

# Tyrosinase Catalyzes Asymmetric Sulfoxidation<sup>†</sup>

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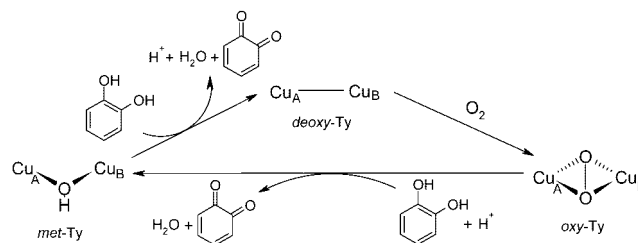
Received December 11, 2007; Revised Manuscript Received January 14, 2008

**ABSTRACT:** Mushroom tyrosinase was found to catalyze the oxidation of organic sulfides to sulfoxides in the presence of a catechol as cosubstrate, in a reaction which is unprecedented for this enzyme and resembles those performed by external monooxygenases. Only the oxy form of the enzyme is in fact capable of oxidizing the sulfide in a two-electron process, while the resulting met form can only be recycled by reduction with catechol. The cosubstrate competes with the sulfide also in the reaction with oxy-tyrosinase. For this reason, the sulfoxidation of thioanisole in the presence of L-3,4-dihydroxyphenylalanine (L-dopa) occurs with moderate yields (~20%) but high enantioselectivity (~85% e.e.), and favors (*S*)-methyl phenyl sulfoxide. The enantioselectivity can be further increased to >90% when excess ascorbic acid is added to the reaction to limit enzyme inactivation by the quinones produced by L-dopa oxidation. An experiment using <sup>18</sup>O<sub>2</sub> showed that 18-O incorporation into methyl phenyl sulfoxide was above 95%, confirming that the mechanism of the sulfoxidation involves oxygen transfer from oxy-tyrosinase to the sulfide.

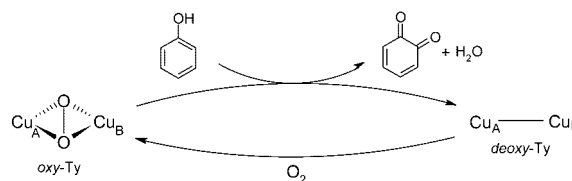
Tyrosinase (Ty<sup>1</sup> EC 1.14.18.1) is a ubiquitous dinuclear copper enzyme, distributed throughout microorganisms, fungi, plants, and animals (1–3). It is of central importance in skin pigmentation, browning of fruits and vegetables, wound healing, and immune response. Tyrosinase catalyzes the *ortho*-hydroxylation of monophenols (monophenolase activity) and the oxidation of *ortho*-diphenols to *ortho*-quinones (diphenolase activity) by O<sub>2</sub> (Scheme 1) (4, 5). The *ortho*-quinones evolve nonenzymatically through several unstable intermediates, which polymerize to melanins. The complex reaction mechanism of the enzyme involves three forms, met-Ty, deoxy-Ty, and oxy-Ty, a  $\mu$ - $\eta^2$ : $\eta^2$ -peroxodicopper(II) species. Deoxy-Ty binds O<sub>2</sub> with high affinity, while the met-Ty and oxy-Ty forms bind monophenols and *ortho*-diphenols. But the met-Ty species is only active on *ortho*-diphenols, while it gives a dead-end complex with monophenols. Therefore, the catalytic cycle of monophenols is simpler because it involves only two enzyme forms, deoxy-Ty and oxy-Ty, in spite of the more energetically demanding chemistry performed. A recent breakthrough in our understanding of this enzyme has been the report by Matoba et al. of the crystal structure of *S. castaneoglobisporus* tyrosinase, in a complex with the Cu transporting caddie protein

Scheme 1: Schematic Representation of the Catalytic Cycle for the Two Activities of Tyrosinase

(1) Diphenolase cycle:  $[2C + O_2 \rightarrow 2Q + 2H_2O]$



(2) Monophenolase cycle:  $[P + O_2 \rightarrow Q + H_2O]$



ORF378 (6). The binding to ORF378 makes the enzyme inactive because ORF378 covers part of the surface of tyrosinase and prevents the binding of substrates to the dicopper site. Actually, an intriguing aspect of this structure is that the side chain of Tyr98 of ORF378 protrudes into the substrate binding pocket of tyrosinase, but the distance of the phenol ring from the coppers is too large to allow a direct attack by oxy-Ty.

Unlike other monooxygenases, such as cytochrome P-450 and dopamine  $\beta$ -hydroxylase, tyrosinase is an internal monooxygenase and does not require a cosubstrate to perform the phenol hydroxylation, which represents the enzyme's most important activity. This has so far restricted the range of substrates investigated with this enzyme to phenolic

<sup>†</sup> This work was supported by PRIN Project of the Italian MIUR, the Italian CNR, the University of Pavia, and CIRCMSB.

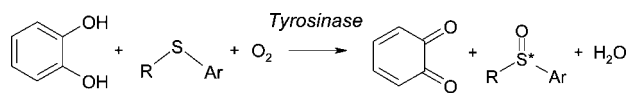
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<sup>1</sup> Abbreviations: Ty, tyrosinase; L-dopa, L-3,4-dihydroxyphenylalanine; MBTH, 3-methyl-2-benzothiazolinone hydrazone; DMF, dimethylformamide; HPLC, high pressure liquid chromatography; e.e., enantiomeric excess; GC, gas chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; C, a generic catecholic substrate; Q, a generic quinone; P, a generic phenolic substrate.

compounds, thus limiting its potential applications. In the present study, we report that tyrosinase catalyzes the asymmetric oxidation of organic sulfides to the corresponding sulfoxides in the presence of a suitable reducing agent, such as a catechol, thus showing that the enzyme can also work as an external monooxygenase.



This activity broadens the specificity of the enzyme with respect to the functionality undergoing oxygenation and places tyrosinase together with cytochrome P-450 (7) and dopamine  $\beta$ -hydroxylase (8), both of which exhibit C-H hydroxylation and S-oxygenation activities.

## EXPERIMENTAL PROCEDURES

**Materials.** Thioanisole, methyl *para*-tolyl sulfide, 2-(4-methylthio)phenylacetic acid, 4-(methylthio)benzaldehyde, methyl phenyl sulfoxide, and L-dopa were from Aldrich. 4-(Methylthio)phenyl methanol and methyl 4-pyridyl sulfide were prepared as described below.  $^{18}\text{O}_2$  gas, at 15 atm and with 97% content of  $^{18}\text{O}$ , was from Aldrich.

**Enzyme Purification.** Mushroom (*Agaricus bisporus*) tyrosinase (3960 units/mg) was obtained from Sigma and purified according to Duckworth and Coleman's procedure (9). As we did in our previous study (10), to rule out the presence of oxidizing activities not related to tyrosinase in the purified enzyme, a 10% native-PAGE was run and then split into two parts that were stained with Coomassie Brilliant Blue to identify proteins and with L-dopa to highlight enzymatic activity. To increase the sensitivity of the assay, the quinone trapping reagent MBTH was used, and the reaction mixture for the zymogram had the following composition: 1 mM L-dopa, 8 mM MBTH, 4% DMF in 100 mM phosphate buffer at pH 7.0 (10); the staining reaction was carried out for 10 min at 37 °C. This assay showed that the small amount of protein impurities were completely inactive in L-dopa oxidation. The enzyme concentration was calculated from the activity of the purified enzyme in the L-dopa oxidation assay (10) and assuming a molecular mass of 120 kDa (11).

**Enzymatic Oxidation of Sulfides.** The reactions were carried out according to standard procedure, where thioanisole (4 mM) and L-dopa (variable amount) were stirred in 50 mM Hepes buffer at pH 6.8 at 25 °C. The reaction was started by the addition of tyrosinase ( $4.4 \times 10^{-7}$  M), and a reddish-brown color slowly developed in the solution. After the given reaction time, a sample of the reaction mixture was withdrawn, and a known amount of 1-naphthol was added as internal standard for HPLC analysis. The sample was extracted three times with dichloromethane, and the organic extracts were combined. The solvent was removed by rotary evaporation (unreacted volatile thioanisole is partially lost in this operation) and the residue dissolved in ethyl acetate and analyzed by HPLC with a Jasco MD-1510 instrument with diode array detection, using a Daicel chiral column OD ( $0.46 \times 25$  cm). The elution mixture contained 85% *n*-hexane and 15% *iso*-propanol and was used at a flow rate of 0.5 mL/min, with optical readings at 242 nm; the peak of the internal standard was evaluated by a reading at

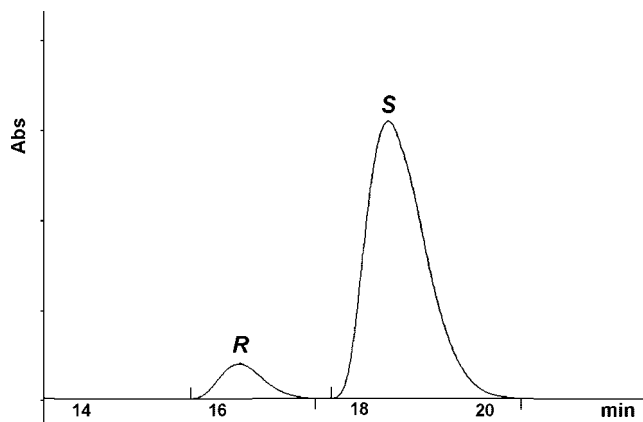


FIGURE 1: HPLC trace showing the separation of (*R*)- and (*S*)-methyl phenyl sulfoxide from the enzymatic oxidation of thioanisole (4 mM) carried out with L-dopa (6 mM) in 50 mM Hepes buffer at pH 6.8, at 25 °C.

233 nm. The (*R*)-sulfoxide eluted at 16.2 min followed by the (*S*)-sulfoxide at 18.5 min (12). No other products (e.g., sulfone) were observed, as confirmed by NMR spectra recorded on samples of the reaction mixtures. A representative HPLC trace of the separation of the enantiomeric sulfoxides is shown in Figure 1. Standard curves were prepared using commercial methyl phenyl sulfoxide and the percent conversion and e.e. determined on the basis of the peak areas in the HPLC traces. Data were obtained at least in duplicate. Although we used purified enzyme in this work, experiments carried out with commercial tyrosinase gave comparable yields and only slightly lower e.e. of sulfoxide.

In the experiments where sulfoxidation of thioanisole was studied in the presence of ascorbic acid (600 mM), this reagent was mixed together with thioanisole (4 mM) and L-dopa (variable amounts) in Hepes buffer as before, but the pH of the buffer was readjusted at 6.8 before adding the enzyme ( $4.4 \times 10^{-7}$  M). The separation and analysis of the reaction products followed the same procedure described above.

The pH dependence of the sulfoxidation was investigated by initially preparing a solution containing thioanisole (4 mM) and L-dopa (0.6 mM) in 50 mM phosphate buffer and subsequently bringing the solution to the desired pH before the addition of tyrosinase ( $4.4 \times 10^{-7}$  M). The separation and analysis of the organic products was carried out following the same procedure described above.

The enzymatic oxidation of methyl *para*-tolyl sulfide, 2-(4-methylthio)phenylacetic acid, 4-(methylthio)benzaldehyde, 4-(methylthio)phenyl methanol, and methyl 4-pyridyl sulfide (1 mM) in the presence of L-dopa (0.6 or 6.0 mM) was performed according to the same general procedure. Standard samples of the various sulfoxides for HPLC analysis were obtained by oxidation of the corresponding sulfides with  $\text{H}_2\text{O}_2$  as described by Baciocchi et al. (13).

**$^{18}\text{O}$  Incorporation into Thioanisole.** A 6-mL sample of a solution containing thioanisole (4 mM) and L-dopa (6 mM) in 50 mM Hepes buffer at pH 6.8 was cooled to 0 °C and degassed through vacuum/argon cycles in a small Schlenk flask. Care was taken in order to prevent loss of the volatile thioanisole in this operation. A carefully degassed solution of tyrosinase ( $4.4 \times 10^{-7}$  M) was then added by a gastight syringe. After raising the temperature to 25 °C, the reaction

was started by slowly bubbling  $^{18}\text{O}_2$  through a gastight syringe (10 mL). The addition of  $^{18}\text{O}_2$  caused the development of a reddish brown color in the solution. The reaction was continued for 4 h. Then it was stopped by the addition of a few drops of concentrated HCl. The sample was extracted with dichloromethane as described before, the organic extracts were evaporated to dryness, and the residue dissolved into 1 mL of methanol and analyzed by GC/MS. The GC/MS analysis was performed with an Agilent 6890 gas chromatograph equipped with an Agilent 5973 MS detector. GC separation of the compounds was performed with an HP.1 (30  $\times$  0.32 mm) column. The temperature was raised with a linear gradient of 20  $^\circ\text{C}/\text{min}$  from the initial 70  $^\circ\text{C}$  up to 270  $^\circ\text{C}$  and then kept at this value for further 6 min.

**Thioanisole Inhibition of L-Dopa Enzymatic Oxidation.** The kinetic experiments of inhibition of the oxidation of L-dopa by thioanisole were performed according to standard procedures (14, 15), operating in 50 mM Hepes buffer at pH 6.8 and 25  $^\circ\text{C}$ . The rate dependence of the enzymatic oxidation of L-dopa was studied as a function of substrate concentration in the presence of variable amounts of thioanisole, from 0 to 1.0 mM, using 5 nM tyrosinase. The oxidation of L-dopa was followed spectrophotometrically by monitoring dopachrome formation at 476 nm; reaction rates were expressed as  $\Delta\text{absorbance}$  units versus time.

**Preparation of Substituted Sulfides. Methyl 4-Pyridyl Sulfide.** 4-Mercaptopyridine (Aldrich) (1.0 g; 9 mmol) was dissolved in 100 mL of 1% methanolic KOH solution, followed by the addition of one equiv. iodomethane. The mixture was heated to reflux for 24 h and then cooled to room temperature and rotary evaporated to dryness. The residue was chromatographed ( $\text{SiO}_2$ ) in  $\text{CH}_2\text{Cl}_2$  with a methanol gradient from 1% to 2%. The product was obtained upon concentrating the eluate to a small volume by rotary evaporation, giving a white powder (yield 59%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.43 (s, 3H;  $\text{CH}_3$ ), 7.05 (dd,  $J$  = 6.23, 1.63 Hz; 2H), 8.35 (dd,  $J$  = 6.24, 1.61 Hz; 2H). Anal. Calcd for  $\text{C}_6\text{H}_7\text{NS}$  (125.19): C, 57.56; H, 5.64; N, 11.19; S 25.61. Found: C, 57.50; H, 5.70; N, 11.10; S, 25.72.

**4-(Methylthio)phenyl Methanol.** To 20 mL of a methanol solution of 4-(methylthio)benzaldehyde (7.5 mmol) kept in an ice bath, 10 equiv.  $\text{NaBH}_4$  dissolved in 30 mL of methanol was added dropwise. The reaction mixture was then refluxed for 30 min, cooled to room temperature and concentrated by rotary evaporation until a white precipitate was obtained. The product was collected by filtration and dried under vacuum (yield 65%).  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  2.51 (s, 3H;  $\text{CH}_3$ ), 4.68 (d,  $J$  = 5.91 Hz; 2H,  $\text{CH}_2$ ), 7.27 (dd,  $J$  = 8.47, 2.14 Hz; 2H), 7.31 (dd,  $J$  = 8.54, 2.18 Hz; 2H). Anal. Calcd for  $\text{C}_8\text{H}_{10}\text{OS}$  (154.23): C, 62.30; H, 6.54; S, 20.79. Found: C, 62.61; H, 6.48; S, 20.61.

## RESULTS AND DISCUSSION

When excess thioanisole is added to mushroom tyrosinase, which is obtained in the met-Ty form after purification, in Hepes buffer at pH 6.8, no reaction occurs because met-Ty is unable to oxidize the sulfide. However, when a reducing agent such as 3,5-di-*tert*-butylcatechol or L-dopa is added, a slow reaction producing sulfoxide occurs. The function of the reducing agent in this process is therefore to reduce met-

Scheme 2: Schematic Representation of the Catalytic Cycle for the Sulfoxidation Catalyzed by Tyrosinase in the Presence of L-dopa As Cosubstrate

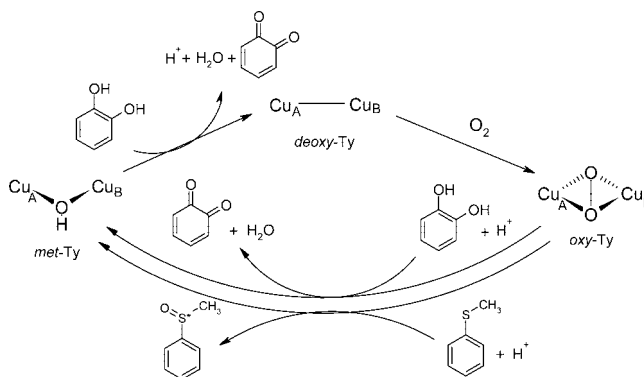


Table 1: Conversion of Thioanisole (4 mM) to Methyl Phenyl Sulfoxide in the Presence of Mushroom Tyrosinase ( $4.4 \times 10^{-7}$  M) and L-dopa in Hepes Buffer at pH 6.8, at 25  $^\circ\text{C}$  after 4 h of Reaction Time<sup>a</sup>

[L-dopa] (mM)	[sulfoxide] (mM)	% vs sulfide	% vs L-dopa	yield (%)	e.e. (%)
0.06	0.04	1	67	67	41
0.3	0.12	3	40	40	52
0.6	0.15	4	25	25	65
1.2	0.28	7	23	23	77
6.0	0.77	20	13	20	86

<sup>a</sup> Yields of isolated sulfoxide are  $\pm 10\%$  and e.e. are  $\pm 5\%$ .

Ty and allow the formation of oxy-Ty, which is the only enzyme form capable of supporting the sulfoxidation (Scheme 2). As the reducing agent is also a substrate for oxy-Ty, there is always a strong competition between the sulfide and reducing agent in this step, which limits the efficiency of the sulfoxidation. Among the reducing agents tested, L-dopa gave the best results in the sulfoxidation, with yields of sulfoxide up to 60%. This is noteworthy, especially considering that tyrosinase suffers strong inactivation by the quinone products derived from L-dopa (16, 17). In addition, it is interesting that methyl phenyl sulfoxide is obtained with a high degree of enantioselectivity (the (*S*)-isomer being favored, with e.e. up to about 90%; Figure 1), showing the important role of the enzyme in the stereochemical control of the reaction. Table 1 reports the yields and e.e. of the sulfoxidation of thioanisole studied in the presence of different L-dopa concentrations. It appears that at low L-dopa concentration this is the limiting reagent, but at high L-dopa concentration, thioanisole becomes limiting. The data in Table 1 give the conversion of sulfide into sulfoxide both in terms of global yield and with respect to L-dopa. Thus, while the amount of sulfoxide increases with L-dopa concentration, the percent conversion referred to the cosubstrate decreases, in agreement with the double role of L-dopa as substrate and competitor.

To probe the monooxygenase mechanism of the sulfoxidation, an experiment was performed in the same conditions as those in the last entry of Table 1 but using  $^{18}\text{O}_2$  (97% enriched). The MS spectrum of the labeled methyl phenyl sulfoxide obtained from  $^{18}\text{O}_2$  is compared with a standard sample in Figure 2. Taking into account the 97% enrichment of  $^{18}\text{O}_2$ , the extent of 18-O incorporation into methyl phenyl sulfoxide was above 95%. This result confirms that sulfoxide can only be formed by reaction of the sulfide with oxy-Ty. In addition, the e.e. for the 18-O-enriched sample of sulfoxide

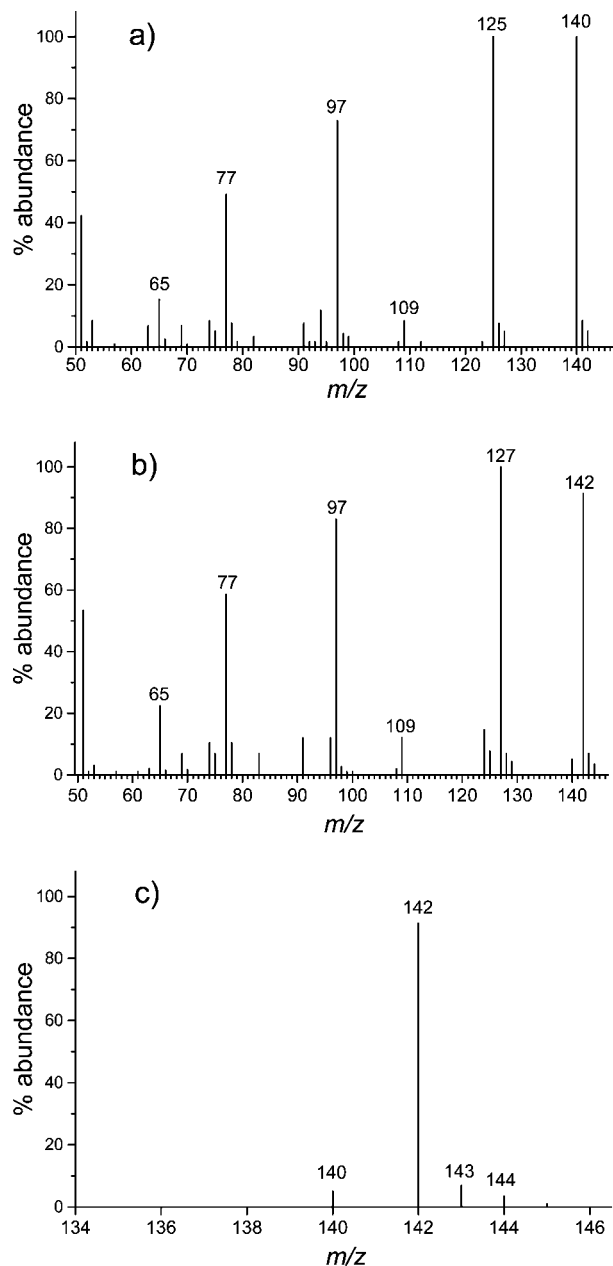


FIGURE 2: MS spectra of (a) standard methyl phenyl sulfoxide and (b) methyl phenyl sulfoxide obtained from the tyrosinase-catalyzed oxidation of thioanisole with the use of 97% enriched  $^{18}\text{O}_2$ . (c) Close up view of spectrum b showing the isotopic pattern of the molecular peak.

was 85%. Both the 18-O atom incorporation and high e.e. seem to exclude a mechanism of sulfide oxidation proceeding through the formation of a sulfur radical intermediate.

The competition between thioanisole and L-dopa was investigated through kinetic studies by measuring the reaction rates for the oxidation of L-dopa to dopachrome by tyrosinase as a function of the concentrations of both L-dopa and thioanisole (Figure 3). The reaction follows a Michaelis-Menten behavior, and the plots clearly show that thioanisole has an inhibitory effect on the reaction. In particular, the observed  $k_{\text{cat}}$  values decrease on increasing the sulfide concentration, although the inhibition is not simply due to competitive binding to the enzyme active site (Figure 4). This behavior can be accounted for by the double action of

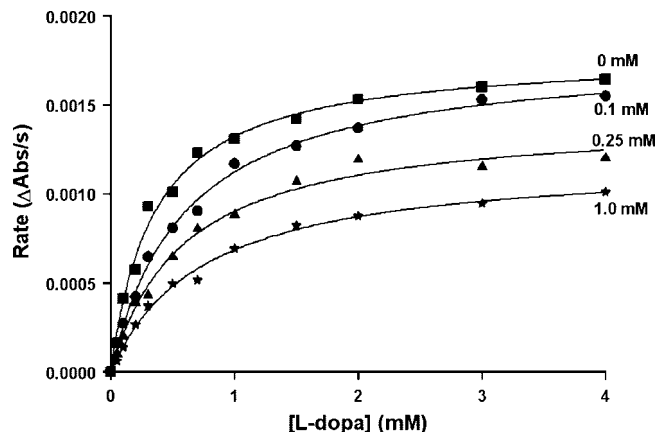


FIGURE 3: Rate dependence on substrate concentration for the tyrosinase-catalyzed oxidation of L-dopa in the presence of different concentrations of thioanisole, measured in 50 mM Hepes buffer at pH 6.8, at 25 °C.

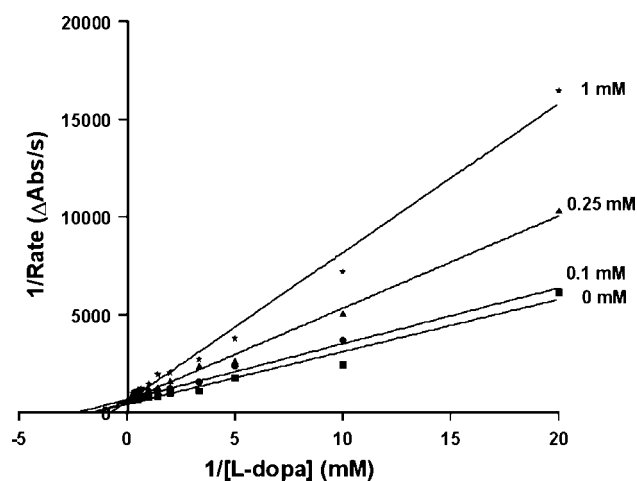


FIGURE 4: Lineweaver-Burk plots for the inhibition of the tyrosinase-catalyzed oxidation of L-dopa by thioanisole; conditions are the same as those described in Figure 3.

Table 2: Conversion of Thioanisole (4 mM) into Methyl Phenyl Sulfoxide in the Presence of Mushroom Tyrosinase ( $4.4 \times 10^{-7}$  M) and L-dopa (0.6 mM), in 50 mM Hepes Buffer at pH 6.8, at 25 °C, at Various Reaction Times<sup>a</sup>

time (h)	[sulfoxide] (mM)	% vs sulfide	% vs L-dopa	e.e. (%)
2	0.03	2	6	90
4	0.07	5	12	68
8	0.08	6	14	68

<sup>a</sup> Yields of isolated sulfoxide are  $\pm 10\%$  and e.e. are  $\pm 5\%$ .

thioanisole: it competes with L-dopa in binding at the active site, but it also reacts with oxy-tyrosinase.

The e.e. of sulfoxide is high at the beginning but decreases with the progress of the reaction. This is shown, for instance, by the data reported in Table 2, which refer to the reaction carried out in the conditions of the third entry of Table 1, where after 2 h the e.e. of sulfoxide is 90%, and then it decreases. This effect is clearly due to the inactivation of the enzyme by the quinones produced by L-dopa oxidation (16, 17), which covalently react with nucleophilic residues of the protein and progressively deplete the selectivity of sulfoxidation. To limit enzyme inactivation, we found it useful to add excess ascorbate, a good reducing agent for the quinones produced by L-dopa but a very poor substrate

Table 3: Conversion of Thioanisole (4 mM) into Methyl Phenyl Sulfoxide in the Presence of Tyrosinase ( $4.4 \times 10^{-7}$  M), L-dopa and Ascorbic Acid (600 mM) in Hepes Buffer at pH 6.8, at 25 °C, after 4 h of Reaction Time<sup>a</sup>

[L-dopa] (mM)	[sulfoxide] (mM)	% vs sulfide	% vs L-dopa	yield (%)	e.e. (%)
0.05	0.05	2	100	100	50
0.3	0.21	8	78	78	88
0.9	0.20	9	22	22	90
1.8	0.27	12	15	15	89
2.75	0.57	21	21	21	93
3.8	0.28	7	7	7	94
4.8	0.45	17	10	17	91
5.3	0.39	15	7	15	88

<sup>a</sup> Yields of isolated sulfoxide are  $\pm 10\%$  and e.e. are  $\pm 5\%$ .

Table 4: pH Dependence of the Conversion of Thioanisole (4 mM) into Methyl Phenyl Sulfoxide in the Presence of Mushroom Tyrosinase ( $4.4 \times 10^{-7}$  M) and L-dopa (0.6 mM), in 50 mM Phosphate Buffer Adjusted at the Desired pH, after 4 h of Reaction Time, at 25 °C<sup>a</sup>

pH	[sulfoxide] (mM)	% vs sulfide	% vs L-dopa	yield (%)	e.e. (%)
3	0.004	0.1	<1	<1	0
4	0.02	0.5	4	4	70
5	0.04	1.5	7	7	77
6	0.07	2.5	12	12	85
7	0.09	3	15	15	81
8	0.1	3.3	16	16	80

<sup>a</sup> Yields of isolated sulfoxide are  $\pm 10\%$  and e.e. are  $\pm 5\%$ .

for tyrosinase (16). Indeed, ascorbate prevents browning of the reaction solution and improves the yield and e.e. of the sulfoxidation, as shown by the data reported in Table 3. The pH of the enzymatic oxidation also affects the output of the reaction, the best conditions being around neutral pH, where the enzyme exhibits optimum activity (18); the relevant data are collected in Table 4.

We also preliminarily investigated the enzymatic oxidation of some other sulfides related to thioanisole. Methyl *para*-tolyl sulfide has very low solubility in aqueous buffer, and in experiments run in the presence of 0.6 and 6.0 mM L-dopa, the sulfoxide was obtained in reduced yield (5% and 2%, respectively) and e.e. (25% and 33%, respectively, in favor of the (*S*)-isomer). Here, the addition of excess ascorbate resulted in almost negligible improvement of the sulfoxidation. With 4-(methylthio)phenyl methanol and 2-(4-methylthio)phenylacetic acid, yields were also low ( $\sim 3\%$ ) and the sulfoxides racemic. As the sulfoxidation strongly depends on the competition with L-dopa oxidation, the conditions would need to be carefully optimized for each substrate, and perhaps a different reducing agent should be searched because sulfides carrying bulky substituents may be very poor competitors toward L-dopa. Sulfides containing electron withdrawing substituents, that is, methyl 4-pyridyl sulfide and 4-(methylthio)benzaldehyde, gave no reaction, with some analogy to phenol hydroxylation, which is prevented when strong electron-withdrawing groups are present on the phenol nucleus (19).

## CONCLUSIONS

Tyrosinase behaves as an external monooxygenase catalyzing the S-oxygenation of organic sulfides in the presence of suitable reducing agents. The basic difference with monooxygenases requiring a cosubstrate is that in the latter

case the cosubstrate selectively reduces the oxidized form of the enzyme but is prevented from reacting with the enzyme active species produced upon reaction with dioxygen. In the sulfoxidation promoted by tyrosinase, the cosubstrate competes with the sulfide also in this step, thus limiting the efficiency of the process. However, reducing agents that are poor tyrosinase substrates, such as ascorbic acid, are also inefficient in the conversion of met-Ty into deoxy-Ty, thereby making the enzyme basically inactive in sulfoxidation. In spite of these limitations, the sulfoxidation activity described here expands the functional competence of tyrosinase beyond the hydroxylation of phenolic C–H bonds and broadens the specificity of the enzyme. Sulfoxidation of thioanisole occurs with high enantioselectivity, particularly when enzyme inactivation is prevented. Actually, in this reaction tyrosinase exhibits higher stereoselectivity than in the oxidation of the natural substrates L-/D-Tyr ( $\sim 75\%$ , in terms of  $k_{cat}/K_m$ ) and L-/D-dopa ( $\sim 70\%$ ) (20), but also in these cases, stereoselectivity is entirely due to substrate recognition (i.e.,  $K_m$ ). These stereochemical features are consistent with our recent finding that the enzyme bears a highly preorganized active site (10). Preference for the (*S*)-sulfoxide indicates that electrophilic attack at the S atom by oxy-Ty occurs from the side of the larger phenyl substituent of the sulfide, a stereochemical output opposite to that of cytochrome P-450 (7, 21) but similar to that of dopamine  $\beta$ -hydroxylase (8) in the same reaction. It is interesting that organic sulfides have been considered so far to act simply as inhibitors of tyrosinase (22, 23).

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BI702421B