Purification and Properties of Peroxisomal Pyruvate (Glyoxylate) Aminotransferase from Rat Liver

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(Received 1 August 1978)

Pyruvate (glyoxylate) aminotransferase from rat liver peroxisomes was highly purified and characterized. The enzyme preparation has a mol.wt. of approx. 80 000 with two identical subunits, an isoelectric point of 8.0 and a pH optimum between 8.0 and 8.5. The enzyme catalysed transamination between a number of L-amino acids and pyruvate or glyoxylate. The effective amino acceptors were pyruvate, phenylpyruvate and glyoxylate with serine, and glyoxylate and phenylpyruvate with alanine as amino donor. These properties and kinetic parameters of the enzyme are remarkably similar to those previously described for mitochondrial alanine–glyoxylate aminotransferase isoenzyme 1 from glucagon-injected rat liver [Noguchi, Okuno, Takada, Minatogawa, Okai & Kido (1978) *Biochem. J.* **169**, 113–122].

Two forms of alanine–glyoxylate aminotransferase (EC 2.6.1.44) are contained in the particulate fraction of rat liver (Noguchi *et al.*, 1978a). One, designated isoenzyme 1, has a pI of 8.0 and a mol.wt. of approx. 80 000, and is present in the mitochondrial fraction. The enzyme is increased in activity by the injection *in vivo* of glucagon and is identical with glucagon-inducible serine–pyruvate aminotransferase (EC 2.6.1.51), histidine–pyruvate aminotransferase and phenylalanine–pyruvate aminotransferase. The other, designated isoenzyme 2, has a pI of 6.0 and a mol.wt. of approx. 175 000 and is also present in the mitochondrial fraction. This enzyme is highly specific for L-alanine and glyoxylate. The mitochondrial isoenzyme 1 has been purified and characterized from liver of glucagon-injected rats.

A non-glucagon-inducible enzyme with a substrate specificity similar to that of isoenzyme 1 has been reported to be present in the peroxisomes and has been designated pyruvate (glyoxylate) aminotransferase (Noguchi *et al.*, 1978b). The present paper describes the identity, with respect to physical and enzymic properties, of the peroxisomal pyruvate (glyoxylate) aminotransferase with the mitochondrial isoenzyme 1 in rat liver.

4 days before rats were killed, and the liver lysosomes from the mitochondria and peroxisomes were separated as described by Rowsell *et al.* (1972).

Methods

Preparation of peroxisomal and mitochondrial extracts. Peroxisomal and mitochondrial fractions were prepared from the particulate fractions of liver (wet wt. about 50g) from Triton WR-1339-injected rats by sucrose-density-gradient centrifugation with a zonal rotor as previously described (Noguchi *et al.*, 1978b). When catalase (EC 1.11.1.6) was assayed as the peroxisomal marker and glutamate dehydrogenase [L-glutamate–NAD⁺ oxidoreductase (deaminating), EC 1.4.1.2] as the mitochondrial marker, about 55% of the particulate serine–pyruvate aminotransferase activity was localized in the peroxisomes, and the remainder in the mitochondria, as previously described (Noguchi *et al.*, 1978b). The peroxisomal fractions with catalase activity and without glutamate dehydrogenase activity were pooled. The mitochondrial fractions with glutamate dehydrogenase activity and without catalase activity were pooled. Each cell fraction was dialysed for 12h against 5mM-potassium phosphate buffer, pH 7.5, containing 100 μ M-pyridoxal 5'-phosphate, sonicated for 4min at 10kHz with a Kubota (Tokyo, Japan) sonicator and then centrifuged at 105 000g for 45min.

Determination of enzyme activities. Transamination between various L-amino acids and pyruvate or glyoxylate, catalase and glutamate dehydrogenase were assayed as previously described (Noguchi *et al.*, 1978a).

A unit of enzyme activity is defined as the amount of enzyme that catalyses a formation of product or a decrease in substrate of 1 μ mol/h at 37°C.

Experimental

Materials

All materials used were obtained as stated previously (Noguchi *et al.*, 1976a,b, 1977b, 1978a,b). Male rats (body wt. about 200g) of the Donryu strain and given free access to food and water were used. Triton WR-1339 at a dose of 85mg/100g body wt. in 0.9% NaCl was injected intraperitoneally

Other methods. Protein determination, polyacrylamide-gel disc electrophoresis, isoelectric focusing, Sephadex G-150 gel filtration and sucrose-density-gradient centrifugation were carried out as described by Noguchi *et al.* (1976a).

Results and Discussion

Isoelectric focusing of peroxisomal and mitochondrial extracts

Mitochondrial and peroxisomal extracts were prepared from livers of Triton WR-1339-injected rats and subjected to isoelectric focusing on a pH 3.5–10 Ampholine gradient. Fractions (2ml) were collected and alanine-glyoxylate aminotransferase, serine-pyruvate aminotransferase and aminotransferase activities towards L-phenylalanine and L-histidine with pyruvate or glyoxylate as amino acceptor were determined. The focusing profiles of these activities are given in Fig. 1. Aminotransferase activities towards phenylalanine and histidine with glyoxylate and histidine-pyruvate aminotransferase activity showed nearly identical focusing profiles with phenylalanine-pyruvate aminotransferase activity. The focusing of the mitochondrial extract resulted in the detection of three activity peaks; the first, with pI 8.0 showed all six aminotransferase activities and has been designated alanine-glyoxylate aminotransferase isoenzyme 1; the second, with pI 6.0, showed only alanine-glyoxylate aminotransferase activity, and has been designated alanine-glyoxylate aminotransferase isoenzyme 2 (Noguchi *et al.*, 1978a); the third, with pI 5.2, showed aminotransferase activities towards L-phenylalanine and L-histidine with pyruvate or glyoxylate but not other aminotransferase activities. The six aminotransferase activities of the enzyme with pI 8.0 (alanine-glyoxylate aminotransferase isoenzyme 1) have been reported to be properties of the same protein (Noguchi *et al.*, 1978a). The enzyme with pI 5.2 may be identical with glutamine-phenylpyruvate aminotransferase (glutamine-oxo acid aminotransferase, EC 2.6.1.15), on the basis of previous reports (Cooper & Meister, 1974; Noguchi *et al.*, 1977a).

In contrast, the focusing of the peroxisomal extract yielded a major peak with pI 8.0 with all six aminotransferase activities coincident and a minor peak of isoenzyme 2 presumably from contaminating mitochondria. These results show that isoenzyme 1 is present both in the mitochondria and in the peroxisomes, and isoenzyme 2 only in the mitochondria.

Purification of peroxisomal pyruvate (glyoxylate) aminotransferase

The peroxisomal extract was prepared from rat livers as described in the Experimental section and

Fig. 1. Isoelectric focusing of the mitochondrial and peroxisomal extracts from rat liver

(a) Mitochondrial and (b) peroxisomal extracts were prepared from rat liver and separately subjected to isoelectric focusing on a pH 3.5–10 Ampholine gradient as described in the text. Fractions of 2ml were collected, and pH values (▲) and alanine-glyoxylate aminotransferase (●), serine-pyruvate aminotransferase (○) and phenylalanine-pyruvate aminotransferase (△) activities were determined.

subjected to a sequence of fractionation procedures: heat treatment, $(\text{NH}_4)_2\text{SO}_4$ fractionation, DEAE-cellulose chromatography, hydroxyapatite chromatography, isoelectric focusing and Sephadex G-150 gel filtration as described for serine-pyruvate aminotransferase (alanine-glyoxylate aminotransferase isoenzyme 1) from liver of glucagon-injected rats (Noguchi *et al.*, 1977b).

The purified serine-pyruvate aminotransferase preparation (in 50mM-potassium phosphate buffer, pH 7.5, containing 100 μM -pyridoxal 5'-phosphate) may be stored at -20°C for at least 4 weeks without loss of activity, and there is little loss with storage at $0-6^\circ\text{C}$ for at least 2 weeks. With the purified enzyme, a single peak with protein and serine-pyruvate aminotransferase activity coincident was obtained by polyacrylamide-gel disc electrophoresis in the absence of sodium dodecyl sulphate, Sephadex G-150 gel filtration and sucrose-density-gradient

Table 1. *Purification of pyruvate (glyoxylate) aminotransferase from rat liver peroxisomes*

Details of purification and assay methods are given in the text. L-Serine (40mM) and pyruvate (10mM) were used as amino donor and amino acceptor respectively

	Total protein (mg)	Specific activity (munits/mg of protein)	Total activity (units)	Recovery (%)	Purification (fold)
Homogenate	8500	3.7	31.45	100	1
Peroxisomal fraction	120	15.7	1.88	6.0	4.2
Peroxisomal extract	27	57	1.54	4.9	15
Heat treatment	20	67	1.34	4.3	18
(NH ₄) ₂ SO ₄	7.6	125	0.95	3.0	34
DEAE-cellulose	2.2	295	0.62	2.1	80
Hydroxyapatite	1.1	530	0.58	1.9	143
Isoelectric focusing and Sephadex G-150	0.12	3500	0.42	1.3	946

centrifugation, and a single protein band by polyacrylamide-gel disc electrophoresis in the presence of sodium dodecyl sulphate.

Results of a typical purification of peroxisomal pyruvate (glyoxylate) aminotransferase are shown in Table 1. About 950-fold purification was achieved, with a recovery of about 1.3% for serine-pyruvate aminotransferase activity.

The purified enzyme preparation catalysed transamination between a number of L-amino acids and pyruvate or glyoxylate (Table 2). The effective amino acceptors were pyruvate, glyoxylate and phenylpyruvate with L-serine as amino donor, and glyoxylate and phenylpyruvate with L-alanine. 2-Oxoglutarate was inactive with either L-serine or L-alanine as amino donor. The mol.wt. was estimated as approx. 80000 ± 7000 by Sephadex G-150 gel filtration and sucrose-density-gradient centrifugation. Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate yielded an estimated mol.wt. of approx. 38000 ± 6000 , suggesting that it probably consists of two identical subunits. Serine-pyruvate aminotransferase activity of the enzyme showed a pH optimum between 8.0 and 8.5; potassium phosphate buffer (0.2M) was used between pH 7.0 and 8.0, and pyrophosphate buffer (0.2M) between pH 8.0 and 9.5. The apparent K_m values were determined from double-reciprocal plots of initial velocity and substrate concentration. The apparent K_m values of the enzyme for L-serine with pyruvate (10mM), for L-alanine with glyoxylate (10mM), for pyruvate with L-serine (100mM) and for glyoxylate with L-alanine (100mM) were 16, 2.1, 0.07 and 0.1mM respectively. The properties of the purified enzyme preparation were remarkably similar to those of mitochondrial alanine-glyoxylate aminotransferase isoenzyme 1 from liver of glucagon-injected rats (Noguchi *et al.*, 1978a). This suggests that in a previous study (Noguchi *et al.*, 1978a) we co-purified the mitochondrial isoenzyme 1 and the peroxisomal pyruvate (glyoxylate) aminotransferase.

Table 2. *Relative activities of peroxisomal pyruvate (glyoxylate) aminotransferase for various L-amino acids or 2-oxo acids*

Assay procedures were as given in the text. (a) shows relative activities for various L-amino acids (40mM) with pyruvate (10mM) or glyoxylate (2mM) as amino acceptor, and (b) shows those for various 2-oxo acids (2mM) with L-serine (100mM) or L-alanine (100mM). Relative activity values are given, signifying transamination rates compared with those for L-serine or glyoxylate.

(a)

L-Amino acid	Relative activity	
	With pyruvate as amino acceptor	With glyoxylate as amino acceptor
Alanine	Not determined	4
Serine	1	1
Glutamate	0.03	0.04
Isoleucine	0.19	0.16
Methionine	2.9	2.7
Glutamine	2.7	2.5
Asparagine	4.4	4.0
Valine	0.20	0.28
Aspartic acid	Not detected	Not detected
Leucine	3.2	2.8
Phenylalanine	5.0	4.8
Tyrosine	2.3	2.2
Histidine	3.0	2.8

(b)

2-Oxo acid	Relative activity	
	With L-serine as amino donor	With L-alanine as amino donor
Pyruvate	1.6	Not determined
Glyoxylate	1	1
Phenylpyruvate	1.9	1.5
2-Oxoglutarate	Not detected	Not detected

Even so, the isoenzyme 1 previously purified would be largely the mitochondrial enzyme; the mitochondrial isoenzyme 1 and the peroxisomal enzyme are contained in the particulate fraction of control rat liver in similar amounts (Noguchi *et al.*, 1978b); however, we have used liver of glucagon-injected rats as the starting material for purification in which mitochondrial isoenzyme-1 activity is increased (about 15-fold) compared with control rat liver but not the peroxisomal activity (Noguchi *et al.*, 1978b).

Hsieh & Tolbert (1976) have partially purified phenylalanine (leucine)-glyoxylate aminotransferase from rat liver peroxisomes. The amino acid specificity is strikingly similar to that of the present peroxisomal enzyme. Further, Cooper (1977) has reported an asparagine aminotransferase from rat liver with a specificity very similar to that of the present peroxisomal enzyme and the mitochondrial liver with a specificity very similar to that of the alanine-glyoxylate aminotransferase isoenzyme 1 from rat liver (Noguchi *et al.*, 1978a). It may be that these aminotransferase activities are all manifestations of the same enzyme.

It has been reported that, in rats, alanine-glyoxylate aminotransferase and serine-pyruvate aminotransferase activities begin to develop in late foetal liver, increase rapidly at birth to a peak during suckling, and then decrease at weaning to the adult

value (Snell & Walker, 1972, 1974). Present findings suggest that an investigation of the separate developmental profiles of rat liver peroxisomal pyruvate (glyoxylate) aminotransferase and the mitochondrial alanine-glyoxylate aminotransferase isoenzyme 1 would be of interest.

References

- Cooper, A. J. L. (1977) *J. Biol. Chem.* **252**, 2032–2038
Cooper, A. J. L. & Meister, A. (1974) *J. Biol. Chem.* **249**, 2554–2561
Hsieh, B. & Tolbert, N. E. (1976) *J. Biol. Chem.* **251**, 4408–4415
Noguchi, T., Okuno, E., Minatogawa, Y. & Kido, R. (1976a) *Biochem. J.* **155**, 107–115
Noguchi, T., Okuno, E. & Kido, R. (1976b) *Biochem. J.* **159**, 607–613
Noguchi, T., Okuno, E. & Kido, R. (1977a) *Biochem. J.* **161**, 177–179
Noguchi, T., Takada, Y. & Kido, R. (1977b) *Biochem. J.* **161**, 609–614
Noguchi, T., Okuno, E., Takada, Y., Minatogawa, Y., Okai, K. & Kido, R. (1978a) *Biochem. J.* **169**, 113–122
Noguchi, T., Minatogawa, Y., Takada, Y., Okuno, E. & Kido, R. (1978b) *Biochem. J.* **170**, 173–175
Rowell, E. V., Snell, K., Carnie, J. A. & Rowell, K. V. (1972) *Biochem. J.* **127**, 155–165
Snell, K. & Walker, D. G. (1972) *Biochem. J.* **128**, 403–413
Snell, K. & Walker, D. G. (1974) *Biochem. J.* **144**, 519–531