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

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Received November 18, 1974

Summary: The following observations strongly suggest that L-kynurenine aminotransferase (KAT) and L-α-amino adipate 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-amino adipate (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 trans-amine 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, C₂, to suberate, C₈ (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...
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 mM with respect to pyridoxal-5'-phosphate (PLP). It was titrated to pH 5.3 with 1.0 N CH₃COOH 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 (NH₄)₂SO₄ 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 mM 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 (NH₄)₂SO₄.
The suspension was centrifuged at 30,000g for 20 min. The precipitates were
dissolved in a minimum volume of 10 mM potassium phosphate buffer, pH 7.0,
which contained 10 mM 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
mM PLP, 5.00 mM αKG, 3.26 mM L-Kyn sulfate, 100 mM imidazole·HCl buffer, pH
6.5, and 0 to 0.10 ml enzyme solution in a total volume of 0.60 ml. The in-
cubation 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 mM CaCl₂ which has been reported
to increase the mitochondrial activity but not that of the supernatant frac-
tions 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 pre-
cipitates 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 mM CaCl₂ which was found to increase mito-
chondrial 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.

<table>
<thead>
<tr>
<th></th>
<th>L-Kynurenine Aminotransferase</th>
<th>L-α-Aminoadipate Aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Units per Organ(^a)</td>
<td>% Activity(^b)</td>
</tr>
<tr>
<td><strong>Kidney fraction:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>1520 ± 341</td>
<td>46.9 ± 4.1</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1700 ± 219</td>
<td>53.1 ± 4.1</td>
</tr>
<tr>
<td><strong>Liver fraction:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>2790 ± 626</td>
<td>81.4 ± 2.5</td>
</tr>
<tr>
<td>Supernatant</td>
<td>633 ± 152</td>
<td>18.6 ± 2.5</td>
</tr>
</tbody>
</table>

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 mM PLP, 4.29 mM αKG, 2.80 mM L-Kyn, 85.7 mM 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 μmoles/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.

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

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

<table>
<thead>
<tr>
<th>Fraction</th>
<th>L-Kynurenine Aminotransferase</th>
<th>L-α-Aminoadipate Aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Units per Organ&lt;sup&gt;a&lt;/sup&gt;</td>
<td>% Activity&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>682 ± 35</td>
<td>37.4 ± 1.9</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1150 ± 134</td>
<td>62.6 ± 1.9</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>2760 ± 68</td>
<td>78.4 ± 1.9</td>
</tr>
<tr>
<td>Supernatant</td>
<td>761 ± 93</td>
<td>21.6 ± 1.9</td>
</tr>
</tbody>
</table>

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.

![Figure 1](image-url)

**Fig. 1.** Inhibition of a partially purified preparation of L-kynurenine aminotransferase (○○○) and L-α-aminoadipate aminotransferase (●●●) with dicarboxylic acids. The preparation had a specific activity of 41.2 μmoles/min/mg protein. Assay conditions are those described in the text with these modifications: KAT assay—0.19 mM PLP, 6.00 mM αKG, 3.92 mM L-Kyn, 100 mM potassium phosphate, pH 7.0, 0.50 ml reaction volume, and 20 min incubation; and AAT assay—6.00 mM αKA, 4.00 mM 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 mM when present. All assays were done in quadruplicate.
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 \( \alpha \)AA are either identical or remarkably similar.

Fig. 2 indicates competition between D,L-\( \alpha \)AA 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).
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 B₆ deficient rats (16).

Acknowledgements: This work was supported in part by The University of Michigan Horace H. Rackham School of Graduate Studies (Faculty Research Grant), The University of Michigan Cancer Research Institute (Pilot Project 24), a Public Health Service research grant (AM 02294), and a Public Health Service training grant (GM 00187). It was also financed in part with Federal funds from the Environmental Protection Agency under grant number R-800637. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

11. Tobes, M. C., unpublished observations.