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SHORT COMMUNICATIONS

A tryptophan hydroxylase in cell-free extracts of malignant mouse mast cells

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CELLS of a transplantable malignant mast cell tumor, known to contain large amounts of serotonin,[1] have recently been shown to hydroxylate tryptophan and phenylalanine *in vitro* if supplemented with glucose.[2] Difficulties were encountered in obtaining a cell-free system, however, since breaking the cells results in a complete loss of hydroxylating activity. It is known that a tetrahydropteridine and ferrous ions enhance the activity of other aromatic ring hydroxylases.[3–6] When a cell-free system of the mouse mast cells was supplemented with these cofactors, it hydroxylated tryptophan and phenylalanine. The mast cell enzyme operated effectively at physiological levels of substrate.

MATERIALS AND METHODS

Mice (Balb/c × DBA/2; F_1 hybrids) bearing the transplantable tumor P815 in the ascitic form during generations 415 through 445 were used in these studies. The cells were transplanted and harvested as described previously.[2]

6,7-Dimethyl-2-amino-4-hydroxytetrahydropteridine ($DMPH_4$) was kindly donated by Drs. Gordon Guroff and Seymour Kaufman for initial studies and subsequently was obtained from California Corp. for Biochemical Research. Tetrahydrofolic acid (THF) was prepared from folic acid by reduction with hydrogen and platinum oxide.[7] DL-Tryptophan-1-^{14}C was obtained from California Corp. for Biochemical Research. The sheep liver enzyme was purified through the first ammonium sulfate step of Kaufman and Levenberg,[8] and glucose dehydrogenase was partially purified from beef liver.[9]

Enzyme activity was estimated by measurement of the rate of formation of tyrosine or 5-hydroxytryptophan (5 HTP). Incubations were carried out aerobically at 37°. Incubation mixtures consisted of: enzyme, 5–10 mg protein; Tris·HCl, pH 7·3, 150 μmoles; NADP, 2 μmoles; ferrous ammonium sulfate, 0·2 μmole; glucose, 150 μmoles; cofactor ($DMPH_4$ or THF in 0·1 M 2-mercaptoethanol), 0·4 μmole; the sheep liver enzyme, 0·2 ml; glucose dehydrogenase, 200 units; final volume 2·0 ml. After preincubation for 10 min the reaction was started by addition of cofactor and 0·2 μmole substrate. At various times, aliquots of the incubation mixture were acidified and assayed for 5-hydroxyindole or tyrosine content as described previously.[2] Protein determinations were done by a modification[10] of the phenol reagent method.

RESULTS

Disruption of the mast cells by a variety of methods resulted in a complete loss of hydroxylating activity, which could be largely restored by addition of Fe^{++} and $DMPH_4$. For routine studies cells were suspended in water and broken by means of a French press. After centrifugation of the broken cell extract at 100,000 *g* for 60 min, all the hydroxylating activity was in the supernatant fraction.

TABLE 1. PURIFICATION OF TRYPTOPHAN HYDROXYLASE*

	Specific activity (mμmole/mg protein/hr)	Total activity (units)
Cell suspension	3·3	7,050
Broken cells	3·4	5,500
Ammonium sulfate, 20%–40%	5·7	2,260

* The activity was determined as described in the text, with tryptophan as a substrate. A unit of enzyme is defined as the amount of enzyme that will catalyze the formation of 1 mμmole 5HTP/hr.

Most of the enzyme activity was in the protein fraction precipitating between 20% and 40% saturation with ammonium sulfate. This fraction was used in subsequent studies after dialysis against 100 volumes of 0·01 M Tris·HCl, pH 7·3, for 2 hr. It could be stored at −20° for up to 2 weeks without loss of activity. The results of a typical purification procedure are summarized in Table 1.

The hydroxylating activity of this enzyme was largely dependent upon the addition of Fe^{++} and cofactor to the incubation (Table 2). The system was also stimulated by sheep liver enzyme and a

TABLE 2. REQUIREMENTS FOR THE CELL-FREE HYDROXYLATION OF TRYPTOPHAN*

	Specific activity
Complete system	9·7
−Cofactor	0·0
$-Fe^{++}$	1·2
−Sheep enzyme	6·2
−NADPH	5·1
−Substrate	2·3
Boiled enzyme	0·0

* The enzyme activity was determined as described in the text. The cofactor used was THF. The specific activity is expressed as mμmoles of product (5HTP) formed/mg protein/hr. The results given are of a typical experiment carried out with duplicate incubations.

NADPH-generating system. Since large amounts of the reduced cofactor were used, a complete dependency was not anticipated. With a cofactor concentration of 10^{-4} M, the hydroxylating activity was 2–3 times greater with $DMPH_4$ than with THF. A broad pH optimum was found; activities varied little between pH 6·5 and pH 7·5; 5HTP was formed at a constant rate for up to 90 min (Fig. 1). Phenylalanine was also hydroxylated at a rate similar to that of tryptophan by the

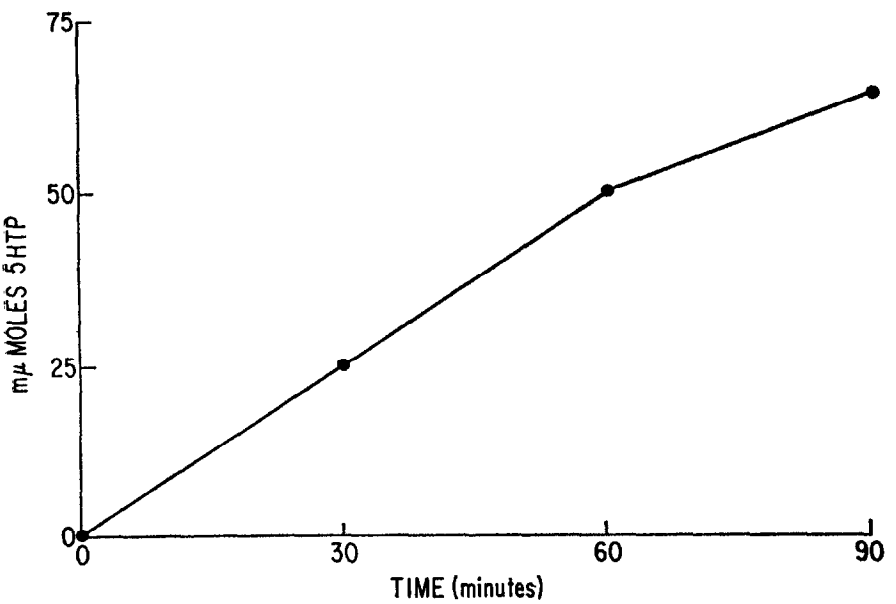

FIG. 1. Rate of 5HTP formation. The reaction was measured in the typical assay system. The enzyme source was the 20%–40% ammonium sulfate fraction (8 mg protein)

enzyme preparation, and this activity had similar requriements. The identity of the product of tryptophan hydroxylation as 5HTP was established as described previously[2] by high-voltage paper electrophoresis of the product formed after incubation with ^{14}C substrate (0·15 μmole of 6·5 $\mu c/\mu$mole).

Since phenylalanine hydroxylase of rat liver also hydroxylates tryptophan[11] when this substrate is present in high concentrations, a rat liver supernatant fraction was examined for hydroxylating activity under the conditions described for the mast cell enzyme. Although phenylalanine was hydroxylated rapidly, tryptophan hydroxylation was not detected. Therefore, although the mast cell hydroxylating enzyme is similar to phenylalanine hydroxylase in cofactor requirement, the two enzymes can be distinguished by the affinity of tryptophan. The K_m values for L-tryptophan are $6·8 \times 10^{-3}$ M for the rat liver enzyme[11] and about 10^{-5} M for the mast cell enzyme.

It has recently been reported [12] that 2-propyl-3,4-dihydroxyphenylacetamide (H2254 of A. B. Hassle & Co.) and α-methyl-3,4-dihydroxyphenylalanine (methyldopa) are good inhibitors of rat liver phenylalanine hydroxylase. These compounds were also found to be inhibitors of the mast cell enzyme; at a concentration of 5×10^{-4} M, H2554 and methyldopa inhibit the reaction 90% and 50% respectively.

DISCUSSION

Little is known of the enzyme that catalyzes the hydroxylation of tryptophan in normal mammalian tissues. The work of Renson *et al.*[11] suggests that, although phenylalanine hydroxylase of liver is capable of hydroxylating tryptophan, this enzyme is not responsible for the majority of the 5-hydroxyindoles produced *in vivo*. Grahame-Smith[13] has recently reported that brain tissue contains a very low level of tryptophan-hydroxylating activity. It is significant, therefore, that a very active tryptophan hydroxylase has been isolated from a tissue containing large amounts of 5-hydroxyindoles. This enzyme operates effectively at physiological substrate concentrations and demonstrates a complete requirement for a reduced pteridine and ferrous ion in broken cell extracts. Since intact cells hydroxylate tryptophan without the addition of these cofactors it would appear that either the cofactors are localized at a specific site within the intact cell of that some stabilizing factor is destroyed. The lack of complete substrate dependence in the partially purified fractions suggests that some proteolysis is occurring during the incubation period and releasing substrate.

While no correlation between this enzyme and the "normal" mammalian enzyme is possible at present, perhaps the properties of the mast cell enzyme will be similar enough to the normal enzyme to aid in its isolation and characterization.

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