Some Properties of Tyrosine Aminotransferase from *Trichoderma viride*

By C. O. ECHETEBU

Department of Biochemistry, University of Nigeria Nsukka, Nsukka, Nigeria

(Received 12 February 1982)

Tyrosine aminotransferase, induced in *Trichoderma viride* by L-tyrosine, was isolated from the culture medium, partially purified and characterized. The enzyme was inducible by both L- and DL-tyrosine, although L-tyrosine was the better inducer. The enzyme required L-tyrosine and α -ketoglutarate as amino donor and amino acceptor, respectively. Its pH optimum in 50 mM-potassium phosphate buffer was 7.6, the apparent Michaelis constants were 0.5 mM for L-tyrosine and 0.25 mM for α -ketoglutarate; it had a molecular weight of 110000.

INTRODUCTION

Tyrosine aminotransferase (TAT; EC 2.6.1.5) catalyses the reversible transfer of an amino group between L-tyrosine and L-glutamate using α -ketoglutarate and 4-hydroxyphenylpyruvate as co-substrates. Aminotransferases in general play an important role in nitrogen metabolism and are said to be responsible for the synthesis, in fungi, of the so-called secondary amino acids from the primary amino acids (Bekker, 1963). TAT has been described by various authors: from dog liver (Canellakis & Cohen, 1956); rat liver (Kenny, 1959, 1962); kidney and heart (Lin & Knox, 1958) and from frog liver (Ohisalo *et al.*, 1977); but has not been reported either from plants or from fungi. This paper describes an attempt at isolation and characterization of extracellular tyrosine aminotransferase from the soil fungus *Trichoderma viride*.

METHODS

Organism. *Trichoderma viride* used in this study was originally isolated from a wooden mortar and identified by standard procedures (Ainsworth, 1971; Clements & Shear, 1973). Stock cultures were grown on slants of Sabouraud agar for 7 d at room temperature and subsequently maintained at 4 °C. Subcultures were made at monthly intervals.

Growth medium. The fungus was grown in modified Fries' medium (1953) containing: KH_2PO_4 , 1.52 g; NaNO_3 , 6.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.52 g; CaCl_2 , 1.0 g and trace amounts of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (about 20 μg) in a litre of distilled water. This salt solution was adjusted to pH 6.5 with 0.1 M-HCl, sterilized at 15 lbf in⁻² (103.5 kPa) for 10 min and mixed with a solution of glucose [sterilized at 10 lbf in⁻² (69 kPa) for 5 min] to give a final concentration of 4% (w/v) glucose. After adding chloramphenicol (500 $\mu\text{g ml}^{-1}$), the medium was dispensed in 200 ml volumes into sterile 3 litre flasks.

Growth of fungus. Six flasks were each inoculated with four loopfuls of conidium-bearing mycelium from a 7 d culture of *T. viride* and incubated at room temperature (28–30 °C) for 7 d without agitation. Good growth was indicated by the formation of a dense mat of green mycelium on the medium. The medium was aseptically decanted and the mycelium was used for enzyme induction.

Induction of TAT. The induction medium was of the same composition as the Fries' medium but without glucose and NaNO_3 , which were replaced by the inducing amino acids, L-tyrosine or DL-tyrosine (6 $\mu\text{g ml}^{-1}$) as the main sources of carbon and nitrogen. Two of the six cultures were induced for TAT with L-tyrosine, two others with DL-tyrosine and the remaining two were grown in Fries' glucose medium as controls.

Induction was commenced by aseptically decanting Fries' glucose medium from the cultures, washing the mycelium with 0.9% (w/v) sterile NaCl at room temperature and then adding the induction medium. Control mycelium was similarly decanted and placed in Fries' glucose medium. All the cultures were incubated at room temperature without shaking, and tyrosine aminotransferase activity in the cultures was measured every 24 h for 14 d.

Extraction and partial purification. The three buffers of Granner & Tomkins (1970) used in the enzyme purification

tion were modified by the addition of 1 mM-2-mercaptoethanol. *Buffer I*. 0.05 M-potassium phosphate, pH 7.6 contained 1 mM-EDTA and 1 mM-2-mercaptoethanol. *Buffer II*. 0.05 M-potassium phosphate, pH 6.5 contained 1 mM-EDTA; 1 mM-2-mercaptoethanol, 2 mM- α -ketoglutaric acid and 0.2 mM-pyridoxal phosphate (PLP). *Buffer III*. 0.125 M-potassium phosphate, pH 7.6 contained 1 mM-EDTA, and 1 mM-2-mercaptoethanol.

After induction with tyrosine, all operations were performed at 0–4 °C unless otherwise stated. Mycelium was filtered from 10 d old cultures through cheese cloth and the filtrate was precipitated with 2 vol. prechilled acetone (–27 °C) added in 30 min with gentle stirring. After standing for another 30 min, the precipitate was collected by centrifugation at 15000 *g* for 30 min, dissolved in 5 ml buffer II and dialysed overnight against 100 vol. of the same buffer. The dialysis residue was further purified by chromatography in a column (2.5 × 50 cm) of Sephadex G-150, preequilibrated with buffer I and eluted with buffer II. Fractions (6 ml) were collected and those with TAT activity were pooled and dialysed against 5 vol. buffer III for 6 h. The dialysis residue, mixed with bovine serum albumin (10 μ g ml^{–1}) in 10% (v/v) glycerol, was used as the partially purified enzyme.

Enzyme assay. Tyrosine aminotransferase activity was measured by the method of Diamondstone (1966). In this method, the amount of *p*-hydroxyphenyl pyruvate formed is measured (after conversion to *p*-hydroxybenzaldehyde with NaOH) spectrophotometrically at 331 nm. One unit of enzyme activity was defined as the amount catalysing the formation of 1 nmol *p*-hydroxyphenyl pyruvate at 37 °C under the assay conditions.

Effect of pH. The pH optimum of the TAT was determined in 0.125 M phosphate buffer pH 6–8. The assay mixtures containing 0.61 mM-L-tyrosine, 10 mM- α -ketoglutarate, 0.05 mM-PLP (final concentrations) and 0.1 ml enzyme solution were incubated at 37 °C for 30 min. The reaction was stopped by adding 0.07 ml of 10 M-NaOH and mixing. The mixtures were kept at 37 °C for another 30 min before reading their absorbances at 331 nm.

Substrate specificity. Amino acids – L-tyrosine, DL-tyrosine, tryptophan, phenylalanine, serine and glycine were tested for their ability to act as amino donors for TAT, while pyruvate and oxaloacetate were tested as amino acceptors. In each case, the reaction mixture (0.93 ml final volume) consisted of 0.61 mM of donor amino acid, 10 mM acceptor, 0.05 mM-PLP and 0.1 ml enzyme solution. Enzyme activities were determined by the standard method.

Effect of substrate concentration. Enzyme activities were determined by varying the concentrations of either the amino donor while keeping the amino acceptor constant, or vice versa.

Molecular weight determination. The molecular weight of TAT from *T. viride* was determined by the method of Whitaker (1963) using myoglobin (mol. wt 17800); cytochrome *c*, (13000); bovine serum albumin, (67000); bovine γ -globulin (160000) as marker proteins. Blue dextran 2000 was used to determine the exclusion volume.

RESULTS AND DISCUSSION

Experiments on the inducibility of tyrosine aminotransferase in *T. viride* (50 ml cultures) showed that the enzyme was inducible by both L- and DL- forms of the amino acid, and that L-tyrosine was the better inducer of the two amino acids (Fig. 1). With L-tyrosine, the culture medium contained about 12 units of TAT activity ml^{–1} after 10 d of growth, while with DL-tyrosine, the activity was only 1.5 units ml^{–1}. The basal level of TAT in the glucose medium was not measurable.

The L-tyrosine induced enzyme was partially purified by acetone precipitation and gel filtration, the elution profile of which is shown in Fig. 2. The enzyme was eluted in one major peak and a preceding shoulder. This shoulder may be due to the presence of the isoenzyme L-aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.1) which is known to be capable of transaminating tyrosine (Ohisalo & Pispá, 1976) and which has been reported to occur in *T. viride* (Eze & Echetebe, 1980). Alternatively, it could be due to the presence of one of the multiple forms of the enzyme as found in higher organisms (Hargrove *et al.*, 1980; Ohisalo *et al.*, 1977; Iwasaki *et al.*, 1973).

When the enzyme was assayed in 0.125 M-potassium phosphate buffer in the pH range 6–8 it showed optimal activity at pH 7.6. Similar pH optima have been reported for the enzyme from higher eukaryotes: pH 7.7 for dog liver (Canellakis & Cohen, 1956); 7.5–7.6 for rat liver (Granner & Tomkins, 1970).

Several amino acids (Table 1), pyruvate and oxaloacetate were used to study the substrate specificity of the enzyme. Neither pyruvate nor oxaloacetate could substitute for α -ketoglutarate as the amino group acceptor and none of the amino acids tested could serve as the amino group donor in the reaction. It appears that in *T. viride*, as in the livers of dog (Canellakis & Cohen, 1956), rat (Kenny, 1959) and frog (Ohisalo *et al.*, 1977), α -ketoglutarate and L-tyrosine are required specifically by this enzyme.

Fig. 1. Induction of extracellular tyrosine aminotransferase in *T. viride*. The fungus was grown in 50 ml Fries' medium (in 250 ml conical flasks inoculated with two loopfuls of 7 d culture per flask) containing L-tyrosine, DL-tyrosine (as main sources of carbon and nitrogen) or glucose as control (each at $6 \mu\text{g ml}^{-1}$). Cultures were harvested every 24 h. Growth was measured by determining the dry weight of the mycelium in L-Tyr (Δ), DL-Tyr ($\blacktriangle$) and glucose ($\bullet$). Enzyme activity (expressed per 50 ml of culture) was assayed as described in Methods in medium containing L-Tyr (∇), DL-Tyr ($\blacktriangle$) or glucose ($\circ$).

Fig. 2. Gel filtration of *T. viride* tyrosine aminotransferase on a Sephadex G-150 column (2.5×50 cm) equilibrated with buffer I and eluted with buffer II. Fractions (6 ml) were collected. Protein content ($\bullet$) and TAT activity ($\circ$) was determined as described in Methods. Active fractions (indicated by the bar) were pooled, dialysed against buffer III, mixed with bovine serum albumin in glycerol and used as the partially purified enzyme.

Table 1. Substrate specificity of *Trichoderma viride* tyrosine aminotransferase

Substrate	Activity (% of control)
Amino donors (0.61 mM)	
L-Tyr (control)	100
DL-Tyr	13
L-Phe	0
L-Ser	0
L-Trp	0
L-Gly	0
Amino acceptors (10 mM)	
α -Ketoglutarate (control)	100
Pyruvate	0
Oxaloacetate	0

The apparent Michaelis constants of this enzyme were 0.5 mM and 0.25 mM for L-tyrosine and α -ketoglutarate, respectively. These figures differ from those reported for dog liver (Cannellakis & Cohen, 1956) and rat liver (Kenny, 1959). The differences may be due to the source and degrees of purification.

A molecular weight of 110000 was found for the fungal enzyme although authors have reported that from one to three forms of the enzyme with different molecular weights occur in higher animals (Ohisalo *et al.*, 1977; Valeriote *et al.*, 1969; Hargrove *et al.*, 1980). It is possible that our enzyme may break into these forms if subjected to similar analysis or it may appear as one of form A enzymes as reported by Rudman & Meister (1953) for *Escherichia coli*.

REFERENCES

- AINSWORTH, G. C. (1971). *Ainsworth and Bisby's Dictionary of the Fungi*, 6th edn. Kew, Surrey: Commonwealth Mycological Institute.
- BEKKER, Z. E. (1963). *Fiziologiya Gribov i ich prakticheskoye Ispolzovaniye*, pp. 72–73. Moscow: Moscow University Press.
- CANNELLAKIS, Z. N. & COHEN, P. P. (1956). Purification studies of tyrosine- α -ketoglutaric acid transaminase. *Journal of Biological Chemistry* **222**, 53–62.
- CLEMENTS, F. E. & SHEAR, C. L. (1973). *The Genera of Fungi*. New York: Hafner Publishing Co.
- DIAMONDSTONE, T. T. (1966). Assay of tyrosine transaminase activity by conversion of *p*-hydroxyphenyl pyruvate to *p*-hydroxybenzaldehyde. *Analytical Biochemistry* **16**, 395–401.
- EZE, L. C. & ECHETEBU, C. O. (1980). Some properties of aspartate and alanine aminotransferases from *Trichoderma viride*. *Journal of General Microbiology* **120**, 523–527.
- FRIES, L. (1953). Factors promoting growth of *Coprinus fimetarius* (L) under high temperature conditions. *Physiologia plantarum* **6**, 551–563.
- GRANNER D. K. & TOMKINS, G. M. (1970). Tyrosine aminotransferase (rat liver). *Methods in Enzymology* **17**, 633.
- HARGROVE, J., DIESTERHAFT, M., NOGUCHI, T. & GRANNER, D. K. (1980). Identification of native tyrosine aminotransferase and explanation for the multiple forms. *Journal of Biological Chemistry* **255**, (1) 71–78.
- IWASAKI, Y., LAMAN, C., DANENBERG, K. & PITOT, H. C. (1973). Studies on the induction and repression of enzymes in rat liver: characterization and metabolic regulation of multiple forms of tyrosine aminotransferase. *European Journal of Biochemistry* **34**, 347–357.
- KENNY, F. T. (1959). Properties of partially purified tyrosine- α -ketoglutarate transaminase from rat liver. *Journal of Biological Chemistry* **234**, 2707–2712.
- KENNY, F. I. (1962). Induction of tyrosine- α -ketoglutarate transaminase in rat liver. II. Enzyme purification and preparation of antitransaminase. *Journal of Biological Chemistry* **237**, 1605–1609.
- LIN, E. C. C. & KNOX, W. E. (1958). Specificity of the adaptive response of tyrosine- α -ketoglutarate transaminase in rat. *Journal of Biological Chemistry* **233**, 1186–1189.
- OHISALO, J. J. & PISPA, J. P. (1976). Heterogeneity of hepatic tyrosine-aminotransferase: separation of the multiple forms from rat and frog by isoelectric focusing and hydroxylapatite column chromatography and their partial characterization. *Acta chemica Scandinavica* **B30**, 491–500.
- OHISALO, J. J., ANDERSSON, S. M. & PISPA, J. P. (1977). Partial purification and properties of frog liver tyrosine aminotransferase. *Biochemical Journal* **163**, 411–417.
- RUDMAN, D. & MEISTER, A. (1953). Transamination in *Escherichia coli*. *Journal of Biological Chemistry* **200**, 591–604.
- VALERIOTE, F. A., AURICCHIO, F., TOMKINS, G. M. & RILEY, D. (1969). Purification and properties of rat liver tyrosine aminotransferase. *Journal of Biological Chemistry* **244**, (13) 3618–3624.
- WHITAKER, J. R. (1963). Determination of molecular weights of proteins by gel filtration on Sephadex. *Analytical Chemistry* **35**, 1950–1953.