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# PARTIAL PURIFICATION AND SOME PROPERTIES OF TRYPTOPHAN DECARBOXYLASE FROM *PHALARIS TUBEROSA*

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**Key Word Index**—*Phalaris tuberosa;* Gramineae; tryptophan decarboxylase; inhibition by indoles; alkaloid biosynthesis.

**Abstract**—A pyridoxal phosphate-dependent tryptophan decarboxylase has been purified 20-fold from seedlings of *Phalaris tuberosa*. The enzyme activity of the seedlings reached a maximum after 4 days. The enzyme activity is maximal at pH 7·6 and could be demonstrated either by the production of $^{14}$C-tryptamine from methylene $^{14}$C-L-tryptophan or of $^{14}CO_2$ from carboxyl-$^{14}$C-L-tryptophan. D-tryptophan was not a substrate but 5-hydroxy- $^{14}$C-tryptophan was. The decarboxylation was inhibited at concentrations of 0·1 mM by a number of indole derivatives including *N,N*-dimethyltryptamine, one of the two major alkaloids produced by the plant. The second alkaloid, 5-methoxy-*N,N*-dimethyltryptamine did not inhibit at this concentration. A possible role of this enzyme in alkaloid biosynthesis is discussed.

## INTRODUCTION

THE FORMATION of tryptamine from tryptophan has been demonstrated in cucumber seedlings[1] but the significance of the reaction is uncertain as the role of tryptamine in plant metabolism has not been defined. Although it has been suggested[2] that it may be involved in the synthesis of indole acetic acid, it appears that other plants synthesise indole acetic acid via indole pyruvic acid.[3–5] Tryptamine, its simple derivatives, and the indole alkaloids which all contain a tryptamine skeleton are widespread in plants. Further, the incorporation of exogenous tryptophan and tryptamine into several indole alkaloids suggests that the decarboxylation of tryptophan with the formation of tryptamine is the first step in the synthesis of these alkaloids. However, tryptophan decarboxylase has not been studied in any plant producing alkaloids although such plants might be expected to have high levels of the enzyme. A suitable plant is *Phalaris tuberosa* which is an important pasture grass in Australia. It produces two major alkaloids, *N,N*-dimethyltryptamine and 5-methoxy-*N,N*-dimethyltryptamine whose biosynthesis has been studied by classical feeding experiments and shown[6] to involve both tryptophan and tryptamine. The production of $^{14}CO_2$ from carboxyl-$^{14}$C-tryptophan has already been demonstrated in cell-free extracts of *P. tuberosa* and briefly reported.[7]

## RESULTS AND DISCUSSION

The demonstration of tryptophan decarboxylase in *P. tuberosa* is significant as this enzyme has not been studied in relation to indole alkaloid synthesis. The dependence of enzyme activity on the stage of seedling development is shown in Fig. 1. Enzyme activity

rose sharply during the 4th day after planting and after reaching a peak at 4 days began to decrease. After 19 days only 1% of the maximum activity remained.

The decarboxylase activity of *P. tuberosa* was measured both by the production of $^{14}$C-tryptamine from methylene-$^{14}$C-L-tryptophan and of $^{14}CO_2$ from carboxyl-$^{14}$C-DL-tryptophan. However, it was not until the decarboxylase had been purified 20-fold that there was agreement between the amount of product formed by each assay (Table 1). This agreement could have been due to the removal of contaminating enzymes during the purification.

Ammonium sulphate fractionation resulted in a 87% loss of the total activity. However, the activity was restored to 65% of that of the original after DEAE cellulose chromatography. A loss of activity (25%) has also been reported in ammonium sulphate fractionation of tryptophan decarboxylase from cucumber seedlings.[1]

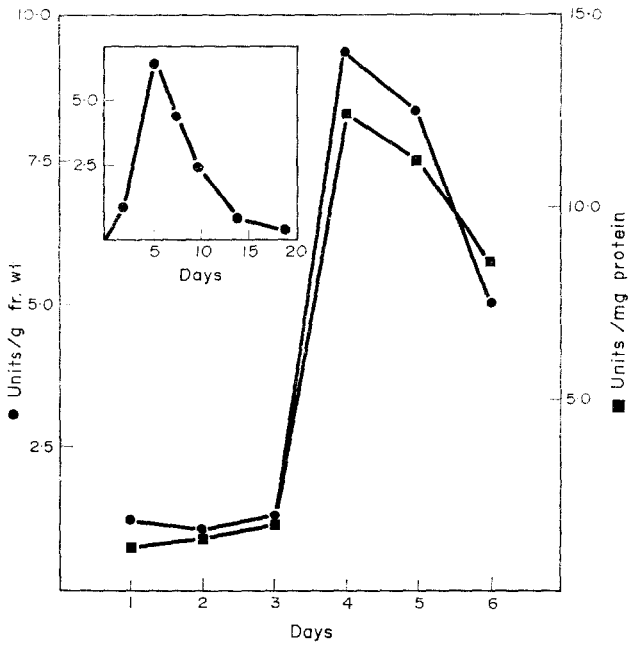


FIG. 1. CHANGE IN ENZYME ACTIVITY (nmol TRYPTAMINE FORMED IN 0·5 ml CRUDE ENZYME PREPARATION) WITH DEVELOPMENT OF SEEDLINGS.

The partially purified enzyme, like other amino acid decarboxylases[8] showed an absolute requirement for pyridoxal phosphate. Only 5% of the activity was observed in the absence of pyridoxal phosphate. The activity had a pH optimum of 7·6 and a $K_m$ of 0·2 mM for tryptophan at a concentration of 5 nmol of pyridoxal phosphate. Enzyme activity was

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TABLE 1. PARTIAL PURIFICATION OF TRYPTOPHAN DECARBOXYLASE

| | Assay | 105,000 *g* dialysed supernatant | 40–50% $(NH_4)_2SO_4$ fraction | DEAE-cellulose eluate |
|---|---|---|---|---|
| Volume (ml) | | 216 | 10 | 24 |
| Activity* | Tryptamine | 4·3 | 12·2 | 25·0 |
| | $CO_2$ | 6·1 | 22·8 | 25·5 |
| Protein (mg/ml) | | 1·6 | 1·70 | 0·50 |
| Specific activity† | Tryptamine | 5·5 | 14·3 | 100 |
| Recovery (total units) | | 1860 | 244 | 1200 |
| Recovery (%) | Tryptamine | 100 | 13 | 65 |

* A unit of activity is defined as *p*mol of product/0·5 ml enzyme/min.
† Units/mg protein.

linear with temperature at least in the range 20–40° and linear with time over at least 4 hr and linear with protein concentration for at least three times the amount of protein used in the standard assay. The specificity of the enzyme was studied using both D-tryptophan and 5-hydroxytryptophan. No tryptamine was produced from D-tryptophan, but 5-hydroxytryptophan was readily decarboxylated to 5-hydroxytryptamine. It was necessary to add 5-hydroxytryptamine to the incubation mixture to prevent the decomposition or other metabolism of the small quantities of $^{14}$C-5-hydroxytryptamine formed by the decarboxylation. In the presence of 10 nmol of 5-hydroxytryptamine, the relative activity of the decarboxylase for 5-hydroxytryptophan was 71% compared with the same concentration of L-tryptophan. This activity fell to 6% in the absence of added 5-hydroxytryptamine. This lability makes it difficult to compare the enzyme activity when using these two substrates. 5-hydroxytryptophan was also an inhibitor of the decarboxylation of L-tryptophan (Table 2). None of these results allows the distinction between the two possibilities that either there is one enzyme which is non-specific for the two compounds or that there are two decarboxylases, one specific for L-tryptophan and one for 5-hydroxy-L-tryptophan.

TABLE 2. INHIBITION OF TRYPTOPHAN DECARBOXYLASE*

| Inhibitor† | % Inhibition |
|---|---|
| *N,N*-dimethyltryptamine | 65 |
| 5-Methoxy-*N,N*-dimethyltryptamine | 0 |
| Tryptamine | 62 |
| 5-Hydroxytryptophan | 45 |
| 5-Methoxytryptophan | 0 |
| Indole acetic acid | 60 |
| Indole pyruvic acid | 0 |
| Indole acetaldehyde | 50 |

* Measured by $^{14}CO_2$ assay.
† Concentration of inhibitors was 1 mM except for indole acetic acid which was 0·5 mM.

Of the other inhibitors in Table 2 both *N,N*-dimethyltryptamine and indole acetic acid were shown to be competitive inhibitors. While the levels of most of these inhibitors in the

plant are unknown and probably small, the concentration of *N,N*-dimethyltryptamine is about 1 $\mu$mol/g fr. wt.[6] The inhibition by 1 mM *N,N*-dimethyltryptamine may therefore be of physiological significance as an important factor in the control of alkaloid biosynthesis in the plant. If most of the 5-methoxy-*N,N*-dimethyltryptamine in the plant is formed from *N,N*-dimethyltryptamine, then the synthesis of this compound could be controlled by the inhibition of tryptophan decarboxylase by *N,N*-dimethyltryptamine. If other pathways are also important, then the control, if any, of 5-methoxy-*N,N*-dimethyltryptamine synthesis must occur at a different step in the biosynthesis.

These preliminary results strongly indicate that the enzyme should be further studied, particularly in relation to its role in alkaloid biosynthesis.

## EXPERIMENTAL

*Plant material.* Seeds of *P. tuberosa* (var. Australian Commercial) were germinated in sand as described previously.[6] With the exception of the experiment relating enzyme activity with seedling development, seedlings were harvested 4–5 days after planting.

*Chemicals.* All radiochemicals were purchased from the Radiochemical Centre, Amersham. Fine chemicals were purchased from Sigma Chemical Co., U.S.A.

*Enzyme extraction and partial purification.* All procedures were carried out at 0–4°. Phosphate buffers at pH 7·6 containing mercaptoethanol (10 mM) were used throughout the purification. Protein was estimated by the Lowry method[9] using bovine serum albumin as a standard. Seedlings were washed with de-ionized water, dried to remove excess moisture, and weighed. The plants were homogenised by grinding with buffer (0·1 M) using a ratio (w/v) of plant material: buffer of 1:1. After straining through 2 layers of muslin, the homogenate was centrifuged at 105 000 *g* for 60 min (Beckman L2-65B ultracentrifuge). The supernatant was dialysed against the same buffer and used as the crude enzyme solution or for further purification. The 105 000 *g* supernatant from 200 g of seedlings was diluted 1:1 with $H_2O$ to give a buffer concentration of 0·05 M. The solution was brought to 40% $(NH_4)_2SO_4$ saturation by the cautious addition of powdered $(NH_4)_2SO_4$ and centrifuged (10 000 *g*; 10 min). The precipitate was discarded and the $(NH_4)_2SO_4$ concentration of the supernatant brought to 50% saturation and centrifuged as before. The pellet was dissolved in 0·05 M buffer (10 ml) and dialysed against the same buffer. The resulting solution was defined as the 40–50% $(NH_4)_2SO_4$ fraction. This was applied to a DEAE cellulose column (Whatman DE-32, 15 × 2 cm) which was equilibrated with 0·05 M buffer. After washing the column with 0·05 M buffer (80 ml) the enzyme was eluted with 0·15 M buffer (flow rate 30 ml/hr) between 50 and 75 ml.

*Incubation mixtures.* Incubation mixtures in standard assays contained in a total volume of 0·6 ml at pH 7·6, 0·5 ml enzyme solution, L-tryptophan (100 nmol including 0·05 $\mu$Ci methylene-$^{14}$C-L-tryptophan, specific activity 54·5 mCi/mmol) and pyridoxal phosphate (5 nmol). Blanks contained boiled enzyme or buffer. The samples were incubated for 3 hr at 40°, except for the dependence of enzyme activity on time.

Variations from the standard incubation mixture were in the substrate. The following substrates were also used: carboxyl-$^{14}$C-DL-tryptophan (0·1 $\mu$Ci, specific activity 31 mCi/nmol) (instead of methylene-$^{14}$C-L-tryptophan); 5-hydroxy-DL-tryptophan (200 nmol including 0·1 $\mu$Ci methylene-$^{14}$C-5-hydroxy-DL-tryptophan, specific activity 55 mCi/mmol); DL-tryptophan (200 nmol with 0·05 $\mu$Ci methylene-$^{14}$C-D-tryptophan, specific activity 18·3 mCi/mmol). The assay system for inhibition studies was the same as the standard except for the addition of inhibitors.

*Estimation of products: tryptamine.* The reaction was stopped by the addition of 3 drops of conc. $NH_3$ solution. 500 nmol of tryptamine was then added followed by anhydrous $Na_2SO_4$ (about 10 g) to completely absorb the solution. The dried reaction mixture was ground in a mortar and pestle, transferred to a small column and washed with $CHCl_3$ (10 ml collected). The $CHCl_3$ was evaporated to near dryness under $N_2$ and the residue was chromatographed quantitatively on silica gel (Merck $F_{254}$ aluminium backed precoated TLC plates) in $CHCl_3$–MeOH–conc. $NH_3$ (70:18:3). Tryptamine was located in UV light and the spots were scraped off into scintillation vials for radioactive counting as described previously.[6] Autoradiograms of randomly selected chromatograms showed that no other radioactive compounds were present near tryptamine. When 5-hydroxytryptophan was used as substrate, 5-hydroxytryptamine was similarly estimated.

$^{14}Co_2$. $CO_2$ formation was measured by the method of Leinweber and Walker.[10]

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