Characteristics of Hepatic Serine–Pyruvate Aminotransferase in Different Mammalian Species

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1. Serine–pyruvate aminotransferase was purified from mouse, rat, dog and cat liver. Each enzyme preparation was homogeneous as judged by polyacrylamide-disc-gel electrophoresis in the presence of sodium dodecyl sulphate. However, isoelectric focusing resulted in the detection of two or more active forms from enzyme preparations from dog, cat and mouse. A single active form was obtained with the rat enzyme. All four enzyme preparations had similar pH optima and molecular weights. 2. Both mouse and rat preparations catalysed transamination between a number of L-amino acids (serine, leucine, asparagine, methionine, glutamine, ornithine, histidine, phenylalanine or tyrosine) and pyruvate. Effective amino acceptors were pyruvate, phenylpyruvate and glyoxylate with serine as amino donor. The reverse transamination activity, with hydroxypyruvate and alanine as substrates, was lower than with serine and pyruvate for both species. Serine–pyruvate aminotransferase activities were inhibited by isonicotinic acid hydrazide. 3. In contrast, both dog and cat enzyme preparations were highly specific for serine as amino donor with pyruvate, and utilized pyruvate and glyoxylate as effective amino acceptors. A little activity was detected with phenylpyruvate. The reverse activity was higher than with serine and pyruvate for both species. Serine–pyruvate aminotransferase activities were not inhibited by isonicotinic acid hydrazide.

It has been suggested that serine–pyruvate aminotransferase (EC 2.6.1.51) of mammalian liver has a role in gluconeogenesis from serine (Williamson & Ellington, 1975; Rowsell *et al.*, 1969; Sallach *et al.*, 1972; Snell, 1975). Rowsell *et al.* (1972a) reported that hepatic serine–pyruvate aminotransferase was distributed in a wide range of animal species and high activity was associated with carnivorous species (dog, cat, common frog and common toad). The enzyme has been partially characterized with crude preparations from dog liver (Sallach, 1956). We have described how highly purified serine–pyruvate aminotransferase from rat liver mitochondria shows a broad specificity for various amino acids with pyruvate or phenylpyruvate as amino acceptor (Noguchi *et al.*, 1976c). The present paper describes the purification and characterization of serine–pyruvate aminotransferase from livers of carnivores (dog and cat) and rodents (mouse and rat).

Materials and Methods

Materials

Lactate dehydrogenase (L-lactate–NAD⁺ oxidoreductase, EC 1.1.1.27), sodium indol-3-ylpyruvate, sodium *p*-hydroxyphenylpyruvate and lithium hydroxypyruvate were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Other

materials were obtained as stated previously (Noguchi *et al.*, 1976a,b,c).

Aminotransferase assays

Assay mixtures contained, unless specified otherwise, 40 mM-L-amino acid, 10 mM-2-oxo acid, 0.2 M-Tris/HCl, pH 9.0, and enzyme preparation. Incubation was at 37°C.

Transamination between an aromatic L-amino acid (histidine, phenylalanine, tyrosine, tryptophan or 5-hydroxytryptophan) and pyruvate was in principle determined by the method of Lin *et al.* (1958). This assay was based on the arsenate-catalysed formation of aromatic 2-oxo acid–enol–borate complexes, which show characteristic absorption spectra in the 300 nm region. Details of this assay have been described (Noguchi *et al.*, 1976a).

Transamination between L-serine and 2-oxo acids (pyruvate, 2-oxoglutarate, phenylpyruvate, *p*-hydroxyphenylpyruvate or indol-3-ylpyruvate) was determined as described by Snell & Walker (1974). The amount of hydroxypyruvate formed was determined with excess of NADH and spinach-leaf D-glycerate dehydrogenase (D-glycerate–NAD⁺ oxidoreductase, EC 1.1.1.29).

Transamination between L-serine and glyoxylate was in principle determined as described for alanine–glyoxylate aminotransferase (EC 2.6.1.44) by Rowsell

et al. (1969, 1972b). In this assay medium, 0.2M-potassium phosphate buffer, pH8.0, was substituted for Tris/HCl buffer, pH9.0, because glyoxylate forms a Schiff base with Tris (Thompson & Richardson, 1968; Rowsell *et al.*, 1972b). After a 1 h incubation, hydroxypyruvate formed was determined with excess of NADH and lactate dehydrogenase by measuring the decrease in A_{340} in the presence of Tris. Under these conditions, hydroxypyruvate is a substrate for lactate dehydrogenase, but not glyoxylate.

Transamination between aliphatic L-amino acids, except L-serine, and pyruvate was determined by measuring the amount of unchanged pyruvate. The reaction was terminated after a 1 h incubation of the assay mixture (0.2 ml) by the addition of 25 μ l of 25% (w/v) trichloroacetic acid. In the control, pyruvate was added after incubation. The incubation mixture was neutralized by the addition of 25 μ l of 2M-KOH. Samples (70 μ l) were then mixed with 70 μ l of NADH (0.86 μ mol) and diluted with 2.5 ml of 0.2M-potassium phosphate buffer, pH7.5. Pyruvate concentrations were determined from the change in A_{340} on the addition of lactate dehydrogenase.

Transamination between alanine and hydroxypyruvate was determined as described by Cheung *et al.* (1969). This assay is based on the quantitative reduction of unchanged hydroxypyruvate in the presence of excess of NADH by spinach D-glycerate dehydrogenase before determination of pyruvate with lactate dehydrogenase.

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.

Determination of protein, polyacrylamide-disc-gel electrophoresis in the presence and absence of sodium dodecyl sulphate, isoelectric focusing and determination of approximate molecular weight by sucrose-density-gradient centrifugation and Sephadex G-150 gel filtration were carried out as described by Noguchi *et al.* (1976a,b,c).

Results

Purification of hepatic mitochondrial serine-pyruvate aminotransferase from mouse, rat, dog and cat

Rat liver mitochondrial serine-pyruvate aminotransferase was purified as previously described (Noguchi *et al.*, 1976c). The same procedure was applied with mouse, cat and dog liver. All operations were carried out at 0–4°C. Potassium phosphate buffer, pH7.5, was used throughout.

Preparation of mitochondrial extracts. A male dog (about 25 kg body wt.), a female cat (about 3.5 kg) and 60 male mice of the ddY strain (15–20 g) were used. The mitochondrial fraction (combined heavy and light mitochondria) was prepared from each liver (dog, 50 g; cat, 45 g; mouse, 60 g) by the method of

de Duve *et al.* (1955). Each mitochondrial fraction was homogenized with 5 vol. of 5 mM buffer in a Waring blender for 2 min. After sonication for 4 min at 10 kHz with a Kubota sonicator (Tokyo, Japan), homogenates were centrifuged at 105 000g for 30 min and the precipitates discarded.

Heat-treatment, $(\text{NH}_4)_2\text{SO}_4$ fractionation, DEAE-cellulose and hydroxyapatite chromatography. Supernatants were then subjected to a sequence of fractionation procedures: heat-treatment, $(\text{NH}_4)_2\text{SO}_4$ fractionation, DEAE-cellulose chromatography and hydroxyapatite chromatography as described for rat liver (Noguchi *et al.*, 1976b,c).

Isoelectric focusing. The concentrated enzyme solution thus obtained was subjected to isoelectric focusing on a pH3.5–10 Ampholine gradient. The focusing resulted in the detection of two or more active forms in enzyme preparations from the three sources (Fig. 1). Similar focusing profiles of the activity were obtained for the two enzyme preparations from cat and dog, showing a peak (pI6.9) with a shoulder (near pI6.6). Indications of a greater degree of heterogeneity were observed with mouse enzyme preparation. The active fractions were collected without separation of multiple forms and concentrated by ultrafiltration.

Sephadex G-150 gel filtration. The concentrated enzyme solution was added to a column (2.5 cm $\times$ 100 cm) of Sephadex G-150, which had been equilibrated with 50 mM buffer. The column was eluted with the same buffer at a flow rate of 25 ml/h. The effluent was collected in 3 ml fractions. A single symmetrical activity peak was obtained with each enzyme preparation from three sources.

Results of typical purifications of serine-pyruvate aminotransferase are shown in Table 1. The enzyme was purified about 148-fold over the dog liver extract, 290-fold over the cat liver extract and 1132-fold over the mouse liver extract. All three enzyme preparations (in 50 mM-potassium phosphate buffer, pH7.5, containing 100 μ M-pyridoxal 5'-phosphate) may be stored at –20°C for at least 4 weeks without loss of activity and there was little loss with storage at 0–6°C for at least 2 weeks.

Criteria of purity

We have previously reported that with the purified serine-pyruvate aminotransferase from rat liver mitochondria, a single peak with protein and activity coincident is obtained by polyacrylamide-disc-gel electrophoresis, Sephadex G-150 gel filtration and sucrose-density-gradient centrifugation, and a single protein band by polyacrylamide-disc-gel electrophoresis in the presence of sodium dodecyl sulphate (Noguchi *et al.*, 1976c).

On polyacrylamide-disc-gel electrophoresis at pH8.9 in 7% gel, a major protein band with a mobility of 0.39 (relative to that of a Bromophenol

Fig. 1. Isoelectric focusing of enzyme preparations obtained after hydroxyapatite column chromatography. Each enzyme preparation of (a) dog, (b) cat or (c) mouse liver from hydroxyapatite columns was separately subjected to isoelectric focusing on a pH3.5-10 Ampholine gradient. Fractions (2ml) were collected. pH values (○) and serine-pyruvate aminotransferase activities (●) were determined as described in the text.

Blue marker) and three minor protein bands with mobility between 0.39 and 0.41 were obtained with mouse enzyme preparation. When unstained gels run in parallel were sliced into 1 mm sections and added to the assay system for the determination of serine-pyruvate aminotransferase activity, the major protein band and the activity were coincident. We could not determine whether activity was also associated with the three minor protein bands; they were located very close to the major protein band. On electrophoresis, both dog and cat enzyme preparations yielded two distinct protein bands (mobilities 0.30 and 0.33) within a diffuse relatively broad band (mobility, 0.26-0.39). The two protein bands coincided with enzyme activity.

Polyacrylamide-disc-gel electrophoresis in the

Table 1. Purification of serine-pyruvate aminotransferase from dog, cat and mouse livers. Details of purification and assay methods are given in the text.

	Total protein (mg)			Total activity (units)			Specific activity (units/mg of protein)			Recovery (%)			Purification (fold)		
	Dog	Cat	Mouse	Dog	Cat	Mouse	Dog	Cat	Mouse	Dog	Cat	Mouse	Dog	Cat	Mouse
Extract	2438	727	600	12921	2108	114	5.3	2.9	0.19	100	100	100	1	1	1
Heat treatment	723	172	72	11568	2098	94	16.0	12.2	1.3	90	100	82	3.0	4.2	6.8
(NH ₄) ₂ SO ₄ fractionation	250	61	25	6075	1720	75	24.3	28.2	3.0	47	82	66	4.6	9.7	16
DEAE-cellulose	25	16	2.4	4600	1363	62	184	85.2	26	36	65	54	35	29	137
Hydroxyapatite	8.2	5.1	0.80	2814	903	46	339	177	57	22	43	40	64	61	300
Isoelectric focusing and Sephadex G-150 gel filtration	1.6	0.6	0.12	1259	504	261	787	840	215	9.7	24	23	148	290	1132

presence of sodium dodecyl sulphate resulted in the detection of a single protein band with all three enzyme preparations.

Sephadex G-150 gel filtration and sucrose-density-gradient centrifugation (for molecular-weight determination) gave a single symmetrical peak with protein and enzyme activity coincident with all three preparations.

Isoelectric focusing on a pH3.5–10 Ampholine gradient resulted in the detection of two or more active forms with each enzyme preparation from dog, cat and mouse. Each focusing profile was nearly identical with that given in Fig. 1. A single activity peak was obtained with rat enzyme preparation (Noguchi *et al.*, 1976a).

Properties of enzyme preparations from mouse, rat, dog and cat

pH optimum. Potassium phosphate buffer (0.2M) was used between pH7.0 and 8.0, and Tris/HCl (0.2M) between pH8.0 and 9.6. Similar pH profiles were obtained for all four enzyme preparations, showing pH optima between pH8.5 and 9.0.

Determination of approximate molecular weight. The molecular weight was estimated as 75000–80000 by Sephadex G-150 gel filtration and 73000–79000 by sucrose-density-gradient centrifugation for all four enzyme preparations. Polyacrylamide-disc-gel electrophoresis in the presence of sodium dodecyl sulphate gave a molecular weight of 36000–40000 for all four enzyme preparations, showing that they probably consist of two identical subunits.

Substrate specificity. The relative activities of the four enzyme preparations with various amino acids (40mM) were determined by using pyruvate (10mM) as amino acceptor. Results are summarized in Table 2. Rat and mouse enzymes both catalysed transamination between pyruvate and serine, leucine, asparagine, methionine, glutamine, ornithine, histidine, phenylalanine or tyrosine. Additional effective amino acids for mouse enzyme were isoleucine, valine and lysine. In contrast, both dog and cat enzymes were highly specific for serine as amino donor with pyruvate as amino acceptor. The amino acceptor specificity of the four enzyme preparations was examined with serine as amino donor (Table 3). Effective 2-oxo acids were pyruvate, phenylpyruvate and glyoxylate for rat and mouse enzyme. Dog and cat enzyme utilized pyruvate, glyoxylate and, less effectively, phenylpyruvate. 2-Oxoglutarate, indol-3-ylpyruvate and *p*-hydroxyphenylpyruvate were not active with any of these enzyme preparations. The apparent K_m values were determined from double-reciprocal plots of initial velocity and substrate concentration. The apparent K_m values for serine with pyruvate (10mM) as amino acceptor and for pyruvate with serine (40mM) as amino donor were respectively as follows: dog, 8.0 and 1.7mM; cat, 10

Table 2. *Relative activities of serine-pyruvate aminotransferase from dog, cat, mouse and rat livers for various amino acids*

Assay conditions were as described in the text. Relative activity values are given, signifying transamination rates compared with that for L-serine. N.D., Not detected. Dog and cat enzyme preparations were highly specific for L-serine. Little or no activity was detected with other amino acids given in Table 2.

L-Amino acid	Relative activity	
	Mouse	Rat
Serine	1	1
Glutamic acid	0.16	N.D.
Leucine	3.9	3.2
Isoleucine	1.8	0.21
Valine	1.6	0.15
Asparagine	3.7	5.0
Lysine	1.4	0.24
Methionine	3.6	3.1
Glutamine	2.8	2.9
Ornithine	1.5	1.3
Aspartic acid	0.18	N.D.
Histidine	3.2	3.0
Phenylalanine	4.2	5.0
Tyrosine	2.7	2.3
Tryptophan	0.26	0.22
5-Hydroxytryptophan	0.21	N.D.

Table 3. *Relative activities of serine-pyruvate aminotransferase from dog, cat, mouse and rat livers 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 pyruvate. Little or no activity was detected with 2-oxoglutarate, indol-3-ylpyruvate, *p*-hydroxyphenylpyruvate for all four enzyme preparations.

2-Oxo acid	Relative activity			
	Dog	Cat	Mouse	Rat
Pyruvate	1	1	1	1
Glyoxylate	0.20	0.71	0.34	0.68
Phenylpyruvate	0.06	0.09	0.65	1.2

and 1.6mM; mouse, 18 and 1.2mM; rat, 16 and 1.0mM.

Reverse reaction. The reverse transamination reaction with alanine (40mM) and hydroxypyruvate (10mM) as substrates was examined as described in the Materials and Methods section (Table 4). The reverse activity was higher than with serine (40mM) and pyruvate (10mM) for both dog and cat enzyme. In the contrast, both mouse and rat enzyme were more active in the direction of alanine and hydroxypyruvate formation.

Inhibition by carbonyl reagents. Serine-pyruvate

aminotransferase activities of all four enzyme preparations were inhibited by the addition of carbonyl reagents, namely semicarbazide and hydroxylamine (Table 5). Isonicotinic acid hydrazide was a strong inhibitor for both rat and mouse enzyme, but not for either dog or cat enzyme.

Discussion

Serine, long known as a gluconeogenic amino acid, has been shown to be one of the best precursors for gluconeogenesis in the perfused rat liver (Ross *et al.*, 1967). Gluconeogenesis from serine may be initiated by serine dehydratase (Snell & Walker, 1974) or serine-pyruvate aminotransferase (Williamson & Ellington, 1975). In the normal fed and starved rats, serine dehydratase seems likely to play a major role in gluconeogenesis (Chan & Freedland, 1971; Snell, 1974; Sandoval & Sols, 1974). In the neonatal rat serine-pyruvate aminotransferase appears to play a greater role (Rowsell *et al.*, 1973; Snell, 1975). However, these studies have been restricted to rats. Rowsell *et al.* (1972a) reported that high activity was associated with carnivorous animal species, in a comparative study for hepatic serine-pyruvate aminotransferase, covering the pig, sheep, rabbit, guinea pig, rat, mouse, cat, dog, common frog and

common toad. In the present study, a comparative study of highly purified serine-pyruvate aminotransferase from rodents (mouse and rat) and carnivores (dog and cat) was carried out. A striking difference in amino donor specificity was observed between rodent and carnivore enzymes. Rodent enzymes showed a broad specificity for amino donors with pyruvate as amino acceptor, whereas carnivore enzymes showed a high specificity for serine. The effective amino acceptors with the rodent enzymes were pyruvate, phenylpyruvate and glyoxylate with serine as amino donor. The carnivore enzymes were active with pyruvate and glyoxylate; little activity was observed with phenylpyruvate.

Rowsell *et al.* (1972a) reported that the reverse transamination activity of liver homogenate from dog and frog with alanine and hydroxypyruvate was higher than with serine and pyruvate. We have obtained the same results with purified enzyme preparations from dog and cat livers. However, both rat and mouse enzyme were more active in the direction of alanine and hydroxypyruvate synthesis. Further studies are required on the purification and characterization of hepatic serine-pyruvate aminotransferase from other animal species, but the present work suggests that the physiological significance of serine-pyruvate aminotransferase may differ between rodents and carnivores.

Enzyme preparations from dog, cat and mouse livers 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. However, isoelectric focusing and electrophoresis in the absence of sodium dodecyl sulphate resulted in the detection of two or more active forms with enzyme preparation from each of the three sources. The same phenomenon has been observed for avian liver 4-hydroxyphenylpyruvate dioxygenase [4-hydroxyphenylpyruvate-oxygen oxidoreductase (hydroxylating, decarboxylating), EC 1.13.11.27] by Wada *et al.*

Table 4. *Reversibility of serine-pyruvate aminotransferase from dog, cat, mouse and rat livers*
Details of assays are given in the text.

Species	Specific activity (units/mg of protein)	
	Serine-pyruvate transamination	Alanine-hydroxypyruvate transamination
Dog	780	1883
Cat	840	998
Mouse	215	134
Rat	245	121

Table 5. *Effect of carbonyl reagents on serine-pyruvate aminotransferase from dog, cat, mouse and rat livers*
Details of assays are given in the text.

Reagent added	Concn. (mM)	Relative activity			
		Dog	Cat	Mouse	Rat
Control		100	100	100	100
Hydroxylamine	0.5	48	52	37	12
	1.0	38	40	21	21
Semicarbazide	1.0	26	32	88	40
	2.0	8	11	51	24
Isonicotinic acid hydrazide	0.5	100	100	68	47
	1.0	96	98	54	31

al. (1975). They suggested that this phenomenon may be explained by non-enzymic deamidation of a single nascent enzyme. The heterogeneity of dog, cat and mouse enzymes might be also explained by this mechanism.

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