Purification and properties of 2-aminoadipate:2-oxoglutarate aminotransferase from bovine kidney

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Previous studies with rat kidney preparations indicated that 2-aminoadipate aminotransferase (AadAT) and kynurenine aminotransferase (KAT) activities are properties of a single protein. We found that bovine kidney contains an appreciable amount of AadAT activity, but lacks KAT activity. AadAT from bovine and rat kidney extracts were purified to electrophoretic homogeneity. The purification procedure included fractionation with $(\text{NH}_4)_2\text{SO}_4$, heat treatment, DEAE-cellulose chromatography and hydroxyapatite chromatography. Physical and kinetic properties, such as pH optima, K_m for substrates, M_r , electrophoretic mobility and inhibition by dicarboxylic acids of bovine kidney AadAT, were similar to those of the rat kidney enzyme. However, bovine kidney AadAT differed from rat kidney AadAT in substrate specificity, amino acid composition and stability when stored. The titration curve of bovine kidney AadAT was also different from that of the rat kidney enzyme. The results suggest that bovine kidney AadAT may have some structural similarity to rat kidney AadAT and that the structural differences observed between the two enzymes may explain the absence of KAT activity in bovine kidney.

INTRODUCTION

2-Aminoadipate aminotransferase (AadAT; 2-aminoadipate:2-oxoglutarate aminotransferase, EC 2.6.1.39) is an enzyme of the lysine catabolic pathway. It catalyses the reversible transamination reaction between 2-aminoadipate and 2-oxoglutarate to produce 2-oxoadipate plus L-glutamate. In mammals, AadAT activity is reportedly found in liver and kidney [1]. Previous studies indicated that in rat liver AadAT is localized in mitochondria, whereas in kidney it is present in mitochondria as well as in the supernatant fraction [1,2]. Kynurenine aminotransferase [KAT; L-kynurenine:2-oxoglutarate aminotransferase (cyclizing), EC 2.6.1.7] is an enzyme of tryptophan catabolism. It catalyses the irreversible transamination reaction between L-kynurenine and 2-oxoglutarate to form kynurenic acid and L-glutamate. In mammals, KAT activity has been reported to be present in liver, kidney, small intestine and brain [3]. In these tissues, KAT is predominantly localized in the inner membrane of mitochondria and plays an important role in the intramitochondrial formation of xanthurenate from 3-hydroxy-L-kynurenine. KAT activity has also been reported in the supernatant fractions of kidney and liver [4].

Tobes & Mason [5] purified AadAT/KAT from rat kidney supernatant to homogeneity, and proposed that AadAT and KAT activities are the properties of a single protein. This hypothesis was based on the observation that both AadAT and KAT co-purified with a constant ratio of specific activities, showed similar chromatographic behaviour and exhibited similar patterns of heat inactivation and inhibition by dicarboxylic acids. This hypothesis was further supported by the studies by

Takeuchi *et al.* [6], who provided similar evidence for AadAT/KAT activities purified from rat liver mitochondria.

Previous attempts to separate AadAT and KAT activities have been unsuccessful [5,6]. We observed that bovine kidney contains an appreciable amount of AadAT activity, but does not contain any KAT activity. To test whether AadAT from bovine kidney is similar to AadAT/KAT from rat kidney, we purified AadAT from bovine and rat kidney supernatants and compared their properties. The results described here show that many of the physical and kinetic properties of bovine kidney AadAT are comparable with those of the rat kidney enzyme. However, bovine kidney AadAT differs from rat kidney AadAT in substrate specificity, stability when stored, amino acid composition and titration curve.

EXPERIMENTAL

Chemicals

2-Aminoadipic acid, 2-oxoglutaric acid, L-kynurenine sulphate, kynurenic acid, pyridoxal 5'-phosphate, Sephadex G-200, standard protein M_r markers and other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals

Adult male albino rats (Sprague–Dawley) weighing 250–300 g were purchased from Charles River Laboratories, Wilmington, ME, U.S.A. Fresh bovine kidneys were obtained from a local store.

Abbreviations used: AadAT, 2-aminoadipate aminotransferase; KAT, kynurenine aminotransferase.

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Enzyme assays

Glutamate dehydrogenase (EC 1.4.1.3) and lactate dehydrogenase (EC 1.1.1.27) activities were assayed spectrophotometrically at 25 °C [7,8]. The standard assay system for AadAT contained 250 mM-potassium phosphate buffer (pH 7.0), 1.7 mM-2-oxoadipate, 16.7 mM-L-glutamate and 0.25 mM-pyridoxal 5'-phosphate in a final volume of 0.6 ml. After the assay mixture incubated for 15 min at 37 °C, the reaction was terminated by adding 0.2 ml of 1 M-HCl. In control tubes, 2-oxoadipate was added after terminating the reaction. The reaction mixture was neutralized by adding 0.2 ml of 1 M-KOH, and the denatured protein was removed by centrifugation. 2-Oxoglutarate formed in the assay system was determined by the glutamate dehydrogenase assay [2].

The reaction mixture for assay of KAT activity contained 500 mM-imidazole buffer (pH 6.5), 3.3 mM-kynurenine sulphate, 16.7 mM-2-oxoglutarate, 0.2 mM-pyridoxal 5'-phosphate and enzyme in a final volume of 0.6 ml. After the assay mixture was incubated for 30 min at 37 °C, the reaction was terminated by adding 10 ml of 1% (w/v) boric acid in 95% (v/v) ethanol. 2-Oxoglutarate was added to control tubes after terminating the reaction. Denatured protein was removed by centrifugation, and the A_{333} corresponding to the formation of kynurenate was measured against boric acid in ethanol. The amount of kynurenic acid formed was calculated as described by Knox [9]. Preliminary experiments were carried out to confirm that the product formation was linear with time for both assays.

Protein determination

Protein concentrations were determined by either the protein-dye-binding assay of Bradford [10], with bovine serum albumin as a standard, or from the absorption coefficient of the enzyme at 280 nm.

Enzyme unit

One unit of enzyme activity is defined as the amount of enzyme that catalysed the formation of 1 μ mol of product/min at 37 °C. Specific activity is expressed as units/mg of protein.

Purification of AadAT/KAT

Identical procedures were used for purifying these enzymes from rat and bovine kidney extracts, except that 0.1 mM-dithiothreitol (instead of 2-mercaptoethanol) was added to the buffer used to purify AadAT from bovine kidney. Unless otherwise indicated, all operations were carried out at 0–4 °C. Potassium phosphate buffers were used routinely.

Rats were killed by decapitation and their kidneys were immediately removed. Rat or bovine kidneys were homogenized (10%, w/v) in 0.25 M-sucrose, pH 7.4, containing 0.1 mM-EDTA and 10 mM-Tris. The homogenate was centrifuged at 600 g for 10 min and the pellet was discarded. Mitochondria were isolated as described by de Duve *et al.* [11]. The supernatant fluid was centrifuged at 5000 g for 10 min. The resulting supernatant was used for the purification of AadAT activities. Other steps of purification, such as acid precipitation, $(\text{NH}_4)_2\text{SO}_4$ and heat fractionations, DEAE-cellulose and hydroxyapatite chromatography, were carried out as described by Tobes & Mason [5].

Polyacrylamide-gel electrophoresis

To check the purity of isolated enzymes, non-gradient polyacrylamide-gel (7.5%) electrophoresis of the native enzymes was carried out at pH 8.5 by the method of David [12]. The M_r of the subunits was estimated by gel electrophoresis in SDS as described by Weber *et al.* [13]. The enzymes were denatured by treatment with 1% SDS solution containing 1% 2-mercaptoethanol at 100 °C for 5 min. The protein standards, lysozyme (M_r 14300), β -lactalbumin (M_r 18400), egg albumin (M_r 45000) and bovine serum albumin (M_r 66000), were treated in the same way.

M_r of the native enzymes

The M_r of AadAT from rat and bovine kidney was determined by chromatography on a standardized Sephadex G-200 column, as described by Andrews [14]. The Sephadex column (1 cm $\times$ 50 cm) was equilibrated at 4 °C with 70 mM-potassium phosphate, pH 7.0, containing 10 mM-2-mercaptoethanol. The column was calibrated with triplicate gel filtration of Blue Dextran and the following standard proteins: cytochrome *c* (M_r 12400), carbonic anhydrase (M_r 29000), bovine serum albumin (M_r 66000), alcohol dehydrogenase (M_r 150000) and β -amylase (M_r 200000). Proteins were eluted in the same buffer, and the elution was monitored by measuring the A_{280} . AadAT and KAT activities were assayed as described above. The void volume (V_0) was assumed to be the elution volume of Blue Dextran. A plot of V_e/V_0 against $\log M_r$ was used to determine the M_r of purified enzymes.

Substrate specificity

The substrate specificity of the purified enzymes from rat and bovine kidney was determined by using a number of α -amino acids as amino donors, with 2-oxoglutarate, pyruvate or glyoxalate as the amino group acceptor. The transamination reaction with aromatic amino acids was assayed by the method of Takada & Noguchi [15]. The amount of keto acid formed was calculated from the molar absorption coefficient of the relevant enol-borate complex. Keto acids were added to control tubes after terminating the reaction. Histidine:pyruvate aminotransferase and 3,5-di-iodotyrosine aminotransferase activities were assayed as described previously [16].

Amino acid analysis

Amino acid analysis was carried out at the Protein Sequencing Facility of the University of Michigan. Freeze-dried samples of purified enzymes were hydrolysed *in vacuo* at 110 °C in 6 M-HCl. The amino acids were analysed as their phenylthiohydantoin derivatives by using the Waters Pico-tag system [17].

Absorption spectra

The absorption spectrum (300–500 nm) for each enzyme was obtained at pH 5.0 and 8.2 in the presence of 0.05 mM-mercaptoethanol as described by Martinez-Carrion & Tiemeier [18]; a Gilford spectrophotometer was used.

Titration curves

Two-dimensional titration curves for the purified enzymes were obtained by using the Pharmacia Phast system and Phastgel IEF media, pH 3–9. The electro-

phoresis and staining procedures were carried out as described in the manufacturer's manual.

Pyridoxal phosphate content

The pyridoxal phosphate content of the pure enzyme was measured colorimetrically with phenylhydrazine as described by Wada & Snell [19].

RESULTS

AadAT in rat and bovine kidney homogenates

The total AadAT activity in homogenates of bovine kidney was lower than that in rat kidney (150 ± 7 versus 400 ± 12 μmol of product formed/h per g of tissue; means $\pm$ S.E.M. of four experiments). However, unlike that of rat kidney (43 ± 1 μmol of product formed/h per g of tissue), bovine kidney homogenate lacked detectable KAT activity. We assayed KAT activity in bovine kidney homogenates at various pH values (6–8.5), in different buffers, and also at different substrate concentrations, but could not detect any such activity. To test whether the absence of KAT activity from bovine kidney homogenates might be due to the presence of an inhibitor, we examined the effect of added bovine kidney homogenate on KAT activity in rat kidney homogenates; no inhibitor was observed (results not shown).

Subcellular distribution

The mitochondrial fractions from bovine and rat kidneys were devoid of lactate dehydrogenase activity, and the supernatant fractions from both sources did not contain any detectable glutamate dehydrogenase activity. These results confirmed that the mitochondrial and supernatant fractions prepared from bovine and rat kidneys were not contaminated by other subcellular components. Some 70% of the total activity in bovine kidney homogenates was localized in mitochondria, and 30% was present in the supernatant. With rat kidney homogenates, 55% of the total activity was present in the mitochondrial fraction and the remaining 45% in the supernatant fraction. Since AadAT from rat kidney has been purified from the supernatant fraction and well characterized in previous studies [5], we purified AadAT from the supernatant of bovine and rat kidney homogenates and compared their properties.

Purification of AadAT

Preliminary results with partially purified enzymes indicated that the AadAT prepared from bovine kidney was less stable than that from rat kidney. For example, with AadAT partially purified from bovine kidney, 100% of the activity was lost when samples were stored at 4 °C for 14 days (in 8 mM-potassium phosphate buffer, pH 7.1), whereas with AadAT partially purified from rat kidney, only 15% of the total activity was lost when stored under identical conditions (results not shown). We tested the effect of various stabilizing agents, such as dithiothreitol, mercaptoethanol and EDTA, on the AadAT activity purified from bovine kidney, and found that 0.1 mM-dithiothreitol improved the stability of the enzyme by 50%. Increasing the concentration of dithiothreitol did not result in better stabilization. In subsequent experiments, therefore, and during purification of bovine kidney AadAT, 0.1 mM-dithiothreitol was included (instead of mercaptoethanol) in all buffers. The activity of AadAT purified from bovine kidney was less stable than that purified from rat kidney. Although both enzymes were stabilized by addition of thiol compounds such as dithiothreitol or mercaptoethanol, the bovine kidney enzyme remained less stable than the enzyme from rat kidney.

AadAT and KAT activities from the supernatant of rat kidney homogenates were purified to electrophoretic homogeneity as described in the Experimental section (results not shown). We purified AadAT from bovine kidney 44-fold by the same method, with 3% yield (Table 1). The degree of purification and the yield of AadAT from bovine kidney was significantly lower than that from rat kidney. Greatest loss of enzyme activity occurred during heat treatment plus $(\text{NH}_4)_2\text{SO}_4$ fractionation.

Comments on enzyme purifications

AadAT and KAT activities in rat kidney extracts copurified with a constant ratio of specific activities, supporting the hypothesis that in rat kidney supernatant AadAT and KAT may be the properties of a single protein. KAT activity was not observed at any stage of purifying bovine kidney AadAT. After $(\text{NH}_4)_2\text{SO}_4$ fractionation, AadAT activity present in bovine and rat

Table 1. Purification of 2-amino adipate aminotransferase from the supernatant fraction of bovine kidney homogenates

Details of the purification procedure are described in the text. One unit of enzyme is the amount of enzyme that catalysed the formation of 1 μmol of product/min at 37 °C.

Step	Total activity (units)	Total protein (mg)	Specific activity (unit/mg)	Recovery (%)	Purification (fold)
Supernatant fraction	35.4	2948	0.01	100	1
Acid precipitation	19.0	824	0.02	68	2
$(\text{NH}_4)_2\text{SO}_4$ and heat fractionation	8.9	262	0.03	25	3
DEAE-cellulose chromatography	7.2	80	0.09	20	8
Hydroxyapatite chromatography I	4.3	14	0.30	12	26
Hydroxyapatite chromatography II	2.3	4	0.58	7	48
Hydroxyapatite chromatography III	1.2	2	0.53	3	44

kidney supernatant fractions was dependent on added pyridoxal phosphate. Tobes & Mason [5] reported that aspartate aminotransferase activity remained with AadAT/KAT activities throughout the purification procedures and could only be separated with great difficulty. Aspartate aminotransferase activity was also associated with bovine kidney AadAT, and was separated only at the last stage of purification.

Purity, M_r and subunit structure

Native polyacrylamide-gel electrophoresis of purified enzymes showed a single band for each protein, indicating that the preparations were homogeneous (Fig. 1). When subjected to Sephadex G-200 column chromatography, the enzyme from both sources was eluted between alcohol dehydrogenase (M_r 150 000) and bovine serum albumin (M_r 66 000), with an apparent M_r of $83\,000 \pm 2000$ ($n = 3$). The subunit structure of the enzymes was examined by polyacrylamide-gel electrophoresis in the presence of 1% SDS (Fig. 2). A single band was obtained in both cases. The M_r of the subunit was estimated to be 41 000 from a semi-logarithmic plot of the M_r values of standard proteins versus their mobilities. These results indicate that bovine kidney AadAT is composed of two apparently identical subunits. The M_r and subunit

Fig. 1. Polyacrylamide-gel electrophoresis of 2-aminoadipate aminotransferase purified from rat and bovine kidney

Enzyme (15 μ g) purified from either rat or bovine kidney was electrophoresed on 7.5%-polyacrylamide gel. The direction of migration was top (cathode) to bottom (anode). Lane 1, rat kidney AadAT; lane 2, bovine kidney AadAT; lane 3, standard M_r markers, including urease, bovine serum albumin, chick egg albumin, carbonic anhydrase and lactalbumin.

Fig. 2. SDS/polyacrylamide-gel electrophoresis of purified samples of 2-aminoadipate aminotransferase

Enzyme (15 μ g) purified from either rat or bovine kidney supernatant fraction was electrophoresed on 10%-polyacrylamide gels in the presence of 1% SDS, as described in the Experimental section. The direction of migration was top (cathode) to bottom (anode). Lane 1, rat kidney AadAT; lane 2, bovine kidney AadAT; lane 3, standard M_r markers: carbonic anhydrase (M_r 29 000), egg albumin (M_r 45 000), bovine albumin (M_r 66 000), phosphorylase b (M_r 97 400), and β -galactosidase (M_r 116 000).

structure of bovine kidney AadAT were the same as those of rat kidney AadAT.

Kinetic parameters

We determined the pH optimum for bovine kidney AadAT activity, using potassium phosphate buffer in the pH range 5–9. Maximum activity was obtained at pH 7.0. The K_m values for substrates were determined by double-reciprocal plots, and were 0.5 mM for 2-oxoadipic acid and 4.6 mM for L-glutamic acid. AadAT from rat kidney supernatant fraction also showed the same pH optimum and K_m values for substrates (results not shown).

Substrate specificity

The ability of the purified enzymes from rat and bovine kidney to catalyse transamination between various amino acids and oxo acids was investigated; the relative rates of activities are shown in Table 2. Maximum activity for rat and bovine kidney AadAT was obtained with 2-aminoadipate as the amino-group donor and 2-oxoglutarate as the amino-group acceptor. With other amino-group donors or acceptors, the relative activity of the bovine kidney enzyme was different from that of rat kidney. For example, AadAT from rat kidney showed considerable activity with kynurenine as an amino-group donor and 2-oxoglutarate as an amino-group acceptor, whereas AadAT from bovine kidney showed no activity with similar substrates (Table 2). Although both enzymes

Table 2. Comparison of the substrate specificity of 2-aminoadipate aminotransferase purified from rat and bovine kidney

In all assays, 15 μ g of purified enzyme and 50 mM of either 2-oxoglutarate or pyruvate was used. The final concentration of aromatic amino acids was 20 mM and that of other amino acids was 40 mM. The final concentration of DL-2-aminopimelic acid was 80 mM. Enzyme activities were assayed as described in the Experimental section and are expressed as the percentage of activity obtained with aminoadipate and 2-oxoglutarate as substrates. The data shown are averages of duplicate analyses.

Amino-group acceptor	Amino-group donor	Rat kidney	Bovine kidney
2-Oxoglutarate	Aminoadipate	100	100
	DL-2-Aminopimelic acid	45	28
	L-Norleucine	31	10
	Kynurenine	10	0
	3,5-Di-iodotyrosine	32	15
	Histidine	4	16
	Phenylalanine, tryptophan, tyrosine, aspartate or alanine	< 6	< 6
Pyruvate	Aminoadipate	4	3
	Kynurenine	20	0
	Histidine	3	32
	Phenylalanine, tryptophan, tyrosine, 3,5-di-iodotyrosine, aspartate or alanine	< 4	< 4

showed some activity with 3,5-di-iodotyrosine and 2-oxoglutarate as substrates, the activity of the rat enzyme was twice that of the bovine enzyme. AadAT from bovine kidney showed significant activity with histidine as an amino-group donor and either pyruvate or 2-oxoglutarate as amino-group acceptor, whereas no activity was seen with rat kidney AadAT for similar substrates.

With 2-aminopimelic acid or norleucine (which resemble aminoadipic acid structurally), the rat kidney enzyme showed a much higher activity than the enzyme from bovine kidney. With other compounds such as aromatic amino acids, neither enzyme showed any activity. Both rat and bovine kidney enzymes did not exhibit any activity with various amino-group donors and glyoxalate as the amino-group acceptor.

The observation that bovine kidney AadAT showed considerable activity with histidine and pyruvate as substrates suggested the possibility that the enzyme from this source may be identical with histidine:pyruvate aminotransferase. To test this hypothesis, we assayed histidine:pyruvate aminotransferase activity at each stage of purification. This specific activity decreased during this process, and the ratio of the specific activities of AadAT to histidine:pyruvate aminotransferase was different at each step, suggesting that the two activities may be the consequence of two different proteins. Since most aminotransferases show wide and overlapping substrate specificities, the histidine:pyruvate aminotransferase activity in these highly purified preparations may represent an intrinsic activity of AadAT, or it may indicate the presence of a contaminant.

Inhibition by dicarboxylic acids

The effect of a number of dicarboxylic acids on the activities of AadAT purified from rat and bovine kidney supernatant fraction is shown in Table 3. Although several dicarboxylic acids inhibited AadAT activity from both sources, the degree of inhibition was frequently quite different for the two enzymes. For example, 3-methylglutaric acid and dimethylglutaric acid caused about 40% inhibition with rat kidney AadAT, whereas

Table 3. Effect of dicarboxylic acids on 2-aminoadipate aminotransferase activity purified from rat and bovine kidney

In each case, 15 μ g of purified enzyme was first incubated with dicarboxylic acid (final concn. 6 mM; neutralized to pH 7.0) at 37 °C for 15 min in potassium phosphate buffer, pH 7.0. Enzyme activity was then determined as described in the Experimental section. The data shown are the averages of duplicate analyses.

Dicarboxylic acid added	Inhibition (%)	
	Rat AadAT	Bovine AadAT
None	0	0
Adipic acid	60	48
Azelaic acid	38	36
Capric (decanoic) acid	45	50
Glutaric acid	5	0
3-Methylglutaric acid	38	2
Dimethylglutaric acid	42	15
Diethylglutaric acid	20	20
Pimelic acid	32	28
Oxaloacetic acid	0	0

less than 15% inhibition was observed with the bovine kidney enzyme. The degree of inhibition caused by adipic acid was also much higher with rat kidney than with bovine kidney AadAT (Table 3).

Effect of metabolites

We tested the effect of various metabolites of the lysine and tryptophan pathways on the AadAT activities from the two sources. These metabolites included lysine, tryptophan, kynurenine, kynurenic acid and saccharopine. Kynurenic acid at 1 mM caused 30% inhibition of bovine kidney AadAT and about 40% inhibition of the rat enzyme. Kynurenine at 1 mM caused more than a 50% decrease in AadAT activity from rat kidney, but had no effect on the bovine enzyme. The inhibitory effect of kynurenine was reversed when 30 mM-L-glutamic acid

Fig. 3. Electrophoretic titration of 2-aminoadipate aminotransferase purified from rat and bovine kidney

Samples (25 ng) of purified enzymes were titrated electrophoretically by using the Pharmacia Phast system and Phastgel media, pH 3–9, as described in the Experimental section. The titration curves shown are for (a) bovine kidney AadAT, (b) rat kidney AadAT and (c) a mixture of AadAT from rat and bovine kidney.

was included in the assay mixture (results not shown). The other compounds had no significant effect on AadAT activity from either rat or bovine kidney.

Titration curves of purified enzymes

The purified enzymes from rat and bovine kidney showed a single titration curve in the pH range 3–9. However, the pattern of the titration curve for the bovine kidney enzyme was slightly different than that of the rat. This observation was further confirmed by titrating a mixture of both enzymes on the same gel (Fig. 3). These results indicate that the net charge on the two enzymes may be different.

Absorption spectra

The absorption spectra for purified AadAT from bovine and rat kidney were identical (Fig. 4). For each enzyme, two absorption maxima (at 330 and 400 nm) were observed at pH 5.3, whereas at pH 8.2 only one maximum (at 330 nm) was seen. These spectra resemble those of aspartate aminotransferase from pig heart [18] and are characteristic of pyridoxal phosphate requiring enzymes.

Amino acid composition

The amino acid composition of AadAT purified from rat and bovine kidney is shown in Table 4. In general, the two enzymes have similar amino acid compositions, with minor differences in the content of alanine, glycine, histidine, leucine, methionine and arginine. Large differences were evident in the content of isoleucine and serine (Table 4).

The pyridoxal phosphate contents of bovine kidney AadAT and rat kidney AadAT/KAT were 1.4 ± 0.05 and 1.9 ± 0.1 mol/mol of enzyme respectively.

Fig. 4. Absorption spectra of 2-aminoadipate aminotransferase purified from rat and bovine kidney

The absorption spectra of bovine and rat kidney AadAT in 0.05 M-Tris, pH 8.2, are shown in curves 1 and 2 respectively. The absorption spectra of bovine and rat kidney AadAT in 0.1 M-acetate, pH 5.3, are shown in curves 3 and 4 respectively.

Immunological cross-reactivity

The immunological cross-reactivity of the purified enzymes was determined by Western-blot analysis. Polyclonal antibodies raised against AadAT from rat kidney supernatant fraction were used to test the antigenicity of purified enzymes. A positive immunoprecipitation band was observed with AadAT from rat kidney, but not with the enzyme from bovine kidney (results not shown).

Table 4. Amino acid composition of 2-aminoadipate aminotransferase purified from rat and bovine kidney

Samples of the purified enzymes were hydrolysed *in vacuo* at 110 °C in constant-boiling HCl for 96 h; the data shown are the average of three independent analyses in duplicate. Abbreviation: N.C., not calculated.

Amino acid	Content (residues/mol)	
	Rat AadAT	Bovine AadAT
Ala	52.9	63.0
Cys	N.C.	N.C.
Asp	93.8	98.9
Glu	69.1	69.3
Phe	35.1	36.5
Gly	94.8	73.3
His	26.3	11.9
Ile	16.9	30.9
Lys	58.1	55.9
Leu	79.7	65.2
Met	11.1	7.9
Pro	67.9	66.0
Arg	31.1	23.9
Ser	34.4	69.8
Thr	33.7	34.3
Val	63.0	57.4
Tyr	24.5	25.7

DISCUSSION

The presence of KAT activity in rat kidney has not been completely understood, because a complete pathway for tryptophan catabolism does not exist in the kidney [20,21]. A previous report suggested that the major function of AadAT/KAT in kidney may involve the lysine catabolic pathway [5]. However, in all species studied so far, KAT activity has been consistently associated with AadAT activity [5,6]. To our knowledge, this is the first report where KAT activity was not associated with AadAT activity.

Previous studies in rat kidney [5], brain [22] and liver [23] indicate that the properties of AspAT are similar to those of AadAT/KAT, thus accounting for the difficulty in separating the two enzymes. We were also unable to separate AspAT from AadAT activity in bovine kidney until the final stage of the purification. Although the specific activity and yield of enzyme decreased during the final step of purification, the step was necessary because it removed the contamination by aspartate aminotransferase activity.

AadAT from different sources show wide and varied substrate specificities. For example, KAT from yeast was able to catalyse the reaction between a large number of L-amino acids and keto acids [24]. KAT from liver and kidney of rat also exhibited wide substrate specificities. A review on the substrate specificity of various aromatic amino acid aminotransferases indicates that almost all of these enzymes possess broad and overlapping substrate specificities [25]. In many of the studies reviewed, enzyme activities that were initially examined as separate entities and referred to as separate aminotransferases were found to be properties of the same protein. For example, it has been proposed that AadAT, KAT and halogenated

tyrosine aminotransferase are properties of a single protein [26]. Similarity alanine:2-oxoglutarate aminotransferase and glutamate:glyoxalate aminotransferase have been reported to be the same protein [27].

KAT activities have been classified into four isoenzymes, based on their substrate specificities, pH optima and isoelectric points. Isoenzyme I is identical with mitochondrial tyrosine:2-oxoglutarate aminotransferase [28], isoenzyme II with AadAT and halogenated aminotransferase [5], isoenzyme III with histidine:pyruvate aminotransferase, phenylalanine:pyruvate aminotransferase and glutamate:oxaloacetate aminotransferase [27], and isoenzyme IV with alanine:glyoxalate aminotransferase [29]. The substrate specificity of bovine kidney AadAT did not resemble any of the reported isoenzymes of KAT or AadAT. The AadAT purified from bovine kidney showed some activity with either halogenated tyrosine and 2-oxoglutarate or with histidine and pyruvate as substrates.

This is the first report on the purification of AadAT activity from bovine kidney, and also, for the first time, AadAT activity has been obtained without any KAT activity. These results show that both the enzymes are similar in many respects. The properties of bovine kidney enzyme, such as M_r of subunits, pH optimum and K_m values, pyridoxal phosphate content and effects of dicarboxylic acids were identical with those of AadAT/KAT from rat kidney. However, the titration-curve analysis, the amino acid composition and the Western-blot analysis indicate that the two proteins may be structurally different. The fact that AadAT and KAT differed in their substrate specificity, inhibition by metabolites, the degree of inhibition by dicarboxylic acids and antigenicity indicates that the structure of the two enzymes may differ at their active sites. The difference may be minor, because of the similarities in the purification procedure and other properties. If these structural differences indeed exist at the active site, they may be responsible for the absence of KAT activity from bovine kidney. Further structural and immunochemical studies are required to confirm the structural similarities and differences between bovine and rat kidney AadAT. Whether the tryptophan metabolic pathway in bovine tissues is different from that in rat tissues also remains to be investigated.

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