Subcellular Distribution and Properties of Kynurenine Transaminase in Rat Liver

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Kynurenine transaminase activity in rat liver was found in both the mitochondrial and supernatant fractions. The mitochondrial and supernatant fractions contained (a) kynurenine-pyruvate transaminase, which showed a preference for pyruvate as amino acceptor and a pH optimum between 8.0 and 8.5, and (b) kynurenine- α -oxoglutarate transaminase, with a preference for α -oxoglutarate and a pH optimum between 6.0 and 6.5. Possible roles of these enzymes in tryptophan metabolism in the liver are discussed.

In mammalian liver L-tryptophan is metabolized in the cytosol to the intermediate *N*'-formyl-L-kynurenine by tryptophan pyrrolase [tryptophan 2,3-dioxygenase, L-tryptophan-oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11, formerly EC 1.13.1.12], and thence to L-kynurenine by kynurenine formamidase (aryl-formylamine amido-hydrolase, EC 3.5.1.9) (Mehler & Knox, 1950; Iria, 1954; Knox, 1955; Greengard & Feigelson, 1962). Subsequently, L-kynurenine is hydroxylated by kynurenine 3-hydroxylase [kynurenine 3-mono-oxygenase, L-kynurenine, NADPH-oxygen oxidoreductase (3-hydroxylating), EC 1.14.13.9, formerly EC 1.14.1.2] in the mitochondria to form 3-hydroxy-L-kynurenine (Okamoto *et al.*, 1967; Okamoto & Hayaishi, 1969). The L-kynurenine and 3-hydroxy-L-kynurenine thus formed may undergo (a) hydrolytic cleavage by kynureninase (L-kynurenine hydrolase, EC 3.7.1.3) (Knox, 1953; Wiss & Weber, 1956) to form anthranilate and 3-hydroxyanthranilate or (b) transamination by kynurenine transaminase [kynurenine aminotransferase, L-kynurenine-2-oxoglutarate aminotransferase (cyclizing), EC 2.6.1.7] (Ueno *et al.*, 1963; Hagino *et al.*, 1965) to produce kynurenate and xanthurenate. Wiss (1953) reported that kynurenine transaminase and kynureninase were associated with the mitochondrial and soluble fractions of swine liver respectively. Later, Ogasawara *et al.* (1962) and Hagino *et al.* (1965) reported that kynurenine- α -oxoglutarate transaminase was distributed in both the mitochondrial and supernatant fractions of rat kidney and liver, but that kynureninase was present only in the supernatant fraction. However, Okamoto & Hayaishi (1970) have observed that kynurenine- α -oxoglutarate transaminase is localized strictly in rat liver mitochondria, whereas kynureninase is in the supernatant fraction. The present study showed that kynurenine transaminase is localized in the mitochondrial and

supernatant fractions of rat liver and that both of these fractions contain kynurenine-pyruvate transaminase and - α -oxoglutarate transaminase.

Experimental

Materials

L-Kynurenine was prepared by ozonolysis of L-tryptophan by the method of Warnell & Berg (1954). α -Oxoglutarate, sodium pyruvate, acetaldehyde and pyridoxal 5'-phosphate were purchased from Nakarai Chemicals Co., Kyoto, Japan, and NADH and 3-hydroxy-L-kynurenine from Calbiochem, San Diego, Calif., U.S.A. DEAE-cellulose and hydroxyapatite were obtained from Seikagaku Kogyo Co., Tokyo, Japan. Other chemicals were of the purest quality available.

Methods

Enzyme assays. (a) Kynurenine transaminase. This was in principle assayed at 37°C by the method described previously (Noguchi *et al.*, 1973; Nakamura *et al.*, 1973), in a total volume of 1 ml, containing (final concentrations) 0.1 M-potassium phosphate buffer, 8 mM-L-kynurenine, 5 μ M-pyridoxal phosphate and 8 mM- α -oxo acid. The α -oxo acid was omitted from the blank. Kynurenine- α -oxoglutarate transaminase activity was assayed at pH 6.5 and kynurenine-pyruvate transaminase activity at pH 8.0. Protein was determined by the biuret method (Chance & Redfearn, 1961) with crystalline bovine serum albumin (moisture content approx. 4%, w/w) as the standard. Enzyme activity was expressed as nmol of kynurenate produced/h and was taken from the linear parts of the reaction curves.

(b) Alcohol dehydrogenase (alcohol-NAD⁺ oxidoreductase, EC 1.1.1.1). This was assayed at 37°C as

described by Büttner (1965). The activity was measured by following the oxidation of NADH spectrophotometrically with acetaldehyde as the substrate. The reaction mixture contained (final concentrations) 50mM-potassium phosphate buffer, pH 7.5, 0.15mM-NADH and 8mM-acetaldehyde. The reaction was started by the addition of acetaldehyde.

(c) Monoamine oxidase [amine-oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4]. This was assayed at 23°C as described by Schnaitman *et al.* (1967), by following the formation of benzaldehyde from benzylamine spectrophotometrically at 250nm in an assay system containing (final concentrations) 50mM-potassium phosphate buffer, pH 7.5, and 2.5mM-benzylamine.

Subcellular fractionation. Donryu-strain male rats (80–100g each) were used. Subcellular fractions of rat liver were obtained by the method of de Duve *et al.* (1955).

Chromatography. All procedures were carried out at about 4°C. Glass-distilled water and potassium phosphate buffer, pH 7.5, were used throughout.

(a) Hydroxyapatite chromatography. The mitochondrial and supernatant fractions were prepared from 5g of rat liver by the method of de Duve *et al.* (1955). The supernatant fraction (450mg of protein) was applied to a column (3.5cm × 7cm) of hydroxyapatite equilibrated with 5mM buffer. Non-adsorbed protein was eluted with 150ml of 5mM buffer, and then the enzymes were eluted with 100ml of 50mM buffer followed by 100ml of 150mM buffer. The flow rate was 150ml/h and the fraction size was 10ml.

The mitochondrial fraction was suspended in 50ml of 5mM buffer, frozen and thawed three times and then centrifuged at 100000g for 30min. The resulting soluble mitochondrial fraction (120mg of protein) was subjected to hydroxyapatite chromatography by the same method as described above.

(b) DEAE-cellulose chromatography. The supernatant and soluble mitochondrial fractions (460mg and 118mg of protein respectively) of rat liver were applied to separate columns (3.5cm × 7cm) of DEAE-cellulose that had been equilibrated with 5mM buffer, and elution was performed with successive 50ml volumes of 5mM, 30mM, 50mM, 70mM and 100mM buffer. The flow rate was 100ml/h. Each eluate was adjusted to 100% saturation with (NH₄)₂SO₄. After centrifugation at 20000g for 30min, each precipitate was dissolved in a small volume of 5mM buffer and dialysed overnight against 6litres of the same buffer.

Results

Subcellular distribution of kynurenine- α -oxoglutarate transaminase

Table 1 shows the distribution of kynurenine transaminase activity in the subcellular fractions of rat liver. When the activity was assayed at pH 6.5 with α -oxoglutarate as amino acceptor, kynurenine transaminase showed the highest specific activity and most of the total activity was in the mitochondrial fraction. The activities in the lysosomal and supernatant fractions were also appreciable. Specific activities at pH 8.0 showed a similar distribution, but those at pH 6.5 were all higher.

Subcellular distribution of kynurenine-pyruvate transaminase

When assays were performed at pH 8.0 with pyruvate as amino acceptor, most of the activity was found in the mitochondrial and supernatant fractions (Table 1). The subcellular distribution at pH 6.5 was similar to that at pH 8.0. The specific activities at pH 8.0 were all higher than those at pH 6.5.

Table 1. Intracellular localization of rat liver kynurenine transaminase activities

Experimental conditions are as described in the Experimental section. All specific activities are expressed as nmol/h per mg of protein.

Fraction	Protein (mg/g of wet tissue)	Kynurenine- α -oxoglutarate transaminase				Kynurenine-pyruvate transaminase			
		At pH 6.5		At pH 8.0		At pH 6.5		At pH 8.0	
		Specific activity	% of total activity	Specific activity	% of total activity	Specific activity	% of total activity	Specific activity	% of total activity
Homogenate	330.0	62.1	100	37.9	100	12.2	100	25.4	100
Nuclei	51.8	25.2	6.4	17.2	7.2	4.4	5.7	7.3	4.5
Mitochondria	80.0	175.0	68.3	90.1	57.6	18.8	37.4	42.5	40.6
Lysosomes	31.0	42.0	10.5	31.3	12.8	5.2	6.6	10.2	6.2
Microsomes	38.5	7.0	1.3	3.9	1.2	1.4	1.3	2.3	1.1
Supernatant	92.5	27.3	12.3	18.1	13.4	13.5	31.0	24.5	27.0
Recovery	313.8	—	98.8	—	92.2	—	82.0	—	79.4

Fig. 1. Chromatography of the supernatant and soluble mitochondrial fractions on hydroxyapatite

The supernatant fraction (a) and soluble mitochondrial fraction (b) were applied to separate hydroxyapatite columns ($3.5\text{cm} \times 7\text{cm}$) and eluted by the method described in the Experimental section. Fractions 1–10 were eluted by 50mM-potassium phosphate buffer, pH 7.5, and fractions 11–20 by 150mM-potassium phosphate buffer, pH 7.5. The fraction size was 10ml. Kynurenine-pyruvate transaminase activity (●) was assayed at pH 8.0 and kynurenine- α -oxoglutarate transaminase activity (○) at pH 6.5 as described in the text.

Subcellular distribution of marker enzymes

Monoamine oxidase was used as the mitochondrial marker enzyme and alcohol dehydrogenase as the cytosol marker enzyme (Tottmar *et al.*, 1973). Recoveries of monoamine oxidase and alcohol dehydrogenase were 101.9 and 99.4% respectively. The percentage distribution of monoamine oxidase was as follows: nuclear fraction, 14.1%; mitochondrial fraction, 62.0%; lysosomal fraction, 17.4%; microsomal fraction, 7.1%; supernatant fraction, 1.3%. Most (95.2%) of the alcohol dehydrogenase was in the supernatant fraction. No activity was detected in the mitochondrial fraction

and very little activity was found in the other particulate fractions. The distribution of the two marker enzymes corresponded well to the results obtained by Tottmar *et al.* (1973).

Hydroxyapatite chromatography

Kynurenine-pyruvate transaminase and kynurenine- α -oxoglutarate transaminase activities were completely separated by hydroxyapatite chromatography, as shown in Fig. 1. The supernatant and mitochondrial kynurenine-pyruvate transaminases were eluted by 50mM-potassium phosphate buffer,

Fig. 2. Chromatography of the supernatant and soluble mitochondrial fractions on DEAE-cellulose

The supernatant fraction (a) and soluble mitochondrial fraction (b) were applied to separate DEAE-cellulose columns (3.5cm×7cm) and eluted by the method described in the Experimental section. Kynurenine-pyruvate transaminase activity (■) was assayed at pH 8.0 and kynurenine-α-oxoglutarate transaminase activity (□) at pH 6.5 as described in the text.

pH 7.5, and the peak activity was in fraction 3. Kynurenine-α-oxoglutarate transaminase activities were not detected in this fraction. The supernatant and mitochondrial kynurenine-α-oxoglutarate transaminases were eluted by 150mM-potassium phosphate buffer and the peak activity was in fraction 13. Kynurenine-pyruvate transaminase activities were not detected in this fraction.

DEAE-cellulose chromatography

Fig. 2 shows the distribution of kynurenine-pyruvate transaminase and kynurenine-α-oxoglutarate transaminase activities in the eluate from the

DEAE-cellulose column. The supernatant and mitochondrial kynurenine-α-oxoglutarate transaminase activities were primarily in the 30mM buffer fraction and showed only very low activity for pyruvate as amino acceptor. Kynurenine-pyruvate transaminase activities of both these subcellular fractions were primarily in the 70mM buffer fraction. In this fraction α-oxoglutarate was about 14–33% as effective as pyruvate as amino acceptor.

pH optimum

Fig. 3 shows pH optima for kynurenine-pyruvate transaminase and kynurenine-α-oxoglutarate trans-

Fig. 3. Effect of pH on the mitochondrial and supernatant kynurenine transaminases

In (a) fractions 3 and 13 from the hydroxyapatite columns were used as kynurenine-pyruvate transaminase and kynurenine- α -oxoglutarate transaminase respectively; in (b) the 30mm- and 70mm-potassium phosphate buffer (pH 7.5) fractions from the DEAE-cellulose columns were used as kynurenine-pyruvate transaminase and kynurenine- α -oxoglutarate transaminase respectively. Chromatographic conditions were as described in Figs. 1 and 2. Assay conditions were as described in the text, except that the buffer used at pH 6.0–8.0 was potassium phosphate buffer and that at pH 8.0–9.0 was Tris-HCl buffer. The pH-activity curves for the supernatant kynurenine-pyruvate transaminase (●) and kynurenine- α -oxoglutarate transaminase (○) and for the mitochondrial kynurenine-pyruvate transaminase (▲) and kynurenine- α -oxoglutarate transaminase (△) are shown.

aminases separated by hydroxyapatite chromatography. The supernatant and mitochondrial kynurenine-pyruvate transaminases showed pH optima between 8.0 and 8.5. pH optima for kynurenine- α -oxoglutarate transaminases in both these subcellular fractions were between 6.0 and 6.5. Kynurenine-pyruvate transaminases and kynurenine- α -oxoglutarate transaminases obtained from the DEAE-cellulose column showed the same pH optima as did those from the hydroxyapatite column.

Substrate specificity

Substrate specificities of kynurenine-pyruvate transaminases and kynurenine- α -oxoglutarate transaminases obtained from the hydroxyapatite column

are shown in Table 2. The supernatant and mitochondrial kynurenine-pyruvate transaminases showed a preference for pyruvate as amino acceptor but not for α -oxoglutarate. Kynurenine- α -oxoglutarate transaminases of both these subcellular fractions showed a preference for α -oxoglutarate but not for pyruvate. All of the mitochondrial and supernatant kynurenine transaminases showed similar apparent K_m values (1.5–2.5 mM) for L-kynurenine. Apparent K_m values (0.2–0.3 mM) of the mitochondrial and supernatant kynurenine- α -oxoglutarate transaminases for α -oxoglutarate were lower than those (1.2–1.3 mM) of kynurenine-pyruvate transaminases for pyruvate. The mitochondrial kynurenine- α -oxoglutarate transaminase was found to catalyse the transamination of 3-hydroxy-L-kynurenine at a rate

Table 2. *Substrate specificities of the supernatant and mitochondrial kynurenine transaminases*

In (a), fractions 3 and 13 from the hydroxyapatite columns were used as kynurenine-pyruvate transaminase and kynurenine- α -oxoglutarate transaminase respectively; in (b), the 30mm- and 70mm-potassium phosphate buffer (pH 7.5) fractions from the DEAE-cellulose columns were used as kynurenine-pyruvate transaminase and kynurenine- α -oxoglutarate transaminase respectively. Chromatographic conditions were as described in Figs. 1 and 2. Kynurenine-pyruvate transaminase activity was assayed at pH 8.0 and kynurenine- α -oxoglutarate transaminase activity at pH 6.5, as described in the Experimental section.

Fraction	Enzyme activity	Apparent K_m (mM)			V_{max} . (nmol/h per mg of protein)
		For kynurenine	For pyruvate	For α -oxoglutarate	
(a) Fractions from hydroxyapatite chromatography					
Mitochondria					
	Kynurenine-pyruvate transaminase	1.5	1.2	—	104
	Kynurenine- α -oxoglutarate transaminase	2.5	—	0.2	452
Supernatant					
	Kynurenine-pyruvate transaminase	1.6	1.3	—	82
	Kynurenine- α -oxoglutarate transaminase	1.6	—	0.3	106
(b) Fractions from DEAE-cellulose chromatography					
Mitochondria					
	Kynurenine-pyruvate transaminase	2.5	1.0	—	63
	Kynurenine- α -oxoglutarate transaminase	2.2	—	0.3	375
Supernatant					
	Kynurenine-pyruvate transaminase	2.9	1.5	—	25
	Kynurenine- α -oxoglutarate transaminase	2.4	—	0.3	42

about one-third of that with L-kynurenine. The apparent K_m value of the enzyme for 3-hydroxy-L-kynurenine was 2.0mM. The 3-hydroxy-L-kynurenine transaminase activities of the mitochondrial and supernatant kynurenine-pyruvate transaminases and the supernatant kynurenine- α -oxoglutarate transaminase were not determined because of these very low activities. Kynurenine-pyruvate transaminase (70mM buffer fraction) and kynurenine- α -oxoglutarate transaminase (30mM buffer fraction) from the DEAE-cellulose column showed similar substrate specificities to those of the enzymes from the hydroxyapatite column.

Discussion

It is well known that kynurenine transaminase is present in mammalian liver and kidney (Mason, 1954, 1957; Ogasawara *et al.*, 1962; Ueno *et al.*, 1963). The enzyme shows a greater preference for α -oxoglutarate than for pyruvate and other amino acceptors and a pH optimum near 6.0. Recently our group has reported the presence in the small intestine (Noguchi *et al.*, 1973; Nakamura *et al.*, 1973) and brain (Minatogawa *et al.*, 1974) of rats of kynurenine-pyruvate transaminase, which uses pyruvate as effective amino acceptor and shows a pH optimum between 8.0 and 8.5. However, there was no report on the presence of kynurenine-pyruvate transaminase in rat liver. The present study shows that kynurenine-pyruvate transaminase is primarily localized in both the mitochondrial and supernatant fractions of rat

livers. Recently we have observed that kynurenine-pyruvate transaminase is found in both the supernatant and mitochondrial fractions of rat kidney (Y. Minatogawa, M. Nakatani, T. Noguchi, M. Morimoto & R. Kido, unpublished work). Kynurenine-pyruvate transaminase of rat liver showed similar pH optimum, oxo acid specificity and elution pattern from the columns of hydroxyapatite and of DEAE-cellulose to those of the brain, small intestine and kidney of rats. It is of interest that kynurenine-pyruvate transaminase is distributed in various tissues. On the other hand, Hagino *et al.* (1965) reported that kynurenine- α -oxoglutarate transaminase is also localized in both the mitochondrial and supernatant fractions of rat liver. However, Okamoto & Hayaishi (1970) described that kynurenine- α -oxoglutarate transaminase is strictly present only in the mitochondrial fraction of rat liver. The present study showed the presence of small amounts of kynurenine- α -oxoglutarate transaminase in the supernatant fraction. Ogasawara *et al.* (1962) reported that in the kidneys of vitamin B₆-deficient rats and of penicillamine-treated rats the supernatant kynurenine- α -oxoglutarate transaminase showed markedly decreased activities, whereas the mitochondrial kynurenine- α -oxoglutarate transaminase remained active. Recently we have observed a decrease in the supernatant kynurenine- α -oxoglutarate transaminase activity but not in that of mitochondrial kynurenine- α -oxoglutarate transaminase in the liver of vitamin B₆-deficient rats (M. Nakatani, T. Noguchi & R. Kido, unpublished work). On the basis of

these data and the subcellular distribution of marker enzymes (monoamine oxidase and alcohol dehydrogenase), the supernatant kynurenine- α -oxoglutarate transaminase is probably not derived from disrupted mitochondria during the subcellular fractionation.

On the basis of the present study, the roles of mitochondrial and supernatant kynurenine transaminases in tryptophan metabolism of rat liver might be explained as follows. The L-kynurenine produced from L-tryptophan via *N'*-formyl-L-kynurenine in the cytosol of the mammalian liver (Mehler & Knox, 1950; Knox, 1955; Greengard & Feigelson, 1962; Okamoto *et al.*, 1967) may undergo two reactions in the cytosol, namely hydrolytic cleavage by kynureninase to form anthranilate and alanine, and transamination by kynurenine-pyruvate transaminase or kynurenine- α -oxoglutarate transaminase to yield kynurenate. Since the K_m value of liver kynureninase for L-kynurenine, about 0.4 mM (Knox, 1953; McDermott *et al.*, 1973), is significantly lower than that (1.3–1.8 mM) of the supernatant kynurenine transaminases, and the V_{max} (63 nmol/h per mg of protein) of kynureninase (Okamoto & Hayaishi, 1970) is higher than those (24.5–27.3 nmol/h per mg of protein) of the supernatant kynurenine transaminases, L-kynurenine may be converted preferentially into anthranilate and alanine.

On the other hand, L-kynurenine that enters the mitochondria from the cytosol is attacked by either

kynurenine hydroxylase (Okamoto & Hayaishi, 1967, 1969) or kynurenine transaminase. Since the K_m value (0.023 mM) of kynurenine hydroxylase for L-kynurenine (Okamoto & Hayaishi, 1969) is much lower than those (1.3 and 1.5 mM) of kynurenine- α -oxoglutarate transaminase and kynurenine-pyruvate transaminase for L-kynurenine, and since kynureninase is not present in mitochondria (Wiss & Fuchs, 1950; Ogasawara *et al.*, 1962; Okamoto & Hayaishi, 1970), L-kynurenine will be hydroxylated preferentially to 3-hydroxy-L-kynurenine. The 3-hydroxy-L-kynurenine thus formed will be converted into xanthurenate by mitochondrial kynurenine- α -oxoglutarate by mitochondrial kynurenine- α -oxoglutarate transaminase, but not by kynurenine-pyruvate transaminase because of its very low activity. Subsequently 3-hydroxy-L-kynurenine that diffuses from the mitochondria into the cytosol may be preferentially attacked by kynureninase as opposed to kynurenine transaminase to give 3-hydroxyanthranilate. Liver kynureninase shows a much lower K_m for 3-hydroxy-L-kynurenine than for L-kynurenine (McDermott *et al.*, 1973), and the supernatant kynurenine-pyruvate transaminase and kynurenine- α -oxoglutarate transaminase show only very low activity for this amino acid. We suggest that supernatant kynurenine transaminase catalyses only the formation of kynurenate from L-kynurenine at high concentrations of L-kynurenine, and the mitochondrial enzyme only

Scheme 1. Main metabolic routes of tryptophan via kynurenine

Main metabolic routes ($\rightarrow$) and minor routes ($\dashrightarrow$) are shown.

the formation of xanthurenate from 3-hydroxy-L-kynurenine. The possible metabolic route of tryptophan via kynurenine in rat liver is summarized in Scheme 1.

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