PURIFICATION AND PROPERTIES OF TYROSINASE INHIBITOR FROM MUSHROOM

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1. Introduction

The control of the enzymic oxidation of phenols has practical significance in the therapy of melanomas [1] and leprosy [2], in senescence [3] and food processing [4]. Extracts inhibitory to mushroom tyrosinase have been obtained from mushrooms [5,6], from guinea pig skin [7,8], from the fungus *Dactylium dendriodes* [9] and from potato tubers [10]. The inhibitors in these extracts have all been described as proteins. Many of their properties however, are dissimilar indicating different entities even from the same sources [7].

Tyrosinase (EC 1.10.3.1) is widely distributed in plants and animals and its activity on phenolic substrates becomes particularly apparent in injured tissues of apple, banana and potato.

This paper describes the properties of a tyrosinase inhibitor purified by column and affinity chromatography.

2. Materials and methods

2.1 Organism

The mushroom *Agaricus hortensis* Cke. was obtained commercially and identified by the late Dr J. Walton Groves, mycologist, Canada Department of Agriculture.

2.2 Extraction of the inhibitor

The inhibitors were extracted from the frozen and thawed sporophores by homogenizing in pyridine–acetate buffer (0.5 M, pH 7.2).

2.3 Purification by affinity chromatography

The active freeze-dried fraction (20.5 mg) obtained by chromatography on a Sephadex G25 column was adsorbed in bulk onto phloroglucinol-hydroxylated agarose [11] coupled with tyrosinase by the cyanogen bromide method [12] for 3 hr at 22°C in pyridine-acetate buffer (0.02 M, pH 7.2). The inhibitors were desorbed from the coupled and adsorbed agarose column (1.4 × 2.8 cm) with pyridine–acetate buffers 0.02 M, pH 8.2 and 0.2 M, pH 5 respectively.

2.4 Electrophoresis

Electrophoresis on 7% acrylamide gel followed the method described by Davis [13]. Immuno-electrophoresis was performed on microslides (L.K.B. Produkter, AB Stockholm) using antiserum developed against *A. hortensis* [5].

2.5 Ultraviolet radiation

A water solution of the inhibitor (1 mg/ml) 1 ml deep in an open container, was irradiated at 336 μm for 1 hr. The light source (Ultra-Violet Products Inc., California) was 10 cm from the solution.

2.6 Assay for the inhibitor

One ml of mushroom tyrosinase (Nutritional Biochemical Corporation) producing a change in 10 units OD/minute at 475 μm with dopa (3,4-dihydroxy-

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phenylalanine) as substrate, and one ml inhibitor sample were incubated for 3 hr at 22°C before reacting with 1 ml of dopa (0.1 M). Controls, omitting the inhibitor, were treated similarly.

2.7 Assay for sulfhydryl groups

Sulfhydryl groups were determined by the method of Sedlak and Lindsay [14].

3. Results

Two inhibitors (Ia, Ib) were desorbed with pyridine-acetate buffers 0.02 M, pH 8.2 and 0.2 M, pH 5

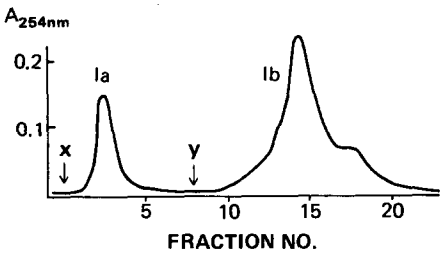

Fig. 1. Desorption of tyrosinase inhibitors (Ia, Ib) from phloroglucinol-hydroxylated-tyrosinase coupled agarose column (1.4 × 2.8 cm). The inhibitors were adsorbed from 20.5 mg, active Sephadex G25 column fraction for 3 hr at 22°C in pyridine–acetate buffer (0.02 M, pH 7.2). Desorption buffers were pyridine–acetate, 0.02M, pH 8.2 (x) and 0.2 M pH 5 (y). Desorbed Ia, Ib were 4.4 mg and 12.8 mg respectively. Rate: 20 ml/hr, fractions 5 ml.

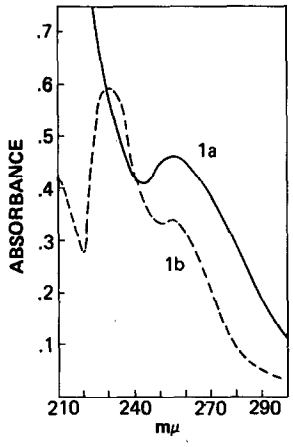

Fig. 2. Absorption spectra of tyrosinase inhibitors (Ia, Ib) separated by affinity chromatography.

respectively (fig. 1). Ia had an absorption maximum at 254 μm (fig. 2) and reacted positively with ninhydrin. Acrylamide gel disc and immunoelectrophoresis disclosed one protein band (fig. 3). Amino acid analysis of the acid hydrolysate of Ia (6N HCl for 12 hr at 110°C) produced three major amino acids, phenylalanine, aspartic and glutamic acids in the approximate ratio 1:1:1 (table 1). The molecular

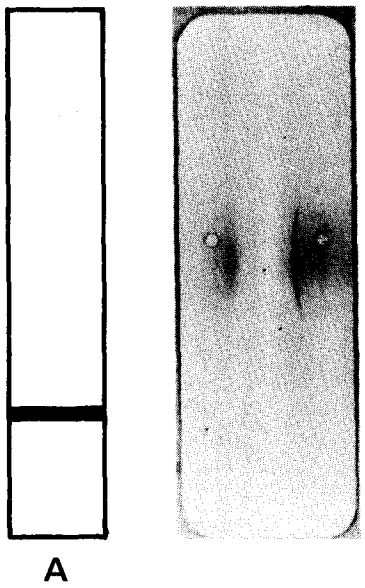

Fig. 3. Electrophoresis [13] of tyrosinase inhibitor (Ia) on 7% acrylamide gel (A) and immuno-electrophoresis [5] of tyrosinase inhibitor (Ia). Antiserum was developed against *A. hortensis* extract.

Table 1
Amino acid analysis of peptide inhibitor of tyrosinase obtained from the mushroom *A. hortensis*

Amino acid	μM/mg
Histidine	.037
Asparagine	.665
Threonine	.052
Serine	.020
Glutamic acid	.574
Glycine	.022
Alanine	.185
Phenylalanine	.710
Ammonia	34.16

weight of Ia, based on amino acid analyses and Sephadex G15 chromatography, was estimated at 1200. The inhibitor was relatively insoluble in organic solvents, was freely soluble in water and does not contain sulfhydryl groups. The inhibitory capacity was not affected by ultraviolet radiation. Ia demonstrated competitive inhibition (fig. 4) with dopa substrate.

The inhibitor Ib, desorbed at pH 5, displayed maximum absorption at 230 μm with a shoulder at 254 μm. The desorption pattern (fig. 1) suggest a heterogenous eluate which inhibited tyrosinase non-competitively (fig. 4).

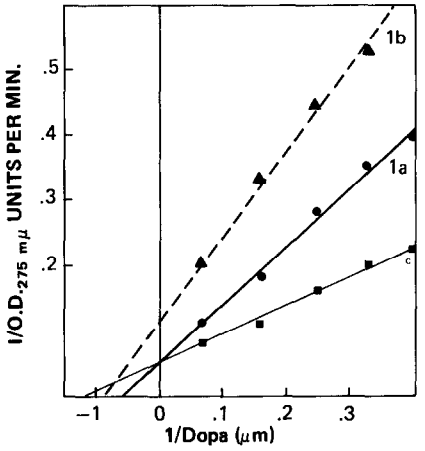

Fig. 4. Lineweaver-Burk plots showing inhibition of tyrosinase catalysis of dopa oxidation by mushroom inhibitors Ia and Ib.

4. Discussion

The inhibitor (Ia) of tyrosinase isolated in these experiments shows some similarity to that described by Flawn and Wilde [7,8] in its UV stability, low molecular weight peptide nature and its apparent sensitivity to ammonium acetate buffers. The inhibitor was not affected by pyridine–acetate buffers and maintained its activity throughout these experiments.

The competitive inhibition by Ia described here was also demonstrated with other tyrosinase inhibitors [15,16].

The phenylalanine content of the peptide undoubtedly accounts for the absorption maximum at 254 μm. The inhibitor eluate (Ib) desorbed at pH 5, with a secondary absorption shoulder at 254 μm, possibly contains some Ia but the major component absorbs at 230 μm.

Incubation of the inhibitor (Ia) with the enzyme for a minimum of 2.5 hr was necessary for optimum activity suggesting possible conformational changes of the enzyme molecule. This phenomenon, also described by Harel et al. [9], is being investigated currently.

Acknowledgements

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