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BRAIN AROMATIC AMINOTRANSFERASE

I. PURIFICATION AND SOME PROPERTIES OF PIG BRAIN L-PHENYLALANINE-2-OXOGLUTARATE AMINOTRANSFERASE*

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SUMMARY

1. Pig brain L-phenylalanine–2-oxoglutarate aminotransferase was purified approximately 900-fold. The purification procedure included preparation of cell-free extract by sonication, $(NH_4)_2SO_4$ fractionation, and adsorption on and elution from calcium phosphate gel chromatography.

2. The assay used throughout the experiments described consisted of two procedures devised in this laboratory. One of these, a spectrophotometric assay, employs a borate–arsenate enolizing mixture; the other, a radiometric procedure, makes use of ion-exchange paper and permits a direct determination of the percent of substrate converted to product.

3. On polyacrylamide gel electrophoresis, the enzyme appears to be made up of one slow-moving component. Maximal activity was observed in the pH range 8.0–9.0 in both phosphate and arsenate buffer. The apparent Michaelis constants for L-phenylalanine and 2-oxoglutarate are $5 \cdot 10^{-2}$ M and $7.4 \cdot 10^{-4}$ M, respectively.

4. Various studies concerning substrate, apoenzyme and coenzyme specificities, the effects of pyridoxal phosphate analogues, sulfhydryl reagents and metal ion chelators were carried out.

INTRODUCTION

The aromatic aminotransferases occupy a significant position in the pathway leading from aromatic 2-oxo acids to biogenic amines. These enzymes play an important role in regulating the rate of biosynthesis of the biogenic amines by their ability to provide a uniform level of amino acid precursors. This regulation may be

Abbreviation: PCMB, *p*-chloromercuribenzoate.

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accomplished by the maintenance of an equilibrium between the aromatic amino acids from circulating aromatic oxo acids, or by the conversion of excess aromatic amino acids to the corresponding 2-oxo acids. Understanding of the mechanism of this step in the pathway for the biosynthesis of the biogenic amines will undoubtedly require a variety of studies on the purified aromatic aminotransferases.

Previous reports on the aromatic aminotransferases have been concerned primarily with liver enzymes[1-5]. The first report on the transamination of aromatic amino acids in nervous tissue was that of HAAVALDSEN[6], who found that rat brain extracts possessed aminotransferase activity towards 5-hydroxytryptophan, tryptophan, 3,4-dihydroxyphenylalanine, tyrosine and phenylalanine. In subsequent publications, HAAVALDSEN and co-workers[7,8] and FONNUM AND LARSEN[9] presented evidence for the occurrence of three aromatic aminotransferases: one for 3,4-dihydroxyphenylalanine, another for phenylalanine and tyrosine and a third for tryptophan and 5-hydroxytryptophan. More recently, partially purified rat brain preparations were shown to possess aminotransferase activity towards phenyl- and indolylamino acids[10]. In all instances, the enzyme preparations were only partially purified, and showed considerable overlap in their specificity.

We have developed a convenient method for the purification of L-phenylalanine aminotransferase from pig brain. The enzyme preparation is about 900-fold purified over the original cortex homogenate and migrates as a single band on acrylamide gel electrophoresis (pH 8.6; 2.7% cross linkage; 40–80 μg protein). The present communication also reports on the specificity and other properties of the enzyme.

MATERIALS AND METHODS

Materials

Reagents. Albumin, *p*-hydroxyphenylpyruvate, DL-norepinephrine were obtained from Sigma Chemical Co., St. Louis, Mo.; pyridoxine hydrochloride, 3,4-dihydroxyphenylethylamine hydrochloride, L-epinephrine and enzyme grade $(NH_4)_2SO_4$ from Mann Research Labs, New York; 2-oxoglutaric acid and sodium arsenate from the Baker Chemical Co., Phillipsburg, New Jersey; boric acid and K_2HPO_4 and KH_2PO_4 from Fisher Chemicals, Fair Lawn, N.J.; and DL-*p*-fluorophenylalanine, L-phenylalanine, L-3,4-dihydroxyphenylalanine, pyridoxamine dihydrochloride, L-glutamine, D-glutamic acid, L-alanine, D-phenylalanine, pyruvic acid, oxalacetic acid, DL-5-hydroxytryptophan, γ-aminobutyric acid, L-aspartic acid, pyridoxamine phosphate, glycine, L-tyrosine, tyramine hydrochloride, isonicotinic acid hydrazide, L-glutamic acid, phenylpyruvic acid, pyridoxal phosphate and calcium phosphate gel from Calbiochem, Los Angeles, California. L-[^{14}C]glutamic acid was obtained from New England Nuclear Corp., Boston, Mass. and [^{14}C]2-oxoglutarate from International Chemical Corp., City of Industry, Calif. Amberlite SA-2 cation paper was obtained from Reeve Angel Co., Clifton, N.J. Cupferron (ammonium salt of *N*-nitrosylphenylhydroxylamine) was a gift of Mallinckrodt Chemical Works, St. Louis, Mo.

Methods

Calcium phosphate gel cellulose was prepared by slowly adding 30 ml of calcium phosphate gel (5.6% solids) to 200 ml of a 10% suspension of Whatman Cromedia CF 11 cellulose powder ('fines' were removed by repeated decantation) with constant

stirring; before column packing, the calcium phosphate gel–cellulose mixture was degassed for 15 min.

Determination of enzyme activity

Enzyme activity was determined either by measuring the rate of aromatic 2-oxo acid formation spectrophotometrically as previously described by GEORGE, TURNER AND GABAY[11] or by the rate of [^{14}C]glutamic acid formation as described by GABAY AND GEORGE[12].

The rate of enzymatic synthesis of phenylalanine and tyrosine from phenylpyruvic and *p*-hydroxyphenylpyruvic acid (the reverse reaction) was determined by a modification of the GABAY AND GEORGE procedure. The reaction mixture contained potassium phosphate buffer (pH 8.0; 120 μmoles), L-[^{14}C]glutamic acid (0.40 μmole; 40 000 counts/min), pyridoxal phosphate (45 μmoles), phenylpyruvic acid (10 μmoles), or *p*-hydroxyphenylpyruvic acid (1.0 μmole) and enzyme (added last) in a final volume of 0.40 ml. After incubation at 37° for 30 min, the reaction was stopped by the addition of 0.10 ml of cold 1 M H_2SO_4. A 0.015-ml aliquot of the reaction mixture was chromatographed and the determination of the percent conversion of [^{14}C]2-oxoglutarate to [^{14}C]glutamic acid was carried out as previously described[12].

Protein was estimated by the method of LOWRY *et al.*[13] or from the absorbance at 210 mμ (ref. 14) using crystalline bovine serum albumin as the standard. A unit of enzyme activity is defined as that amount of enzyme which catalyzes the formation of 1 μmole of phenylpyruvic acid (or glutamic acid) per h under the above conditions. Specific activity is defined as the units of enzyme per mg of protein.

Purification of the enzyme

Step 1: Preparation of cell-free extract. The cortex (100 g) was scraped from a partially thawed pig brain (partially thawed by immersion in 0.10 M potassium phosphate buffer, pH 7.0), and suspended in 100 ml of cold 0.10 M potassium phosphate buffer (that was 65% saturated with respect to $(NH_4)_2SO_4$ at 4°, pH 8.0 (unless indicated otherwise, all operations were carried out at 4°)) by means of a pre-cooled Waring Blendor (30 sec). 25-ml aliquots of the suspension were placed in the pre-cooled, refrigerated chamber of a Raytheon 250 W sonic oscillator; the cells were sonicated at maximum power (10 kcycles) for 7 min. After sonication, the combined extracts were mixed with 300 ml of the phosphate–ammonium sulfate buffer, stirred thoroughly and filtered overnight through Whatman No. 12 filter paper.

Step 2: $(NH_4)_2SO_4$ fractionation. The clear, pink filtrate was brought to 100% of saturation by gradual addition of solid $(NH_4)_2SO_4$; after standing for 30 min the precipitate was collected by centrifugation and extracted with 15-ml portions of 90, 80 and 70% satd. $(NH_4)_2SO_4$, respectively. After each extraction, the precipitate was collected by centrifugation and the supernatant was discarded. The remaining precipitate was then dissolved in 30 ml of 0.10 M potassium phosphate buffer (pH 8.0) and dialyzed overnight against 15 vol. of the same buffer. The dialyzed solution was treated with solid $(NH_4)_2SO_4$ to obtain 68% of saturation; after standing overnight the precipitate was collected by centrifugation, dissolved in 10 ml of 0.10 M potassium phosphate buffer (pH 8.0), and then dialyzed overnight against 100 vol. of the same buffer.

Step 3: Adsorption on and elution from calcium phosphate gel. The dialyzed solution was added to the top of a calcium phosphate gel–cellulose column (20 cm ×

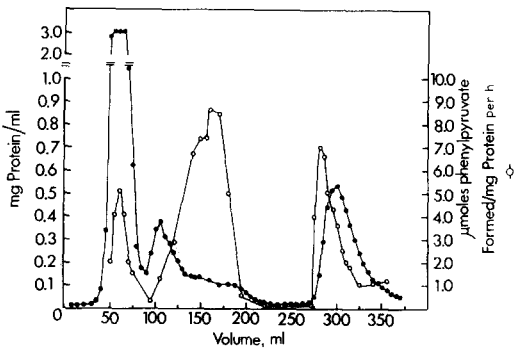


Fig. 1. Phenylalanine aminotransferase—adsorption on and elution from calcium phosphate gel–cellulose. Purified phenylalanine aminotransferase (200 mg, 350 units) was adsorbed on and eluted from calcium phosphate gel–cellulose (1.0 cm × 20 cm), as described in the text.

1.0 cm) previously equilibrated with 0.01 M potassium phosphate buffer (pH 8.0). The protein was eluted with the following solutions: 0.01 M, 0.05 M, 0.075 M and 0.10 M potassium phosphate buffers (pH 8.0). Fractions of 5 ml were collected at a flow rate of approx. 10 ml per h. Enzyme activity was eluted with 0.01 M, 0.05 M and 0.10 M buffer (Fig. 1). In each of the three fractions, the tubes containing most of the activity exhibited constant specific activity; these were respectively pooled, concentrated by use of an Amicon ultrafiltration cell (Diaflo XM-50 membrane ultrafiltrator: Amicon Corporation, Cambridge, Mass.), made 0.40 M in potassium phosphate buffer (pH 8.0) and frozen. The purification of the enzyme is summarized in Table I.

When fractions containing 100-fold purified enzyme were made 0.40 M in potassium phosphate buffer (pH 8.0) and then frozen, there was no detectable loss

TABLE I

PURIFICATION OF THE ENZYME

Enzyme from 225 g of frozen cortex was purified; other details are given in text.

Step No.	*Fraction*	*Protein (mg)*	*Activity*		*Yield (%)*	*Purification (fold)*
			Total (units)	*Specific (units/mg)*		
	Crude homogenate	16 000	975	0.061	(100)	6.2*
1	Cell-free extract	1 470	850	0.58	88	58
2	$(NH_4)_2SO_4$ (after dialysis)	248	436	1.76	45	176
3	Calcium phosphate column: 0.01 M	32.2	178	5.53	18	553
	0.05 M	1.4	13	9.28	1.3	928
	0.10 M	2.7	19	7.04	2.0	704

* This represents an approx. 6-fold purification over the original starting material when homogenized as originally described in phosphate buffer from which $(NH_4)_2SO_4$ has been excluded.

of activity after storage for 9 months at − 20°. The most highly purified enzyme was similarly stored and when assayed after two weeks of storage, no loss in activity could be detected. The enzyme in the 0.05 M and 0.10 M potassium phosphate buffer (pH 8.0) migrated as a single band (40–80 μg protein) on polyacrylamide-gel electrophoresis at pH 8.6 following the procedure of DAVIS[15].

RESULTS

Purification of the enzyme

The purification of the enzyme is summarized in Table I. It is apparent from MATERIALS AND METHODS that each of the three distinct steps actually represents the combination of a number of individual steps, their combination decreasing the work and increasing the yield. Consider, for example, the first two steps. In Step 1, the "cell-free extract" represents a pooling of the following steps: (a) homogenation, (b) sonication, (c) extraction, and (d) $(NH_4)_2SO_4$ fractionation (0–65% satd. $(NH_4)_2SO_4$ soluble fraction). Similarly in Step 2, the "$(NH_4)_2SO_4$ fractionation" represents a combination of the following steps: (a) concentration by $(NH_4)_2SO_4$ precipitation, (b) selective extraction of non-enzymatic protein by back extraction with decreasing concentrations of $(NH_4)_2SO_4$, (c) dissolving of the remaining enzyme containing protein and extensive dialysis, and (d) taking a very narrow $(NH_4)_2SO_4$ fraction, *i.e.* the equivalency of a 65–68% $(NH_4)_2SO_4$ fraction. Lastly, Step 3 represents the adsorption on, and desorption off, the chromatographic column.

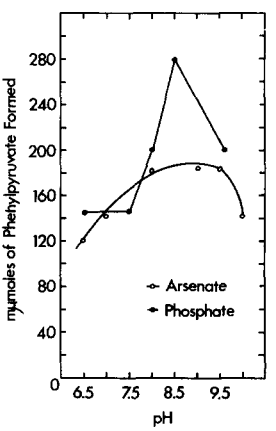


Fig. 2. Effect of pH on phenylpyruvate formation. The reaction mixtures contained L-phenylalanine (15 μmoles; pH as indicated), 2-oxoglutaric acid (10 μmoles; pH as indicated), pyridoxal phosphate (90 mμmoles), buffer (●—●, potassium phosphate; ○—○, potassium arsenate; 188 μmoles; pH as indicated) and enzyme (60 μg) in a final volume of 0.80 ml; incubated at 37° for 1 h.

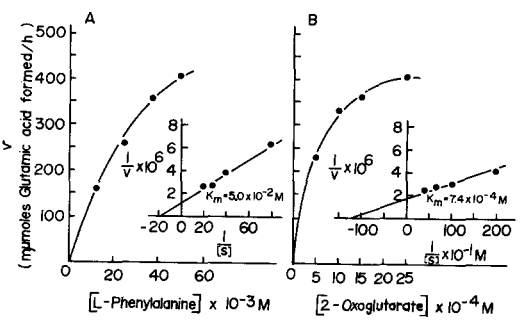
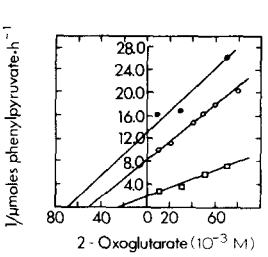


Fig. 3. Effect of substrates on phenylalanine aminotransferase. Activity curves and double reciprocal plots are shown. The reaction mixture contained: (A) potassium phosphate buffer (188 μmoles), pyridoxal phosphate (90 mμmoles), [^{14}C]2-oxoglutarate (0.4 μmole; 80 000 counts/min), L-phenylalanine (concentrations varied as indicated) and enzyme (20 μg) in a final volume of 0.80 ml; incubated at 37° for 30 min (B) all components and conditions were identical with those of (A), except that L-phenylalanine concentration was 10 μmoles and 2-oxoglutarate concentrations varied as indicated.



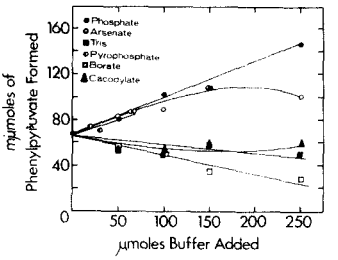


Fig. 4. Effect of high concentrations of 2-oxoglutarate on phenylpyruvate formation. The reaction mixtures contained potassium phosphate (188 μmoles; pH 8.0), pyridoxal phosphate (90 mμmoles), L-phenylalanine (●—●, 8 μmoles; ○—○, 15 μmoles; □—□, 52 μmoles; pH 8.0), 2-oxoglutarate (pH 8.0) as indicated and enzyme (500–1000 μg) in a final volume of 0.80 ml; incubated at 37° for 1 h.

Fig. 5. Effect of different buffers on phenylpyruvate formation. The reaction mixtures contained L-phenylalanine (30 μmoles; pH 8.0), 2-oxoglutaric acid (0.6 μmole; pH 8.0), pyridoxal phosphate (90 mμmoles), buffer (pH 8.0) as indicated (●—●, phosphate; ○—○, arsenate; ■—■, Tris; ◐—◐, pyrophosphate; □—□, borate; and ▲—▲, cacodylate) and enzyme (60 μg) in a final volume of 0.8 ml; incubated at 37° for 1 h. In each case, an aliquot of enzyme was dialyzed against 200 vol. of 0.10 M of the respective buffer, pH 8.0, overnight and then diluted 3-fold with water before use. The volume of enzyme taken was 0.10 ml, which therefore meant that 3 μmoles of the appropriate buffer were also added.

General catalytic properties of the enzyme

The time course of both phenylpyruvate and glutamate formation was linear for at least 1 h under the conditions of assay described above. Maximal activity was observed in the pH range 8.0–9.0 in both phosphate and arsenate buffer (Fig. 2).

Michaelis constants. The apparent Michaelis constants for phenylalanine (K_m $5 \cdot 10^{-2}$ M) and 2-oxoglutarate (K_m $7.4 \cdot 10^{-4}$ M) were determined by the usual graphical procedure as shown in Figs. 3A and 3B: High concentrations of 2-oxoglutarate were found to be inhibitory with the degree of inhibition being a function of phenylalanine concentration (Fig. 4).

Metal ion chelators (10^{-2} M EDTA and Cupferron, 10^{-3} M diethyldithiocarbamate and 10^{-4} M cuprizone) had little or no effect on enzymatic activity, nor did high concentrations of $(NH_4)_2SO_4$ (0.10 M).

TABLE II

EFFECT OF POTASSIUM PHOSPHATE AND Tris–HCl BUFFERS ON PHENYLPYRUVATE FORMATION

The reaction mixtures contained phenylalanine (pH 8.0, 15 μmoles), α-ketoglutaric acid (pH 8.0, 10 μmoles), pyridoxal phosphate (90 mμmoles), enzyme (1.4 mg), buffer (pH 8.0, 213 μmoles) in a final volume of 0.80 ml, incubated at 37° for 1 h. The enzyme was dialyzed overnight against 0.10 M potassium phosphate or Tris–HCl buffers, pH 8.0.

Dialysis buffer	*Buffer added to reaction mixture (μmoles)*		*Phenylpyruvate formed (mμmoles/h)*
	Tris	*Phosphate*	
Phosphate	0	188	168
Phosphate	188	0	128
Tris	188	0	86
Tris	0	188	138

Effect of buffers

Maximal enzyme activity is obtained only in the presence of phosphate buffer. In the absence of added phosphate the reaction proceeded at only 50% of that observed in optimal phosphate concentration. Dialysis overnight against Tris buffers results in 50% inhibition that can be partially reversed by the addition of phosphate to the reaction mixture. The effect of excluding phosphate from the reaction mixture of a phosphate-dialyzed enzyme or adding phosphate to the reaction mixture of a Tris-dialyzed enzyme are approximately the same; each results in approx. 20% inhibition (Table II). The effects of various concentrations of phosphate, pyrophosphate, arsenate, cacodylate, borate, glycine and Tris buffers on the reaction are given in Fig. 5. Under the conditions employed, phosphate, pyrophosphate and arsenate were appreciably active, while much lower activity was observed with cacodylate, borate and Tris. Glycine buffer had no effect on the enzyme activity.

Apoenzyme formation and coenzyme specificity

Pyridoxal phosphate serves as a coenzyme for the phenylalanine-2-oxoglutarate aminotransferase. The enzyme exhibits no lag period and approx. 70–80% of its normal activity is observed when pyridoxal phosphate is omitted from the reaction mixture (Fig. 6A). The partially resolved apoenzyme (formed by 5 min 50° pre-

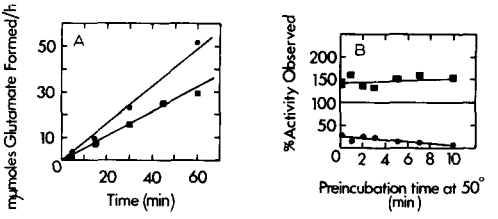


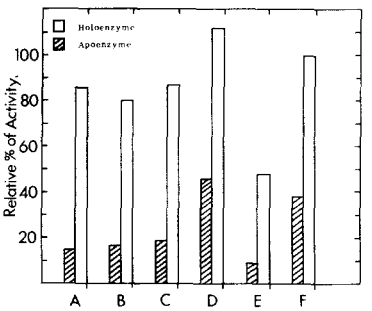
Fig. 6. A. The effect produced on the rate of [^{14}C]glutamate formation by the deletion of pyridoxal phosphate from the reaction mixture. The reaction mixtures contained L-phenylalanine (20 μmoles; pH 8.0), [^{14}C]2-oxoglutarate (0.2 μmole; 40 000 counts/min), potassium phosphate (140 μmoles; pH 8.0) and enzyme (25 μg) in a final volume of 0.40 ml; incubated at 37° for 30 min. In experiment 1, pyridoxal phosphate (●—●, 90 mμmoles) was also added and in experiment 2, pyridoxal phosphate was omitted (■—■, no pyridoxal phosphate). B. The effect of preincubation time on apoenzyme formation and the ability of pyridoxal phosphate to reactivate the enzyme. The reaction mixtures contained L-phenylalanine (15 μmoles; pH 8.0), 2-oxoglutarate (5 μmoles; pH 8.0), potassium phosphate (188 μmoles; pH 8.0) and enzyme (30 μg) in a final volume of 0.80 ml; incubated at 37° for 1 h. Immediately before assay, the enzyme was diluted with an equal volume of 1 M potassium phosphate buffer (pH 5.7) and preincubated at 50° (in water with constant mixing) for the time intervals shown. In addition, in experiment 1, the enzyme was made $1.12 \cdot 10^{-4}$ M in pyridoxal phosphate (■—■) and preincubated (preincubation II) at 37° for 10 min before the reaction was initiated. In experiment 2, the 10-min 37° preincubation (preincubation II) was carried out in the absence of pyridoxal phosphate (●—●).

incubation in 0.5 M phosphate buffer, pH 5.7) possesses less than 20% of the holoenzyme activity but can be rapidly and completely reactivated by preincubation with pyridoxal phosphate. Not only can the apoenzyme be completely restored to the holoenzyme state (as judged from velocity measurements), but the preincubation appears to enhance its activity as compared to that of the control; similar results were also observed with the native enzyme (Fig. 6B).

Analogues of pyridoxal phosphate

The ability of a number of pyridoxal phosphate analogues to act as coenzyme for aminotransferase activity was determined with the apoenzyme prepared as described above. The apoenzyme was added directly to the "pyridoxal phosphate analogue containing" reaction mixture in order to initiate the reaction without any preliminary apoenzyme–coenzyme preincubation.

The hydrochloride salts of pyridoxine, pyridoxamine and pyridoxal had no cofactor properties under the conditions used—pyridoxal hydrochloride actually appeared to be slightly inhibitory (Fig. 7). Both pyridoxamine 5'-phosphate and pyri-



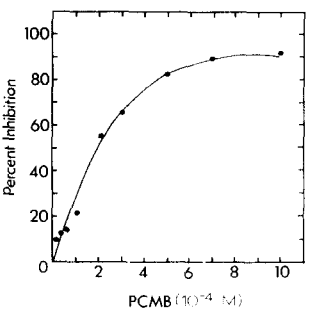


Fig. 7. Effect of various pyridoxal phosphate analogues on the activity of apo- and holo- aminotransferase. The reaction mixtures contained L-phenylalanine (155 μmoles; pH 8.0), 2-oxoglutarate (0.3 μmole; 60 000 counts/min), potassium phosphate (140 μmoles; pH 8.0), the pyridoxal phosphate analogue to be tested (0.4 μmole) and the enzyme (12.5 μg) in a final volume of 0.40 ml; incubated at 37° for 30 min. (A) None, (B) pyridoxine·HCl, (C) pyridoxamine·2 HCl, (D) pyridoxamine phosphate, (E) pyridoxal·HCl, and (F) pyridoxal phosphate.

Fig. 8. Effect of *p*-chloromercuribenzoate on phenylpyruvate formation. The reaction mixtures contained potassium phosphate (188 μmoles; pH 8.0), pyridoxal phosphate (90 mμmoles), phenylalanine (15 μmoles; pH 8.0), 2-oxoglutaric acid (10 μmoles; pH 8.0), enzyme (1.2 mg) and *p*-chloromercuribenzoate as indicated in a final volume of 0.80 ml, incubated at 37° for 1 h.

doxal 5'-phosphate were effective in partially restoring the apoenzyme activity and "in stimulating" the holoenzyme—pyridoxamine phosphate was somewhat more effective than pyridoxal phosphate.

Thermostability of phenylalanine and tyrosine aminotransferases

When a 100-fold purified enzyme fraction, containing both phenylalanine and tyrosine aminotransferase activity, is heated for 10 min at various temperatures, the tyrosine activity possesses the greater stability. Over 80% of the tyrosine activity remains after 10 min at 60° as compared to only 60% of the phenylalanine aminotransferase activity.

Effect of sulfhydryl reagents

The transamination of phenylalanine was markedly inhibited by *p*-chloromercuribenzoate (PCMB). Inhibition was virtually complete at a concentration of $5 \cdot 10^{-4}$ M; half maximal inhibition was observed with $2 \cdot 10^{-4}$ M (Fig. 8). Inhibition by PCMB could be reduced by preincubation of the enzyme with L-phenylalanine and pyridoxal phosphate and reversed by a subsequent preincubation with 2-mer-

TABLE III

EFFECT OF 2-MERCAPTOETHANOL AND PCMB ON PHENYLPYRUVATE FORMATION

The reaction mixtures contained phenylalanine (pH 8.0, 15 μmoles), α-ketoglutaric acid (pH 8.0, 10 μmoles), pyridoxal phosphate (90 mμmoles), enzyme (1.0 mg), potassium phosphate buffer (225 μmoles, pH 8.0), PCMB (0.16 μmole), 2-mercaptoethanol (8.0 μmoles) in a final volume of 0.80 ml, incubated at 37° for 1 h.

Preincubation conditions	*Phenylpyruvate formed (mμmoles/h)*
None	130
2-Mercaptoethanol	142
PCMB	64
PCMB; 2-mercaptoethanol*	112

* The enzyme was preincubated for two successive 10-min periods at 0° in volumes of 0.33 ml and 0.41 ml.

captoethanol (Table III). Preincubation with this reducing reagent (10^{-2} M 2-mercaptoethanol) only slightly stimulated the enzyme (10%).

Substrate specificity

The activity of a partially purified preparation of the enzyme (500-fold purified) towards a number of amino acids was determined by measuring the rate of formation of [^{14}C]glutamic acid as described under MATERIALS AND METHODS (Table IV). Whereas in the forward direction (phenylalanine → phenylpyruvic acid) 2-oxosuccinic acid appears to be approx. 70% as effective as 2-oxoglutaric acid in promoting transamination by accepting the alpha amino group, in the reverse direction (phenylpyruvic acid → phenylalanine), L-aspartic acid is only about 10% as effective as L-glutamic acid. L-glutamine is similarly ineffective (Table V). The enzyme exhibited optical specificity in both the forward and the reverse direction; no transaminase

TABLE IV

ACTIVITY OF THE ENZYME TOWARD VARIOUS AMINO ACIDS

The reaction mixtures consisted of enzyme (6 μg), amino acid, potassium phosphate (pH 8.0; 120 μmoles), pyridoxal phosphate (45 mμmoles), and α-keto[^{14}C]glutarate (0.3 μmoles; 37 000 counts/min) in a final volume of 0.4 ml. The incubation was carried out and the formation of [^{14}C]glutamate was determined as described under MATERIALS AND METHODS. All K_m values are expressed with respect to L isomer only.

Amino acid	*Concn. (mM)*	*Glutamate formed (mμmoles)*	*$v_{max.}$ (μmoles/h)*	*K_m (mM)*	*Relative activity (%)*
L-Phenylalanine	50	71	0.30	50	100
L-Tyrosine	36	35	0.02	3.8	49
L-3,4-dihydroxyphenylalanine	5.8	33	0.10	6	46
DL-*p*-fluorophenylalanine	15	29	0.08	7	41
L-Tryptophan	15	37	0.17	15	52
DL-5-hydroxytryptophan	16	44	—	—	62
L-Histidine	23	32	—	—	35

TABLE V

EFFECT OF KETOSUCCINATE–ASPARTATE *versus* KETOGLUTARATE–GLUTAMATE ON ENZYME ACTIVITY IN FORWARD (a) AND REVERSED (b) DIRECTIONS

(a). The reaction mixture consisted of enzyme (43 μg), L-phenylalanine (40 μmoles), pyridoxal phosphate (90 mμmoles), potassium phosphate (pH 8.0, 240 μmoles) and α-keto acid (0.6 μmole) in a final volume of 0.80 ml. The incubation was carried out and the formation of phenylpyruvate was determined spectrophotometrically as described under MATERIALS AND METHODS. (b) The reaction mixture consisted of enzyme (25 μg), phenylpyruvate (8 μmoles), pyridoxal phosphate (45 mμmoles), potassium phosphate (pH 8.0; 120 μmoles) and L-amino acid (1.2 μmoles; 40 000 counts/min) in a final volume of 0.4 ml. The incubation was carried out and the formation of α-keto[^{14}C]glutarate or α-keto[^{14}C]succinate was determined as described under MATERIALS AND METHODS.

Amino group donor	*Amino group acceptor*	*v (mμmoles/h)*	*Relative activity (%)*
Forward			
Phenylalanine	None	4	0
Phenylalanine	α-Ketoglutarate	206	100
Phenylalanine	α-Ketosuccinate	146	70
Reversed			
None	Phenylpyruvate	34	0
Glutamate	Phenylpyruvate	400	100
Aspartate	Phenylpyruvate	72	10

activity was observed with D-phenylalanine (forward direction) or D-glutamic acid (reverse direction).

Tyramine, DOPA, dopamine, norepinephrine and epinephrine (intermediates or end products in the catecholamine biosynthetic pathway) were tested at a final concentration of $1.25 \cdot 10^{-3}$ M and found to have no effect on the transamination of tyrosine (K_m $3.8 \cdot 10^{-3}$ M).

High concentrations of *p*-fluorophenylalanine are inhibitory; although the K_m

TABLE VI

ADDITIVE EFFECT OF PHENYLALANINE AND TYROSINE ON ENZYME ACTIVITY

The reaction mixture consisted of enzyme (6 μg), amino acid, potassium phosphate (pH 8.0; 120 μmoles), pyridoxal phosphate (45 mμmoles), and α-keto[^{14}C]glutarate (0.4 μmole; 37 000 counts/min) in a final volume of 0.4 ml. The incubation was carried out and the formation of [^{14}C]glutamate was determined as described under MATERIALS AND METHODS.

Amino acid (μmoles)		*Glutamate formed (μmoles/h)*	
L-*Phenylalanine*	L-*Tyrosine*	*Observed*	*Predicted**
0	1.0	136	
5	0	186	
5	1.0	292	322
40	0	589	
50	0	589	
50	1.0	634	752

* Predicted on the basis of the additive effect of a single enzyme capable of reacting with both phenylalanine and tyrosine or two distinct enzymes.

is $7 \cdot 10^{-3}$ M, $3.2 \cdot 10^{-2}$ M *p*-fluorophenylalanine resulted in an approx. 50% inhibition in enzyme velocity. Similar substrate inhibition was not observed with phenylalanine, 3,4-dihydrophenylalanine or histidine. The limited solubilities of tyrosine, tryptophan and 5-hydroxytryptophan did not permit similar investigations. Preliminary data in the case of phenylalanine and tyrosine are consistent with the idea of a single enzyme with dual or multiple substrate specificity (Table VI).

DISCUSSION

The present report on the purification of phenylalanine–2-oxoglutarate aminotransferase is the first for the isolation of this enzyme from pig brain, and preliminary electrophoretic data indicate that the enzyme migrates as a single component on polyacrylamide gel at pH 8.6. Available data indicate that the purified enzyme is stable on storage for at least 9 months. The preparation should, therefore, be useful for detailed mechanism and structural studies. The general catalytic properties of the present enzyme preparation, *i.e.*, 2-oxoglutarate and pyridoxal phosphate dependency, are in accord with current ideas on transamination. High concentrations of 2-oxoglutarate are inhibitory for the pig brain phenylalanine–2–oxoglutarate aminotransferase. Such inhibition is similar to the effect observed with other aromatic aminotransferases, for example, tyrosine–2-oxoglutarate transaminase from dog liver[1] and rat brain[8], but is in contrast to that reported for the tyrosine–2-oxoglutarate transaminase from rat liver[16] and the halogenated tyrosine transaminase from rat kidney[17]. Other aminotransferases have also been reported to be inhibited by high concentrations of 2-oxoglutarate—consider, for example, the kinetic studies of VELICK AND VARVA[18] as well as those of HENSON AND CLELAND[19]. The observation that the highly purified preparation utilizes tyrosine, *p*-fluorophenylalanine, 3,4-dihydroxyphenylalanine, tryptophan, 5-hydroxytryptophan as well as histidine, supports the conclusion of the broad substrate capability of this preparation. The possibility of contamination of this preparation by other enzymes, although unlikely due to the degree of enzyme purity, cannot be excluded at this time. Studies that should conclusively answer the question concerning the occurrence of single versus multiple aromatic aminotransferases are in progress. To date, such studies have been hampered by the low solubilities of the aromatic amino acids. However, preliminary substrate saturation experiments with phenylalanine and tyrosine support a single-enzyme concept (*cf.* Table VI). It is noteworthy that the enzyme exhibits definite anion requirements. Phosphate, arsenate and pyrophosphate (in order of decreasing ability) were the most effective in promoting transamination, whereas borate, cacodylate and Tris were either not as effective or slightly inhibitory. The observation that the enzyme was unaffected by such common chelating agents as EDTA, Cupferron and diethyldithiocarbamate (preincubated up to 36 h) at concentrations as high as 10^{-2} M argues against the requirement of metal ions for its catalytic activity.

The enzyme is sensitive to PCMB. This sensitivity can be reduced by a preincubation of the enzyme with phenylalanine plus pyridoxal phosphate. PCMB inhibition can be partially reversed by preincubation of the enzyme with 2-mercaptoethanol. However, the presence of sulfhydryl reducing reagents, *e.g.*, 2-mercaptoethanol, is not required for enzyme stability.

The holoenzyme is dissociable into its apo- and coenzyme moities. Restoration

of the holoenzyme activity is readily accomplished by pyridoxal phosphate preincubation, and preliminary data indicate that pyridoxamine can also act as a coenzyme.

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