L-KYNURENINE AMINOTRANSFERASE AND L-α-AMINOADIPATE AMINOTRANSFERASE. I. EVIDENCE FOR IDENTITY

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Summary: The following observations strongly suggest that L-kynurenine aminotransferase (KAT) and L-α-aminoadipate aminotransferase (AAT) activities are associated with the same protein: 1. a similar distribution in the supernatant and mitochondrial fractions of kidney and liver of male rats, 2. a similar distribution in these fractions of kidney and liver of female rats, 3. a similar sex difference in the supernatant and mitochondrial fractions of kidney with no sex difference in liver fractions, 4. a similar pattern of inhibition with a homologous series of dicarboxylic acids with adipate being most effective, 5. competitive inhibition of KAT with D,L-α-aminoadipate (αAA), 6. substrate inhibition of KAT with α-ketoadipate (αKA), and 7. AAT activity in a highly purified preparation of KAT. A scheme for the interaction of the two activities in lysine and tryptophan metabolism is presented.

L-Kynurenine aminotransferase (E.C. 2.6.1.7) catalyzes the conversion of L-kynurenine (Kyn) or L-3-hydroxykynurenine to kynurenic acid or xanthurenic acid with the concomitant conversion of α-ketoglutarate (αKG) to L-glutamate (Glu). Several observations have suggested to us that it might also transaminate L-αAA.

Inhibition of KAT with dicarboxylic acids of varying chain length was reported; adipate was the most inhibitory of those acids in the homologous series from oxalate, C_2, to suberate, C_8 (1). Adipate was a competitive inhibitor with respect to L-Kyn but not to αKG. This maximal inhibition was postulated to be reflective of the distance between two cationic groups at the active site of the enzyme. Similar observations with aspartate aminotransferase (2-4) demonstrated that carboxylic acids which were analogs of the best keto acid substrates were the strongest inhibitors.

Applying this generalization to KAT, one would expect αKA to be the best keto acid substrate. Recently αKA has been shown to be a good substrate for

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Abbreviations: KAT, L-Kynurenine Aminotransferase; AAT, L-α-Aminoadipate Aminotransferase; Kyn, Kynurenine; αAA, α-Aminoadipate; αKG, α-Ketoglutarate; αKA, α-Ketoadipate; Glu, Glutamate; PLP, Pyridoxal-5'-Phosphate.

KAT with an apparent Km similar to that of αKG (5). It was inhibitory at higher concentrations, possibly because it was transaminated to αAA which then competed with Kyn for the enzyme. It has been reported that D,L-αAA substantially inhibits KAT from kidney mitochondria, but the type of inhibition was not studied (6).

The AAT of rat liver was located in the mitochondria (7). It catalyzed the conversion of L-αAA to αKA with the concomitant conversion of αKG to L-Glu. A substrate specificity study was done with a partial purified preparation, but Kyn was not tested.

In this communication, we present evidence that strongly supports the concept that the two activities are associated with the same protein.

MATERIALS AND METHODS

Tissue Fractionation: Adult albino rats, weighing approximately 250 gms, were sacrificed by guillotine. The kidneys and livers were immediately placed in ice and were then homogenized and fractionated as described previously (8). The supernatant and mitochondrial fractions were frozen immediately, and were used within 3 weeks. Little activity was lost over 3 months at -15°C.

Preparation of Partially Purified Enzyme: All procedures were carried out at 0-4°C unless stated otherwise. Kidney supernatant fractions from male rats were thawed to obtain 50.0 ml. The solution was made 0.01 m*M* with respect to pyridoxal-5'-phosphate (PLP). It was titrated to pH 5.3 with 1.0 *N* CH_3COOH and allowed to stand for 5 min with occasional stirring. The suspension was centrifuged at 30,000g for 10 min. The pooled supernatant fluid was adjusted to pH 6.5 with 1.0 *N* NaOH. Solid $(NH_4)_2SO_4$ was added (9) until the solution was 35% saturated. After stirring for 1 hr and maintaining the pH at 6.5, the suspension was made 0.05 m*M* with respect to PLP. While constantly stirring, the suspension was brought to 55° in a boiling water bath. After 5 min at 55°, it was brought back to 4°C by stirring in an ice bath. It was centrifuged at 30,000g for 20 min. While stirring for 1-2 hrs and maintaining at pH 6.5, the

pooled supernatant fluid was made 65% saturated with additional $(NH_4)_2SO_4$. The suspension was centrifuged at 30,000g for 20 min. The precipitates were dissolved in a minimum volume of 10 m*M* potassium phosphate buffer, pH 7.0, which contained 10 m*M* 2-mercaptoethanol. The dissolved precipitates were pooled and dialyzed overnight against 2.1 liters of the buffer with one change of the buffer.

L-Kynurenine Aminotransferase Assay: The incubation mixture contained 0.20 m*M* PLP, 5.00 m*M* αKG, 3.26 m*M* L-Kyn sulfate, 100 m*M* imidazole·HCl buffer, pH 6.5, and 0 to 0.10 ml enzyme solution in a total volume of 0.60 ml. The incubation mixture was held for 10 min at room temperature before the addition of L-Kyn, and it was then incubated for 30 min at 37°C. The reaction was stopped with the addition of 10 ml of 1% boric acid in 95% ethanol. The tubes were mixed well, and the precipitate was removed by centrifugation at 5000g for 5 min. Kynurenic acid formation was estimated spectrophotometrically (10) using a reaction blank containing everything but αKG. Crude mitochondrial fractions were assayed in the presence of 1.42 m*M* $CaCl_2$ which has been reported to increase the mitochondrial activity but not that of the supernatant fractions or of the disrupted mitochondrial preparations (6,8).

L-α-Aminoadipate Aminotransferase Assay: The assay system was a modification of the assay with L-Glu and αKA as substrates described by Nakatani *et al.* (7). The buffer was at pH 7.0. After stopping the reaction with 0.10 ml of 1.0 *N* HCl, 0.10 ml of 1.1 *N* KOH was added to raise the pH to 7.5. The precipitates were removed by centrifugation at 5000g for 5 min. A reaction blank contained everything but αKA. Assays of crude mitochondrial fractions were done in the presence of 2.00 m*M* $CaCl_2$ which was found to increase mitochondrial activity but not that of the supernatant fractions and to have no effect on glutamate dehydrogenase (11).

Protein Determination: Protein was measured according to Lowry *et al.* with bovine serum albumin as the standard (12).

Chemicals: L-Kyn sulfate, αKG, αKA, D,L-αAA, PLP, the dicarboxylic acids, and

TABLE I. Distribution of L-Kynurenine Aminotransferase and L-α-Aminoadipate Aminotransferase in the Kidney and Liver of Male Rats.

	L-Kynurenine Aminotransferase		L-α-Aminoadipate Aminotransferase	
	Total Units per Organ[a]	% Activity[b]	Total Units per Organ[a]	% Activity[b]
Kidney fraction:				
Mitochondrial	1520 ± 341	46.9 ± 4.1	11900 ± 3220	42.4 ± 5.1
Supernatant	1700 ± 219	53.1 ± 4.1	15800 ± 2050	57.6 ± 5.1
Liver fraction:				
Mitochondrial	2790 ± 626	81.4 ± 2.5	31500 ± 6940	76.3 ± 1.6
Supernatant	633 ± 152	18.6 ± 2.5	9690 ± 1480	23.7 ± 1.6

Four male rats were used: body wt., 251.9 ± 4.1 gms; kidney wt., 1.97 ± 0.19 gms; liver wt., 9.89 ± 0.50 gms. Each fraction was analyzed in duplicate. The mean and standard deviation are presented. Assay conditions are those described in the text with the following modifications: KAT assay - 0.17 m*M* PLP, 4.29 m*M* αKG, 2.80 m*M* L-Kyn, 85.7 m*M* imidazole·HCl buffer, pH 6.5, and a reaction volume of 0.70 ml; and AAT assay - 5 min incubation of kidney fractions. Linearity was maintained in all rate determinations.

[a] Units are defined as mμmoles/min.

[b] Activity is based on the activity recovered in the two fractions. The two fractions accounted for 80 to 90% of the two activities in the kidney homogenate and for 60 to 70% of the two activities in the liver homogenate.

glutamate dehydrogenase were purchased from Sigma Chemical Co. All other chemicals were obtained from other reputable firms.

RESULTS AND DISCUSSION

The distribution of hepatic and renal AAT between the supernatant and mitochondrial fractions of male rats was similar to that of KAT (Table I). The kidney supernatant fraction had slightly more of both activities than the mitochondrial fraction, whereas most of both activities of the liver was in the mitochondrial fraction. Distribution of KAT has been reported earlier (13,14).

Table II shows the distribution of AAT between the supernatant and mitochondrial fractions of female rats to be similar to that of KAT. Both activity

TABLE II. Distribution of L-Kynurenine Aminotransferase and L-α-Aminoadipate Aminotransferase in the Kidney and Liver of Female Rats.

	L-Kynurenine Aminotransferase		L-α-Aminoadipate Aminotransferase	
	Total Units per Organ[a]	% Activity[b]	Total Units per Organ[a]	% Activity[b]
Kidney fraction:				
Mitochondrial	682 ± 35	37.4 ± 1.9	6460 ± 156	36.3 ± 3.0
Supernatant	1150 ± 134	62.6 ± 1.9	11400 ± 1290	63.7 ± 3.0
Liver fraction:				
Mitochondrial	2760 ± 68	78.4 ± 1.9	26600 ± 1900	72.6 ± 2.9
Supernatant	761 ± 93	21.6 ± 1.9	10000 ± 1170	27.4 ± 2.9

Four female rats were used: body wt., 249.8 ± 1.6 gms; kidney wt., 1.72 ± 0.09 gms; liver wt., 9.20 ± 0.84 gms. Each fraction was analyzed in duplicate. The mean and standard deviation are presented. Assay conditions and footnotes are presented in Table I.

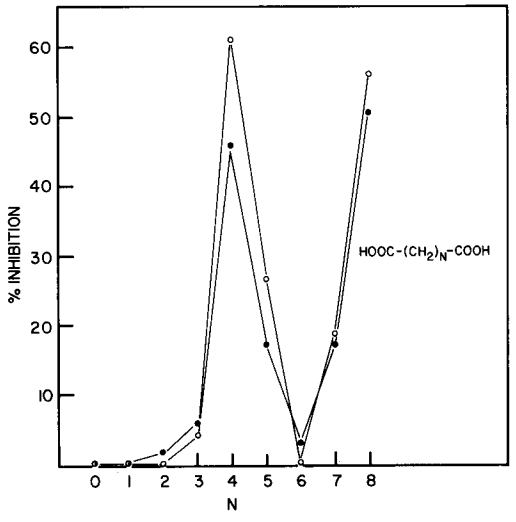

Fig. 1. Inhibition of a partially purified preparation of L-kynurenine aminotransferase (- o -) and L-α-aminoadipate aminotransferase (- ● -) with dicarboxylic acids. The preparation had a specific activity of 41.2 mμmoles/min/mg protein. Assay conditions are those described in the text with these modifications: KAT assay--0.19 m*M* PLP, 6.00 m*M* αKG, 3.92 m*M* L-Kyn, 100 m*M* potassium phosphate, pH 7.0, 0.50 ml reaction volume, and 20 min incubation; and AAT assay--6.00 m*M* αKA, 4.00 m*M* L-Glu, and 7 min incubation. No inhibition of glutamate dehydrogenase was observed with any of the dicarboxylic acids at the conditions described. Linearity was maintained. Dicarboxylic acids were 6.00 m*M* when present. All assays were done in quadruplicate.

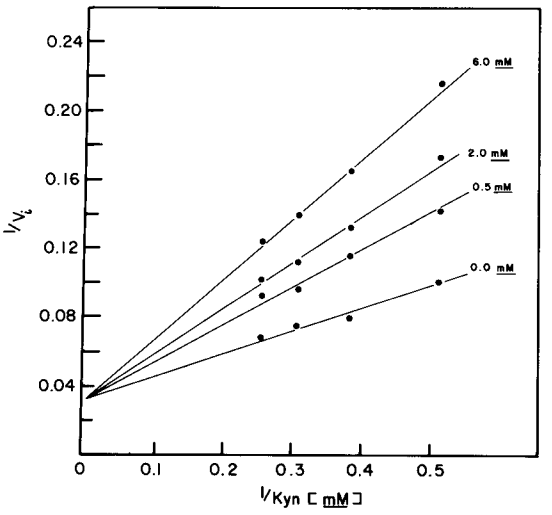

Fig. 2. Inhibition of a partially purified preparation of L-kynurenine aminotransferase with D,L-α-aminoadipate at the concentrations indicated. The preparation had a specific activity of 59.4 mμmoles/min/mg protein. Assay conditions were those described in the text except that L-Kyn concentrations were varied. All assays were done in triplicate.

levels were substantially lower in the supernatant and mitochondrial kidney fractions of females than those of males but were similar to those of the males in liver fractions. Sex differences of KAT were reported earlier (15). The distribution of the two activities was different in the kidney of females as compared to males, but no sex difference in distribution was observed in the liver.

Partially-purified kidney supernatant KAT and AAT were inhibited by a homologous series of dicarboxylic acids of increasing chain length from oxalate, C_2, to sebacate, C_{10}, as shown in Fig. 1. The patterns of inhibition of the two activities are identical, and the inhibition with adipate was maximal. These results suggest that the active sites for Kyn and αAA are either identical or remarkably similar.

Fig. 2 indicates competition between D,L-αAA and L-Kyn for the KAT of a partially purified kidney supernatant fraction. This competition would be expected if both substrates are transaminated by the same protein. Substantial inhibition of AAT with L-Kyn has been observed also but will be reported later (11).

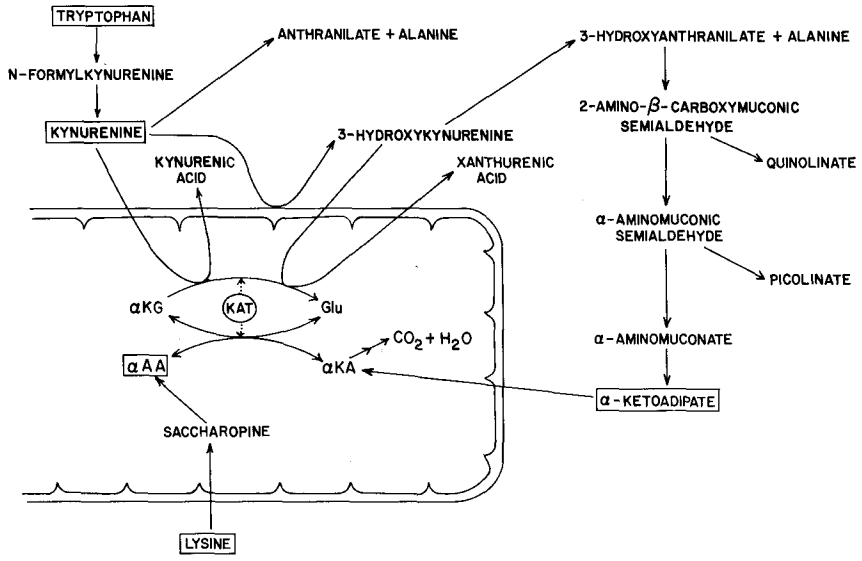

Fig. 3. Abbreviated scheme of potential interactions of tryptophan and lysine catabolism, based on the view that kynurenine aminotransferase and α-aminoadipate aminotransferase are identical.

A preparation of KAT, which was 900-fold purified from the kidney homogenate activity and which appeared homogeneous by polyacrylamide gel electrophoresis, exhibited strong AAT activity. The method of purification will be published elsewhere. Using this preparation, a substrate specificity study was conducted, using imidazole·HCl buffer, pH 6.5, at 37°C for 2 hrs. The products were separated using thin layer chromatography and detected with the ninhydrin reaction or UV light. The only two activities detected were that of KAT using L-Kyn and αKG or αKA as substrates and that of AAT assayed in either direction. All common α-amino acids but valine, threonine, methionine, asparagine and glutamine were tested. These results demonstrate copurification of the two activities.

The evidence presented is strongly suggestive of the identity of KAT and AAT and we are unable to present a reasonable alternative interpretation of the data. Additional evidence for identity will be presented elsewhere in publications concerning the purification and purity of KAT.

The view that KAT and AAT are identical suggests a scheme, shown in Fig.

3, where lysine and tryptophan catabolism can interact via this transaminase. If limiting amounts of KAT(AAT) are shared with the two sets of substrates in lysine and tryptophan catabolism, one can predict that feeding increased amounts of lysine will competitively interfere with the formation of kynurenic and xanthurenic acids during tryptophan catabolism. Such inhibitions have been observed with vitamin B_6 deficient rats (16).

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