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Characteristics of Hepatic Alanine–Glyoxylate Aminotransferase in Different Mammalian Species

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Mitochondrial extracts of dog, cat, rat and mouse liver contain two forms of alanine–glyoxylate aminotransferase (EC 2.6.1.44): one, designated isoenzyme 1, has mol.wt. approx. 80000 and predominates in dog and cat liver; the other, designated isoenzyme 2, has mol.wt. approx. 175000 and predominates in rat and mouse liver. In rat and mouse liver, isoenzyme 1 activity was increased by the injection *in vivo* of glucagon, but not isoenzyme 2 activity. Isoenzyme 1 was purified and characterized from liver mitochondrial extracts of the four species. Both rat and mouse enzyme preparations catalysed transamination between a number of L-amino acids and glyoxylate, and with L-alanine as amino donor the effective amino acceptors were glyoxylate, phenylpyruvate and hydroxypyruvate. In contrast, both dog and cat enzyme preparations were specific for L-alanine and L-serine with glyoxylate, and used glyoxylate and hydroxypyruvate as effective amino acceptors with L-alanine. Evidence that isoenzyme 1 is identical with serine–pyruvate aminotransferase (EC 2.6.1.51) was obtained. Isoenzyme 2 was partially purified from mitochondrial extracts of rat and mouse liver. Both enzyme preparations were specific for L-alanine and glyoxylate. On the basis of physical properties and substrate specificity, it was concluded that isoenzyme 2 is a separate enzyme. Some other properties of isoenzymes 1 and 2 are described.

It has been reported that hepatic alanine–glyoxylate aminotransferase (EC 2.6.1.44) is distributed in a wide range of animal species, and relatively high activity is associated with carnivorous species (dog, cat, common frog and common toad) (Rowsell *et al.*, 1969, 1972*a*). The enzyme of rat liver is mainly localized in the mitochondria (Rowsell *et al.*, 1972*b*) and is increased in activity by the injection of glucagon, possibly by a cyclic AMP-dependent mechanism (Snell, 1971; Snell & Walker, 1972). The enzyme activity in rat liver mitochondria begins to develop in late-foetal liver, increases rapidly at birth to a peak during suckling and then decreases at weaning to the adult value (Snell & Walker, 1972). An enzyme catalysing an irreversible reaction between alanine and glyoxylate has been partially purified and characterized from human liver extract (Thompson & Richardson, 1967). However, previous investigations of alanine–glyoxylate aminotransferase have not involved extensive purification or detailed analysis of the enzymic properties.

In the present paper, it is reported that mitochondrial extracts of dog, cat, rat and mouse liver contain two forms of alanine–glyoxylate aminotransferase: one, isoenzyme 1, predominates in dog and cat liver; the other, isoenzyme 2, predominates in rat and mouse. Purification and properties of isoenzyme 1 from dog, cat, rat and mouse liver, and of isoenzyme 2 from rat and mouse liver, are described.

Materials and Methods

Materials

Lactate dehydrogenase (L-lactate–NAD^+ oxidoreductase, EC 1.1.1.27), D-glycerate dehydrogenase (D-glycerate–NAD^+ oxidoreductase, EC 1.1.1.29), sodium phenylpyruvate and sodium hydroxypyruvate were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. and glutamate dehydrogenase [L-glutamate–NAD^+ oxidoreductase (deaminating), EC 1.4.1.2] was from Calbiochem, San Diego, CA, U.S.A. Hydroxylamine, semicarbazide and isonicotinic acid hydrazide were obtained from Nakarai Chemicals, Kyoto, Japan. Other materials used were obtained as stated previously (Noguchi *et al.*, 1976*a*,*b*).

Aminotransferase assays

Assay mixtures contained, unless specified otherwise, 40mM-L-amino acid, 2mM-2-oxo acid, 40μM-pyridoxal phosphate, 0.2M-potassium phosphate buffer, pH8.2, and enzyme preparation in a total volume of 0.4ml.

Transamination between L-alanine or L-serine and glyoxylate was assayed by the method of Rowsell *et al.* (1972*c*). Pyruvate or hydroxypyruvate formed was determined in the presence of Tris by using lactate dehydrogenase and NADH. In the presence of Tris, these two 2-oxo acids formed are substrates for lactate dehydrogenase, but glyoxylate is not.

Transamination between L-glutamate and glyoxylate or pyruvate was assayed as described by Rowsell *et al.* (1972*c*). 2-Oxoglutarate formed was determined with NADH, NH_4Cl and glutamate dehydrogenase.

Transamination between L-methionine or L-asparagine and glyoxylate or pyruvate was determined as described by Cooper & Meister (1972). This assay is based on the formation of the ferric ion complex of the corresponding 2-oxo acid.

Transamination between L-glutamine and glyoxylate was determined as described by Cooper & Meister (1972). This assay is based on the formation of the glycine complex with phthalaldehyde.

Transamination between aromatic L-amino acids (histidine, phenylalanine, tyrosine, tryptophan or 5-hydroxytryptophan) and 2-oxo acid (glyoxylate, 2-oxoglutarate or pyruvate) was in principle determined by the method of Lin *et al.* (1958). This assay is based on the arsenate-catalysed formation of aromatic 2-oxo acid enol–borate complexes. Details of this assay have been described (Noguchi *et al.*, 1976*a*).

Transamination between branched-chain L-amino acids (leucine, valine or isoleucine) and glyoxylate, 2-oxoglutarate or pyruvate was assayed as described by Ichihara & Koyama (1966). The 2-oxo acid formed was determined as its 2,4-dinitrophenylhydrazone.

Transamination between L-aspartate and 2-oxo acid (glyoxylate, 2-oxoglutarate or pyruvate) was assayed as described by Boyd (1961). Oxaloacetate formed was determined with NADH and malate dehydrogenase.

Transamination between L-alanine and 2-oxoglutarate was assayed as described by Swick *et al.* (1965). Pyruvate formed was determined with NADH and lactate dehydrogenase.

Transamination between L-methionine, L-glutamine or L-asparagine and 2-oxoglutarate was assayed as described by Nakatani *et al.* (1970). This assay is based on the determination of unchanged 2-oxo glutarate in the presence of NADH and NH_4Cl with glutamate dehydrogenase.

Transamination between L-serine and 2-oxo acid (pyruvate, 2-oxoglutarate or phenylpyruvate), except glyoxylate, was assayed as described by Snell & Walker (1974). Hydroxypyruvate formed was determined with NADH and spinach D-glycerate dehydrogenase.

Transamination between L-glutamine and phenylpyruvate was assayed as described by Kupchik & Knox (1970). The disappearance of the enol–borate complex of phenylpyruvate was continuously measured at 300nm.

Transamination between L-alanine and phenylpyruvate was assayed by measuring the amount of pyruvate formed with NADH and lactate dehydrogenase in the presence of borate/arsenate buffer. After incubation, the reaction mixture (0.4ml) was inactivated by the addition of 50μl of 25% (w/v) trichloroacetic acid and then neutralized with 40μl of 2M-KOH. The mixture was diluted with 0.5ml of 1M-borate/1M-arsenate buffer, pH6.5, containing 0.1mg of NADH. Pyruvate concentration was determined from the change in A_{340} on the addition of lactate dehydrogenase. Phenylpyruvate is not a substrate for lactate dehydrogenase in the presence of borate/arsenate buffer owing to its enol–borate complex-formation.

Transamination between L-alanine and hydroxypyruvate was assayed by measuring the amount of unchanged hydroxypyruvate with spinach D-glycerate dehydrogenase and NADH as described by Cheung *et al.* (1969).

Transamination between glycine or L-glutamine and pyruvate was determined by measuring the amount of unchanged pyruvate with lactate dehydrogenase in the presence of Tris. The reaction was terminated after a 1h incubation of the assay mixture (0.2ml) by the addition of 25μl of 25% (w/v) trichloroacetic acid. In the control, pyruvate was added after incubation. The incubation mixture was then neutralized by the addition of 20μl of 2M-KOH. Samples (70μl) were mixed with 70μl of NADH (0.86μmol) and diluted with 3.5ml of 2M-Tris/HCl, pH8.4. Pyruvate concentration was determined from the change in A_{340} on the addition of lactate dehydrogenase. Under these conditions, neither glyoxylate nor 2-oxoglutaramate formed are substrates for lactate dehydrogenase; glyoxylate forms a complex with Tris (Rowsell *et al.*, 1972*c*) and 2-oxoglutaramate exists in the cyclic ketalactam form (Cooper & Meister, 1972).

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

Other methods

Polyacrylamide-disc-gel electrophoresis in the presence of sodium dodecyl sulphate was carried out by the method of Weber & Osborn (1969). Determination of protein, polyacrylamide-disc-gel electrophoresis in the absence of sodium dodecyl sulphate, isoelectric focusing on a pH3.5–10 Ampholine gradient and determination of approximate molecular weight by sucrose-density-gradient centrifugation and Sephadex G-150 gel filtration were carried out as previously described (Noguchi *et al.*, 1976*a*,*b*).

Results

Gel filtration of liver mitochondrial extract

Figs. 1(*a*)–1(*d*) show the elution profiles of alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities from a Sephadex G-150 column with a liver mitochondrial extract from dog, cat, rat and mouse. Two peaks of alanine–glyoxylate aminotransferase activity were obtained in each case. The slower moving enzyme (mol.wt. approx. 80000) is hereafter designated isoenzyme 1 and the faster moving (mol.wt. approx. 175000) isoenzyme 2: approximate molecular weights of isoenzymes were estimated by their rate of movement through Sephadex G-150 relative to catalase, bovine serum albumin, ovalbumin and cytochrome *c* (Noguchi *et al.*, 1976*a*). Isoenzyme 1 predominated in dog and cat liver, whereas isoenzyme 2 predominated in rat and mouse liver. Isoenzyme 1 from all four species and cat isoenzyme 2 has serine–pyruvate aminotransferase activity, but not isoenzyme 2 of dog, rat and mouse. The activity ratio, alanine–glyoxylate aminotransferase/serine–pyruvate aminotransferase, for the peak fractions was 6.7 (dog), 6.0 (cat), 4.3 (rat) and 5.9 (mouse) for isoenzyme 1 and 7.0 (cat) for isoenzyme 2.

Isoenzyme 1 from both rat and mouse after glucagon treatment showed virtually identical percentage increases in both alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities (about 33-fold and 19-fold respectively) (Figs. 1*e* and 1*f*), suggesting that the two activities of isoenzyme 1 are properties of the same protein. However, isoenzyme-2 activity was not affected by glucagon in either species. The effect of glucagon on dog and cat isoenzymes was not examined.

Purification of hepatic mitochondrial isoenzymes 1 *and* 2

Isoenzyme 1 was purified from dog and cat liver and from liver of glucagon-injected rats and mice, and

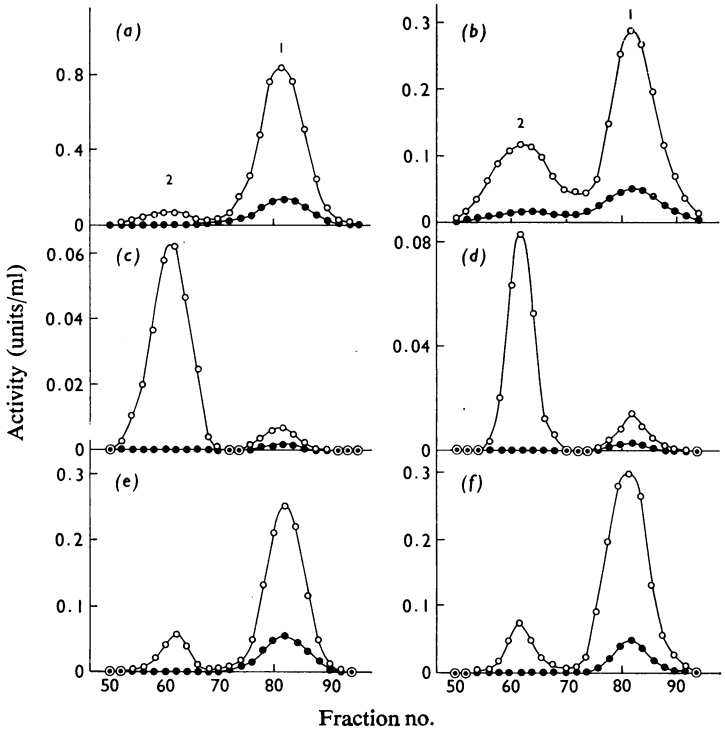

Fig. 1. *Elution profiles of alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities from a Sephadex G-150 column with a liver mitochondrial extract from dog, cat, rat or mouse*

Each mitochondrial extract from dog, cat, rat and mouse liver, and liver of glucagon-injected rat and mouse, was prepared, applied to a Sephadex G-150 column in amount corresponding to 1 g of liver and eluted as previously described (Noguchi *et al.*, 1976*a*, 1977*b*). The effluent was collected in 3 ml fractions. The Figure shows elution profiles of alanine–glyoxylate aminotransferase (○) and serine–pyruvate aminotransferase (●) with a liver mitochondrial extract from (*a*) dog, (*b*) cat, (*c*) rat, (*d*) mouse, (*e*) glucagon-injected rat or (*f*) glucagon-injected mouse.

isoenzyme 2 was from control rat and mouse liver. All manipulations were carried out at 0–4°C. Potassium phosphate buffer, containing 100 μM-pyridoxal phosphate and 1 mM-2-mercaptoethanol, was used throughout. At each stage in the purification aminotransferase activity towards L-alanine with glyoxylate as amino acceptor was determined.

Purification of isoenzyme 1. The same procedure was applied with dog, cat, rat and mouse liver. A male dog (about 25 kg body wt.), a female cat (about 3.5 kg), five male rats (50–60 g) of the Donryu strain and ten male mice (12–15 g) of the ddY strain were used. Rats and mice were injected subcutaneously with glucagon (0.17 mg/100 g body wt. every 8 h for 3 days) suspended in 0.15% KCl. The crude mitochondria (combined heavy and light mitochondria) were prepared from liver samples (dog, 13 g; cat, 13 g; rat, 14 g; mouse, 12 g) by the method of de Duve *et al.* (1955). Each mitochondrial fraction was homogenized with 5 vol. of 5 mM-phosphate buffer in a Waring blender for 2 min. After sonication for 4 min at 10 kHz with a Kubota Sonicator (Tokyo, Japan), each homogenate was centrifuged at 105000*g* for 30 min and the precipitate discarded.

The supernatant was applied to a column (5.0 cm × 6.0 cm) of DEAE-cellulose, equilibrated with 5 mM-phosphate buffer. The column was washed with 800 ml of 5 mM-phosphate buffer and the enzyme was eluted with 25 mM-phosphate buffer. The active fractions were pooled and concentrated by Diaflo ultrafiltration (Bioengineering Co., Tokyo, Japan) by using a G10T filter.

The concentrated enzyme solution was added to a column (2.5 cm × 100 cm) of Sephadex G-150, which had been equilibrated with 50 mM-phosphate buffer. The column was eluted with the same buffer at a flow rate of 25 ml/h. The effluent was collected in 3.0 ml fractions. Two peaks of activity, the slower moving isoenzyme 1 and the faster moving isoenzyme 2, were obtained for enzyme preparations from four species. The active isoenzyme-1 fractions were pooled and concentrated by ultrafiltration. Isoenzyme-2 fractions obtained were not subjected to further purification, owing to their low activities.

The concentrated isoenzyme-1 solution was subjected to a sequence of fractionation procedures: heat-treatment, hydroxyapatite chromatography, isoelectric focusing on a pH 3.5–10 Ampholine gradient and Sephadex G-150 gel filtration as described for mitochondrial serine–pyruvate aminotransferases from dog, cat, rat and mouse (Noguchi *et al.*, 1976*c*, 1977*b*).

Results of typical purifications of alanine–glyoxylate aminotransferase are shown in Table 1. Isoenzymes 1 and 2 were eluted together from a DEAE-cellulose column, but completely separated by the first gel filtration. Alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase

Table 1. *Purification of alanine–glyoxylate aminotransferase isoenzyme* 1 *from dog, cat, rat and mouse liver*

Details of purification and assay methods are given in the text. D, Dog; C, cat; R, rat; M, mouse.

	Total protein (mg)				Specific activity (units/mg of protein)				Total activity (units)				Recovery (%)				Purification (fold)				Alanine–glyoxylate aminotransferase/serine–pyruvate aminotransferase activity ratio			
	D	C	R	M	D	C	R	M	D	C	R	M	D	C	R	M	D	C	R	M	D	C	R	M
Extract	366	312	365	350	0.388	0.593	0.290	0.257	142	185	106	89.8	100	100	100	100	1	1	1	1	7.3	7.2	5.1	7.8
DEAE-cellulose	53	41	44	39	1.92	3.88	1.90	1.93	102	159	83.6	75.4	72	86	79	84	5	6.5	6.6	7.5	6.5	5.8	4.3	5.9
Sephadex G-150	17	12	21	10	4.88	10.1	3.39	6.18	83.0	121	71.1	61.8	58	65	67	69	13	17	12	24	6.7	5.7	4.3	5.7
Heat treatment	9.2	6.3	7.8	4.9	9.18	19.4	9.68	12.6	84.5	122	75.5	61.7	60	66	71	69	24	33	33	49	6.9	6.0	4.4	5.8
Hydroxyapatite	2.5	2.0	3.2	1.9	28.9	48.6	17.5	16.3	72.2	97.1	56.0	31.0	51	52	53	35	74	82	60	63	6.7	6.1	4.5	5.7
Isoelectric focusing and Sephadex G-150 gel filtration	0.50	0.43	0.91	0.63	91.0	86.3	21.4	23.2	45.5	37.1	19.5	14.6	32	20	18	16	235	146	74	90	6.8	6.2	4.6	5.9

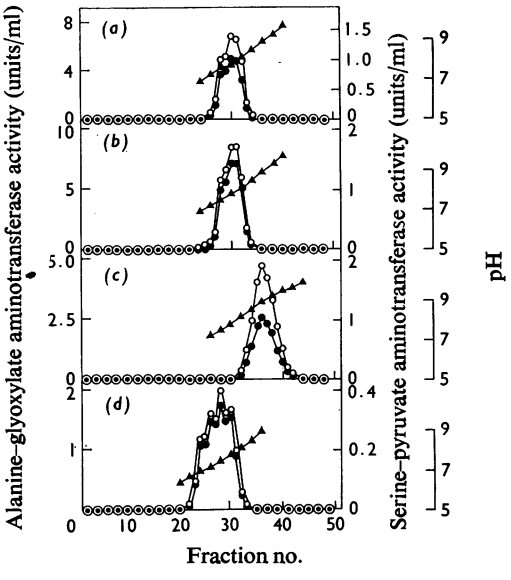

Fig. 2. *Isoelectric-focusing profiles of isoenzyme-1 preparations obtained after hydroxyapatite chromatography*
Enzyme preparation (*a*, dog; *b*, cat; *c*, rat; *d*, mouse) obtained after hydroxyapatite chromatography was subjected to isoelectric focusing on a pH3.5–10 Ampholine gradient. Fractions (2ml) were collected. pH values (▲), alanine–glyoxylate aminotransferase activity (○) and serine–pyruvate aminotransferase activity (●) were determined as described in the text.

activities were not separable by any fractionation step after the first gel filtration. As shown in Fig. 2, the two enzyme activities from the four sources were identically focused in preparations obtained after hydroxyapatite chromatography, suggesting that the two activities are associated with the same protein. Alanine–glyoxylate aminotransferase activity was purified 700-fold for dog liver, 508-fold for cat liver, 178-fold for rat liver and 258-fold for mouse liver, compared with the original homogenate. Similar specific activities were obtained for dog and cat isoenzyme-1 preparations, and for rat and mouse isoenzyme-1 preparations. In each case the activity ratio, alanine–glyoxylate aminotransferase/serine–pyruvate aminotransferase, remained constant throughout the purification after the removal of isoenzyme 2 by the first Sephadex G-150 gel filtration: dog, 6.5–6.9; cat, 5.7–6.2; rat, 4.3–4.6; mouse, 5.7–5.9. All four enzyme preparations (in 50mM-potassium phosphate buffer containing 100μM-pyridoxal phosphate and 1mM-2-mercaptoethanol) may be stored at −20°C for at least 4 weeks without loss of either activity, and there was little loss with storage at 0–6°C for at least 2 weeks.

All four enzyme preparations were homogeneous as judged by polyacrylamide-disc-gel electrophoresis in the presence of sodium dodecyl sulphate, Sephadex G-150 gel filtration and sucrose-density-gradient centrifugation as described for serine–pyruvate aminotransferase preparations from dog, cat, rat and mouse liver (Noguchi *et al.*, 1976*c*, 1977*b*). However, polyacrylamide-disc-gel electrophoresis in the absence of sodium dodecyl sulphate and isoelectric focusing on a pH3.5–10 Ampholine gradient resulted in the detection of multiple forms of alanine–glyoxylate aminotransferase for each enzyme preparation from dog, cat and mouse liver, and a single form for rat liver enzyme. These results were nearly identical with those obtained for serine–pyruvate aminotransferase preparations (Noguchi *et al.*, 1977*b*).

With each isoenzyme-1 preparation, alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities were not separable by polyacrylamide-disc-gel electrophoresis in the absence of sodium dodecyl sulphate, Sephadex G-150 gel filtration, sucrose-density-gradient centrifugation and isoelectric focusing.

Purification of isoenzyme 2. The same procedure was applied, unless specified otherwise, with rat and mouse liver. Hepatic mitochondrial extracts were prepared from livers (about 144g) of 15 male rats (body wt. 200–250g) and livers (about 24g) of 16 male mice (body wt. 15–20g) as described for purification of isoenzyme 1.

Each extract was warmed rapidly to 60°C and maintained at this temperature, with constant stirring, for 1min, after which it was quickly chilled in a 0.9% NaCl/ice bath at about −7°C. The precipitate was removed by centrifugation at 5000*g* for 20min.

Solid $(NH_4)_2SO_4$ was added to the supernatant, with gentle stirring, to 30% saturation. After 30min, the precipitate was removed by centrifugation for 20min and discarded. $(NH_4)_2SO_4$ was added to the supernatant to 70% saturation, and after 30min, the precipitate was collected by centrifugation at 5000*g* for 20min, dissolved in 5mM-phosphate buffer and desalted by dialysis against the same buffer overnight. The inactive precipitate formed during dialysis was removed by centrifugation at 10000*g* for 1h. About 80% of the aspartate–2-oxoglutarate aminotransferase activity was removed by this treatment.

The non-diffusible solution was applied to a column (3.3cm × 4.5cm for mouse enzyme, 5.0cm × 6.0cm for rat enzyme) of DEAE-cellulose equilibrated with 5mM-phosphate buffer. The column was washed with 5mM- and then 15mM-phosphate buffer (250ml of each for mouse enzyme; 500ml of each for rat enzyme), and then the enzyme was eluted with 50mM-phosphate buffer. The active fractions were pooled and concentrated by ultrafiltration by using a G-10T filter.

The enzyme solution was added to a column (2.5cm×100cm) of Sephadex G-150 and eluted as described for isoenzyme 1. Two peaks of activity (isoenzymes 1 and 2) were obtained for both rat and mouse enzyme preparations. Active isoenzyme-2 fractions were collected, concentrated by ultrafiltration and diluted with water to adjust the buffer concentration to 5mM.

The concentrated enzyme solution was applied to a column (1.5cm×3.5cm for mouse enzyme; 2.5cm×7cm for rat enzyme) of hydroxyapatite previously equilibrated with 5mM-phosphate buffer and elution was performed with successive equal volumes (100ml for mouse enzyme and 250ml for rat enzyme) of 5, 15, 50, 100 and 250mM-phosphate buffer. Fractions (10ml) were collected and monitored for the enzyme activity. Rat isoenzyme 2 was eluted with 50mM buffer and mouse isoenzyme 2 with 100mM-phosphate buffer. Active fractions were pooled and concentrated by ultrafiltration.

The concentrated enzyme solution was subjected to isoelectric focusing on a pH3.5–10 Ampholine gradient as previously described (Noguchi *et al.*, 1976*a*). The focusing resulted in the detection of a single peak of activity with the enzyme preparation from each source (rat, pI6.0; mouse, pI5.9) (Fig. 3). In each case alanine–glyoxylate aminotransferase was separated from alanine–2-oxoglutarate aminotransferase (rat, pI7.6; mouse, pI6.7), aspartate–2-oxoglutarate aminotransferase (rat and mouse, pI9.3), glutamine–phenylpyruvate aminotransferase (rat and mouse, pI5.2) and glutamate–glyoxylate aminotransferase (rat, pI5.1; mouse, pI4.9). The alanine–glyoxylate aminotransferase fractions were pooled and concentrated by ultrafiltration.

The concentrated enzyme solution was applied to a Sephadex G-150 column and eluted as described above. A single peak of activity was obtained with each enzyme preparation from the two species. Active fractions were pooled and concentrated by ultrafiltration.

Results of typical purifications of rat and mouse isoenzyme 2 are shown in Table 2. The enzyme was purified 147-fold over the rat liver homogenate and about 115-fold over the mouse liver homogenate.

Polyacrylamide-disc-gel electrophoresis at pH8.9 in 7% (w/v) acrylamide gel yielded a major band of protein which represented about 60% and 65% of the total protein for rat and mouse isoenzyme-2 preparations respectively. In each case, the major band of protein coincided with the enzyme activity.

The two enzyme preparations (in 50mM-potassium phosphate buffer, pH7.5, containing 100μM-pyridoxal phosphate and 2mM-2-mercaptoethanol) may be stored at −20°C for at least 4 weeks without loss of activity, and there was little loss with storage at 0–4°C at least for 2 weeks.

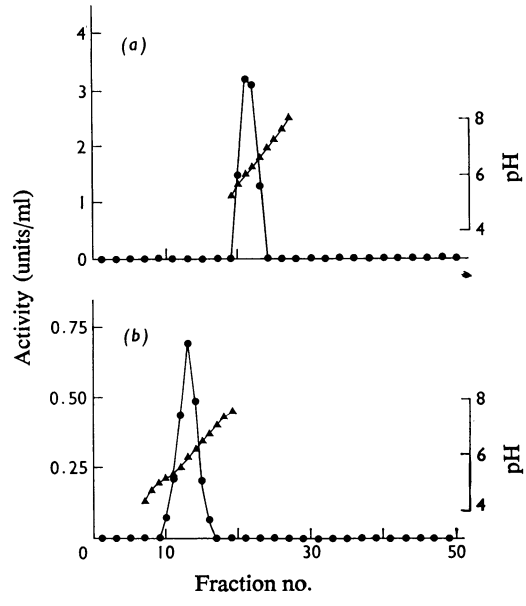

Fig. 3. *Isoelectric-focusing profiles of isoenzyme-2 preparations obtained after hydroxyapatite chromatography*
Enzyme preparation of (*a*) rat or (*b*) mouse liver from a hydroxyapatite column was subjected to isoelectric focusing on a pH3.5–10 Ampholine gradient. Fractions (2ml) were collected. pH values (▲) and alanine–glyoxylate aminotransferase activity (●) were determined as described in the text.

Properties of isoenzymes 1 *and* 2

pH optimum. Potassium phosphate buffer (0.2M) was used between pH7.0 and 8.0, and sodium pyrophosphate buffer (0.2M) between pH8.0 and 9.6. Alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities showed similar pH profiles, with pH optima between 8.3 and 8.6, for all four isoenzyme-1 preparations. Rat and mouse isoenzyme 2 had pH optima between 8.5 and 8.8 and between 8.1 and 8.4 respectively.

Determination of approximate molecular weight. The mol.wt. was estimated as 77000±4000 by Sephadex G-150 gel filtration and as 80000±6000 by sucrose-density-gradient centrifugation for isoenzyme 1 from each of the four sources, and 170000±5000 and 175000±7000 respectively for rat and mouse isoenzyme 2.

Polyacrylamide-disc-gel electrophoresis in the presence of sodium dodecyl sulphate yielded mol.wts. of 38000±6000 for all four isoenzyme-1 preparations, showing that they probably consist of two identical subunits. The molecular weight of subunits of rat and mouse isoenzyme 2 were not determined, owing to their impurity.

Table 2. *Purification of alanine–glyoxylate aminotransferase isoenzyme 2 from rat and mouse liver*
Assay methods are as described in the text, except that L-alanine and glyoxylate were used at 120 and 5 mM respectively.

	Total protein (mg)		Specific activity (units/mg)		Total activity (units)		Recovery (%)		Purification (fold)	
Purification step	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse
Homogenate	22700	3820	0.020	0.023	455	87.8	100	100	1	1
Mitochondrial extract	3700	889	0.054	0.053	200	47.1	44	54	2.7	2.3
Heat treatment	1940	343	0.10	0.13	194	44.6	43	51	5.0	5.7
$(NH_4)_2SO_4$ fractionation	670	106	0.18	0.32	121	33.9	27	38.6	9.0	13.9
DEAE-cellulose	210	32.4	0.36	0.50	75.6	16.2	17	18.5	18.0	21.7
Sephadex G-150	95.3	10.3	0.44	0.89	41.9	9.2	9.2	10.4	22.0	38.7
Hydroxyapatite	38.4	3.0	0.59	2.00	22.7	6.0	5.0	6.8	29.5	87.0
Isoelectric focusing and Sephadex G-150	1.80	0.50	2.93	2.64	5.3	1.3	1.2	1.5	147	115

Table 3. *Glyoxylate aminotransferase activities of alanine–glyoxylate aminotransferase isoenzyme 1 from dog, cat, rat and mouse liver for various amino acids*
Assay conditions were as described in the text, except the concentration (6.5 mM) of L-tyrosine. Relative activity values are given, signifying transamination rate compared with that for L-alanine. N.D., Not detected. Dog and cat enzyme preparations were specific for L-alanine and L-serine; little or no activity was detected with other amino acids listed in the Table.

	Relative activity			
L-Amino acid	Dog	Cat	Rat	Mouse
Alanine	1	1	1	1
Serine	0.15	0.15	0.22	0.17
Glutamic acid			N.D.	N.D.
Isoleucine			0.04	0.28
Methionine			0.62	0.58
Glutamine			0.59	0.45
Asparagine			1.0	0.62
Valine			0.1	0.22
Aspartic acid			0.06	N.D.
Leucine			0.68	0.63
Phenylalanine			1.0	0.71
Tyrosine			0.5	0.46
Histidine			0.66	0.54
Tryptophan			0.05	0.04
5-Hydroxytryptophan			0.02	0.03

Substrate specificity. The relative activities of isoenzymes 1 and 2 with various amino acids (40 mM) were determined with glyoxylate (2 mM) as amino acceptor. A striking difference in amino donor specificity was observed between rodent (rat and mouse) and carnivore (dog and cat) isoenzyme 1 (Table 3). Rat and mouse isoenzyme-1 preparations showed a broad specificity for L-amino acids with glyoxylate. The effective L-amino acids for both enzymes were alanine, phenylalanine, asparagine, leucine, methionine, glutamine, histidine, tyrosine and serine. Additional effective L-amino acids with mouse isoenzyme 1 were isoleucine and valine. In contrast, both dog and cat isoenzyme-1 preparations were highly specific for L-alanine and L-serine. Glyoxylate, phenylpyruvate and hydroxypyruvate were all effective 2-oxo acids with L-alanine for rat and mouse isoenzyme 1 (Table 4). Dog and cat isoenzyme 1 utilized glyoxylate, hydroxypyruvate and, less effectively, phenylpyruvate (Table 4). With 2-oxoglutarate as amino acceptor, all L-amino acids listed in Table 3 were inactive with any of these isoenzyme-1 preparations. All four isoenzyme-1 preparations catalysed transamination between L-serine (40 mM) and pyruvate (2 mM) (specific activity: dog, 13.4 units/mg of protein; cat, 13.9 units/mg; rat, 4.65 units/mg; mouse, 3.93 units/mg). With isoenzyme-1 preparation from any of the four sources, the activity ratio alanine–glyoxylate aminotransferase/serine–pyruvate aminotransferase was unchanged after isoelectric focusing, polyacrylamide-disc-gel electrophoresis in the absence of sodium dodecyl sulphate, Sephadex G-150 gel filtration and sucrose-density-gradient centrifugation.

On the other hand, rat and mouse isoenzyme-2 preparations were highly specific for L-alanine and glyoxylate. Little or no activity with glyoxylate was detected with other L-amino acids listed in Table 3 and with L-alanine as amino donor neither enzyme showed activity with hydroxypyruvate, 2-oxoglutarate or phenylpyruvate. With 2-oxoglutarate and pyruvate as amino acceptors, the two isoenzyme-2 preparations did not show transaminase activity towards any L-amino acid listed in Table 3.

The apparent K_m values were determined from double-reciprocal plots of initial velocity and substrate concentration. The apparent K_m values of isoenzyme 1 for L-alanine with glyoxylate (2 mM) as amino acceptor and for glyoxylate with L-alanine (40 mM) as amino donor were respectively, as follows: dog, 3.9 and 0.13 mM; cat, 3.0 and 0.10 mM; rat, 2.1 and 0.07 mM; mouse, 1.4 and 0.2 mM.

The apparent K_m values of isoenzyme 2 for alanine

Table 4. *Alanine aminotransferase activities of isoenzyme 1 from dog, cat, rat and mouse liver and isoenzyme 2 from rat and mouse liver for various 2-oxo acids*

Details of assays are given in the text. Relative activity values are given, signifying transamination rates compared with that for glyoxylate. Little or no activity was detected with 2-oxoglutarate for all four enzyme preparations.

	Relative activity					
	Isoenzyme 1				Isoenzyme 2	
2-Oxo acid	Dog	Cat	Rat	Mouse	Rat	Mouse
Glyoxylate	1	1	1	1	1	1
Hydroxypyruvate	0.29	0.35	0.25	0.28	0.02	0.03
Phenylpyruvate	0.02	0.03	1.1	1.5	0.05	0.04

Table 5. *Effect of carbonyl reagents on alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities of isoenzyme 1 from dog, cat, rat and mouse liver and alanine–glyoxylate aminotransferase activity of isoenzyme 2 from rat and mouse liver*

Details of assays are given in the text.

		Relative activity					
		Isoenzyme 1				Isoenzyme 2	
Reagent added ...	(mM)	Dog	Cat	Rat	Mouse	Rat	Mouse
Control		1	1	1	1	1	1
Hydroxylamine	0.5	0.50	0.54	0.34	0.39	0.70	0.45
	1.0	0.36	0.40	0.23	0.25	0.65	0.32
Semicarbazide	1.0	0.29	0.37	0.44	0.53	0.75	0.33
	2.0	0.10	0.14	0.31	0.32	0.47	0.19
Isonicotinic acid hydrazide	0.5	0.96	1.0	0.53	0.62	0.30	0.75
	1.0	0.92	0.96	0.29	0.49	0.17	0.51

with glyoxylate (2mM) were 25 and 30mM for mouse and rat respectively. Corresponding K_m values for glyoxylate with alanine (120mM) were 0.72 and 1.0mM respectively.

Reverse reaction. The reverse reaction with glycine and pyruvate is barely detectable in frog liver homogenates (Rowsell *et al.*, 1969), and not at all with L-alanine–glyoxylate aminotransferase partially purified from human liver extract (Thompson & Richardson, 1967). The reverse transamination reaction between glycine (40mM) and pyruvate (2mM) as substrates was examined as described in the Materials and Methods section. Negative results were obtained; all preparations of isoenzymes 1 and 2 were found to transfer irreversibly the amino group from L-alanine to glyoxylate.

Inhibition by carbonyl reagents. Alanine–glyoxylate aminotransferase and serine–pyruvate aminotransferase activities of all four isoenzyme-1 preparations, each to the same degree, were inhibited by the addition of carbonyl reagents, namely semicarbazide and hydroxylamine (Table 5). Isonicotinic acid hydrazide was a strong inhibitor for rat and mouse isoenzyme 1, but not for either dog or cat enzyme. Alanine–glyoxylate aminotransferase activities of isoenzyme-2 preparations from rat and mouse were strongly inhibited by all three carbonyl reagents.

Discussion

Alanine–glyoxylate aminotransferase (Rowsell *et al.*, 1969, 1972*b*,*c*; Snell & Walker, 1972), serine–pyruvate aminotransferase (Rowsell *et al.*, 1972*a*; Snell & Walker, 1974; Sallach *et al.*, 1972; Snell, 1975), phenylalanine–pyruvate aminotransferase (Civen *et al.*, 1967; Brown & Civen, 1969; Fuller *et al.*, 1972, 1974) and histidine–pyruvate aminotransferase (Spolter & Baldridge, 1964; Budillon *et al.*, 1971; Morris *et al.*, 1973) of rat liver have been studied as separate enzymes. All four enzymes are mainly localized in the mitochondrial fraction and induced by glucagon or cyclic AMP. We have reported that rat liver mitochondrial fraction contains two forms of histidine–pyruvate aminotransferase; one is induced by glucagon, and is identical with glucagon-inducible phenylalanine–pyruvate aminotransferase and also with serine–pyruvate aminotransferase; the other is not affected by glucagon, and

is identical with glutamine–phenylpyruvate aminotransferase (Noguchi *et al.*, 1976*a*,*b*,*c*, 1977*a*,*b*). The present study showed that crude mitochondrial extracts of liver contain two forms of alanine–glyoxylate aminotransferase with mol.wts. approx. 80000 (isoenzyme 1) and 175000 (isoenzyme 2) respectively. Liver extracts of carnivores (dog and cat) contain predominantly isoenzyme 1 whereas isoenzyme 2 predominates in rodents (rat and mouse). Rodent isoenzyme 1 is increased in activity by glucagon, but isoenzyme 2 is not. Isoenzyme 1 was highly purified from dog and cat liver, and from liver of glucagon-injected rats and mice by the same procedure. In each case, the activity ratio alanine–glyoxylate aminotransferase/serine–pyruvate aminotransferase maintained a constant value during all purification steps after the removal of isoenzyme 2 by gel filtration. In each case, this ratio was unchanged after a variety of treatments of the purified isoenzyme 1, namely gel filtration, inhibition by carbonyl reagents, polyacrylamide-disc-gel electrophoresis, sucrose-density-gradient centrifugation and isoelectric focusing. These results suggest that the two activities of isoenzyme 1 are properties of the same protein. A striking difference in substrate specificity was observed between carnivore (dog and cat) and rodent (rat and mouse) isoenzyme 1. With glyoxylate as amino acceptor, rodent enzymes showed a broad specificity for L-amino acids, whereas carnivore enzymes were specific for L-alanine and L-serine. Glyoxylate, phenylpyruvate and hydroxypyruvate were effective amino acceptors for rodent enzymes, and glyoxylate and hydroxypyruvate for carnivore enzymes.

Alanine–glyoxylate aminotransferase has been partially purified from human liver extract (Thompson & Richardson, 1967). Feld & Sallach (1973) suggested that hydroxypyruvate–alanine aminotransferase and alanine–glyoxylate aminotransferase activities were co-purified from rabbit liver cytosol. Both human and rabbit enzyme preparations show an amino-donor specificity similar to that of carnivore isoenzyme 1, but not to that of rodent isoenzymes 1 and 2. The mol.wt. (41000) and isoelectric point (pI 7.9) of rabbit enzyme differ from those of isoenzymes 1 and 2. These values have not been determined with human enzyme preparation (Thompson & Richardson, 1967).

Hsieh & Tolbert (1976) have partially purified a glyoxylate aminotransferase from rat liver peroxisomes. The amino acid substrate specificity is strikingly similar to that of rat liver isoenzyme 1. Further, Cooper (1977) has reported an asparagine transaminase from rat liver with a very similar amino acid specificity to that of rat liver isoenzyme 1. It may be that these aminotransferase activities are all manifestations of the same enzyme. Further studies on the identity of these enzymes are required.

Alanine–glyoxylate aminotransferase isoenzyme 2, which differs from isoenzyme 1 in physical and enzymic properties, was also purified from liver mitochondrial extracts of rats and mice. Both enzyme preparations were specific for L-alanine with glyoxylate as amino acceptor and for glyoxylate with L-alanine as amino donor.

It has been reported that in rats alanine–glyoxylate aminotransferase activity begins to develop in late foetal liver, increases rapidly at birth to a peak during suckling and then decreases at weaning to the adult values (Snell & Walker, 1972). Present findings suggest that an investigation of the separate developmental profiles of rat liver isoenzymes 1 and 2 would be of interest.

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