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Purification and Characterization of Kynurenine–2-Oxoglutarate Aminotransferase from the Liver, Brain and Small Intestine of Rats

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1. Kynurenine–2-oxoglutarate aminotransferase (isoenzyme 1) was purified to homogeneity from the liver, brain and small intestine of rats by the same procedure. The three enzyme preparations had nearly identical pH optima, substrate specificities and molecular weights. Isoenzyme 1 was active with 2-oxoglutarate but not with pyruvate as amino acceptor, and utilized a wide range of amino acids as amino donors. Amino acids were effective in the following order of activity: L-aspartate ≫ L-tyrosine > L-phenylalanine > L-tryptophan > 5-hydroxy-L-tryptophan > L-kynurenine. The molecular weight was approximately 88000 as determined by sucrose-density-gradient centrifugation. The pH optimum was between 8.0 and 8.5. On the basis of substrate specificity, substrate inhibition, subcellular distribution and polyacrylamide-disc-gel electrophoresis, it is suggested that liver, brain and small intestinal kynurenine–2-oxoglutarate aminotransferase (isoenzyme 1) is identical with mitochondrial tyrosine–2-oxoglutarate aminotransferase and also with mitochondrial aspartate–2-oxoglutarate aminotransferase. 2. An additional kynurenine–2-oxoglutarate aminotransferase (isoenzyme 2) was purified from the liver. This enzyme was specific for 2-oxoglutarate and L-kynurenine. Sucrose-density-gradient centrifugation gave a molecular weight of approximately 100000. The pH optimum was between 6.0 and 6.5. This enzyme was not detected in the brain or small intestine.

It is well known that kynurenine–2-oxoglutarate aminotransferase [L-kynurenine–2-oxoglutarate aminotransferase (cyclizing), EC 2.6.1.7] is present in mammalian liver and kidney (Mason, 1954, 1957; Ogasawara *et al.*, 1962; Ueno *et al.*, 1963; Nakatani *et al.*, 1974). The enzyme catalyses the formation of kynurenate from kynurenine, and shows a greater preference for 2-oxoglutarate than for pyruvate and other amino acceptors. We have reported the presence of kynurenine–2-oxoglutarate aminotransferase activity in the small intestine (Noguchi *et al.*, 1973; Nakamura *et al.*, 1973) and brain of rats (Minatogawa *et al.*, 1973). However, previous investigations of kynurenine–2-oxoglutarate aminotransferase have not involved extensive purification or detailed analysis of the enzymic properties. The present report describes the purification and characterization of kynurenine–2-oxoglutarate aminotransferases from the liver, brain and small intestine of rats.

Experimental

Materials

L-Kynurenine was prepared by ozonolysis of L-tryptophan by the method of Warnell & Berg (1954). L-Tyrosine, L-phenylalanine, L-tryptophan and L-aspartate were purchased from Tanabe Amino Acid Foundation, Osaka, Japan, and 2-oxoglutarate and pyridoxal 5′-phosphate from Nakarai Chemicals, Kyoto, Japan. 5-Hydroxy-L-tryptophan was obtained from Calbiochem, San Diego, Calif., U.S.A., and DEAE-cellulose and hydroxyapatite from Seikagaku Kogyo Co. Ltd., Tokyo, Japan. Sephadex C-25 and Sephadex G-100 were products from Pharmacia Fine Chemicals, Uppsala, Sweden. 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 Roy & Price (1959). Other chemicals were of the purest quality available.

Methods

Enzyme assays. Enzyme assays were carried out at 37°C.

Kynurenine–2-oxoglutarate aminotransferase was assayed by measuring the amount of kynurenate formed by the Dowex 1-column method (Noguchi *et al.*, 1973; Nakamura *et al.*, 1973). The reaction mixture contained, in a final volume of 0.8 ml, 20 mM-L-kynurenine, 8 mM-2-oxoglutarate and 40 μM-pyridoxal 5′-phosphate in 0.1 M-potassium phosphate

buffer, pH 8.0, with isoenzyme 1 or pH 6.5 with isoenzyme 2. 2-Oxoglutarate was omitted from the blank. Enzyme activity was calculated from the linear parts of the progress curves. A unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of 1 μmol of kynurenate/h under the above conditions. Specific activity is defined as enzyme units per mg of protein.

Tyrosine, phenylalanine, tryptophan and 5-hydroxytryptophan aminotransferase assays were based on the arsenate-catalysed formation of aromatic 2-oxo acid–enol–borate complexes that show characteristic absorption spectra in the 300 nm region (George *et al.*, 1967; Oja, 1968). The reaction mixtures contained, in a final volume of 0.8 ml, 6.5 mM-L-amino acid, 8 mM-2-oxoglutarate and 40 μM-pyridoxal 5′-phosphate in 0.1 M-potassium phosphate buffer, pH 8.0.

Aspartate–2-oxoglutarate aminotransferase assay was based on the increase in extinction at 280 nm resulting from the formation of oxaloacetate (Nisonoff & Barnes, 1962). Incubation was carried out in a total volume of 3.0 ml containing 0.1 M-potassium phosphate buffer, pH 8.0, 6.5 mM-L-aspartate, 8 mM-2-oxoglutarate and 40 μM-pyridoxal 5′-phosphate.

Protein determination. The protein content was determined by the biuret method (Gornall *et al.*, 1949) in crude extracts or by the method of Lowry *et al.* (1951) in purified preparations with bovine serum albumin as the standard.

Polyacrylamide-disc-gel electrophoresis. Electrophoresis was carried out at 4°C for 90 min with a current of 2 mA/column by the following two methods. The purified enzyme (about 15 μg) was applied in 20% (w/v) sucrose solution and protein was stained with Amido Black.

One method used a continuous 5% (w/v) total acrylamide-gel column (0.5 cm × 8 cm) containing 0.25% bisacrylamide by the method of Barisas (1974). The running buffer was 0.025 M-Tris–acetate buffer, pH 6.0. Crystal Violet was used as a tracking dye. For enzyme assay immediately after electrophoresis the gels were cut into 2 mm slices and these were added individually to the assay medium as enzyme source.

A second method used a continuous 7% (w/v) total acrylamide-gel column (0.5 cm × 8 cm) containing 0.184% bisacrylamide by the original procedures of Davis (1964). A Tris–glycine buffer, pH 8.9 (11.0 g of Tris and 14.4 g of glycine per l) was used as the running buffer and Bromophenol Blue as a tracking dye.

Sucrose-density-gradient centrifugation. Approximate molecular-weight determinations were made on sucrose gradients (5–20%, w/v) by the method of Martin & Ames (1961). Centrifugation was carried out at 39000 rev./min with a RP 50 swinging-bucket rotor in a Hitachi 65P (Hitachi, Tokyo, Japan) at 4°C for 15 h. Fractions (3 drops) were collected by upward displacement of the centrifuge tube contents with a Mitsumi Gradienter SJ-1300UD (Mitsumi Scientific Industry Co., Tokyo, Japan). Bovine catalase was used as internal standard with an assumed $s_{20,w}$ = 11.3 S.

Results

Separation of kynurenine–2-oxoglutarate aminotransferases by DEAE-cellulose chromatography

Donryu strain male rats (60–80 g) were killed by decapitation, and livers, brains and small intestines were removed. Six livers and 70 brains were rinsed with ice-cold 5 mM-potassium phosphate buffer, pH 7.5, and weighed. Small intestines (32) were cut longitudinally, washed with tap water and weighed. Each tissue was homogenized in a Waring blender for 2 min with 2 vol. of ice-cold 5 mM-potassium phosphate buffer, pH 7.5. After three cycles of freezing and thawing, each homogenate was centrifuged at 100000 *g* for 30 min and each resulting supernatant was diluted with 5 mM-potassium phosphate buffer, pH 7.5, to a total volume of 340 ml. Subsequent procedures were carried out at about 4°C. Each supernatant solution was applied to a separate column (5.5 cm × 17 cm) of DEAE-cellulose equilibrated with 5 mM-potassium phosphate buffer, pH 7.5, and elution was performed with successive 500 ml volumes of 5, 15 and 250 mM-potassium phosphate buffer, pH 7.5. The flow rate was adjusted to about 200 ml/h. Fractions (20 ml) were collected and monitored for kynurenine–2-oxoglutarate aminotransferase activity. DEAE-cellulose column separations are shown in Fig. 1. Kynurenine aminotransferase activities of the liver, brain and small intestinal extracts appeared in the non-adsorbed fraction (the first effluent and 5 mM buffer fraction). However, chromatography of the liver extract resulted in the appearance of additional kynurenine–2-oxoglutarate aminotransferase activity in the adsorbed fraction (15 mM buffer fraction).

The non-adsorbed kynurenine aminotransferase is referred to hereafter as isoenzyme 1 and the adsorbed kynurenine aminotransferase as isoenzyme 2.

Purification of isoenzyme 1 from the liver, brain and small intestine

Liver, brain and small intestinal isoenzyme 1 were purified to homogeneity by the same procedure. All procedures were carried out at 0–4°C. Potassium phosphate buffer, pH 7.5, containing 200 μM-pyridoxal 5′-phosphate was used throughout.

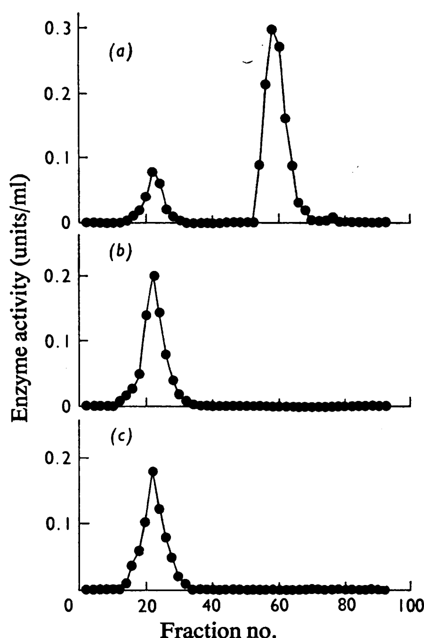

Fig. 1. *Chromatography of rat liver, brain and small intestinal extract on DEAE-cellulose*

The extract (340ml), (*a*) from six rat livers, (*b*) from 70 rat brains or (*c*) from 32 rat small intestines was applied to a column (5.5cm×17cm) of DEAE-cellulose and eluted as described in the text. The fraction size was 20ml. Fractions 18–42 were eluted by 5mM-potassium phosphate buffer, pH7.5, fractions 43–67 by 15mM-potassium phosphate buffer, pH7.5, and fractions 68–92 by 250 mM-potassium phosphate buffer, pH7.5. Kynurenine–2-oxoglutarate aminotransferase was assayed at pH8.0 by the method described in the Experimental section.

Heat treatment. Active DEAE-cellulose isoenzyme 1 fractions (Fig. 1) were pooled, brought rapidly to 60°C and maintained at this temperature, with constant stirring, for 15s, after which they were quickly chilled in a cold-water bath. The resulting precipitate was removed by centrifugation.

$(NH_4)_2SO_4$ fractionation. Solid $(NH_4)_2SO_4$ was added with gentle stirring to the supernatant from the previous step to 60% saturation. After being left for 30min, the precipitate was removed by centrifugation and discarded. $(NH_4)_2SO_4$ was added to the supernatant to 95% saturation, and the precipitate was collected by centrifugation after 30min. The precipitate was dissolved in a minimum volume of 5mM buffer and desalted by dialysis against the same buffer overnight.

CM-Sephadex C-25 chromatography. The non-diffusible solution was placed on a column (3.0cm× 30cm) of Sephadex C-25 equilibrated with 5mM buffer. The column was eluted in sequence with 100ml portions of 5 and then 15mM buffer. The flow rate was adjusted to 130ml/h and fractions (4ml) were collected. The eluate was assayed for isoenzyme 1 activity that appeared in the 15mM buffer fraction. Active fractions were pooled and concentrated in Diaflo ultrafiltration cells with a G-10T membrane.

Hydroxyapatite chromatography. The concentrated enzyme solution was applied to a hydroxyapatite column (1.3cm×5cm) previously equilibrated with 15mM buffer. The column was washed in sequence with 50ml portions of 15 and 50mM buffer, and then isoenzyme 1 was eluted with 100mM buffer. The flow rate and fraction size were 90ml/h and 4ml respectively. The active fractions were pooled and concentrated by ultrafiltration.

Sephadex G-100 gel filtration. The concentrated enzyme solution was added to a column (2.5cm× 100cm) of Sephadex G-100 equilibrated with 50mM buffer. The column was eluted with the same buffer at a flow rate of 25ml/h. The effluent was collected in 3.6ml fractions and monitored for enzyme activity. The active fractions were pooled and concentrated by ultrafiltration.

Results of typical purifications of isoenzyme 1 from the liver, brain and small intestine are shown in Tables 1, 2 and 3 respectively. Isoenzyme 1 was purified about 217-fold over the liver extract, 370-fold over the brain extract and 724-fold over the intestinal extract. Liver isoenzyme 1 activity especially was recovered in very low yield, owing to the removal of an additional liver kynurenine–2-oxoglutarate aminotransferase (isoenzyme 2) by DEAE-cellulose chromatography (Fig. 1). All three enzyme preparations were of nearly identical specific activity, suggesting the homogeneity and identity of these enzyme preparations.

Purification of liver isoenzyme 2

All procedures were carried out at 0–4°C. Potassium phosphate buffer, pH7.5, containing 200μM-pyridoxal 5′-phosphate was used throughout.

Heat treatment. Active DEAE-cellulose isoenzyme 2 fractions (Fig. 1) were pooled. The enzyme solution was brought rapidly to 60°C in the presence of 2-oxoglutarate and 200μM-pyridoxal 5′-phosphate and maintained at this temperature, with constant stirring, for 30s, after which it was quickly chilled in a cold-water bath. After centrifugation, the resulting supernatant was concentrated by ultrafiltration and diluted with distilled water to adjust the buffer concentration to 5mM.

Second DEAE-cellulose chromatography. The concentrated enzyme solution from the previous step was applied to a column (2.5cm×7cm) of DEAE-cellulose equilibrated with 5mM buffer. After

Table 1. *Purification of kynurenine–2-oxoglutarate aminotransferase (isoenzyme 1) from rat liver*

Details of the purification and assay method are given in the text.

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
Extract	2679.0	56.3	0.021	100.0	1.0
DEAE-cellulose	280.5	10.1	0.036	17.9	1.7
Heat treatment	140.3	8.56	0.061	15.2	2.9
$(NH_4)_2SO_4$	65.2	6.39	0.098	11.3	4.7
CM-Sephadex C-25	3.1	1.27	0.410	2.3	19.5
Hydroxyapatite	0.54	0.88	1.63	1.6	77.6
Sephadex G-100	0.12	0.55	4.56	0.98	217.1

Table 2. *Purification of kynurenine–2-oxoglutarate aminotransferase (isoenzyme 1) from rat brain*

Details of the purification and assay method of the enzyme are given in the text.

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
Extract	2254.6	27.1	0.012	100.0	1.0
DEAE-cellulose	296.2	26.1	0.088	96.3	7.3
Heat treatment	203.1	18.7	0.092	69.0	7.7
$(NH_4)_2SO_4$	68.2	18.8	0.276	69.4	23.0
CM-Sephadex C-25	3.48	2.70	0.776	10.0	64.7
Hydroxyapatite	0.66	1.23	1.86	4.5	155.0
Sephadex G-100	0.21	0.93	4.44	3.4	370.0

Table 3. *Purification of kynurenine–2-oxoglutarate aminotransferase (isoenyzme 1) from rat small intestine*

Details of the purification and assay method are given in the text.

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
Extract	3096.0	21.1	0.0068	100.0	1.0
DEAE-cellulose	424.6	22.1	0.052	104.7	7.6
Heat treatment	273.4	17.5	0.064	82.9	9.4
$(NH_4)_2SO_4$	81.3	16.3	0.200	77.3	29.4
CM-Sephadex C-25	5.6	4.61	0.824	21.8	121.2
Hydroxyapatite	1.1	2.93	2.66	13.9	391.2
Sephadex G-100	0.26	1.28	4.92	6.1	723.5

washing with 200ml of 5mM buffer, the column was eluted by a 300ml linear gradient from 5 to 50mM buffer. A flow rate of about 120ml/h was maintained. Fractions (5.0ml) were collected and assayed for isoenzyme 2 activity. The active fractions were pooled and concentrated by ultrafiltration.

Hydroxyapatite chromatography. The concentrated enzyme solution was placed on a column (2.0cm × 7.5cm) of hydroxyapatite previously equilibrated with 20mM buffer. The column was washed with 100ml portions of 20 and 50mM buffer, at a flow rate of 70ml/h and then the enzyme was eluted with 100mM buffer. The active fractions were pooled, concentrated by ultrafiltration and diluted with distilled water to adjust the buffer concentration to 5mM.

CM-Sephadex C-25 chromatography. The concentrated enzyme solution was added to a column (2.5cm × 25cm) of Sephadex C-25 previously equilibrated with 5mM buffer. The enzyme was eluted with the same buffer at a flow rate of 100ml/h. The active fractions were pooled and concentrated by ultrafiltration.

Sephadex G-100 gel filtration. The concentrated enzyme solution was placed on a column (2.5cm × 100cm) of Sephadex G-100 previously equilibrated

Table 4. *Purification of kynurenine-2-oxoglutarate aminotransferase (isoenzyme 2) from rat liver*

Details of the purification and assay method of the enzyme are given in the text.

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
Extract	2679.0	109.8	0.041	100.0	1.0
First DEAE-cellulose	179.2	68.1	0.38	62.0	9.3
Heat treatment	121.0	61.7	0.51	56.2	12.4
Second DEAE-cellulose	62.1	49.7	0.80	45.3	19.5
Hydroxyapatite	24.3	29.4	1.21	26.8	29.5
CM-Sephadex C-25	11.2	25.1	2.24	22.9	54.6
Sephadex G-100	2.2	17.2	7.80	15.7	190.2

with 50mM buffer. The column was eluted with the same buffer at a flow rate of 25ml/h. Fractions (3.6ml) were collected and assayed for isoenzyme 2 activity.

A typical purification procedure is shown in Table 4. Purification was about 190-fold and yield about 16%.

Criteria of purity

On Sephadex G-100 columns (the last step of purification), the purified isoenzyme 1 from the liver, brain and small intestine was eluted as a single symmetrical peak with protein and kynurenine aminotransferase activity coincident. Sephadex G-100 gel filtration of the purified liver isoenzyme 2 gave a single symmetrical peak with protein and enzyme activity coincident.

The sedimentation pattern of isoenzyme 1 in a sucrose density gradient (for molecular-weight determinations) was a single activity peak for enzyme preparation from any of the three tissues. Also liver isoenzymes 2 yielded a single activity peak under the same conditions.

On polyacrylamide-disc-gel electrophoresis at pH6.0 in 5% (w/v) gels, the purified isoenzyme 1 from the liver, brain or small intestine moved toward the cathode with the same mobility as a single protein band, and this coincided with all the measured aminotransferase activities, i.e. kynurenine, tyrosine or aspartate with 2-oxoglutarate. The activity ratio of isoenzyme 1 towards these amino acids was unchanged after electrophoresis. On electrophoresis at pH8.3 in 5% gels isoenzyme 1 remained as a single protein band at the starting point, as did activities toward all three amino donors. Electrophoresis of purified liver isoenzyme 2 at pH8.9 in 7% (w/v) gel did not give a single protein band and kynurenine aminotransferase activity was found to move toward the anode.

Properties of isoenzyme 1 and isoenzyme 2

Liver, brain and small intestinal isoenzyme 1 preparations had nearly identical pH optima and molecular weights, and showed similar substrate specificities. Marked differences were observed with liver isoenzyme 2.

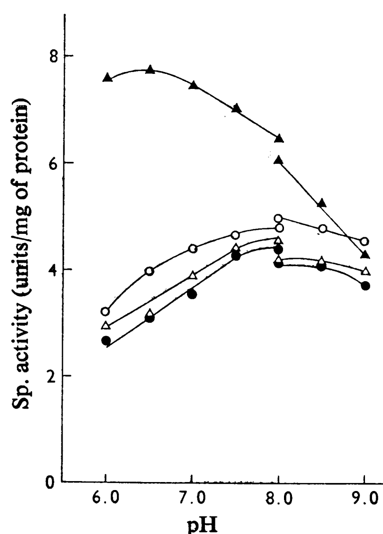

Fig. 2. *Effect of pH on kynurenine-2-oxoglutarate aminotransferase (isoenzymes 1 and 2)*

Assay conditions were as described in the Experimental section, except that potassium phosphate buffer was used at pH6.0–8.0 and Tris–HCl buffer at pH8.0–9.0. Isoenzyme 1 activity of the brain (●), small intestine (○) and liver (△), and isoenzyme 2 activity of the liver (▲) are shown.

pH optimum. The relationship between pH and the enzymic activity was studied and the results are presented in Fig. 2. The purified isoenzyme 1 had a pH optimum between 8.0 and 8.5, and isoenzyme 2 between 6.0 and 6.5.

Determination of molecular weight. Sucrose-density-gradient centrifugation gave molecular weights of about 88000 and 100000 for isoenzyme 1

Table 5. *Specificity of kynurenine–2-oxoglutarate aminotransferase (isoenzymes* 1 *and* 2) *for various* L-*amino acids*

Enzyme assays were at pH 8.0, except for kynurenine aminotransferase activity of liver isoenzyme 2 which was assayed at pH 6.5. The final concentration of L-amino acids was 6.5 mM. For further details see the text. Relative activity signifies transamination rate compared with that for kynurenine.

	Relative activity			
	Kynurenine–2-oxoglutarate aminotransferase 1			Kynurenine–2-oxoglutarate aminotransferase 2
L-Amino acid	Brain	Small intestine	Liver	Liver
Aspartate	671.0	693.0	687.0	0
Tyrosine	6.7	7.0	7.2	0
Phenylalanine	2.3	2.5	2.6	0
Tryptophan	1.7	2.0	1.9	0
5-Hydroxytryptophan	1.2	1.4	1.3	0
Kynurenine	1.0	1.0	1.0	1.0

Table 6. *Inhibition of kynurenine–2-oxoglutarate aminotransferase (isoenzymes* 1 *and* 2) *by* L-*aspartate and aromatic* L-*amino acids*

L-Kynurenine concentration was 6.5 mM. L-Aspartate and aromatic amino acids were 5 mM and added to the preincubation medium along with the enzyme for 5 min before the reaction was initiated with L-kynurenine. Isoenzyme 1 activity was assayed at pH 8.0 and isoenzyme 2 activity at pH 6.5 as described in the text.

	Inhibition (%)			
	Kynurenine–2-oxoglutarate aminotransferase 1			Kynurenine–2-oxoglutarate aminotransferase 2
Addition	Brain	Small intestine	Liver	Liver
Aspartate	96	93	95	0
Tyrosine	78	60	76	2.0
Phenylalanine	62	63	69	1.9

and isoenzyme 2 respectively, with bovine catalase ($s_{20,w}$ = 11.3S) as internal standard.

Substrate specificity. With L-kynurenine, L-tyrosine and L-phenylalanine, isoenzyme 1 of the liver, brain and small intestine was specific for 2-oxoglutarate as amino acceptor exhibiting no measurable activity with pyruvate. The relative initial velocities of isoenzyme 1 and isoenzyme 2 with various amino acids (6.5 mM) were determined by using 2-oxoglutarate (5 mM) as amino acceptor. Results are summarized in Table 5. Isoenzyme 1 utilized a wide range of amino acids. These amino acids were effective as amino donors in the following order of activity: L-asparate ≫ L-tyrosine > L-phenylalanine > L-tryptophan > 5-hydroxy-L-tryptophan > L-kynurenine. These results suggest that isoenzyme 1 may be identical with tyrosine–2-oxoglutarate aminotransferase (tyrosine–2-oxoglutarate aminotransferase, EC 2.6.1.5) and also with aspartate aminotransferase (L-aspartate–2-oxoglutarate aminotransferase, EC 2.6.1.1). Further evidence was obtained with the addition of 5 mM each of L-aspartate and various aromatic amino acids to the complete system of the isoenzyme 1 reaction. As shown in Table 6, L-aspartate, L-tyrosine and L-phenylalanine all strongly inhibited the catalysis by isoenzyme 1 preparations from the liver, brain and small intestine on transamination between kynurenine and 2-oxoglutarate.

Liver isoenzyme 2 was specific for 2-oxoglutarate as amino acceptor showing no activity with pyruvate. But it was also specific for L-kynurenine; little or no activity was observed with L-tyrosine, L-phenylalanine, L-tryptophan, 5-hydroxy-L-tryptophan and L-aspartate.

Subcellular distribution of kynurenine–2-oxoglutarate aminotransferase activity

Liver kynurenine–2-oxoglutarate aminotransferase. We have reported that 68.3% of kynurenine–2-oxoglutarate aminotransferase was in the mitochondrial fraction and 12.3% in the supernatant fraction (Nakatani *et al.*, 1974). The supernatant fraction was prepared from 5g of rat liver by the method of de Duve *et al.* (1955) and was applied to a column (2.5cm×5cm) of DEAE-cellulose equilibrated with 5mM-potassium phosphate buffer, pH7.5. When elution was performed with successive 50ml volumes of 5, 15, 50 and 250mM of the same buffer, kynurenine–2-oxoglutarate aminotransferase activity was detected in the adsorbed isoenzyme 2 (15mM buffer) fraction but not in the non-adsorbed isoenzyme 1 (first effluent and 5mM buffer) fraction.

Brain kynurenine–2-oxoglutarate aminotransferase. Subcellular fractionation of rat brain was carried out by the method of Lapetina *et al.* (1967). The distribution of the enzyme activity was as follows: nuclear fraction, 13.9%; crude mitochondrial fraction, 79.4%; microsomal fraction, 1.7%; supernatant fraction, 2.1%. The total recovery of kynurenine–2-oxoglutarate aminotransferase was 97.1%. The distribution of the enzyme corresponded well with that of brain tyrosine–2-oxoglutarate aminotransferase activity described by Miller & Litwack (1969).

Small intestinal kynurenine–2-oxoglutarate aminotransferase. Subcellular fractionation of rat small intestine was performed as described by Noguchi *et al.* (1970). The distribution was as follows: nuclear fraction, 34.9%; mitochondrial fraction, 54.7%; microsomal fraction, 0%; supernatant fraction, 3.1%. The total recovery of kynurenine–2-oxoglutarate aminotransferase activity was 92.7%. This distribution corresponded well to that of monoamine oxidase [amine–oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4] described by Noguchi *et al.* (1970).

The results suggest that isoenzyme 1 is localized largely in the mitochondrial fraction of the liver, brain and small intestine.

Discussion

Mammalian liver and kidney kynurenine–2-oxoglutarate aminotransferases show a greater preference for 2-oxoglutarate than for pyruvate or other amino acceptors, and have a pH optimum near 6.0 (Mason, 1954, 1957; Ogasawara *et al.*, 1962; Ueno *et al.*, 1963; Okamoto & Hayaishi, 1970; Nakatani *et al.*, 1974). Recently our group reported the presence of kynurenine–2-oxoglutarate aminotransferase activity in the brain (Minatogawa *et al.*, 1973) and small intestine (Noguchi *et al.*, 1973; Nakamura *et al.*, 1973) of rats with a pH optimum near 8.0. In the present study, kynurenine–2-oxoglutarate aminotransferase (isoenzyme 1) with a pH optimum between 8.0 and 8.5 was purified to homogeneity and characterized from the brain, small intestine and liver of rats. This enzyme was active with 2-oxoglutarate but not with pyruvate; it utilized a wide range of amino acids as amino donors. All the amino acids tested donated their amino group more effectively than kynurenine, and aspartate was the most effective.

Miller & Litwack (1971) demonstrated that hepatic mitochondrial tyrosine–2-oxoglutarate aminotransferase was identical with hepatic mitochondrial asparate–2-oxoglutarate aminotransferase. Subsequently, Rosenberg *et al.* (1974) reported that heart tyrosine–2-oxoglutarate aminotransferase and hepatic mitochondrial aspartate-2-oxoglutarate aminotransferase were similar with regard to kinetics and specificity. Isoenzyme 1 showed a similar substrate specificity and molecular weight (approx. 90000) to the hepatic mitochondrial asparate–2-oxoglutarate aminotransferase described by Miller & Litwack (1971).

On the basis of our observations on substrate specificity, substrate inhibition, polyacrylamide-gel electrophoresis, subcellular distribution and the report by Miller & Litwack (1971), it is suggested that isoenzyme 1 is identical with mitochondrial tyrosine–2-oxoglutarate aminotransferase and also with mitochondrial aspartate–2-oxoglutarate aminotransferase.

Moreover we have applied the isoelectric-focusing technique with mitochondrial extracts of rat liver, brain and small intestine and detected a single aspartate–2-oxoglutarate aminotransferase activity peak with isoelectric point at pH9.4 that has also kynurenine– and tyrosine–2-oxoglutarate aminotransferase activities; and isoenzyme 1 from these tissues purified in the present study in each case has the same isoelectric point (unpublished work). This observation and the present data suggest that isoenzyme 1 is the major mitochondrial aspartate–2-oxoglutarate aminotransferase of the liver, brain and small intestine.

On the other hand, an additional kynurenine–2-oxoglutarate aminotransferase (isoenzyme 2) with a pH optimum between 6.0 and 6.5 was purified and characterized from the liver in the present study. Isoenzyme 2 is specific for 2-oxoglutarate and kynurenine, and in other respects also shows different properties from isoenzyme 1. On the basis of pH optimum and substrate specificity, isoenzyme 2 appears to be identical with kynurenine–2-oxoglutarate aminotransferase previously described in mammalian liver and kidney. DEAE-cellulose chromatography (Fig. 1) indicates that the brain and small intestinal concentration of isoenzyme 2 is very small or zero. Our present data suggest the need to

investigate the role of aspartate–2-oxoglutarate aminotransferase in tryptophan metabolism of brain and small intestine.

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