Subcellular Distribution of Pyruvate (Glyoxylate) Aminotransferases in Rat Liver

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The distribution of pyruvate (glyoxylate) aminotransferases in the particulate fraction of rat liver homogenates was examined by centrifugation in a sucrose density gradient. Aminotransferase activities towards serine, phenylalanine and histidine with pyruvate and those towards phenylalanine and histidine with glyoxylate were nearly identically distributed. Some 50–55% of the particulate activity was localized in the peroxisomes and the remainder in the mitochondria. Most of alanine–glyoxylate aminotransferase activity was localized in the mitochondria, with some activity in the peroxisomes. Glucagon injection resulted in increases of these enzyme activities in the mitochondria, but not in the peroxisomes.

Aminotransferases that utilize pyruvate or glyoxylate as effective amino acceptor are distributed in livers of a wide range of animal species (Rowell *et al.*, 1969, 1972a; Minatogawa *et al.*, 1977). It has been reported that alanine–glyoxylate aminotransferase (EC 2.6.1.44), serine–pyruvate aminotransferase (EC 2.6.1.51), histidine–pyruvate aminotransferase and phenylalanine–pyruvate aminotransferase activities are mainly localized in rat liver mitochondrial fraction, and these activities are increased by the injection of glucagon, possibly by a cyclic AMP-dependent mechanism (Rowell *et al.*, 1972b; Snell, 1975; Snell & Walker, 1972, 1974; Morris *et al.*, 1973; Noguchi *et al.*, 1976a, 1977). We have previously described that these glucagon-inducible enzyme activities are associated with the same protein (Noguchi *et al.*, 1976a,b, 1977, 1978). For a proper consideration of the role of these enzymes in cellular metabolism reliable knowledge of their subcellular location is required. The present paper describes the subcellular distribution of various pyruvate (glyoxylate) aminotransferases in rat liver.

Experimental

Materials

Reagents. All materials used were obtained as stated previously (Noguchi *et al.*, 1976a,b, 1977, 1978). Triton WR-1339 was from Nakarai Chemicals, Kyoto, Japan.

Animals. Male rats (body wt. about 200 g) of the Donryu strain with free access to food and water were used. Glucagon dissolved in 0.9% (w/v) NaCl was given by subcutaneous injection (0.2 mg/100 g body wt., every 8 h for 24 h) under conditions previously

described (Noguchi *et al.*, 1976a). Triton WR-1339 at a dose of 85 mg/100 g body wt. in 0.9% (w/v) NaCl was injected intraperitoneally 4 days before rats were killed to separate liver lysosomes from the mitochondria and peroxisomes, as described by Rowell *et al.* (1972b).

Methods

Preparation of subcellular fractions. All procedures were carried out at 0–4°C. Rats were decapitated by a guillotine and bled. Livers were quickly removed, cut into small pieces and homogenized by one excursion in 0.25 M-sucrose in 20 mM-glycylglycine, pH 7.5, in a Potter–Elvehjem homogenizer with a Teflon pestle at 600 rev./min. The homogenate (10%, w/v) was centrifuged at 500g for 3 min to sediment nuclei and any whole cells and the supernatant fraction was then centrifuged at 15000g for 20 min. The resulting pellet was washed with 0.25 M-sucrose in 20 mM-glycylglycine, pH 7.5, and suspended in the same sucrose solution at a final concentration of 0.4 g wet wt. of the original tissue/ml. Then 30 ml of the suspension was applied at the core over a 650 ml linear sucrose gradient (27–62%, w/w) in a B-14 zonal rotor in an MSE Super-Speed 75 centrifuge. The gradient was centrifuged at 58000g for 3 h. At the end of centrifugation, the rotor was unloaded by pumping water into the core at the rate of 10 ml/min and collecting 10 ml fractions from the edge of the rotor. The same procedure was used for livers from glucagon- or Triton WR-1339-injected rats.

Determination of enzyme activities. L-Serine–pyruvate aminotransferase and L-alanine–glyoxylate aminotransferase were assayed as described, respectively, by Snell & Walker (1974) and Rowell *et al.*

(1972c). Aminotransferase activities towards L-phenylalanine and L-histidine with pyruvate or glyoxylate were determined as described by Noguchi *et al.* (1976a). Reaction mixture contained 100 mM-L-amino acid, 20 mM-pyruvate or 5 mM-glyoxylate, 40 μ M-pyridoxal 5'-phosphate, enzyme preparation and 0.2 M-potassium phosphate buffer, pH 8.0.

Catalase (EC 1.11.1.6) (Sinha, 1972), rhodanese (EC 2.8.1.1) (Sorbo, 1955; Rowsell *et al.*, 1972b) and acid phosphatase (Gianetto & de Duve, 1955) were assayed as described in the cited references.

Results and Discussion

Snell (1975) has reported that rat liver serine-pyruvate aminotransferase is predominantly localized in the mitochondria and that peroxisomes are not a major subcellular site. Hsieh & Tolbert (1976) have described that phenylalanine (leucine)-glyoxylate aminotransferase of rat liver was located only in the

peroxisomes and not in the mitochondria. However, different results were obtained in the present study. A representative sedimentation in a sucrose density gradient for the particulate fraction from control rat liver is presented in Fig. 1. Aminotransferase activities towards serine, phenylalanine and histidine with pyruvate as amino acceptor and those towards phenylalanine and histidine with glyoxylate showed nearly identical distribution profiles. Two activity peaks were obtained: one coincided with rhodanese activity as the mitochondrial marker; the other coincided with catalase activity as the peroxisomal marker. Some 50–55% of the particulate activity (fractions 10–25) was recovered in the peroxisomal fraction and the remainder in the mitochondrial fraction. Alanine-glyoxylate aminotransferase activity is predominantly localized in the mitochondrial fraction, confirming the result by Rowsell *et al.* (1972b). However, a minor activity peak, which coincided with catalase activity, was also obtained,

Fig. 1. Subcellular distribution of rat liver pyruvate (glyoxylate) aminotransferases

The particulate fraction was prepared from rat liver homogenates, placed over a linear sucrose gradient and centrifuged for 3 h at 58 000g in a B-14 zonal rotor as described in the text. At the end of centrifugation, 10 ml fractions were collected from the edge of the rotor. (a) Rhodanese (○), catalase (●), phenylalanine-glyoxylate aminotransferase (▲) and phenylalanine-pyruvate aminotransferase (△) activities. (b) Serine-pyruvate aminotransferase (○), histidine-pyruvate aminotransferase (▲), histidine-glyoxylate aminotransferase (△) and alanine-glyoxylate aminotransferase (●) activities.

suggesting some peroxisomal localization of alanine-glyoxylate aminotransferase. When the particulate fraction was prepared from livers of rats pretreated with Triton WR-1339 and subjected to sucrose-density-gradient centrifugation, all five aminotransferase activities described above showed different distribution profiles from acid phosphatase activity as the lysosomal marker. Compared with control rats, liver from glucagon-injected rats showed virtually identical increases (about 7.5-fold) in the activities of the mitochondrial pyruvate (glyoxylate) aminotransferases except alanine-glyoxylate aminotransferase (for which an increase about 3-fold was observed). None of the peroxisomal aminotransferase activities was affected by glucagon. We have previously described that rat liver crude mitochondrial fraction contains two types of alanine-glyoxylate aminotransferase, designated isoenzyme 1 and isoenzyme 2 respectively: isoenzyme 1 with pI 8.0 is induced by glucagon and identical with the following glucagon-inducible enzymes, phenylalanine-pyruvate aminotransferase, histidine-pyruvate aminotransferase, serine-pyruvate aminotransferase and alanine-glyoxylate aminotransferase; the other with pI 6.4 is not affected by glucagon and is specific for alanine and glyoxylate (Noguchi *et al.*, 1976a,b, 1977, 1978). These reports and the present findings suggest that the mitochondrial phenylalanine-glyoxylate aminotransferase and histidine-glyoxylate aminotransferase are also identical with isoenzyme 1. Hsieh & Tolbert (1976) have partially purified phenylalanine (leucine)-glyoxylate aminotransferase from rat liver peroxisomes. The amino acid substrate specificity is strikingly similar to that of the mitochondrial isoenzyme 1. Furthermore, Cooper (1977) has reported an asparagine aminotransferase from rat liver homogenates with a very similar amino acid specificity to that of isoenzyme 1. Studies on the purification and characterization of peroxisomal

pyruvate (glyoxylate) aminotransferase and comparison with the mitochondrial isoenzyme 1 are required.

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