Tryptophan Hydroxylase

PURIFICATION AND SOME PROPERTIES OF THE ENZYME FROM RABBIT HINDBRAIN

JEFFREY H. TONG* AND SEYMOUR KAUFMAN
From the Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, Maryland 20014

SUMMARY

Tryptophan hydroxylase from rabbit hindbrain has been extensively purified. It is estimated that the enzyme is between 85 and 90% pure and has a molecular weight of 230,000. Sodium dodecyl sulfate gel electrophoresis shows that the enzyme is composed of two subunits very close in molecular weight (57,500 and 60,900). The substrate specificity and the reaction stoichiometry catalyzed by the enzyme in the presence of 6,7-dimethyltetrahydropterin, 6-methyltetrahydropterin, and tetrahydrobiopterin have been determined. The effect of some natural occurring phospholipids on the purified enzyme was investigated.

The conversion of L-tryptophan to 5-hydroxy-L-tryptophan is the first step in the hydroxyindole pathway of L-tryptophan metabolism. Since the first evidence for a specific tryptophan hydroxylase in mammalian brain (1), the enzyme has been extensively studied in vitro with extracts from some mammalian brain tissues (2-9), and is generally accepted as the rate limiting enzyme in the biosynthesis of the putative neurotransmitter, serotonin (10, 11).

With the development of a rapid and sensitive fluorometric assay for tryptophan hydroxylase, the partial purification of the enzyme from rabbit hindbrain has been described. The enzyme was shown to catalyze the conversion of L-tryptophan to 5-hydroxy-L-tryptophan according to Equation 1 (13). The present study was undertaken to purify further the enzyme from this source and to extend the findings on the properties of the enzyme previously reported by Friedman et al. (13).

\[ \text{L-Tryptophan} + \text{tetrahydrobiopterin} + \text{O}_2 \rightarrow \]
\[ \text{5-hydroxy-L-tryptophan} + \text{quinonoid dihydrobiopterin} + \text{H}_2\text{O} \]  \hspace{1cm} (1)

EXPERIMENTAL PROCEDURES

Material

Sepharose 4B and 6B were purchased from Pharmacia. Polyethylene glycol 6000 (MW 6,000 to 7,500) was obtained from J. T.

* Recipient of Canadian Medical Research Council Fellowship. Present address: Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada.

Baker Chemical Co. L-[U-14C]phenylalanine (specific activity 46.0 mCi/mmol), and L-[3,5-3H]tyrosine (specific activity 48.2 Ci/mmol) were purchased from New England Nuclear. DEAE-cellulose (DE52) was obtained from Whatman Biochemicals Ltd., Maidstone, Kent, England. 6-Fluoro-DL-tryptophan was obtained from Sigma, 3-iodo-L-tyrosine from Aldrich and Ampholine pH 3.5 to 10 from LKB Instruments Inc., Rockville, Md. Other materials were obtained from previously described sources (13).

Methods

Assay Methods and Definition of Unit—Tryptophan hydroxylase activity was usually assayed by the fluorometric method as described by Friedman et al. (13). Since the \( K_a \) of the hydroxylase for tryptophan is lower in the presence of 6-methyltetrahydropterin (6MPHa) than it is in the presence of DMFH (13), the former pterin was used in place of DMFH. The complete reaction mixture contained the following components (in micromoles) in a final volume of 0.37 ml: Tris-acetate pH 7.5, 50; L-tryptophan, 0.2; 6MPHa, 0.1; TPNH, 0.05; glucose 6-phosphate, 0.5; N-methyl-D-glucose 6-phosphate dehydrogenase and dihydropteridine reductase in excess, and an appropriate amount of hydroxylase, usually 0.04 ml to 0.2 ml. After a 30 min incubation in a shaking water bath at 37°, the reaction was stopped by the addition of 0.03 ml of 60% perchloric acid and the mixture was centrifuged. Exactly 0.2 ml of the supernatant fraction was added to 0.06 ml of concentrated hydrochloric acid. The amount of product, 5-hydroxytryptophan, was measured fluorometrically with an Amino-Bowman spectrophotofluorometer (excitation 295 nm, emission 538 nm). Boiled enzyme served as the control. One unit of activity is defined as the amount of enzyme necessary to catalyze the formation of 1 nmol of product per min. Specific activity was then defined as units of activity per mg of protein. Protein concentrations were measured by the method of Lowry et al. (14), with bovine serum albumin as the standard.

The hydroxylation of L-phenylalanine or L-tyrosine was carried out under identical assay conditions for tryptophan hydroxylase except that L-phenylalanine containing 0.2 \( \mu \)Ci of L-[U-14C]phenylalanine or L-tyrosine containing 0.45 \( \mu \)Ci of 3,5-dinitro-L-tyrosine was substituted for L-tryptophan. The hydroxylation of L-phenylalanine was measured by the conversion of L-[U-14C]phenylalanine to L-[U-14C]tyrosine. Tyrosine and phenylalanine were separated by thin layer chromatography with the following solvent system: chloroform-methanol-ammonium hydroxide-water (58:32:8:2). The hydroxylation of tyrosine was measured by the tritium release method (15) as employed in this laboratory (16).

The tryptophan-dependent oxidation of DMFH and 6MPH was measured indirectly by following the oxidation of TPNH at 340 nm in the presence of sheep liver dihydropteridine reductase (17). For this assay, the TPNH-regenerating system (glucose 6-
phosphate and glucose-6-phosphate dehydrogenase) was omitted. Electrophoresis—Analytical polyacrylamide disc electrophoresis was carried out in the cold (4°) at each purification step using 7% polyacrylamide gel (pH 8.9) according to the method described by Davis (18) and Ornstein (19). The gels were stained for protein with Coomassie blue and for glycoprotein with the periodic acid-Schiff reagent stain according to the methods of Kapitany and Zebrowski (20), Segrest and Jackson (21), and Glossmann and Neville (22). In experiments to determine the purity of enzyme where quantitation of protein bands was needed, the gels were stained with Amido black 10B (Canalco, Inc., Rockville, Md.) and scanned at 280 nm as described by Davis et al. (23). To determine the position of tryptophan hydroxylase, the gels were polymerized with 0.0005% riboflavin in the place of persulfate (24). After electrophoresis, the gels were sliced into 1.5-mm sections and assayed for enzyme activity. Determination of the molecular weight of tryptophan hydroxylase by polyacrylamide gel electrophoresis was carried out according to the method of Hedrick and Smith (25). Electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate was carried out according to the method of Neville (26).

Electrofocusing—Partially purified tryptophan hydroxylase (13) (30 to 90 mg of protein) was dialyzed against 400 volumes of 1% glycine buffer to 3 liters. The dialyzed sample was centrifuged and the clear supernatant fraction was focused on a 400-ml Ampholine column (LKB Instruments Inc., Rockville, Md.) as described by Vesterberg (27).

Preparation of 6-fluoro-tryptophan-substituted Sepharose—Water was added to 100 g of Sepharose 4B (wet weight) to a volume of 500 ml, and the mixture was allowed to stand at room temperature for 24 hr. The mixture was then centrifuged and the clear supernatant fraction was focused on a 400-ml Ampholine column (LKB Instruments Inc., Rockville, Md.) as described by Vesterberg (27).

Preparation of 6-fluoro-tryptophan-substituted Sepharose—Water was added to 100 g of Sepharose 4B (wet weight) to a volume of 500 ml, and the mixture was allowed to stand at room temperature for 24 hr. The mixture was then centrifuged and the clear supernatant fraction was focused on a 400-ml Ampholine column (LKB Instruments Inc., Rockville, Md.) as described by Vesterberg (27).

Preparation of 6-fluoro-tryptophan-substituted Sepharose—Water was added to 100 g of Sepharose 4B (wet weight) to a volume of 500 ml, and the mixture was allowed to stand at room temperature for 24 hr. The mixture was then centrifuged and the clear supernatant fraction was focused on a 400-ml Ampholine column (LKB Instruments Inc., Rockville, Md.) as described by Vesterberg (27).

Preparation of 6-fluoro-tryptophan-substituted Sepharose—Water was added to 100 g of Sepharose 4B (wet weight) to a volume of 500 ml, and the mixture was allowed to stand at room temperature for 24 hr. The mixture was then centrifuged and the clear supernatant fraction was focused on a 400-ml Ampholine column (LKB Instruments Inc., Rockville, Md.) as described by Vesterberg (27).

Nickel Gradient Centrifugation—Linear gradients of sucrose from 5 to 20% in 0.05 M Tris-acetate, pH 7.5, and 2 mM dithiothreitol were prepared and used in the further purification of the enzyme.

Step 2: Adsorption and Elution from Calcium Phosphate Gel—One hundred milliliters of a calcium phosphate gel suspension (30 mg/ml) were added over a period of 20 min to 350 ml of the extract. Stirring was continued for another 15 min. The gel collected by centrifugation was resuspended in 350 ml of 0.05 M Tris-acetate, pH 7.5, containing 2 mM dithiothreitol. After 10 min of stirring and 5 min of centrifugation, the supernatant fraction was discarded and the calcium phosphate gel was resuspended with 180 ml of 0.125 M potassium phosphate, pH 6.8, 2 mM dithiothreitol. The mixture was stirred for another 10 min and centrifuged. The supernatant fraction containing the hydroxylase was saved and the gel was discarded.

Step 3: Fractionation with Polyethylene Glycol (MW 6000)—The calcium phosphate gel eluate from Step 1 was brought to pH 7.5 by dropwise addition of 1.0 M Tris. A 50% (w/v) solution of polyethylene glycol in 0.05 M Tris-acetate, pH 7.5, was added dropwise to the calcium phosphate gel eluate to a concentration of 4.5% (w/v) with stirring. Stirring was continued for an additional 15 min. The precipitate was removed by centrifugation at 27,000 × g for 15 min and discarded. To the supernatant fraction, 50% polyethylene glycol solution was added slowly to give a final concentration of 10% (w/v). Stirring was continued for an additional 30 min. The precipitate was collected by centrifugation at 27,000 × g for 15 min and discarded. The supernatant fraction containing the hydroxylase could be quick-frozen in a Dry Ice-acetone bath and stored at −80° with no loss of enzyme activity for a period of 3 weeks.

Step 4: Gel Filtration—The enzyme fraction from Step 3 (protein 5 mg/ml) was applied to a column of Sepharose 6B (2.6 × 45 cm) equilibrated with 0.05 M Tris-acetate, pH 8.5, 2 mM dithiothreitol. The same buffer was used as eluant. Gel filtration was performed as described above. The flow rate of 25 ml/hour, 0.5-ml fractions were collected. Fractions with high enzyme activity were pooled.

Step 5: DEAE-cellulose Chromatography—A column of DEAE-cellulose (2.5 × 10 cm) was equilibrated with 0.05 M Tris-acetate, pH 8.5, 2 mM dithiothreitol. Combined fractions (40 ml) from the previous step were applied to the column. The column was eluted with a stepwise sodium chloride gradient, and 5-ml fractions were collected. The enzyme was quickly frozen (acetone-Dry Ice) and stored at −80°. Little loss of enzyme activity was observed over a period of 3 weeks.

Prior to being used for the first time, the column of Sepharose was treated with 25 ml of brain extract and washed with eluting buffer. This procedure was repeated twice.
FIG. 1. Sucrose density gradient centrifugation pattern of tryptophan hydroxylase. Detailed experimental conditions were as described under “Methods.” The sample (250 μg of purified tryptophan hydroxylase in 250 μl) was layered on a 5 to 20% sucrose gradient (the solvent was 0.05 M Tris-acetate, pH 7.5, 2 mM dithiothreitol) and centrifuged for 17 hours at 32,000 rpm and 2°C in an SW 50.1 rotor. The total enzyme activity recovered was between 30 to 40% (●—●), protein in μg per fraction; (▲—▲) hydroxylase activity. SHTP, 5-hydroxytryptophan.

FIG. 2. Electrophoretic pattern of purified tryptophan hydroxylase on 7% polyacrylamide disc gel (pH 8.9). The electrode buffer was Tris-glycine, pH 8.2. To determine the position of hydroxylase activity, the gels (200 μg of purified enzyme/gel) were sliced into 1.5-mm sections. Each section was transferred to an assay tube, crushed in 0.5 ml of reaction mixture, and assayed under standard conditions (see “Methods”) except that incubation was increased to 60 min. The total activity recovered was about 10%. Control gels (25 to 50 μg of purified enzyme/gel) were stained for protein with Coomassie brilliant blue.

FIG. 3. Calibration curve for the determination of the molecular weight of rabbit hindbrain tryptophan hydroxylase on Sepharose 6B. Proteins of known molecular weight were chromatographed on the column as described in Fig. 1. The void volume was determined with blue dextran 2000. The Kav values of each protein were determined using the equation: Kav = Vp - Vv/Vt - Vv (in which Vp is the elution volume for the protein, Vt that for blue dextran 2000, and Vv the total bed volume).

FIG. 4. Calibration curve for the determination of molecular weight of rabbit hindbrain tryptophan hydroxylase by disc gel electrophoresis according to the method described by Hedrick and Smith (25). Proteins of known molecular weight were run on disc gel and the slope of a log Rp versus gel concentration plot determined. The slope values obtained from such plots were then related to their respective molecular weights. Standard proteins used were: ovalbumin (OVA); bovine serum albumin monomer (BSA1); bovine serum albumin dimer (BSA2); catalase; apoferritin.

Criteria of Purity

The purity of the enzyme preparation was established by sucrose gradient centrifugation and disc gel electrophoresis procedures. Sucrose density gradient centrifugation showed a single peak containing enzyme with constant specific activity (Fig. 1). Disc gel electrophoresis of the purified enzyme with 7% gel at pH 8.3 showed one major band which coincided with the enzyme activity (Fig. 2). A single major band was also observed with 6%, 8%, and 10% gels. Minor contaminants that were detected in disc gel electrophoresis were quantitated by scanning the gels which had been previously stained with Amido black 10B at 280 nm (23). We estimate that the enzyme purified by the present procedure is 85 to 90% pure.

We are using the word “pure” to mean freedom from protein that has no hydroxylase activity.
Molecular Weight

From Sepharose 6B gel filtration and from the polyacrylamide gel electrophoretic technique of Hedrick and Smith (25) (Figs. 3 and 4), a molecular weight of 240,000 was obtained. From sucrose density centrifugation (30) experiments of the essentially pure hydroxylase using ovalbumin (\(\eta_p, \omega, 3.55\)), bovine serum albumin (\(\eta_p, \omega, 4.55\)) and catalase (\(\eta_p, \omega, 11.30\)) as standards, the sedimentation constant (\(\eta_p, \omega\)) of the hydroxylase was calculated to be 10.86. This value, together with the diffusion constant calculated from the Stokes radius (obtained from gel filtration), yielded a molecular weight for tryptophan hydroxylase of 220,000 (assuming a partial specific volume of 0.72 cm\(^3\) per g). From the three independent methods used to determine the molecular weight of rabbit hindbrain hydroxylase, an average of 230,000 was obtained.

Subunit Structure

Rat liver phenylalanine hydroxylase has been shown to consist of two isozymes, each of which is capable of existing as a monomer (MW 55,000), a dimer (MW 110,000), and a tetramer (MW 210,000) (24). In light of these findings, it was of interest to determine whether the purified rabbit hindbrain tryptophan hydroxylase (MW 230,000) is composed of subunits.

The electrophoresis pattern of the purified enzyme on sodium dodecyl sulfate polyacrylamide gel showed two major bands (MW 57,500 and MW 60,900) of approximately equal intensity (Fig. 5). Thus, it is likely that the active tryptophan hydroxylase is a tetramer composed of two subunits very close in molecular weight. So far, we have not been able to demonstrate the existence of a dimeric form for this enzyme. In addition, there is no evidence for the existence of isozymes.

It should be noted that we have not ruled out the possibility that the enzyme is composed of only a single type of subunit, and the two subunits that we have observed on electrophoresis in sodium dodecyl sulfate gels are the result of partial proteolysis of the subunits during the isolation procedure.

Glycoprotein

The purified tryptophan hydroxylase was stained for glycoprotein with three periodic acid-Schiff reagent staining methods (20-22) after disc gel electrophoresis. No carbohydrate was detected. We conclude that the amount of bound carbohydrate in purified tryptophan hydroxylase is less than 1%.

Electrofocusing and 6-Fluoro-tryptophan-substituted Sepharose 4B

In electrofocusing experiments with the 10-fold purified enzyme (15) over a pH range of 3.5 to 10, the approximate isoelectric point of tryptophan hydroxylase was found to be between pH 5.2 to 5.7. Since there was extensive loss of total enzyme activity during electrofocusing, the technique was not used as a purification step.

Tryptophan hydroxylase was found to bind tightly to Sepharose 4B to which 6-fluoro-tryptophan, an inhibitor of the enzyme (31), was attached through its amino group (see “Methods”). A column of 6-fluoro-tryptophan-substituted Sepharose 4B was equilibrated with 0.05 M Tris-acetate, pH 7.5, and the sample was applied to the column in the same buffer. After washing the column with 5 to 10 bed volumes of the above buffer, the enzyme was eluted from the gel with 25 mM Tris base. The eluate containing the enzyme was adjusted to pH 7.5 immediately with 50 mM acetic acid. The total recovery of enzyme activity was between 75 to 85%. A 6- to 8-fold purification of the enzyme from the crude extract was achieved by this method. Even though the 6-fluoro-tryptophan-substituted Sepharose 4B did not give any further purification when combined with other steps in the purification scheme and was not used as a purification step, it is still a useful gel for concentrating the enzyme and for desalting purposes.

Effect of Phospholipids

It has been observed that lyssolecithin and several other naturally occurring phospholipids stimulate rat liver phenylalanine hydroxylase (32). More recently, phosphatidylserine was found to stimulate bovine caudate tyrosine hydroxylase (33). Unlike the stimulation observed with these two enzymes, however, we have been unable to demonstrate any effect of phospholipids on tryptophan hydroxylase from rabbit hindbrain. The following compounds at 0.2 mM or 0.4 mM did not show any stimulation of tryptophan hydroxylase when 6MPH\(_4\) or tetrahydrobiopterin was used as cofactor: lecithin, lyssolecithin, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine, lysophosphatidylserine, and sphingomyelin.

Stoichiometry with Model Tetrahydropterins

The reaction stoichiometry for the rat liver phenylalanine hydroxylase and bovine adrenal tyrosine hydroxylase have been previously determined in this laboratory. It was shown that the ratio of DMPH\(_4\), 6MPH\(_4\), and tetrahydrobiopterin oxidized to tyrosine formed by phenylalanine hydroxylase was about one (34, 35). For tyrosine hydroxylase, the ratio of tetrahydropterin oxidized to 3,4-dihydroxyphenylalanine formed was 1.0 for 6MPH\(_4\), and for tetrahydrobiopterin, and 2 for DMPH\(_4\) (16). Recently Friedman et al. (13) have determined the stoichiometry for the rabbit hindbrain tryptophan hydroxylase and demonstrated that for each mol of 5-hydroxy-L-tryptophan formed, 1 mol of tetrahydrobiopterin is oxidized.

Although most of the studies published on tryptophan hydroxylase utilized DMPH\(_4\) or 6MPH\(_4\) as the tetrahydropterin cofactor, the molar ratio of these two cofactors oxidized to 5-hydroxy-L-tryptophan formed has never been demonstrated. It was therefore important to determine the reaction stoichiometry with these two cofactors. The results of these experiments (Table II) show that the molar ratio of DMPH\(_4\), or 6MPH\(_4\), oxidized to 5-hydroxy-L-tryptophan formed is very nearly 1:1.
Stoichiometry with DMHP and 6MPH

The reaction mixture in a final volume of 1 ml contained the following components (in micromoles): Tris-acetate, pH 7.5, 50; N-methyl-N-3-hydroxyphenylalanine, 0.7; L-tryptophan, 0.85; TPNH, 0.1, and catalase and dihydropteridine reductase (in excess). The hydroxylase was freed of dithiothreitol by passing it through a Sephadex G-25 or a 6-fluoro-tryptophan-substituted Sepharose 6B column (see text). The reaction was started by the addition of 0.15 μmol of 6MPH or 0.16 μmol of DMHP. The amount of 6MPH, or DMHP, consumed in 30 min at 37° was measured indirectly by following the oxidation of TPNH at 340 nm (17). At the end of the reaction, 0.03 ml of 60% perchloric acid was added to 0.37-ml aliquots of the reaction mixture, and the amount of 6MPH or DMPH, consumed in 30 min at 37° was determined fluorometrically as described under “Methods.” A complete reaction mixture in which the L-tryptophan had been omitted served as the control. In Experiments 1, 2, 4, 5, and 6, 0.7 mg of purified hydroxylase was used and 0.4 mg was used in Experiment 3.

Substrate Specificity

It has been reported that in the presence of DMHP, the partially purified tryptophan hydroxylase from the beef pineal gland can catalyze the hydroxylation of L-phenylalanine and L-tryptophan at equivalent rates, whereas the ratio of the rate of phenylalanine hydroxylation to that of tryptophan hydroxylation in the 30,000 X g supernatant fraction from rat brainstem is less than 1:100 (12). From the studies with phenylalanine hydroxylase and tyrosine hydroxylase in this laboratory, it has been established that the catalytic properties of these two aromatic amino acid hydroxylases vary depending on the pterin cofactor used (36). We examined the phenylalanine and tyrosine hydroxylating activity (see “Methods") of our highly purified tryptophan hydroxylase preparation from rabbit hindbrain with different pterin cofactors. The results of these experiments are summarized in Table III. We found that the rate of hydroxylation of L phenylalanine was 28.8% (DMHP), 44.3% (6MPH), and 85.9% (tetrahydrobiopterin) of that observed with L-tryptophan.

That p-tyrosine was the hydroxylation product of phenylalanine to tyrosine is due to a contamination with tyrosine hydroxylase (Table III). The rate of hydroxylation of tyrosine was approximately 5% (DMHP), 3% (6MPH) and 1% (tetrahydrobiopterin) that observed with tryptophan. Also, experiments with 3-I-tyrosine, a specific inhibitor for tyrosine hydroxylase, at concentrations of 5 X 10⁻⁴ to 5 X 10⁻³ M (K, value, 5 X 10⁻⁷ M, for bovine caudate tyrosine hydroxylase) did not show significant inhibition of the conversion of tryptophan to 5-hydroxy-tryptophan, or phenylalanine to tyrosine.

Values for the Kₘ of the purified enzyme toward L-phenylalanine and L-tryptophan using tetrahydrobiopterin (0.15 mM) as cofactor were determined from conventional Lineweaver-Burk plots. The Kₘ values obtained were 280 μM for L-phenylalanine and 32 μM for L-tryptophan (Figs. 6 and 7). Both L-tryptophan and L-phenylalanine showed substrate inhibition with tetrahydrobiopterin as the cofactor. In agreement with our previous finding, substrate inhibition was observed when L-tryptophan concentration was greater than 0.2 mM (13). Substrate inhibition was observed with L-phenylalanine at concentrations greater than 0.5 mM (Fig. 6).

T. Lloyd and S. Kaufman, unpublished observations.
have previously shown that the substrate specificity of pterin-substrate for tryptophan hydroxylase from pineal glands (12). We and tetrahydrobiopterin but is close to 2.0 with DMPH (16). Enzyme, the ratio of tetrahydropterin oxidized to hydroxylated product is higher than it does adrenal tyrosine hydroxylase; with the former enzyme, the ratio of tetrahydropterin oxidized to 5-hydroxy-L-tryptophan product formed is close to 1.0 with all three pterins (34, 35), whereas with tyrosine hydroxylase, the ratio is 1.0 with 6MPPH and tetrahydrobiopterin but is close to 2.0 with DMPPH (16).

In 1969, Jequier et al. (12) reported that a crude preparation of tryptophan hydroxylase from rat brain in the presence of DMPPH was unable to catalyze the hydroxylation of phenylalanine (phenylalanine was hydroxylated at less than 1% the rate observed with tryptophan). Phenylalanine was, however, a substrate for tryptophan hydroxylase from pineal glands (12). We have previously shown that the substrate specificity of pterin-dependent hydroxylases can vary markedly with the pterin co-factor used, e.g., bovine adrenal tyrosine hydroxylase catalyzes the hydroxylation of tyrosine and phenylalanine at comparable rates in the presence of tetrahydrobioperin (16), whereas in the presence of DMPPH, the rate of hydroxylation of phenylalanine is only 5% that of tyrosine (39). In view of these earlier results, we were prompted to reinvestigate the substrate specificity of brain tryptophan hydroxylase in the presence of tetrahydrobioperin. Our results (Table III) are indeed reminiscent of those with tyrosine hydroxylase: in the presence of tetrahydrobioperin, phenylalanine and tryptophan are hydroxylated by tryptophan hydroxylase at comparable rates. Even in the presence of DMPPH, however, the rate of hydroxylation of phenylalanine is significant, being about one-third the rate with tryptophan.

It is not clear why previous workers failed to detect the phenylalanine hydroxylase activity of brain tryptophan hydroxylase. It is possible that the assay method (40) used was not sensitive enough to detect it. Alternatively, it is conceivable that there is a species difference in the substrate specificity of the brain enzyme, i.e. phenylalanine is a substrate for the enzyme from rabbit brain but it is not for the enzyme from rat brain. Against the latter possibility is the work of Bagchi and Zarycki (41) that suggests that an enzyme other than tyrosine hydroxylase is present in certain areas of rat brain (such as midbrain) that is capable of converting phenylalanine to tyrosine.

The phenylalanine hydroxylating activity of brain tryptophan hydroxylase has implications for our understanding of aromatic amino acid metabolism in the disease, phenylketonuria. Although as mentioned previously, the $V_{\text{max}}$ values for tryptophan and phenylalanine hydroxylation in the presence of tetrahydrobioperin are comparable, their $K_m$ values are not (32 $\mu$M for tryptophan and 287 $\mu$M for phenylalanine). The reported concentrations for these two amino acids in nonphenylketonuric human brains are 120 $\mu$M for tryptophan and 170 $\mu$M for phenylalanine (42). Assuming that these two amino acids are uniformly distributed, our results suggest that under normal physiological conditions L-tryptophan will be used almost exclusively by the enzyme; the hydroxylation rate of L-phenylalanine would be approximately 10% that of L-tryptophan. When the concentration of phenylalanine is very high with respect to tryptophan, however, as is the case with phenylketonuric patients (850 $\mu$M for phenylalanine and 50 $\mu$M for tryptophan) (42), the prediction is that the hydroxylation rate of phenylalanine in the brain will be higher than normal with a subsequent decrease in 5-hydroxytryptophan biosynthesis. Our results therefore support the idea that the reduction in 5-hydroxyindole metabolites in phenylketonuric patients is due in part to competition for phenylalanine by tryptophan hydroxylase (8). They do not support the speculation (43) that phenylalanine hydroxylation in the brains of phenylketonuric patients is defective and that this defect is a contributing factor in the development of mental retardation in this disease. Indeed, as the above considerations indicate, the likelihood is that the rate of phenylalanine hydroxylation in the brains of these patients may be higher than in those of normal subjects.

As has been discussed previously (13, 36), phenylalanine, tyrosine, and tryptophan hydroxylases have so many kinetic properties in common that they can be regarded as a family of enzymes. This notion has predictive value: a kinetic property exhibited by one of the pterin-dependent aromatic amino acid hydroxylases is likely to be shared by the others.

The present work adds additional support to this idea and also provides the first evidence that the resemblance extends to the area of physical properties. The resemblance between brain tryptophan hydroxylase and hepatic phenylalanine hydroxylase...
is particularly striking. Thus, phenylalanine hydroxylase can exist as monomers (51,000 to 55,000 molecular weight), dimer (110,000), and tetramer (210,000) (24); the size of the monomers of tryptophan hydroxylase is 58,000 to 61,000 and the enzyme appears to exist as a tetramer of molecular weight 230,000. (The reason for the discrepancy between our results and those of Youdim et al. (38), who reported a molecular weight of about 60,000 for crude tryptophan hydroxylase from pig brainstem, is not apparent). Furthermore, as the present work has demonstrated, tryptophan hydroxylase can utilize phenylalanine as a substrate, just as phenylalanine hydroxylase can utilize tryptophan. To avoid stretching this generalization beyond its usefulness, it should be emphasized that the two hydroxylases are distinct enzymes that can be readily distinguished, e.g. a specific antiserum to rat liver phenylalanine hydroxylase does not inhibit rat brain tryptophan hydroxylase (it does, however, cross-react with tyrosine hydroxylase) (44).

REFERENCES
42. McKean, C. M. (1972) Brain Res. 47, 409-470
43. Bessman, S. (1972) J. Pediatr. 81, 834-842
Tryptophan hydroxylase. Purification and some properties of the enzyme from rabbit hindbrain.
J H Tong and S Kaufman


Access the most updated version of this article at http://www.jbc.org/content/250/11/4152

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/11/4152.full.html#ref-list-1