Aromatic Amino Acid Transaminase in Rat Intestine

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The transamination of aromatic L-amino acids (5-hydroxytryptophan, tryptophan, tyrosine, phenylalanine and kynurenine) was shown to be catalysed by enzyme preparations from rat small intestine. On the basis of the partial purification and characterization of these aromatic amino acid transaminases, it is suggested that rat small intestine contains several kinds of aromatic amino acid transaminases.

Aromatic amino acid transaminases have been studied extensively with enzyme preparations from mammalian liver, kidney and brain (Mason, 1959; Kenney, 1962; Wurzman & Larin, 1968; Civen *et al.*, 1970; Tangen *et al.*, 1965; Miller & Litwack, 1969). Particularly, the transamination of aromatic amino acids in the brain has received much attention because of its significance in the biosynthesis of catechol and indole amines. Haavaldsen (1962) reported that aromatic amino acids such as 5-hydroxytryptophan, tryptophan, tyrosine, phenylalanine and 3,4-dihydroxyphenylalanine could be readily transaminated by rat brain extract, suggesting that the aromatic keto acids might act as precursors of the corresponding biological amines.

5-Hydroxytryptamine, synthesized from tryptophan via 5-hydroxytryptophan (Noguchi *et al.*, 1971, 1973), is present in appreciable amounts in mammalian intestine. The transamination of 5-hydroxytryptophan and tryptophan might play a role in the regulation of the concentration of 5-hydroxytryptamine in the intestine. However, there is no report on the presence of aromatic amino acid transaminases in the intestine. In the present investigation, aromatic amino acid transaminases were found in rat small intestine, partially purified and characterized.

Experimental

Materials

L-Kynurenine was prepared by ozonolysis of L-tryptophan (Brown & Price, 1955). L-Tryptophan, L-tyrosine and L-phenylalanine were purchased from Tanabe Amino Acid Foundation, Osaka, Japan. 5-Hydroxy-L-tryptophan was obtained from Calbiochem, San Diego, Calif., U.S.A. Sodium pyruvate, oxaloacetic acid, protamine sulphate and pyridoxal phosphate were from Nakarai Chemicals Co. Ltd., Kyoto, Japan. Analytical-grade Dowex AG1 resin (X8; 200-400 mesh; Cl⁻ form) was from Bio-Rad Laboratories, Richmond, Calif., U.S.A. The acetate form of Dowex 1 was prepared as described by Price (1954) and Roy & Price (1959). DEAE-

cellulose and calcium phosphate gel were purchased from Seikagaku Kogyo Co. Ltd., Tokyo, Japan.

Methods

Enzyme assays. (a) Transaminase assay with L-kynurenine. The incubation mixture contained 100mM-potassium phosphate buffer (pH 8.0), 45mM-L-kynurenine, 48 μ M-pyridoxal phosphate, 45mM- α -oxo acid and enzyme preparation in a total volume of 1.0ml. The control incubation mixture was the same except that boiled enzyme was added. After incubation at 37°C, 0.1ml of 10% (w/w) HClO₄ was added. The solution was adjusted to pH 6.7 with 6ml of 0.15M-potassium phosphate buffer and centrifuged at 15000g for 10min. The supernatant and two rinses with 3ml of distilled water were applied to a column (4cm $\times$ 0.8cm) of Dowex 1 (acetate form), which was washed with 100ml of deionized water and 50ml of 4M-acetic acid; then kynurenic acid was eluted with 40ml of 12M-acetic acid. The eluate was evaporated to dryness at reduced pressure and the residue was dissolved in 3.5ml of 0.2M-potassium phosphate buffer, pH 7.5, and filtered. The absorbance of the filtrate was measured at 333nm against that of the control. For calculation of the amount of kynurenic acid formed its molar absorption coefficient at 333nm was taken as 8850 (Knox, 1953). Protein was determined by the biuret method with crystalline bovine serum albumin (moisture, approximately 4% w/w) as the standard (Chance & Redfearn, 1961). Enzyme activity was expressed as nmol of kynurenic acid produced/h and was taken from the linear parts of the reaction curves.

(b) Transaminase assay with 5-hydroxy-L-tryptophan, L-tryptophan, L-tyrosine and L-phenylalanine. The transaminase assay was based on the ability of aromatic α -oxo acids to form enol-borate complexes showing characteristic spectra in the 300nm region (Knox & Pitt, 1957). The assay was carried out at 37°C in a Hitachi model EPS type-3 recording spectrophotometer by the methods of Haavaldsen (1962) and Fonnum *et al.* (1964). The reaction mixture consisted of 670mM-borate buffer,

pH 8.2, 3.3 mM aromatic amino acid, 4 μ M-pyridoxal phosphate, 2.3 mM α -oxo acid and enzyme preparation in a total volume of 3.0 ml. α -Oxo acid was omitted from the blank. Incubation was carried out at 37°C and the increase in absorbance was measured at 320 nm with L-phenylalanine and L-tyrosine, and at 328 nm with L-tryptophan and 5-hydroxy-L-tryptophan. Enzyme activity was expressed as nmol of the corresponding α -oxo acid formed/h.

Enzyme preparation. All procedures were carried out at about 0°C. Glass-distilled water and potassium phosphate buffer, pH 7.5, were used throughout.

The procedure for preparation A was as follows.

Step 1, extraction. Donryu strain male rats (45, 80–100 g each) were stunned, decapitated and bled. The small intestines were removed, cut open longitudinally and washed several times with ice-cold 5 mM buffer. They were then cut into small pieces and homogenized in a Waring Blendor for 2 min with 2 vol. of the same buffer. The homogenate was centrifuged at 10000g for 120 min and the precipitate discarded.

Step 2, protamine treatment. Protamine sulphate (1.5 g) was added to the extract. The solution was stirred for 30 min and then centrifuged at 10000g for 120 min and the precipitate was discarded.

Step 3, $(\text{NH}_4)_2\text{SO}_4$ fractionation. The protamine supernatant was subjected to $(\text{NH}_4)_2\text{SO}_4$ fractionation to prepare the 30–60% and 60–100%-saturated fractions. Each precipitate was collected by centrifugation and then dissolved in a small volume of 5 mM buffer. Each solution was dialysed overnight against 10 litres of the same buffer and then the insoluble materials were removed by centrifugation at 20000g for 60 min.

Step 4, calcium phosphate gel fractionation. A calcium phosphate gel suspension in 5 mM buffer was added to the 30–60%-saturated- $(\text{NH}_4)_2\text{SO}_4$ fraction (10 mg of gel/mg of protein). The mixture was stirred twice for 15 min with 100 ml of buffer and centrifuged, and the two supernatants were combined and brought to 65% saturation with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was collected by centrifugation and dissolved in a small vol. of 5 mM buffer, and dialysed overnight against 6 litres of the same buffer.

Step 5, DEAE-cellulose chromatography. The non-diffusible fraction was applied to a DEAE-cellulose column (2.5 cm $\times$ 10 cm) equilibrated with 5 mM buffer. Non-absorbed protein was eluted with 200 ml of the same buffer, and then the enzymes were eluted with 100 ml of 0.1 M buffer. The eluate was brought to 65% saturation with $(\text{NH}_4)_2\text{SO}_4$. After centrifugation, the precipitate was dissolved in a small volume of 5 mM buffer and dialysed overnight against 6 litres of the same buffer.

For preparation B, the same procedures as for preparation A were employed until step 3.

Step 4, calcium phosphate gel fractionation. The

60–100%-saturated- $(\text{NH}_4)_2\text{SO}_4$ fraction of step 3 was treated with calcium phosphate gel by the method described for preparation A. The gel was washed with 100 ml of 5 mM buffer and the enzymes were eluted with 100 ml of 0.2 M buffer. The eluate was brought to 100% saturation with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was collected by centrifugation, dissolved in a small volume of 5 mM buffer and dialysed overnight against 10 litres of the same buffer.

Step 5, DEAE-cellulose chromatography. The non-diffusible fraction was applied to a column of DEAE-cellulose by the procedure described for preparation A. The column was washed with 200 ml of 5 mM buffer. The effluents were combined and precipitated by addition of $(\text{NH}_4)_2\text{SO}_4$ to 100% saturation. After centrifugation, the precipitate was dissolved in a small volume of 5 mM buffer and dialysed overnight against 6 litres of the same buffer.

Results and Discussion

Table 1 shows the aromatic amino acid transaminase activities of the fractions at the five stages of preparation A. The purification (about 23-fold) and yield (about 60%) of transaminase activities with L-kynurenine were similar with either pyruvate or oxaloacetate as acceptors. This suggests that the two types of transaminations were catalysed by the same enzyme. L-Kynurenine-2-oxoglutarate transaminase was purified 6-fold with a yield of 15%. Kynurenine transaminases in the DEAE-cellulose fraction (step 5 of preparation A) showed similar activities with pyruvate and oxaloacetate; however, 2-oxoglutarate was only about 26% as effective.

Both the purification (0.5–3.8-fold) and recovery (0.07–0.5%) of the transaminase activities towards other aromatic amino acids (5-hydroxy-L-tryptophan, L-tryptophan, L-tyrosine and L-phenylalanine) were very low in comparison with those of kynurenine transaminase activities. Pyruvate and oxaloacetate were equally effective with the DEAE-cellulose fraction, but 2-oxoglutarate was not active. Transaminase activities towards all the amino acids tested with the three oxo acid substrates were scarcely detected in the 0–30%-saturated- $(\text{NH}_4)_2\text{SO}_4$ fraction (step 3), the 0.1 M buffer extract from calcium phosphate gel after 5 mM buffer extraction (step 4) and the 5 mM buffer effluent from DEAE-cellulose column (step 5).

The aromatic amino acid transaminase activities of the fractions at the five stages of preparation B are shown in Table 2. Both kynurenine-2-oxoglutarate and oxaloacetate transaminase activities were purified about 18-fold with a yield of about 8%, suggesting that the two transaminations were catalysed by a single enzyme. The purification and yield of kynurenine-pyruvate transaminase were low.

Table 1. *Purification of aromatic amino acid transaminases by preparation A*

For further details see the text.

Substrate	Specific activity (nmol product/h per mg of protein)					Purification	Yield (%)
	Step 1	Step 2	Step 3	Step 4	Step 5		
Kynurenine-pyruvate	8.8	14	9.8	26	209	23	59
Kynurenine-2-oxoglutarate	8.6	14	4.1	8.0	52	6.0	15
Kynurenine-oxaloacetate	8.6	14	10	25	199	23	57
5-Hydroxytryptophan-pyruvate	16	16	33	20	22	1.4	0.2
5-Hydroxytryptophan-2-oxoglutarate	16	16	0	0	0	0	0
5-Hydroxytryptophan-oxaloacetate	14	14	22	18	20	1.4	0.2
Tryptophan-pyruvate	12	13	19	23	20	1.7	0.2
Tryptophan-2-oxoglutarate	13	16	0	0	0	0	0
Tryptophan-oxaloacetate	13	13	16	20	16	1.2	0.2
Tyrosine-pyruvate	30	19	30	46	113	3.8	0.5
Tyrosine-2-oxoglutarate	29	16	0	0	0	0	0
Tyrosine-oxaloacetate	28	17	25	42	95	3.4	0.5
Phenylalanine-pyruvate	15	16	5.3	5.9	8.0	0.5	0.07
Phenylalanine-2-oxoglutarate	13	17	0	0	0	0	0
Phenylalanine-oxaloacetate	11	13	4.4	5.6	6.0	0.5	0.07

Table 2. *Purification of aromatic amino acid transaminases by preparation B*

For further details see the text.

Substrate	Specific activity (nmol product/h per mg of protein)					Purification	Yield (%)
	Step 1	Step 2	Step 3	Step 4	Step 5		
Kynurenine-pyruvate	8.8	14	15	32	63	7.1	3.1
Kynurenine-2-oxoglutarate	8.6	14	23	62	152	18	7.7
Kynurenine-oxaloacetate	8.6	14	23	65	146	17	7.5
5-Hydroxytryptophan-pyruvate	16	16	12	3.2	2.8	0.2	0.2
5-Hydroxytryptophan-2-oxoglutarate	16	16	18	13	14	0.9	1.0
5-Hydroxytryptophan-oxaloacetate	14	14	17	8.0	10	0.7	1.0
Tryptophan-pyruvate	12	13	14	4.8	1.9	0.2	0.2
Tryptophan-2-oxoglutarate	13	16	18	35	9.7	0.7	1.0
Tryptophan-oxaloacetate	13	15	16	24	8.0	0.6	0.8
Tyrosine-pyruvate	30	19	20	9.2	12	0.4	0.5
Tyrosine-2-oxoglutarate	29	16	35	60	58	2.0	2.6
Tyrosine-oxaloacetate	28	17	33	44	48	1.7	2.2
Phenylalanine-pyruvate	15	16	4.6	2.6	2.1	0.1	0.2
Phenylalanine-2-oxoglutarate	13	17	5.3	14	13	1.0	1.3
Phenylalanine-oxaloacetate	11	13	5.2	8.4	9.2	0.8	1.0

As in preparation A, both the purification (0.1–2-fold) and yield (0.2–2.6%) of transaminase activities towards other aromatic amino acids were very low compared with those of kynurenine transaminase activities. In the fraction from DEAE-cellulose, both 2-oxoglutarate and oxaloacetate were equally effective as amino acceptors whereas pyruvate activity was low. The 5 mM buffer extract from calcium phosphate gel (step 4), and the 0.1 M buffer eluate

from the DEAE-cellulose column (step 5) showed hardly any aromatic transaminase activity.

The above data show that aromatic amino acid transaminases in rat small intestine consist of at least two types of enzymes; their properties are summarized in Table 3. The type 1 enzyme exhibits higher preference for pyruvate and oxaloacetate as amino acceptors, whereas type 2 shows higher preference for 2-oxoglutarate and oxaloacetate. The type 1

Table 3. *Properties of aromatic amino acid transaminases*

For further details see the text.

	Type 1 enzyme		Type 2 enzyme	
	Kynurenine-pyruvate (oxaloacetate) transaminase	Aromatic amino acids-pyruvate (oxaloacetate) transaminases	Kynurenine-2- oxoglutarate (oxaloacetate) transaminase	Aromatic amino acids-2-oxoglutarate (oxaloacetate) transaminases
(NH ₄) ₂ SO ₄ -saturated fraction	30-60%	30-60%	60-100%	60-100%
Adsorption on calcium phosphate gel	Not adsorbed	Not adsorbed	Adsorbed	Adsorbed
Adsorption on DEAE-cellulose	Adsorbed	Adsorbed	Not adsorbed	Not adsorbed
Effective amino acceptors	Pyruvate, oxaloacetate	Pyruvate, oxaloacetate	2-Oxoglutarate, oxaloacetate	2-Oxoglutarate, oxaloacetate
Purification factor	About 23-fold	0.5-3.8-fold	About 18-fold	0.1-2.0-fold
Yield	About 60%	0.07-0.5%	About 8%	0.2-2.6%

enzyme was partially purified by preparation A: it was mainly fractionated in the 30-60%-saturated-(NH₄)₂SO₄ precipitates, not absorbed on calcium phosphate gel but adsorbed on DEAE-cellulose. In contrast, the type 2 enzyme was partially purified by preparation B: it was mainly recovered in the 60-100%-saturated fraction of (NH₄)₂SO₄, adsorbed on calcium phosphate gel and not adsorbed on DEAE-cellulose.

On the basis of the difference of the purification ratio, factors and yields, it is suggested that each type contains at least two kinds of enzymes, specific for L-kynurenine and other amino acids respectively. It is of course entirely possible that each type consists of several enzymes.

Generally, aromatic amino acid transaminases in mammalian liver and kidney show higher preference for 2-oxoglutarate than for pyruvate and oxaloacetate (Canellakis & Cohen, 1956; Lin & Civen, 1959; Ueno *et al.*, 1963; Wurzman & Larin, 1968). Tangen *et al.* (1965) reported that rat brain extract contained at least three aromatic amino acid transaminases. Transaminase I had a high affinity for 3,4-dihydroxyphenylalanine, transaminase II an affinity for phenylalanine and tyrosine and transaminase III an affinity for tryptophan and 5-hydroxytryptophan. The preferred amino acceptor of these enzymes was 2-oxoglutarate. The α -oxo acid substrate specificity of intestinal aromatic amino acid transaminases found by us appears to differ from that of transaminases in other mammalian tissues.

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