Tryptophan aminotransferase activity in rat liver

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1. By using an antiserum raised against rat liver tyrosine aminotransferase, it was shown that about 60% of tryptophan aminotransferase activity in rat liver extracts is catalysed by this enzyme. 2. Induction of tryptophan aminotransferase activity by intraperitoneal injections of tryptophan or triamcinolone can be entirely attributed to the effects of these agents on tyrosine aminotransferase. 3. The origin of the other 40% of tryptophan aminotransferase activity remains to be established. This activity increases after starvation for 48 h. 4. It is unlikely that tryptophan transamination plays a quantitatively important role in the metabolism of tryptophan by the liver.

The major pathway for tryptophan catabolism in the liver is initiated by the enzyme tryptophan 2,3-dioxygenase [L-tryptophan: oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11]. Tryptophan aminotransferase (L-tryptophan:2-oxoglutarate aminotransferase, EC 2.6.1.27) activity has, however, been demonstrated in crude extracts of rat liver (Lin *et al.*, 1958a). This activity is induced when intraperitoneal injections of cortisol or tryptophan are given to intact or adrenalectomized rats (Civen & Knox, 1959); this gives credence to the suggestion that the enzyme may have a role in tryptophan metabolism *in vivo*.

Tryptophan aminotransferase activity in crude rat liver extracts may not be catalysed by a distinct enzyme. Studies of the substrate specificity of purified tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) suggest that tryptophan aminotransferase activity may represent, at least in part, a minor activity of tyrosine aminotransferase (Jacoby & La Du, 1964). By contrast, Lin *et al.* (1958b) concluded that the two aminotransferase activities are catalysed by distinct enzymes, since tyrosine aminotransferase activity, measured in crude liver extracts from pyridoxine-deficient rats, is increased more by cortisol and shows a greater dependence on pyridoxal 5'-phosphate than does tryptophan aminotransferase activity.

We have studied the effect of a monospecific antiserum raised against rat liver tyrosine amino-

transferase on tryptophan aminotransferase activity in crude extracts of liver from fed and starved rats and from rats injected intraperitoneally with tryptophan or triamcinolone. The results reported here permit conclusions to be drawn about the nature of hepatic tryptophan aminotransferase activity.

Materials and methods

Animals

Male Sprague-Dawley rats (200–250 g) were used throughout. The animals were either allowed free access to food or were deprived of food for 48 h before use. Some fed animals were injected intraperitoneally with a suspension of triamcinolone in 0.9% (w/v) NaCl (10 mg/kg body wt.) 6 h and 3 h before being killed. Other fed animals were injected intraperitoneally with a micro-crystalline suspension of tryptophan in 0.9% NaCl containing 0.1% (w/v) Tween 80 (750 mg/kg body wt.) 12 h and 6 h before use.

Materials

L-Tyrosine, L-tryptophan, pyridoxal 5'-phosphate and triamcinolone were from Sigma. Norit GS-X was from Clydesdale Co., Glasgow, Scotland, U.K. Bovine serum albumin (fraction V), obtained from International Enzymes, Windsor, Berks., U.K., was freed from fatty acids and other impurities as described by Chen (1967). L-[*side-chain*-2,3-³H]Tyrosine was obtained from Amersham International, Amersham, Bucks., U.K. PCS scintillation 'cocktail' was from B.D.H.

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2-Oxoglutarate was from Boehringer Corp. (London). All other chemicals, of the purest grade available, were from standard suppliers.

Preparation of extracts

Livers were rinsed in 0.9% NaCl, chopped into small pieces with scissors and then homogenized in 4 vol. of 50 mM-potassium phosphate/2 mM-2-oxoglutarate/1 mM-EDTA/0.2 mM-pyridoxal 5'-phosphate, pH 7.6, by using an Ystral tissue disperser ('Polytron'-type) (Ystral G.m.b.H., Ballrechten-Dottingen, Germany, through the Scientific Instrument Centre, Liverpool, U.K.). For extraction of tryptophan aminotransferase the 2-oxoglutarate was omitted. The extracts were then centrifuged at 12000g for 4 min at 4°C (Eppendorf model 3200) to remove cell debris, which interfered with the spectrophotometric assay for tryptophan aminotransferase (see below). The supernatants were kept on ice before assay.

Enzyme assays

Tryptophan aminotransferase was assayed at 30°C by following the formation of a complex between borate and the enol form of indol-3-ylpyruvate at 328 nm. A tautomerase present in rat liver extracts catalyses the interconversion of the keto and enol forms of indol-3-ylpyruvate (Spencer & Knox, 1962). The conditions used by Lin *et al.* (1958a) were modified to give an assay medium containing 0.5 M-sodium borate, 40 mM-L-tryptophan, 6.7 mM-2-oxoglutarate, 0.4 mM-pyridoxal 5'-phosphate, pH 8.0, and 300 µl of liver supernatant in a final volume of 3 ml. After preincubation of the liver supernatant for 5 min, the reaction was started by addition of 2-oxoglutarate, which was omitted from the blanks.

Tyrosine aminotransferase was assayed at 30°C by measuring the rate of release of ³H from L-[side-chain-2,3-³H]tyrosine. The method described by Marston & Pogson (1977) was slightly modified. The assay medium contained 100 mM-potassium phosphate, 100 mM-2-oxoglutarate, 0.05% (w/v) bovine serum albumin, 0.425 mM-2-oxoglutarate, 0.05% (w/v) bovine serum albumin, 0.425 mM-L-tyrosine (containing 0.2 µCi of L-[side-chain-2,3-³H]tyrosine) and 5 µl of liver supernatant in a total volume of 50 µl at pH 7.6. After separate preincubation of the liver supernatant and assay medium for 5 min at 30°C, the incubations were started with supernatant and stopped with 300 µl of Norit GS-X (20 mg/ml suspension in water) after 60 min. After thorough mixing, the samples were centrifuged at 12000g for 2 min at 4°C (Eppendorf model 3200). A supernatant sample (250 µl) was counted for radioactivity in 2 ml of PCS. Blanks were measured by adding the Norit GS-X before the supernatant.

Anti-(tyrosine aminotransferase)

Rat liver tyrosine aminotransferase was purified as described by Donner *et al.* (1978). The enzyme was homogeneous as judged by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, giving a single band of *M_r* 48000–50000. The specific activity of this enzyme preparation was 120 units/mg of protein. An antiserum was raised in rabbits by injection of 0.5 mg of antigen 9, 6 and 3 weeks before bleeding the animal. Antigen was injected in 250 µl of 100 mM-potassium phosphate, pH 7.6, together with 250 µl of Freund's complete adjuvant. The antiserum was concentrated by (NH₄)₂SO₄ precipitation and stored at –80°C in 100 mM-potassium phosphate/0.9% NaCl, pH 7.6, until required. The antiserum gave a single line in the Ouchterlony double-diffusion test against both purified enzyme and centrifuged liver extract. Analysis of material precipitated from crude liver extracts by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis indicated that a single antigenic species had been precipitated by the antiserum and that the *M_r* of this component was identical with that of purified tyrosine aminotransferase.

Complete precipitation of rat liver tyrosine aminotransferase was accomplished by incubating 100 µl of liver supernatant with 3 µl of antiserum at 25°C for 1 h and then at 4°C for 2 h. The immunoprecipitates were removed by centrifugation at 12000g for 4 min at 4°C (Eppendorf model 3200) and the supernatants retained for enzyme assay. Corresponding incubations of liver supernatant with rabbit serum were used as controls.

Results and discussion

Measurements of tryptophan aminotransferase activity in crude rat liver extracts as a function of substrate concentration (4–40 mM) gave a *K_m* for L-tryptophan of 27.0 ± 2.6 mM (mean ± s.e.m. for three determinations). The tryptophan concentration range used was far from ideal, but was determined at the upper end by the solubility of tryptophan and at the lower end by the activity of tryptophan aminotransferase. This high *K_m* suggests that tryptophan aminotransferase activity may represent a minor activity of one or more transaminases. Furthermore, this value compares favourably with the *K_m* of purified rat liver tyrosine aminotransferase for L-tryptophan of 30 mM (Jacoby & La Du, 1964).

The activities of tyrosine aminotransferase and tryptophan aminotransferase in crude rat liver extracts are increased by intraperitoneal injections of tryptophan (Kenney & Flora, 1961; Civen & Knox, 1959) or steroid hormones such as cortisol

(Lin & Knox, 1958; Civen & Knox, 1959). Since the major part of tryptophan aminotransferase activity was attributable to tyrosine aminotransferase (see Fig. 1), it might have been that induction of tryptophan aminotransferase by tryptophan or steroid hormones represented an increase only in that portion of its activity catalysed by tyrosine aminotransferase. In agreement with previous work (see above), we observed an increase in the activities of tyrosine and tryptophan aminotransferase after administration of tryptophan or triamcinolone, but no change after starvation for 48 h (Table 1). In addition, the increases in tryptophan aminotransferase activity were abolished after incubation of the liver extracts with anti-(tyrosine aminotransferase) serum. This suggested that the enzyme or enzymes which catalyse the residual tryptophan aminotransferase activity were not inducible by tryptophan or triamcinolone, though starvation for 48 h did cause an increase (Table 1).

Measurements of tryptophan aminotransferase activity remaining in crude liver extracts after incubation with antiserum as a function of substrate concentration (4–40 mM) gave a K_m for L-tryptophan of 42.8 ± 1.9 mM (mean $\pm$ S.E.M. of three determinations). This residual activity could have represented minor activities of other transaminases such as aspartate aminotransferase, whose mitochondrial and cytosolic forms in pig heart catalyse transamination of tryptophan (Shrawder & Martinez-Carrion, 1972). However, we were unable to detect any inhibition of the residual tryptophan aminotransferase activity by aspartate or indeed by any of 15 other amino acids tested (results not shown). We were unable to determine the effect of kynurenine on tryptophan aminotransferase activity, because kynurenine absorbs strongly at the same wavelength as the enol-borate complex. It therefore remains possible that one of the forms of kynurenine aminotransferase [L-kynurenine:2-oxoglutarate amino-

Fig. 1. Activities of tyrosine aminotransferase and tryptophan aminotransferase titrated against anti-(tyrosine aminotransferase) serum
Procedures for the preparation of liver extracts, assay of enzymes and immunoprecipitation of tyrosine aminotransferase are described in the Materials and methods section. Results are means $\pm$ S.E.M. for three independent observations. ○, Enzyme activity in the presence of antiserum; ●, enzyme activity in the presence of control serum.

Table 1. Effects of anti-(tyrosine aminotransferase) serum on the induction of tyrosine aminotransferase and tryptophan aminotransferase activities

Procedures for treatment of animals, preparation of liver extracts, assay of enzymes and immunoprecipitation of tyrosine aminotransferase are described in the Materials and methods section. The results are means $\pm$ S.E.M. from three independent observations. Means that are significantly different by Student's *t* test from those for fed control rats are shown by: **P* < 0.05; ***P* < 0.01. Other differences are not significant.

	Tyrosine aminotransferase activity (nmol/min per g wet wt. of liver)		Tryptophan aminotransferase activity (nmol/min per g wet wt. of liver)	
	No antiserum	+ antiserum	No antiserum	+ antiserum
Fed control	1029 $\pm$ 66	113 $\pm$ 10	51.9 $\pm$ 5.4	17.8 $\pm$ 0.2
48 h-starved	1549 $\pm$ 195	121 $\pm$ 15	60.9 $\pm$ 0.5	30.2 $\pm$ 3.8*
Tryptophan-treated	4073 $\pm$ 575**	267 $\pm$ 68	241.7 $\pm$ 64.5*	26.5 $\pm$ 3.6
Triamcinolone-treated	4646 $\pm$ 664**	203 $\pm$ 32	255.1 $\pm$ 55.1*	26.8 $\pm$ 4.6

transferase (cyclizing), EC 2.6.1.7] found in rat liver (Noguchi *et al.*, 1975) could catalyse the transamination of tryptophan.

We therefore conclude that the major fraction of tryptophan aminotransferase activity in crude rat liver extracts is catalysed by tyrosine aminotransferase. At physiological concentrations of tyrosine and tryptophan this activity is unlikely to be quantitatively important in hepatic tryptophan metabolism.

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