to peptide bond absorption, was analyzed by an Aviv model 215 Spectropolarimeter (Lakewood, NJ, USA) to give the content of regularly secondary structure in MT. Protein solutions were prepared in the phosphate buffer. The protein solutions of 0.2 mg/mL without and with incubation by different concentrations of benzenethiol (0.12, 0.20 mM) for at least 4 min were used to obtain the spectra. All spectra were collected in triplicate from 190 to 260 nm against a background-corrected buffer blank. The results were expressed as ellipticity ($^\circ cm^2 dmol^{-1}$) based on a mean amino acid residue weight (MRW) of 125 for MT having the average molecular weight of 120 kDa [13]. The molar ellipticity was determined as $[\theta] = (100 \times (MRW) \times \theta_{obs}/cl)$, where θ_{obs} is the observed ellipticity in degrees at a given wavelength, c is the protein concentration in mg/ml and l is the length of the light path in cm.

Intrinsic fluorescence. Intrinsic fluorescence intensity measurements were carried out using a Hitachi spectrofluorimeter, MPF-4 model, equipped with a thermostatically controlled cuvette compartment. The intrinsic emission of protein, 0.17 mg/ml, was seen at the excitation wavelength of 280 nm. The experiments were repeated in the presence of different concentrations of benzenethiol (0.12 and 0.20 mM).

All graphs here were obtained by using Microsoft Excel.

Results And Discussion

The inhibitory effect of benzenethiol on both MT activities was examined at two different pH values (5.3 and 6.8) and temperatures (20°C and 30°C). Double reciprocal Lineweaver-Burk plots for the cresolase activity of MT on hydroxylation of *p*-coumaric acid, as the substrate, in the presence of different concentrations of benzenethiol are shown in Figures 1 (at pH 5.3) and 2 (at pH 6.8). A series of straight lines intersect each other exactly on the vertical axis. The maximum velocity (V_{max}) has not been changed by benzenethiol but the apparent Michaelis constant (K'_m) value has been increased, which confirms the competitive mode of the inhibition. The intersects on Figures 1 and 2 show the secondary plots, the slope (K'_m/V_{max}) at any concentration of the inhibitor versus concentration of inhibitor, which gives the inhibition constants (K_i) from the abscissa-intercepts. The K_i values have been summarized in Table I indicating that the inhibition constant was smaller at the lower pH 5.3, but increased when the temperature increased from 20 to 30°C.

Double reciprocal Lineweaver-Burk plots for the catecholase activity of MT on oxidation of caffeic acid, as the substrate, in the presence of different concentrations of benzenethiol are shown in Figures 3 (at pH 5.3) and 4 (at pH 6.8). Here again the straight lines intersect on the vertical axis due to competitive inhibition of the enzyme by the inhibitor. The K_i values obtained from the abscissa-intercept of the secondary plots, the intersects on Figures 3 and 4, are also summarized in Table I. As shown in Table I, the value of K_i is smaller at pH 5.3 and its value decreased when the temperature increased from 20 to 30°C. Moreover, comparison of the K_i values in Table I reveals that benzenethiol has inhibited the cresolase activity more strongly than the catecholase activity of MT.

Considering the structural resemblance between benzenethiol and the mono-substituted aromatic ring of the phenolic substrates as well as the mode of inhibition, it is not surprising that the cresolase reaction has been inhibited more strongly than the catecholase reaction of MT. Besides, lowering the pH of the reaction medium affects directly the extent of ionization of both the substrate and the inhibitor. Since benzenethiol has a lower $pK_a = 6.6$ [57] than

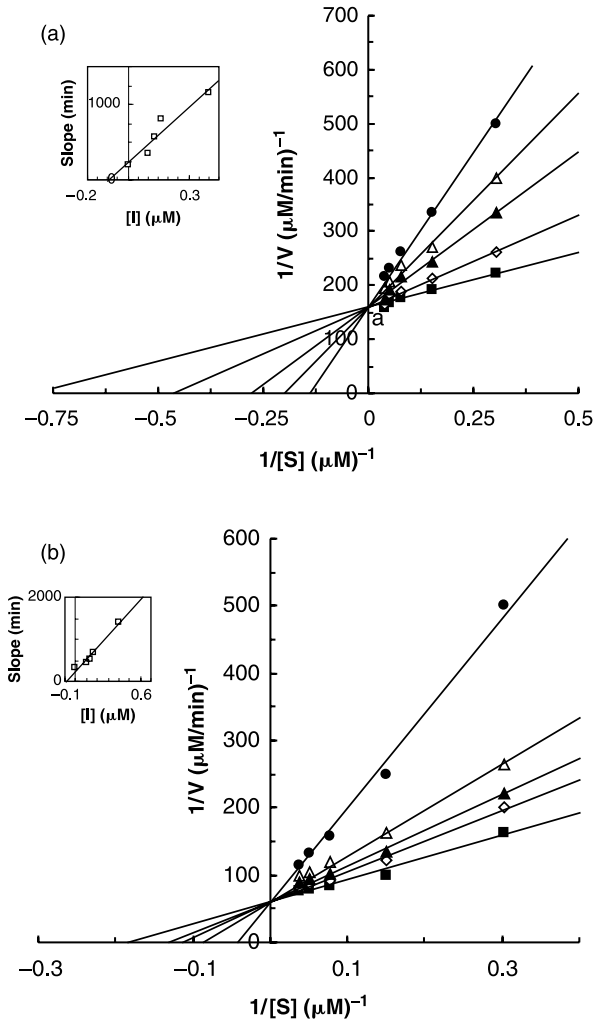

Figure 1. Double reciprocal Lineweaver-Burk plots of MT kinetic assays for cresolase reactions of *p*-coumaric acid in 10 mM phosphate buffer, pH = 5.3, at two temperatures of 20°C (a) and 30°C (b) and 11.8 μM enzyme concentration, in the presence of different fixed concentrations of benzenethiol: 0 μM (■), 0.099 μM (◇), 0.132 μM (▲), 0.162 μM (Δ), 0.400 μM (◆).

the phenolic and catecholic substrates, the former wins the competition at lower pH.

In order to understand the effect of temperature on the inhibition and to shed light into the nature of the benzenethiol binding with MT, the change of the Gibbs standard free energy of binding (ΔG°) was calculated using the association binding constant (K), obtained from the inverse of the K_i value, in Equation (1) [58]:

$$\Delta G^\circ = -R\,T\ln K \tag{1}$$

where R is the gas constant, and T is the absolute temperature. The standard enthalpy change of binding (ΔH°) was also calculated using K values at two temperatures in the van't Hoff equation [58]:

$$\ln\frac{K_2}{K_1} = -\frac{\Delta H^\circ}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right) \tag{2}$$

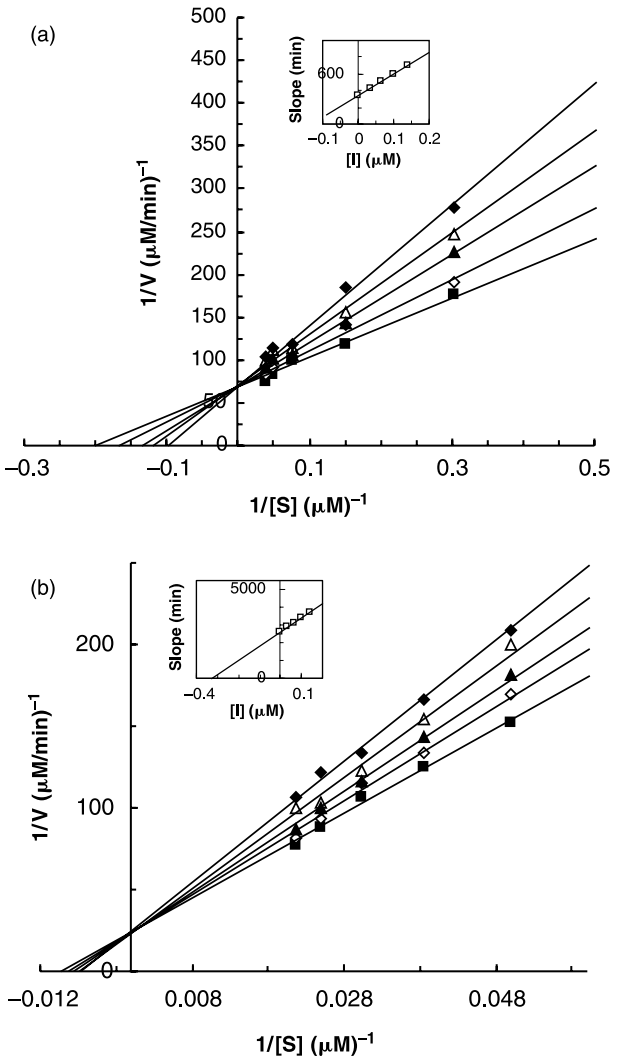

Figure 2. Double reciprocal Lineweaver-Burk plots of MT kinetic assays for cresolase reactions of *p*-coumaric acid in 10 mM phosphate buffer, pH = 6.8, at two temperatures of 20°C (a) and 30°C (b) and 11.8 μM enzyme concentration, in the presence of different fixed concentrations of benzenethiol: 0 μM (■), 0.033 μM (◇), 0.066 μM (▲), 0.100 μM (Δ), 0.140 μM (◆).

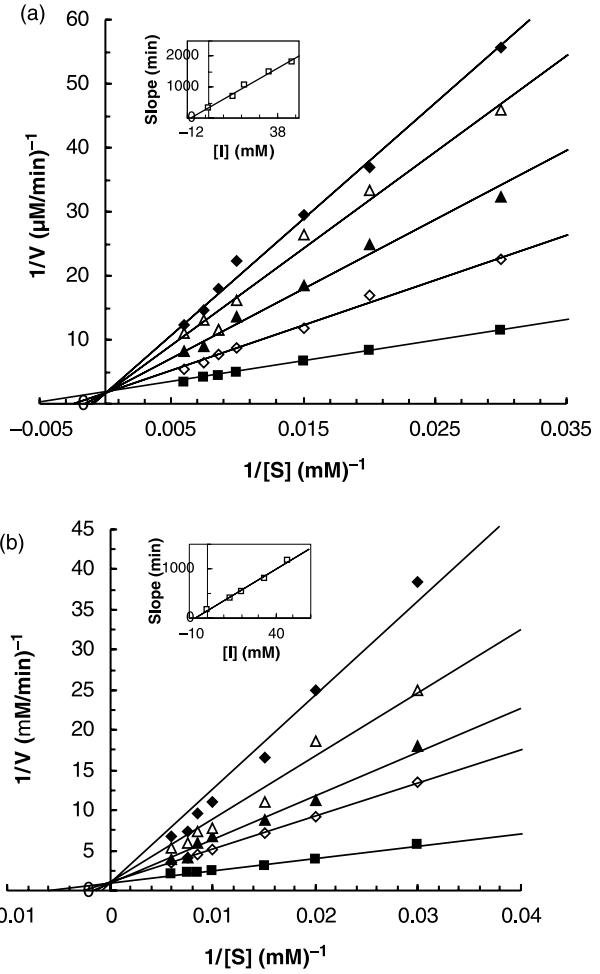

Figure 3. Double reciprocal Lineweaver-Burk plots of MT kinetic assays for catecholase reactions of caffeic acid in 10 mM phosphate buffer, pH = 5.3, at two temperatures of 20°C (a) and 30°C (b) and 11.8 μM enzyme concentration, in the presence of different fixed concentrations of benzenethiol: 0 μM (■), 13.33 μM (◇), 20.00 μM (▲), 33.33 μM (Δ), 46.67 μM (◆).

where K_1 and K_2 are association binding constants at two temperatures of T_1 and T_2, respectively. Finally the standard entropy change (ΔS°) was calculated using Equation (3) [58]

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \qquad (3)$$

All the calculated thermodynamics parameters (K, ΔG°, ΔH° and ΔS°) are summarized in Table I. This data suggests that the binding process for the inhibitor is spontaneous at both pHs and temperatures ($\Delta G^\circ < 0$). Besides, the binding process of benzenethiol seems to be only entropy driven ($\Delta S^\circ > 0$) in the catecholase inhibition. This is the reason why raising the temperature during the catecholase

Table I. Thermodynamic parameters of binding benzenethiol on mushroom tyrosinase at two different temperatures and pHs.

ΔH° (kJ mol^{-1})	$T\Delta S^\circ$ (kJ mol^{-1})	ΔG°(kJ mol^{-1})	K_i(μM)	$K_a(M)^{-1}$	T(°C)	pH	Reaction type
43.31	71.35	−28.04	10.2	$.098 \times 10^6$	20	5.3	Catecholase activity
	73.58	−30.27	6.07	$.16 \times 10^6$	30		
46.73	73.41	−26.68	17.37	$.057 \times 10^6$	20	6.8	
	75.73	−29	9.87	$.1 \times 10^6$	30		
−13.26	26.52	−39.78	.081	12.34×10^6	20	5.3	Cresolase activity
	27.47	−40.73	.095	10.52×10^6	30		
−77.43	−38.75	−38.68	.13	7.69×10^6	20	6.8	
	−39.83	−37.6	.33	3.03×10^6	30		

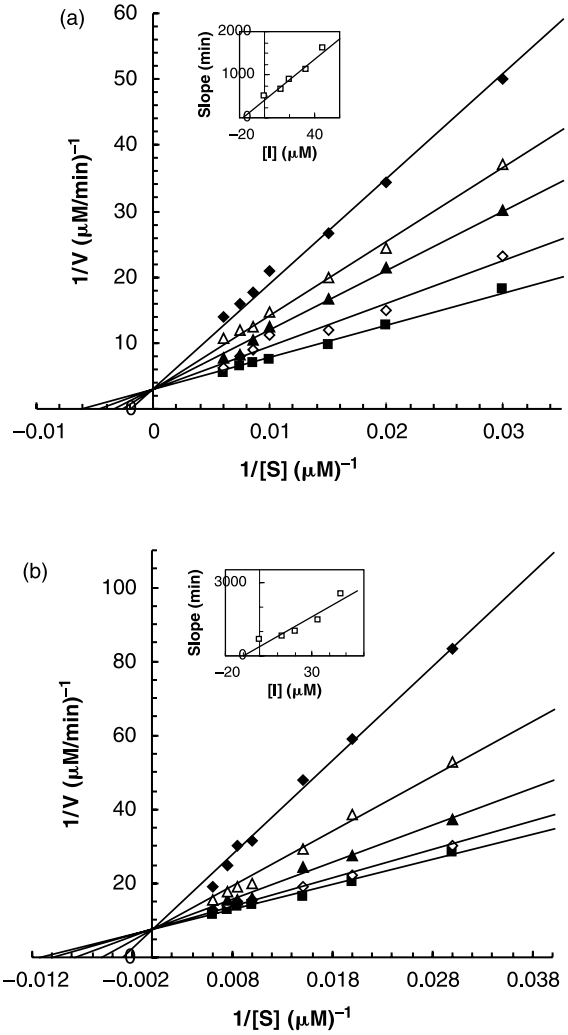

Figure 4. Double reciprocal Lineweaver-Burk plots of MT kinetic assays for catecholase reactions of caffeic acid in 10 mM phosphate buffer, pH = 6.8, at two temperatures of 20°C (a) and 30°C (b) and 11.8 μM enzyme concentration, in the presence of different fixed concentrations of benzenethiol: 0 μM (■), 13.33 μM (◇), 20.00 μM (▲), 33.33 μM (Δ), 46.67 μM (◆).

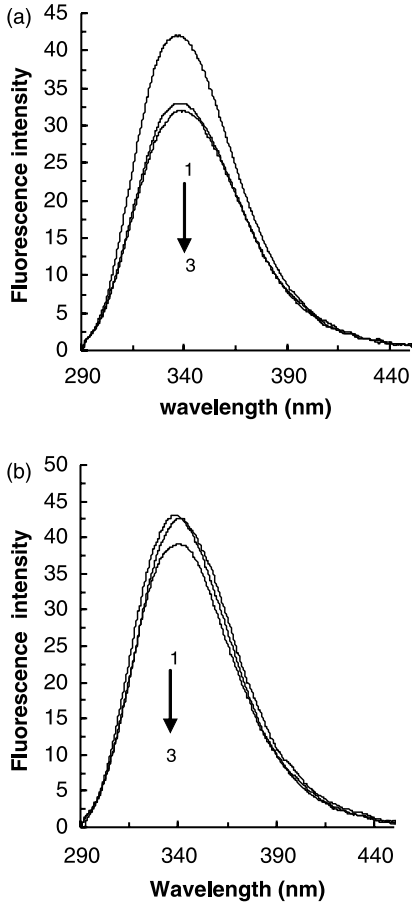

Figure 5. Intrinsic fluorescence emission spectra of MT (1) and MT in the presence of benzenethiol at two concentrations of 0.12 mM (2) and 0.20 mM (3) at pH = 5.3 (a) and pH = 6.8 (b). The excitation wavelength was 280–nm. The concentration of the enzyme is 0.17 mg/mL.

inhibition has caused a decrease in the K_i value of the inhibitor. In contrast, the raising of the temperature has brought about an increase in the K_i value during the cresolase inhibition which is mostly enthalpy driven ($\Delta H^\circ < 0$).

The impact of pH on the enthalpy changes as well as the diverse effect of pH on the entropy changes of the cresolase inhibition by benzenethiol are worthy of attention. The significant change in the enthalpy after increasing the pH from 5.3 to 6.8, which is close to the optimum pH of the MT activities, indicates the growth of the constructive electrostatic interactions in the cresolase reaction [59–60]. These interactions help the enzyme to sculpture an optimized, but vulnerable, conformer which can account for the negative entropy changes at pH = 6.8. Similar evidence was obtained from the fluorescence spectroscopic studies. While Figure 5a supports observable changes in the tertiary structure of MT in the presence of benzenethiol at pH = 5.3, Figure 5b shows little structural changes at pH = 6.8. It means that the formation of the EI complex at pH = 6.8 can hardly cause further changes beyond that which pH has achieved. It is also worthy of note that the CD spectra rules out possible changes in the secondary structure of MT in the presence of benzenethiol at pH = 5.3 (see Figure 6).

In contrast to the cresolase reaction, catecholase inhibition by benzenethiol has experienced less entropy and enthalpy changes upon alterations in pH or temperature. Besides, the binding process in the catecholase inhibition is only entropy driven. It means that the inhibitor interaction with the active site of the enzyme is predominantly hydrophobic [61]. Since the raise in entropy after the temperature increase is not so high, it can be concluded that the overall conformational changes during the binding process in the catecholase reaction must not be large. Such a result had also been extracted from studies on the inactivation of MT [50,53].

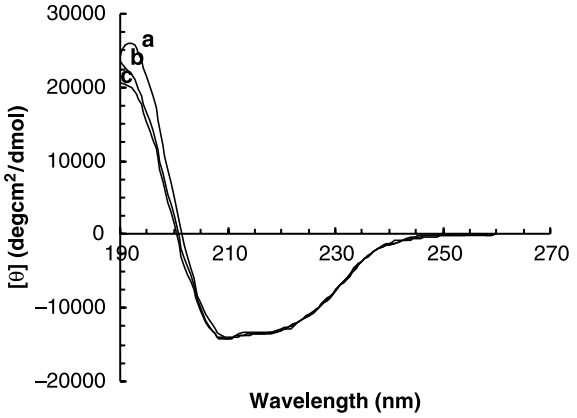

Figure 6. UV-CD spectra registered for MT in the absence (a) and in the presence of different two concentrations of benzenethiol; 0.12 mM (b) and 0.20 mM (c) at pH = 5.3. The concentration of the enzyme is 0.20 mg/mL.

The results of our analysis support the idea that the monophenolase activity of MT involves electrostatic interactions, while the diphenolase activity develops through hydrophobic interactions. It means that the type of binding process is different in the two types of MT activities. Based on an extended Huckel theory calculation for a tyrosinase active site model, it has been shown that the ionization of the hydroxyl group of the phenolic substrate is a crucial step in its interaction with the positively charged copper of the active site in the cresolase reaction [62]. Besides, electrophilic attack on the aromatic ring of the phenolic substrate seems to be an important step towards formation of the C-O bond in the cresolase reaction [63]. In contrast, oxidation of o-diphenolic compounds by MT has fewer electronic requirements than the oxidation of monophenolic substrates [51]. In fact, proper docking of the catechol substrate and its interactions with a hydrophobic pocket close to the MT active site, similar to what has been reported for phenylthiourea in the catechol oxidase active site [64–65], apparently plays a crucial role in the catecholase reaction.

Acknowledgements

The financial support given by the University of Tehran and the Iran National Science Foundation (INSF) are gratefully acknowledged.

References

[1] Seo Sy, Sharma VK, Sharma N. Mushroom tyrosinase: Recent prospects. J Agric Food Chem 2003;51:2837–2853.
[2] Van Gelder CWG, Flurkey WH, Wichers HJ. Sequence and structural features of plant and fungal tyrosinases. Phytochem 1997;45:1309–1323.
[3] Winder AJ, Harris H. New assays for tyrosine hydroxylase and dopa oxidase activities of tyrosinase. Eur J Biochem 1991;198:317–326.
[4] Robb DA. Copper proteins and copper enzymes. Boca Raton: CRC Press; 1984.
[5] Riley PA. Tyrosinase kinetics: a semi-quantitative model of the mechanism of oxidation of monohydric and dihydric phenolic substrates. J Theor Biol 2000;203:1–12.
[6] Fenoll LG, Rodriguez-Lopez JN, Garcia-Sevilla F, Tudela J, Garcia-Ruiz PA, Varon R, et al. Oxidation by mushroom tyrosinase of monophenols generating slightly unstable o-quinones. Eur J Biochem 2000;267:5865–5878.
[7] Land EJ, Ramsden CA, Riley PA. Tyrosinase autoactivation and the chemistry of ortho-quinone amines. Acc Chem Res 2003;36:300–308.
[8] Whitaker JR. In: Wong DWS, ed. Food enzymes, structure and mechanisms. New York: Chapman and Hall; 1995. p 271–307.
[9] Jaenicke E, Decker H. Tyrosinases from crustaceans form hexamers. Biochem J 2003;371:515–523.
[10] Sanchez-Ferrer A, Rodriguez-Lopez JN, Garcia-Canovas F, Garcia-Carmona F. Tyrosinase: A comprehensive review of its mechanism. Biochim Biophys Acta 1995;1247:1–11.
[11] Xie LP, Chen QX, Huang H, Wang HZ, Zhang RQ. Inhibitory effects of some flavonoids on the activity of mushroom tyrosinase. Biochemistry (Mosc) 2003;68:487–491.
[12] Martinez MV, Whitaker JR. The biochemistry and control of enzymatic browning. Trends Food Sci Technol 1995;6: 195–200.
[13] Strothkemp KJ, Jolley RL, Mason Hs. Quaternary structure of mushroom tyrosinase. Biochem Biophys Res Commun 1976;70:519–524.
[14] Yong G, Leone C, Strothkemp KJ. Agricus bisporus metapotyrosinase: preparation, characterization, and conversion to mixed-metal derivatives of the binuclear site. Biochemistry 1990;29:9684–9690.
[15] Orlow SJ, Bao-Kang Z, Chakraborty AK, Dricker M, Pifko-Hirst S, Pawelek JM, et al. High-molecular weight forms of tyrosinase and the tyrosinase-related proteins: evidence for a melanogenic complex. J Investig Dermatol 1994;103:196–201.
[16] Eicken C, Krebs B, Sacchettini JC. Catechol oxidase-structure and activity. Curr Opin Struct Biol 1999;9:677–683.
[17] Cooksey CJ, Garratt PJ, Land EJ, Ramsden CA, Riley PA. Tyrosinase kinetics: failure of the auto-activation mechanism of monohydric phenol oxidation by rapid formation of a quinomethane intermediate. Biochem J 1998;333:685–691.
[18] Rescigno A, Sollai F, Pisu B, Rinaldi A, Sanjust E. Tyrosinase inhibition: general and applied aspects. J Enz Inhib Med Chem 2002;17:207–218.
[19] Nihei kI, Yamagiwa Y, Kamikawa T, Kubo I. 2-Hydroxy-4-isopropylebenzaldehyde, a potent partial tyrosinase inhibitor. Bioorgan Med Chem Lett 2004;14:681–683.
[20] Khan SB, Haq AU, Afza N, Malik A, Khan MTH, Shah MR, Choudhary MI. Tyrosinase-inhibitory long chain Esters from Amber boa ramose. Chem Pharm Bull 2005;53:86–89.
[21] Shimizu K, Kondo R, Sakai K. Inhibition of tyrosinase by flavonoids, stilbenes and related 4-substituted resorcinol: Structure-activity investigations. Planta Medica 2000; 66:11–15.
[22] Hashimoto A, Ichihashi M, Mishima Y. The mechanism of depigmentation by hydroquinone; a study on suppression and recovery processes of tyrosinase activity in the pigment cells in vivo and in vitro. Jpn J Dermatol 1984;94:794–804.
[23] Khan V. Effect of kojic acid on the oxidation of D, L-dopa, norepinephrine, and dopamine by mushroom tyrosinase. Pigment Cell Res 1995;8:234–240.
[24] Tasaka K, Kamei C, Nakano S, Takeuchi Y, Yamato M. Effects of certain resorcinol derivatives on the tyrosinase activity and the growth of melanoma cells. Meth Find Exp Clin Pharmacol 1998;20:99–109.

[25] Katagiri T, Yokoyama K, koiso I, Matukami M, nakano H. Inhibitory effect of esculin on melanogenesis. Jpn J Dermatol 1994;104:1367–1372.
[26] Kumano Y, Sakamoto T, Egawa M, Iwai I, Tanaka M, Yamamato I. In vitro and in vivo prolonged biological activities of novel vitamin c derivative. J Nutr Sci Vitaminol (Tokyo) 1998;44:345–359.
[27] Akiu S, Suzuki Y, Asahara T, Fujinuma Y, Fukuda M. Inhibitory effect of arubutin on melanoma cells. Jpn J Dermatol 1991;101:609–613.
[28] Ichihashi M, Funasaka Y, Oashi A, Chacruborty A, Ahmad NU, Ueda M, Osawa T. The inhibitory effect of D, L-alpha-tocopheryl ferulate in lecithin on melanogenesis. Anticancer Res 1999;19:3769–3774.
[29] Taylor SL, Bush RK. Sulfites as food ingredients. Food Technol 1986;40:47–52.
[30] Palumbo A, Ischia M, Misuraca G, Parota G. Mechanism of inhibition of melanogenesis by hydroquinone. Biochim Biophys Acta 1991;1073:85–90.
[31] Maeda K, Fukuda M. In vitro effectiveness of several whitening cosmetic components in human melanocytes. J Soc Cosmet Chem 1991;42:361–368.
[32] Friedman M. Food browning and its prevention: An overview. J Agric Food Chem 1996;44:631–653.
[33] Shi Y, Chen OX, Wang Q, Song KK, Qiu L. Inhibitory effects of cinnamic acid and its derivatives on the catecholase activity of mushroom tyrosinase. Food Chemistry 2005;92:707–712.
[34] Huang XH, Chen QX, Wang Q, Song KK, Wang J, sha L, Guan X. Inhibition of the activity of mushroom tyrosinase by alkyl benzoic acids. Food Chem 2006;4:1–6.
[35] Khatib S, Nerya O, Musa R, Shmuel M, Tamir S, Vaya J. Chalcones as potent tyrosinase inhibitors: the importance of a 2, 4-substituted resorcinol moiety. Bioorgan Med Chem 2005;13:434–441.
[36] Gasowsaka B, Kafarski P, Wojtasek H. Interaction of mushroom tyrosinase with aromatic amines, o-diamines and o-aminophenoles. Biochim Biophys Acta 2004;1673: 170–177.
[37] Espin JC, Wichers HG. Effect of captopril on mushroom tyrosinase activity in vitro. Biochim Biophys Acta 2001;1554:289–300.
[38] Andrawis A, Khan V. Effect of methimazole on the activity of mushroom tyrosinase. Biochim J 1996;235:91–96.
[39] Kubo I, Kinst-Hori I. Flovonols from saffron flower: Tyrosinase inhibitory activity and inhibition mechanism. J Agric Food Chem 1999;47:4121–4125.
[40] Kubo I, Kinst-Hori I, Ishiguro K, Chaudhuri SK, Sanchez Y, Ogura T. Tyrosinase inhibitory flvonoids from heterotheca inuloides and their structural functions. Bioorg Med Chem Lett 1994;4:1443–1446.
[41] Chen QX, Kubo I. Kinetics of mushroom tyrosinase inhibition by quercetin. J Agric Food Chem 2002;50:4108–4112.
[42] Kubo I, Kinst-Hori I, Chaudhuri Sk, Kubo Y, Sanchez Y, Ogura T. Flovonols from Heterotheca inuloides: Tyrosinase inhibitory activity and structural criteria. Bioorg Med Chem 2000;8:1749–1755.
[43] Kubo I, Kinst-Hori I. Tyrosinase inhibitors from cumin. J Agric Food Chem 1988;46:5335–5341.
[44] Pierpoint WS. Thenzymic oxidation of cholorogenic acid and some reactions of the quinine produsued. Biochem J 1966;98:567–580.
[45] Seiji M, Yashida T, Itakura H, Irimajiri T. Inhibition of melanin formation bye sulfhydryl compounds. J Investig Dermatol 1969;52:280–286.
[46] Hanlon DP, Shuman S. Copper ion binding and enzyme inhibitory properties of the antithyroid drug methimazole. Experientia 1975;31:1005–1006.
[47] Anderson JW. Extraction of enzyme and sub cellular organelles from plant tissues. Phytochem 1968;7:1973–1988.
[48] Palmer JK, Robbert JB. Inhibition of banana polyphenol oxidase by 2-mercaptobenzothiazole. Science 1967;157: 200–201.
[49] Haghbeen K, Saboury AA, Karbassi F. Substrate share in the suicide inactivation of mushroom tyrosinase. Biochim Biophys Acta 2004;1675:139–146.
[50] Karbassi F, Haghbeen K, Saboury AA, Ranjbar B, Moosavi-Movahedi AA. Activity, structural and stability changes of mushroom tyrosinase by sodium dodecyl sulfate. Colloids and Surface B: Biointerfaces 2003;32:137–143.
[51] Shareefi Borojerdi S, Haghbeen K, Karkhane AA, Fazli M, Saboury AA. Successful resonance raman study of cresolase activity of mushroom tyrosinase. Biochem Biophys Res Commun 2004;314:925–930.
[52] Gheibi N, Saboury AA, Haghbeen K, Moosavi-Movahedi AA. Activity and structural changes of mushroom tyrosinase induced by n-alkyl sulfates. Colloids and Surface B: Biointerfaces 2005;45:104–107.
[53] Karbassi F, Haghbeen K, Saboury AA, Ranjbar B, Moosavi-Movahedi AA, Farzami B. Stability, structural and suicide inactivation changes of mushroom tyrosinase after acetylation by N-acetylimidazole. Int J Biol Macromol 2004;34:257–262.
[54] Karbassi F, Saboury AA, Hassan Khan MT, Iqbal Choudhary M, Saifi ZS. Mushroom tyrosinase inhibition by two potent uncompetitive inhibitors. J Enz Inhib Med Chem 2004;19:349–353.
[55] Gheibi N, Saboury AA, Mansury-Torshizy H, Haghbeen K, Moosavi-movahedi AA. The inhibition effect of some n-alkyl dithiocarbamates on mushroom tyrosinase. J Enz Inhib Med Chem 2004;20:393–399.
[56] EI-Bayoumi MA, Frieden EA. Spectrophotometric method for the determination of the catecholase activity of tyrosinase and some of its applications. J Amer Chem Soc 1957;79: 4854–4858.
[57] Serjeant EP, Dempsey B. Ionization Constants of Organic Acids in Aqueous Solution. Pergamon: Oxford; 1979.
[58] Atkins P, DePaula J. Physical Chemistry. 7th Ed. Chap 9, New York: WH Freeman & Company; 2002.
[59] Hegde SS, Kumar AR, Ganesh KN, Swaminathan CP, Khan MI. Thermodynamics of ligand (substrate/end product) binding to endoxylanas from chainia sp.isothermal calorimetry and fluorescence titration studies. Biochim Biophys Acta 1998;1388:93–100.
[60] Tellez-Sanz R, Bernier-Villamor V, Garcia-Fuentes L, Gonzalez-Pacanowska D, Baron C. Thermodynamic characterization of the binding of dCMP to the Asn229Asp mutant of thymidylate synthase. FEBS Lett 1997;409:385–390.
[61] Coassolo P, Sarrazin M, Sari JC, Briand C. Microcalorimetric studies on the binding of some benzodiazepine derivatives to human serum albumin. Biochem Pharmacol 1978;27: 2787–2792.
[62] Giessner-Prettre C, Maddaluno M, Stussi D, Webber J, Eisenstein O. Theoretical study of oxyhemocyanine. A plausible insight on the first step of phenol oxidation by tyrosinase. Arch Biochem Biophys 1992;296:247–253.
[63] Solomon EI, Winkler ME, Lerch K, Hwang YT, Porras AG, Wilcox DG. Substrate analogue binding to the coupled binuclear copper active site. J Am Chem Soc 1985;107: 4015–4027.
[64] Klabunde T, Eicken C, Sacchettini JC, Krebs B. Crystal structure of a plant catechol oxidase containing a dicopper center. Nature Struct Biol 1998;5:1084–1090.
[65] Decker H, Tuczek F. Tyrosinase/catechol oxidase activity of hemocyanins: structural basis and molecular mechanism. Trends Biochem Sci 2000;392:39.