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Identity of Rat Kidney Histidine–Pyruvate Aminotransferase with Glutamine–Oxo Acid Aminotransferase

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Histidine–pyruvate aminotransferase and glutamine–phenylpyruvate aminotransferase were purified from rat kidney by the same procedure. The ratio of the two activities remained constant during purification and was unchanged by a variety of treatments of the purified enzyme. Glutamine was found to act as a competitive inhibitor of histidine–pyruvate aminotransferase. These results suggest that rat kidney histidine–pyruvate aminotransferase is identical with glutamine–oxo acid aminotransferase. Identity of the two enzymes in other tissues of rats is discussed.

Rat liver contains two forms of histidine–pyruvate aminotransferase. One, designated isoenzyme 1 (pI 8.0), is present only in the liver and is induced by the injection of glucagon; the other, designated isoenzyme 2 (pI 5.2), is found in the kidney, liver, brain and heart, and is not affected by glucagon (Noguchi *et al.*, 1976*a*,*b*). Isoenzyme 1 has been reported to be identical with serine–pyruvate aminotransferase (EC 2.6.1.51) (Noguchi *et al.*, 1976*c*).

It has also been documented that glutamine aminotransferase (glutamine–oxo acid aminotransferase, EC 2.6.1.15) is present in various mammalian tissues (Kupchik & Knox, 1970). The enzyme has been characterized and highly purified from the kidney, liver and brain of rats.

The present report describes the identity of rat kidney histidine–pyruvate aminotransferase (isoenzyme 2) with glutamine–oxo acid aminotransferase.

Materials and Methods

All materials used were obtained as stated previously (Noguchi *et al.*, 1976*a*,*b*,*c*). Histidine–pyruvate aminotransferase activity was determined as described by Noguchi *et al.* (1976*a*). The assay mixture (0.8 ml) contained 20 mM-L-histidine, 20 mM-sodium pyruvate, enzyme preparation and 0.2 M-Tris/HCl, pH 9.0. Glutamine–oxo acid aminotransferase activity was determined by the method of Kupchik & Knox (1970). The reaction mixture (0.8 ml) contained 20 mM-glutamine, 1.4 mM-sodium phenylpyruvate, enzyme preparation and 0.2 M-Tris/HCl, pH 9.0. The disappearance of the enol–borate complex of phenylpyruvate was continuously measured at 300 nm.

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

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

Histidine–pyruvate aminotransferase was purified from kidneys (about 8 g wet wt.) of four male rats (about 250 g body wt.) of the Donryu strain as previously described (Noguchi *et al.*, 1976*b*).

Results and Discussion

Fig. 1 shows the isoelectric-focusing profile of rat kidney extract. Histidine–pyruvate aminotransferase and glutamine–phenylpyruvate aminotransferase activities were identically focused, showing a single activity peak with pI 5.2. The ratio of the two activities was about 9.0.

The results of the purification of histidine–pyruvate aminotransferase and glutamine–phenylpyruvate aminotransferase are shown in Table 1. Both enzyme activities were found in the same fractions in all purification steps. About 160-fold purification was achieved, with a recovery of about 23% for both enzyme activities. The activity ratio remained constant during the purification.

On polyacrylamide-disc-gel electrophoresis at pH 8.9 in 7% gel, a major component which represented about 85% of the total protein, and two other protein bands, were obtained with the purified enzyme preparation. Unstained gels run in parallel were sliced into 2 mm sections and added to the assay system for determination of activities; the major protein band and the two aminotransferase activities were coincident. Sephadex G-150 gel filtration, sucrose-density-gradient centrifugation and isoelectric focusing on a pH 4–6 Ampholine gradient gave a single symmetrical peak, possessing the two enzyme activities. The activity ratio of the enzyme

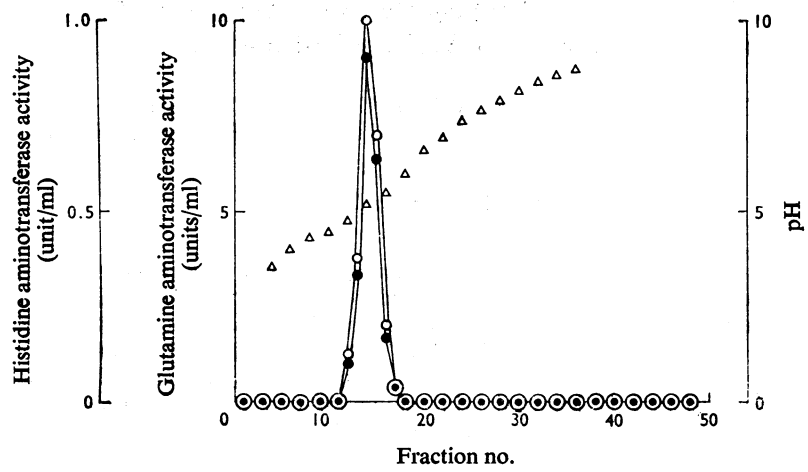

Fig. 1. *Isoelectric-focusing profiles of rat kidney extract*
A male rat (200g body wt.) of the Donryu strain was decapitated with a guillotine and kidneys were removed. Kidneys were homogenized in 5vol. of ice-cold 5mM-potassium phosphate buffer, pH7.5, in a Potter–Elvehjem tissue grinder with a Teflon pestle. After sonication at 20kHz for 4min, the homogenate was centrifuged at 105000*g* for 30min. A portion of the resulting supernatant (corresponding to 1g of kidney) was subjected to isoelectric focusing on a pH3.5–10 Ampholine gradient. Fractions (2ml) were collected. pH values (△), histidine–pyruvate aminotransferase activity (○) and glutamine–phenylpyruvate aminotransferase activity (●) were determined as described in the text.

Table 1. *Purification of histidine–pyruvate aminotransferase and glutamine–phenylpyruvate aminotransferase from rat kidney*
The final column shows the ratio of glutamine–phenylpyruvate aminotransferase to histidine–pyruvate aminotransferase activity.

		Histidine–pyruvate aminotransferase			Glutamine–phenylpyruvate aminotransferase			
	Total protein (mg)	Specific activity (units/mg)	Total activity (units)	Purification (fold)	Specific activity (units/mg)	Total activity (units)	Purification (fold)	Activity ratio
Extract	328	0.14	46.0	1	1.23	403	1	8.8
$(NH_4)_2SO_4$ fraction	165	0.24	39.6	1.7	2.18	360	1.8	9.1
Heat treatment	34	1.0	34.0	7.1	8.70	296	7.1	8.7
DEAE-cellulose	4.1	5.9	24.2	42	49.6	203	40	8.4
Hydroxyapatite	1.4	12.2	17.0	87	104	146	85	8.5
Isoelectric focusing and Sephadex G-150	0.47	22.0	10.3	157	198	93	161	9.0

towards histidine and glutamine was unchanged after these treatments.

The two enzyme activities were inhibited, each to the same extent, by carbonyl reagents, namely 1mM-isonicotinic acid hydrazide (42% inhibition), 2mM-hydroxylamine (54%) and 1mM-semicarbazide (62%). Histidine–pyruvate aminotransferase activity of the enzyme was competitively inhibited by the addition of glutamine (6.7mM).

These results suggest that kidney histidine–pyruvate aminotransferase is identical with rat kidney glutamine–oxo acid aminotransferase. Glutamine aminotransferase, which uses phenylpyruvate as an effective amino acceptor, has been highly purified and characterized from the kidney (Cooper & Meister, 1974), brain (Vanleuven, 1975, 1976) and liver (Cooper & Meister, 1972). We have reported that highly purified enzyme preparations (histidine–pyruvate aminotransferase isoenzyme 2) from the kidney, brain, liver and heart of rats were remarkably similar in isoelectric point, pH optimum, molecular weight, electrophoretic migration, K_m values for histidine and pyruvate, and degree of inhibition of phenylalanine aminotransferase and

tyrosine aminotransferase activities by histidine, suggesting the identity of isoenzyme-2 preparations from these four tissues. On the basis of the above reports and the present results, it is suggested that histidine–pyruvate aminotransferase (isoenzyme 2) of rat brain, kidney, heart and liver is also identical with glutamine aminotransferase.

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