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INHIBITION OF MUSHROOM TYROSINASES BY LAMPRENE AND THIAMBUTOSINE

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Abstract—The inhibition by lamprene of the oxidation of L-dopa, catalysed by tyrosinase from the wild mushroom, *Xerocomus badius*, was of the mixed type with a K_i of 30 μM. The inhibition by thiambutosine of the tyrosinase from the cultivated mushroom, *Agaricus campestris*, was competitive with a K_i of 15 μM.

Since the introduction of the mouse foot-pad model by Shepard [1] as an experimental basis for the chemotherapy of leprosy, many drugs have been developed against this disease. Nevertheless, their mechanism remains still unknown. Lamprene (B663, clofazimine), a riminophenazine compound, is considered as one of the most promising drugs, while thiambutosine (Ciba 1906) is widely used when dapsone (diaminodiphenyl sulphone), the drug of choice, is not tolerated [2].

Prabhakaran and Kirchheimer [3] demonstrated the existence of an active phenoloxidase in *Mycobacterium leprae*. This enzyme might be involved in a key metabolic process in this mycobacterium [4]. Inhibitors of the phenolase also suppressed the multiplication of the bacteria in the mouse foot-pad [5].

This paper reports the inhibition by lamprene and by thiambutosine of tyrosinases (phenoloxidase, E.C. 1.14.18.1), isolated from mushrooms.

MATERIALS AND METHODS

Materials. Tyrosinases were prepared from mushrooms by the method of L. H. Evans, T. N. Wagner and H. S. Mason*. Mushrooms were immersed in liquid nitrogen for a few minutes and ground in a Waring Blender. The endogenous substrates were extracted with a saturated sodium benzoate–ammonium sulphate solution (700 g ammonium sulphate and 2.125 g sodium benzoate per liter of water, pH 5.6). Protein was solubilized from the remaining pulp with 0.1 M sodium benzoate adjusted to pH 5.6 with 1 N sulphuric acid. Ammonium sulphate was added, the precipitate at 35–45% saturation was submitted to chromatography on Sephadex G-100 and on hydroxylapatite. In the experiments with thiambutosine tyrosinase from *Agaricus campestris* was used. The inhibition by lamprene was studied on tyrosinase from *Xerocomus badius*.

Lamprene and thiambutosine were a gift from Ciba-Geigy, Basle, Switzerland. 3,4-Dihydroxy-L-phenylalanine (L-dopa) was purchased from Aldrich-Europe, Janssen Pharmaceutica, Beerse, Belgium and dimethyl sulphoxide p.a. was obtained from Merck, Darmstadt, Germany.

Methods. The reaction rates were determined spectrophotometrically [6] or by measuring the oxygen consumption.

The increase in absorbance at 475 nm was followed in a Beckman DB-G spectrophotometer (Fullerton, CA, U.S.A.). The reaction cuvette was filled with 3 ml air-saturated tyrosinase solution, incubated with inhibitor. By means of a micro syringe 50–200 μl L-dopa were injected (final concentrations 0.025–1 mM). Activities were calculated from initial velocities and expressed as the change in absorbance per min, multiplied by 1000. With thiambutosine reaction rates were calculated per μl tyrosinase stock solution.

Oxygen consumption was measured with a Clark oxygen electrode (Radiometer, Copenhagen, Denmark), placed in a plexiglass holder provided with a stopper and equipped with a magnetic stirrer. Solutions of L-dopa were added through a capillary in the stopper (final concentrations 0.025–1 mM). The oxygen consumption was registered with a recorder, activities were calculated from initial velocities and expressed as μmol oxygen per min.

Inhibitor concentrations in the reaction mixture were limited to 20 μM, owing to the low solubility of lamprene and thiambutosine in water. These inhibitors were dissolved in dimethyl sulphoxide. The experiments were carried out at 25° in 0.1 M phosphate buffer, pH 6.93, 5% (v/v) dimethyl sulphoxide. Up to this final concentration there was no influence of dimethyl sulphoxide on the reaction rate.

RESULTS AND DISCUSSION

Both methods yielded similar results for the inhibition of tyrosinase by lamprene (Figs. 1 and 2). Lineweaver–Burk plots of velocity data for a series of concentrations of lamprene indicated changes of both K_m and V (Tables 1 and 2). This inhibition was of a mixed type, the dissociation constant of the enzyme-inhibitor complex K_i was calculated from the slopes of the straight lines, as described by Webb [7]. A second constant α, which measures the influence of the inhibitor on the dissociation of the enzyme-sub-

*Personal communication.

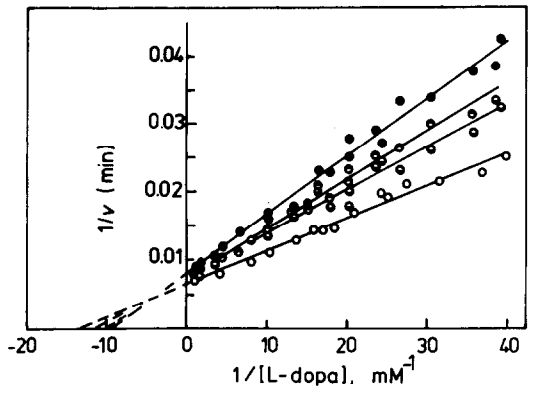

Fig. 1. Kinetic patterns of inhibition of tyrosinase (*Xerocomus badius*) by lamprene with respect to L-dopa in phosphate buffer 0.1 M, pH 6.93, 5% (v/v) dimethyl sulphoxide. Reaction rates were determined spectrophotometrically. ○, Blank; ◒, 8.6 μM, ◓, 17.8 μM, and ●, 18.7 μM lamprene.

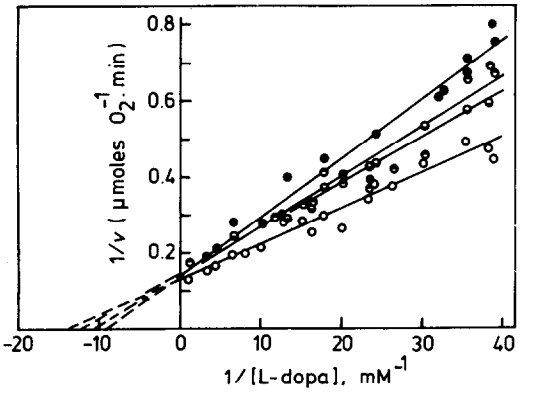

Fig. 2. Kinetic patterns of inhibition of tyrosinase (*Xerocomus badius*) by lamprene with respect to L-dopa (conditions of Fig. 1). Reaction rates were determined with the oxygen electrode. ○, Blank; ◒, 10.2 μM, ◓, 12.2 μM, and ●, 18.0 μM lamprene.

Table 1. Kinetic parameters for the inhibition of the tyrosinase of *Xerocomus badius* by lamprene with L-dopa as a substrate in phosphate buffer 0.1 M, pH 6.93, 5% (v/v) dimethyl sulphoxide. Reaction rates determined by the spectrophotometric method

Lamprene μM	V min^{-1} 10^2	K_m μM	K_i μM	α
0	1.55	74.7	—	—
8.6	1.36	86.5	26.0	2.3
17.8	1.40	100	35.4	4.3
18.7	1.30	110	23.9	3.2

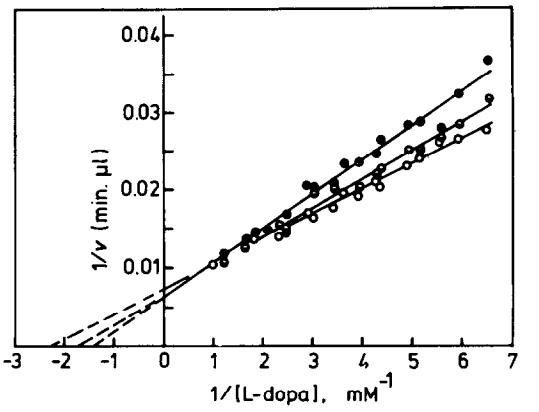

Fig. 3. Kinetic patterns of inhibition of tyrosinase (*Agaricus campestris*) by thiambutosine with respect to L-dopa (conditions of Fig. 1). Reaction rates were determined spectrophotometrically. ○, Blank; ◒, 4.02 μM and ●, 8.30 μM thiambutosine.

strate complex and thereby indicates the extent of competitive ($\alpha = \infty$) versus non-competitive inhibition ($\alpha = 1$), was also obtained graphically. The values listed in Table 1 and 2 show that the competitive character of the inhibitor is more pronounced, an average K_i value of 30 μM was derived.

Double reciprocal plots, calculated from the data obtained with thiambutosine, proved the inhibition to be competitive (Fig. 3). An average K_i value of 15 μM was obtained (Table 3).

These results can be compared with the conclusions of other papers, dealing with the inhibition of tyrosinases. Duckworth and Coleman [8] reported a competitive inhibition of the catecholase activity of mushroom tyrosinase by benzoic acid (K_i = 1 μM), Pomerantz [9] found a non-competitive inhibition of tyrosinase from hamster melanoma by diethyldithiocarbamate (K_i = 80 μM). Methimazole, another inhibitor of the multiplication of *M. leprae* [10], inhibited tyrosinase with a K_i value in the range of 1 μM [11].

These data indicate that mushroom tyrosinases are inhibited by the anti-leprosy drugs lamprene and

Table 3. Kinetic parameters for the inhibition of the tyrosinase of *Agaricus campestris* (conditions of Table 1). Reaction rates determined by the spectrophotometric method

Thiambutosine μM	V min^{-1} μl^{-1} 10^2	K_m μM	K_i μM
0	1.38	446	—
4.02	1.52	562	16.5
8.30	1.52	658	14.1

Table 2. Kinetic parameters for the inhibition of the tyrosinase of *Xerocomus badius* by lamprene (conditions of Table 1). Reaction rates determined with the oxygen electrode

Lamprene μM	V μmol O_2 min^{-1}	K_m μM	K_i μM	α
0	31.3	73	—	—
10.2	27.3	81	37.4	1.9
12.2	28.8	94	36.0	5.0
18.0	29.6	110	28.4	7.4

thiambutosine. We intend to compare the inhibition of both enzymes by these substances and to extend these data to other drugs, like dapsone.

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