Tyrosine aminotransferase activity in a soluble extract of ergot fungus

The numerous ergoline alkaloids produced by the ergot fungus, *Claviceps purpurea* (Fries) Tulasne, and their several synthetic derivatives include a number of medicinally significant agents (Stoll, 1965). Medical, biological and chemical interests in these alkaloids have led to extensive use of saprophytic cultures of various strains of *C. purpurea* and *C. paspali* in many studies of the biosynthesis of the ergoline alkaloids (Stoll & Hofmann, 1965; Ramstad, 1968). Recently, Cavender & Anderson (1970) demonstrated the formation of clavine alkaloids from known precursors by a cell-free preparation of *C. purpurea*. However, few individual enzymes or enzyme reactions in claviceps have been established by direct experiments with cell-free fractions, and the relation between particular enzyme reactions and alkaloid formation remains to be elucidated.

Staba, Speaker & Schwarting (1961), using suspensions of washed mycelium of three different strains of claviceps for incubation with substrates, reported the association of alkaloid formation with aspartic: glutamic transaminase activity. We should like to report preliminary experiments with a soluble extract made from cultured mycelium of *Claviceps purpurea* which showed activity of tyrosine aminotransferase (tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5).

An *Agropyron* strain of *C. purpurea* which produced clavine alkaloids (cultured from sclerotium obtained from Dr. Matazo Abe of Tokyo University of Education, Japan) was grown in a synthetic medium (Abe, Yamano & others, 1952) in 500 ml Erlenmeyer flasks as still cultures at room temperature for 14–20 days. Seventy to 100 g (fresh wt) of mycelial tissue from 25–35 flasks of cultures (150 ml of growth medium per flask) were separated from the liquid by centrifugation, washed three times with water, and pressed dry between filter papers by hand, and weighed. This tissue was then frozen overnight, thawed at room temperature, and homogenized for 3 min at 4° in a pre-chilled Servall Omni-mixer with M/15 phosphate buffer, pH 7·3, containing 0·001 M ethylenediamine tetra-acetic acid tetrasodium salt (EDTA), using 5 ml of buffer for each g of mycelial tissue fresh wt. All subsequent steps from this point on until incubation with the substrates were carried out with pre-chilled containers at 0–4°. The homogenate was then centrifuged in a Servall RC-2 centrifuge at 1085 *g* for 30 min, and the residue discarded. The supernatant liquid was again centrifuged at 5090 *g* for 10 min and the residue again discarded. The 5090 *g* supernatant was re-centrifuged in a Beckman Spinco L-2 ultracentrifuge at 105 000 *g* for 30 min. The supernatant liquid was then dialysed against polyethylene glycol PEG 20M (Union Carbide) for 15–20 h at 4° to reduce the volume to about one-third. The resulting opaque liquid, with or without further dilution with buffer, constituted the soluble enzyme preparation which contained 2–4 mg of protein per ml (Miller, 1959).

The enzyme preparation was tested and assayed for tyrosine aminotransferase activity (Diamondstone, 1966) by determining the amount of *p*-hydroxyphenylpyruvate (pHPP) formed in the incubation mixture at 3, 6, 9, 12, 15, 20 and 30 min of incubation in a water bath at 30° with shaking at 90 strokes/min. The incubation mixture had a total volume of 4·5 ml, including 2 ml of the crude enzyme preparation, and containing (μmol) of L-tyrosine 11·25, of α-ketoglutaric acid (KG) 45, pyridoxal phosphate (PLP) 0·18, sodium diethyldithiocarbamate (DDC) 18, 2-mercaptoethanol 4·5, and M/15 phosphate buffer (pH 7·3) to make up to volume. The tyrosine was dissolved in 0·03N NaOH, DDC in water, and all the other components of the incubation mixture were each dissolved in an appropriate amount of the same buffer. The reagents were from commercial sources: L-tyrosine (Schwarz Bioresearch); KG (Nutritional Biochemical Corp.); PLP monohydrate (Mann Research Labs.); DDC sodium salt

(Fisher reagent); EDTA tetrasodium salt (British Drug Houses); 2-mercaptoethanol (Eastman); pHPP (Nutritional Biochemical Corp.).

For each incubation mixture, all the components excluding α-KG but including the enzyme preparation were incubated in the water bath shaker for 5 min before zero time when the reaction was started with the addition of α-KG. The reaction was stopped at each time interval by adding 0·3 ml of 10 N NaOH solution to the incubation mixture, with immediate shaking, and then followed by addition of 0·2 ml of the same buffer to make up to 5 ml volume. After standing at room temperature for 20 min, the mixture was centrifuged at 12 000 *g* for 10 min. This last centrifugation was found unnecessary by Diamondstone (1966) with mammalian preparations, but was essential with our

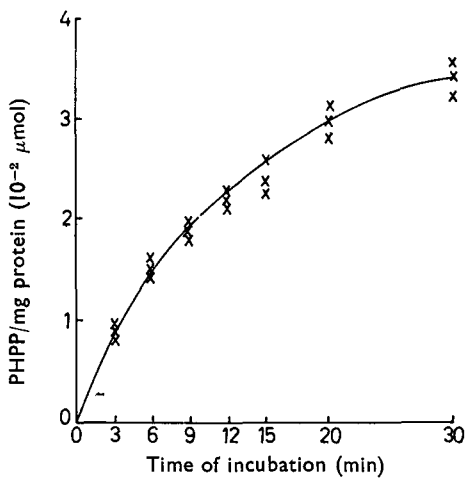

FIG. 1. Progress curve: Increase of *p*-hydroxyphenylpyruvate with time (per mg of protein of enzyme preparation). Each incubation mixture contained 2 ml of enzyme preparation, equivalent to 6·9 mg of protein, and other components as described in the text.

claviceps preparation to obtain a clear solution. Aliquots of the supernatant were then read for absorbance at 331 nm. This absorbance reading for each sample, minus the absorbance reading of a zero time control, was then converted to μmol of pHPP by referring to a standard curve made from assaying known concentrations of pHPP in the same buffer and carried through the same procedure in the presence of all the other components as the incubation mixtures but without the enzyme preparation. The zero time control sample contained the complete incubation system including the enzyme preparation, and at zero-time the 10 N NaOH solution was added to it immediately before adding the α-KG. After that, it was then carried through the same treatments of standing, centrifugation, and spectrophotometric reading as the other samples. Within the working range of concentrations of pHPP used in our experiments, the extinction coefficient was 20 530 M^{-1} (compared to 19 900 M^{-1} obtained by Diamondstone, 1966).

A progress curve, Fig. 1, shows the increase of reaction product (pHPP) with time of incubation. The specific activity (rate of pHPP formed per mg protein per unit time) determined from the slope of the initial portion of the progress curve was 0·16 μmol of pHPP formed/mg protein h^{-1}.

Vining (1970) recently reported evidence which showed that the influence of tryptophan on alkaloid formation in claviceps involved not merely the utilization of tryptophan as a precursor but had wider metabolic connections. Evidence for tryptophan: 2-oxoglutarate transamination has also been reported recently (Teuscher, 1970).

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