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KYNURENINE AMINOTRANSFERASE AND α-AMINOADIPATE AMINOTRANSFERASE: III. EVIDENCE FOR IDENTITY WITH HALOGENATED TYROSINE AMINOTRANSFERASE

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Summary

A nearly homogeneous preparation of α-aminoadipate (kynurenine) aminotransferase exhibited substantial activity with 3,5-diiodo-L-tyrosine, a major substrate for halogenated tyrosine aminotransferase. The new activity was found, according to heat inactivation and several inhibition studies, not to be attributable to contamination. Many of the properties previously reported for the two enzymes are identical or very similar. This paper lists these similarities and reports our observations of additional similarities of these activities in the supernatant and mitochondrial fractions of both rat kidney and liver. The properties of the purified enzyme and the noted similarities suggest that α-aminoadipate aminotransferase, kynurenine aminotransferase, and halogenated tyrosine aminotransferase activities are associated with the same protein. These activities are discussed in terms of a possible role in thyroid hormone metabolism.

We have reported evidence that kynurenine aminotransferase and α-aminoadipate aminotransferase activities are associated with the same protein (1,2). Copurification of the two activities from the rat kidney supernatant fraction yielded a preparation that was determined to be nearly homogeneous by analytical disc gel electrophoresis at pH 8.9 and pH 7.5, isoelectric focusing on polyacrylamide gels, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2). This preparation exhibited activity with a broad range of substrates including minor activities with tryptophan, phenylalanine, and tyrosine (3). The properties of the purified preparation (2,3) clearly distinguish it from enzymes isolated from rat tissues that have been designated aminotransferases of tryptophan, phenylalanine, and tyrosine (4-7) but not from all aminotransferases known to be active with aromatic amino acids.

We recently observed an activity with the purified and characterized preparation towards 3,5-diiodo-L-tyrosine which was slightly better than towards L-kynurenine. 3,5-Diiodo-L-tyrosine is a major substrate for halogenated tyrosine aminotransferase, an enzyme that was purified 30-fold from a sonic extract of rat kidney mitochondria (8). Many of the properties reported for that enzyme (8) are identical or very similar to those reported for α-aminoadipate (kynurenine) aminotransferase (2,3).

In this communication, we provide evidence of further similarities of the kynurenine aminotransferase and halogenated tyrosine aminotransferase activities

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in the kidney mitochondrial and supernatant fractions. We also provide evidence that the activity towards 3,5-diiodo-L-tyrosine is intrinsic to the characterized enzyme from the rat kidney supernatant fraction and is not due to contamination by a physically similar but different enzyme. This and previously reported observations strongly suggest that α-aminoadipate aminotransferase, kynurenine aminotransferase, and halogenated tyrosine aminotransferase activities are associated with the same protein.

Experimental Procedures

Tissue Fractionation: The kidneys and livers of adult albino rats were immediately placed in ice and then homogenized, fractionated, and frozen as described previously (1,9).

Preparation of Kidney Supernatant Enzyme: A partially purified preparation (1) and a highly purified and characterized preparation (2) from the kidney supernatant fraction were used in this study.

Preparation of Partially Purified Kidney Mitochondrial Enzyme: All procedures were done at 0 to 4°, and all solutions were prepared with redistilled and deionized water. All buffer solutions were potassium phosphate buffers containing 10 mM 2-mercaptoethanol.

Step 1: Sonication - Frozen mitochondria from adult male rats were thawed at room temperature. The suspension was diluted with 0.25 M sucrose such that it was 10.0 mg protein per ml and 0.20 mM pyridoxal-P at pH 7.0. A total of 200 ml of the mitochondrial suspension was sonciated in 100 ml batches in a salt-ice bath with a sonifier cell disruptor W-350 (Branson Sonic Power Co.). The sonifier was set at duty cycle of 50%, an output control of 6, and a power output of 135 watts. The sonication of a batch was done by pulsing for 4 treatments of 2.5 min each for a total time of 10 min. The temperature of the suspension, immediately after sonication, did not go higher than 11°. The suspension was cooled to below 5° before the next sonication. The sonicated solution was centrifuged at 30,000 x g for 20 min. The supernatant was collected, and the precipitates were discarded.

Step 2: Acid Precipitation - The pooled supernatant fluid, at an initial pH of approximately 6.8 was titrated to pH 5.2 with 1.0 M acetic acid and allowed to stand 5 min with occassional stirring. The suspension was centrifuged at 30,000 x g for 10 min. The pooled supernatant fluid was adjusted to pH 7.1 with 1.0 M NaOH and concentrated to 20 ml by ultrafiltration in a 500 ml Diaflo cell with a PM-30 membrane at a working pressure of 45 psi N_2. The concentrated enzyme was dialyzed for 19 h against 2.1 liters of 8 mM buffer, pH 7.1, containing 0.20 mM pyridoxal-P, with one change of buffer.

Step 3: DEAE-Cellulose Chromatography - A column (2.7 x 15.0 cm) was equilibrated with 8 mM buffer, pH 7.1. The dialyzed sample was applied to the column at a rate of approximately 40 ml per h. The enzyme was eluted with the initial buffer at the same flow rate. Fractions of 3 ml were collected. Kynurenine aminotransferase activity appeared after the initial protein peak. The active fractions were combined.

Assays: Protein was measured according to Lowry et al. with bovine serum albumin as the standard (10). Kynurenine aminotransferase was assayed as previously described (1,2). 3,5-Diiodo-L-tyrosine transamination was assayed as described by Nakano but at pH 6.5 (8).

Materials: L-Kynurenine sulfate, 3,5-diiodo-L-tyrosine, α-ketoglutarate, α-ketoadipate, pyridoxal-P, the dicarboxylic acids, and DEAE-cellulose

(medium mesh) were purchased from Sigma Chemical Co. L-α-Aminoadipate was purchased from Calbiochem. All other chemicals were of reagent grade and of the finest quality available. DEAE-cellulose (medium mesh) was washed, equilibrated, and packed into a column under 10 psi N_2 as described by Peterson (11).

Results

The distribution of hepatic and renal 3,5-diiodo-L-tyrosine aminotransferase activity of male and female rats is shown in Table I. The kidney supernatant fraction had more activity than the mitochondrial fraction, whereas most of the activity of the liver was in the mitochondrial fraction. The activity was substantially lower in the supernatant and mitochondrial kidney fractions of females than those of males but the female levels were similar to those of males in liver fractions. The distribution was different in the kidney of females as compared to males, but no sex difference in distribution was observed in the liver. These observations are similar to those reported earlier for α-aminoadipate and kynurenine aminotransferase activities (1).

TABLE I

Distribution of 3,5-Diiodo-L-tyrosine Aminotransferase Activity In the Kidney and Liver of Male and Female Rats

	Males		Females	
	Total Units per Organ[a]	% Activity[b]	Total Units per Organ[a]	% Activity[b]
Kidney Fraction:				
Mitochondrial	1760 ± 283	35.7 ± 4.5	606 ± 90	25.3 ± 0.3
Supernatant	3170 ± 302	64.3 ± 4.5	1780 ± 245	74.7 ± 0.3
Liver Fraction:				
Mitochondrial	3730 ± 1080	79.0 ± 5.8	3430 ± 501	77.5 ± 1.0
Supernatant	1090 ± 755	21.0 ± 5.8	1000 ± 159	22.5 ± 1.0

Four male rats were used: body wt., 256.3 ± 1.6 gms; kidney wt., 1.94 ± 0.09 gms; liver wt., 11.08 ± 0.97 gms. Four female rats were used: body wt., 256.1 ± 1.1 gms; kidney wt., 1.66 ± 0.05 gms; liver wt., 8.91 ± 0.87 gms. Each fraction was analyzed in duplicate. The mean and standard deviation are presented for both the weights and the activities. Assay conditions were those described in Experimental Procedures.

[a] Units are defined as mμmoles/min.

[b] % Activity is based on the activity recovered in the two fractions. The two fractions accounted for 90 to 100% of the activity in the kidney homogenate and for 65 to 85% of the activity in the liver homogenate.

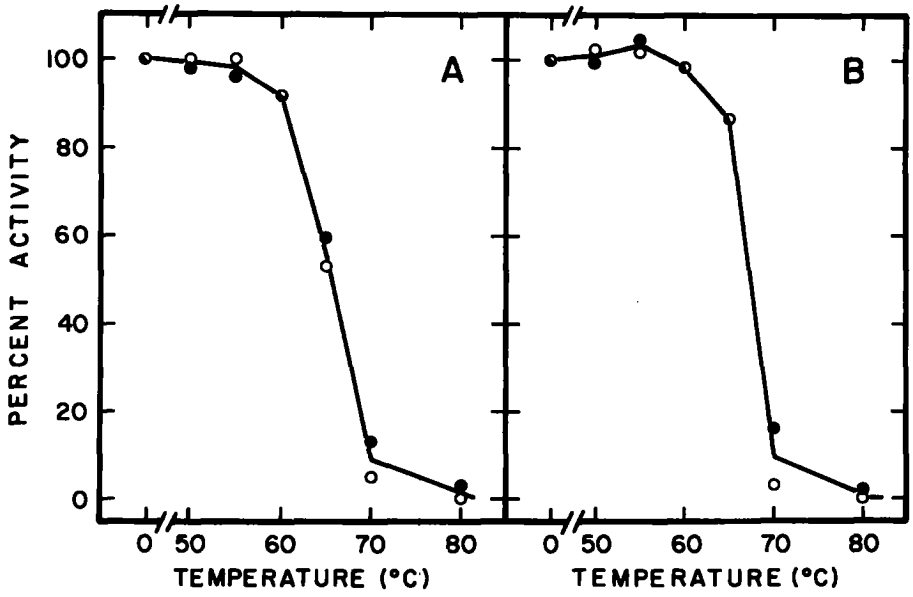

FIG. 1

Heat inactivation of 3,5-diiodo-L-tyrosine aminotransferase (-O-) and kynurenine aminotransferase (-●-) activities. A. A partially-purified enzyme preparation from the rat kidney supernatant was used (1). Each sample (3.0 ml) contained 4.0 mg protein/ml in 10 mM potassium phosphate buffer, pH 7.0, containing 10 mM 2-mercaptoethanol and 0.20 mM pyridoxal-P. The samples were brought to a given temperature, maintained for 5 min., cooled immediately on ice at 4°, and centrifuged at 30,000 x g for 10 min. The supernatants were assayed in duplicate for the aminotransferase activities as described in Experimental Procedures. B. A partially-purified enzyme preparation from the rat kidney mitochondria was used after treatment through the first two steps of the scheme (Experimental Procedures) but without concentrating the preparation by ultrafiltration and dialyzing against the buffer. Each sample (3.0 ml) contained 2.1 mg protein/ml in 10 mM potassium phosphate buffer, pH 7.0, containing 0.25 M sucrose and 0.20 mM pyridoxal-P. The samples were treated and assayed as described for A.

Using partially purified preparations from both the kidney mitochondrial and supernatant fractions, a similar pattern of heat inactivation was observed for kynurenine and 3,5-diiodo-L-tyrosine aminotransferase activities (Fig. 1). Inactivation of both fractions was initiated near 60° and was near completion at 70°. The heat inactivation pattern is very similar to that reported earlier for the kidney supernatant α-aminoadipate and kynurenine aminotransferase activities (2,3).

After the first two steps of the purification procedure (2), 3,5-diiodo-L-tyrosine aminotransferase activity from the kidney supernatant fraction was totally dependent on added pyridoxal-P. This is consistent with what has been observed for α-aminoadipate and kynurenine aminotransferase activities (2) and for halogenated tyrosine aminotransferase activity (8).

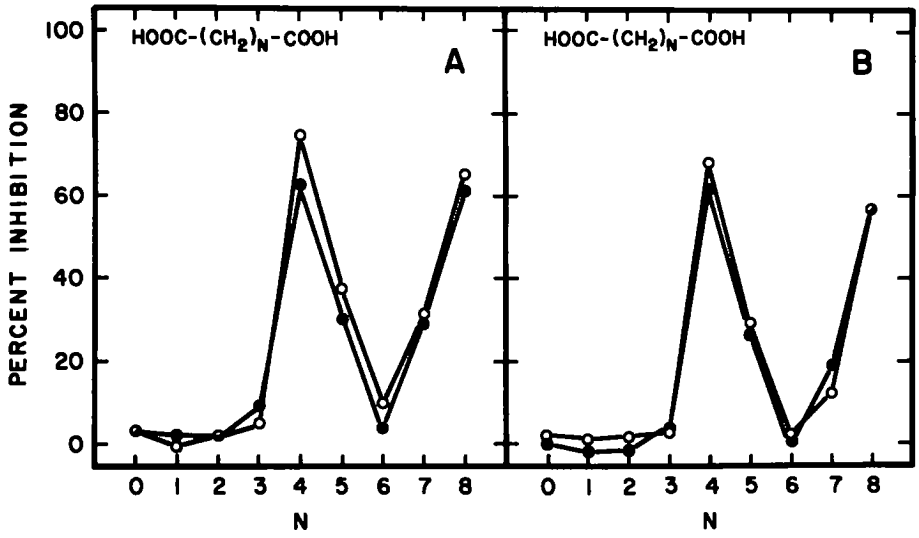

FIG. 2

Inhibition of 3,5-diiodo-L-tyrosine aminotransferase (-O-) and kynurenine aminotransferase (-●-) activities by dicarboxylic acids. The activities were assayed in duplicate as described in Experimental Procedures. A. A preparation from rat kidney mitochondria was used after purification through the three steps of the scheme (Experimental Procedures). Dicarboxylic acids were 4.0 mM. B. The purified and characterized preparation from the rat kidney supernatant (2) was used in the study of the inhibition of 3,5-diiodo-L-tyrosine aminotransferase with 4.0 mM dicarboxylic acids. This is compared with the study of the inhibition of kynurenine aminotransferase with 6.0 mM dicarboxylic acids with a previously described rat kidney supernatant preparation (1).

Using a partially purified preparation from the kidney mitochondrial fraction, both kynurenine and 3,5-diiodo-L-tyrosine aminotransferase activities were inhibited by a homologous series of dicarboxylic acids of increasing chain length from oxalate, C_2, to sebacate, C_{10} (Fig. 2A). The patterns of inhibition of the two activities are identical, and the inhibition with adipate was maximal. These results suggest similar or identical active sites for L-kynurenine and 3,5-diiodo-L-tyrosine transamination.

The purified and characterized preparation of the kidney supernatant α-aminoadipate (kynurenine) aminotransferase, which was determined to be nearly homogeneous (2), demonstrated substantial activity towards 3,5-diiodo-L-tyrosine. The analytical techniques utilized to characterize this preparation did not demonstrate a protein band that might account for this activity. This did not rule out the possibility that this activity was due to contamination by a physically similar protein as opposed to an intrinsic activity of the characterized protein. Several experiments were designed to discriminate between these possibilities with the purified preparation.

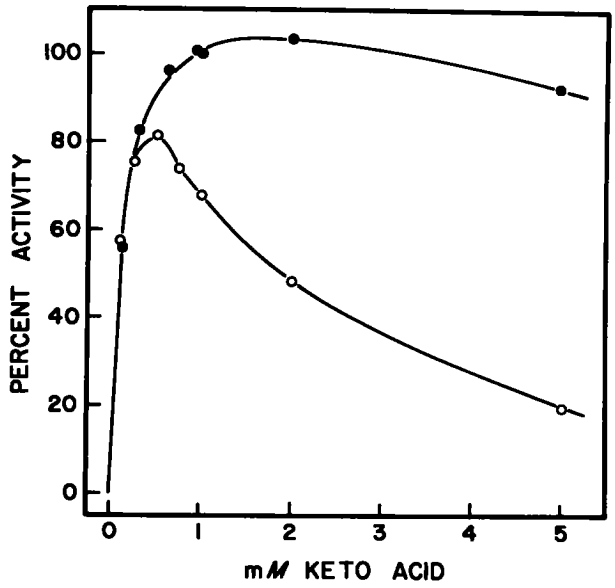

FIG. 3

Effect of varying the concentration of keto acids on 3,5-diiodo-L-tyrosine aminotransferase activity. The purified and characterized α-aminoadipate (kynurenine) aminotransferase preparation from the rat kidney supernatant (2) was used. The activity was assayed in duplicate as described in Experimental Procedures except the concentration of α-ketoglutarate (-●-) or α-ketoadipate (-O-) was varied.

The activity towards 3,5-diiodo-L-tyrosine was inhibited by a homologous series of dicarboxylic acids of increasing chain length from oxalate, C_2, to sebacate, C_{10} (Fig. 2B). The inhibition with adipate was maximal. The inhibition pattern is similar to that observed for the mitochondrial kynurenine and 3,5-diiodo-L-tyrosine aminotransferase activities (Fig. 2A) and that reported for the supernatant α-aminoadipate and kynurenine aminotransferase activities (1). This suggests that the active site for 3,5-diiodo-L-tyrosine transamination is identical or remarkably similar to that for L-kynurenine and L-α-aminoadipate transamination.

The spacing of negative charges yielding maximal inhibition has been postulated to be reflective of the distance between two cationic groups at the active site (12). This led to the discovery that α-ketoadipate was a good substrate for kynurenine aminotransferase from both kidney fractions (13). Both α-ketoglutarate and α-ketoadipate were observed in this study to be good substrates for the transamination of 3,5-diiodo-L-tyrosine as well; α-ketoadipate was inhibitory at higher concentrations (Fig. 3). These results are very similar to those observed for kynurenine aminotransferase (13).

Activity towards 3,5-diiodo-L-tyrosine was competitively inhibited with L-α-aminoadipate (Fig. 4). This competition would be expected if both substrates were transaminated by the same protein. Similarly activity towards L-kynurenine was competitively inhibited with L-α-aminoadipate (1). Kynurenine aminotransferase activity was inhibited by 3,5-diiodo-L-tyrosine, but the type of inhibition was not studied.

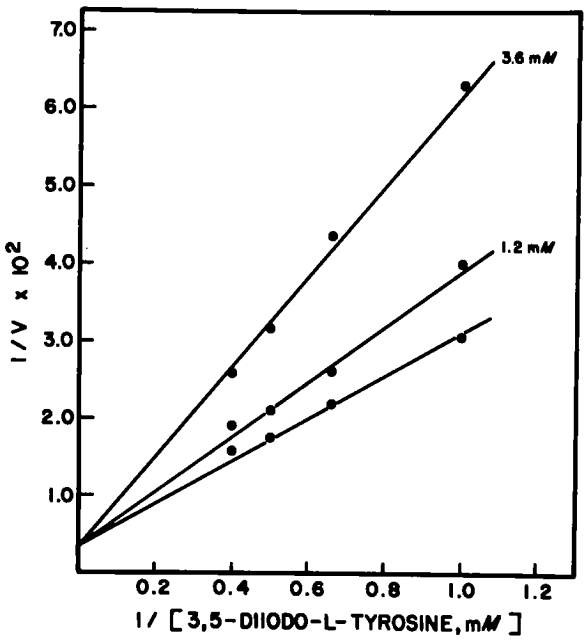

FIG. 4

Inhibition of 3,5-diiodo-L-tyrosine aminotransferase activity (-●-) with L-α-aminoadipate. The purified and characterized α-aminoadipate (kynurenine) aminotransferase preparation from the rat kidney supernatant (2) was used. The activity was assayed in duplicate as described in Experimental Procedures except that 3,5-diiodo-L-tyrosine concentrations were varied. The initial velocity, V, is defined as mmoles aromatic keto acid formed per liter per min.

Discussion

The following properties reported for halogenated tyrosine aminotransferase (8) are very similar to those reported for the highly purified α-aminoadipate (kynurenine) aminotransferase from the rat kidney supernatant (2,3) and for partially purified preparations from the rat kidney mitochondria: 1. the chromatographic behavior on DEAE-cellulose and hydroxylapatite, 2. the range for ammonium sulfate fractionation, 3. the molecular weight, 4. the complete dependence on added pyridoxal-P for activity, and 5. the broad substrate specificity. There are also several similarities of the activities of the two enzymes towards their nominal substrates, 3,5-diiodo-L-tyrosine and L-kynurenine (8,12,14): 1. the relative Km values, 2. the pH dependence, and 3. the keto acid specificity.

The following additional similarities of the kynurenine aminotransferase and halogenated tyrosine aminotransferase activities in the mitochondrial and supernatant fractions were observed in this study: 1. a similar distribution in the supernatant and mitochondrial fractions of kidney and liver, 2. a similar sex difference in the supernatant and mitochondrial fractions of kidney with no sex difference in liver fractions, and 3. a similar pattern of heat inactivation. A similar total dependence on added pyridoxal-P for activity was observed for both aminotransferase activities in the kidney supernatant fraction. A similar inhibition pattern with a homologous series of dicarboxylic acids with adipate being most effective was observed for both aminotransferase activities in the kidney mitochondrial fraction. The above similarities strongly suggest that the two aminotransferase activities are associated with the same protein in the kidney mitochondrial and supernatant fractions. Furthermore, the evidence suggests that there are no readily detectable differences between the kidney supernatant and mitochondrial enzymes. We are presently purifying and characterizing the mitochondrial enzyme to determine if we can further support these concepts.

The following data strongly support the view that the activity towards 3,5-diiodo-L-tyrosine is intrinsic to the protein purified and characterized for α-aminoadipate and kynurenine aminotransferase activities from the rat kidney supernatant fraction and is not due to contamination by a physically similar but different enzyme: 1. a similar inhibition pattern of the three activities with a homologous series of dicarboxylic acids with adipate being most effective, 2. substrate inhibition of 3,5-diiodo-L-tyrosine transamination with α-ketoadipate, 3. competitive inhibition of 3,5-diiodo-L-tyrosine transamination with L-α-aminoadipate, and 4. inhibition of L-kynurenine transamination with 3,5-diiodo-L-tyrosine. These observations and previously reported similarities provide very strong support for the view that α-aminoadipate aminotransferase, kynurenine aminotransferase, and halogenated tyrosine aminotransferase activities are associated with the same protein.

In the preparation of the highly-purified α-aminoadipate (kynurenine) aminotransferase used in this study, specific activities were determined for α-aminoadipate, kynurenine, and aspartate transamination at each stage of purification (2). Specificity for diiodotyrosine was not suspected at the time, however, so its activity was not followed during purification. Evidence for the copurification of this activity with the others will be sought in further purification of the mitochondrial enzyme although some complications may be anticipated in interpreting activity ratios in view of (1) the overlapping specificities of aminotransferases, (2) the present lack of knowledge concerning the specificities of other aminotransferases for diiodotyrosine, and (3) Nakano's early report that the substrates of the halogenated tyrosine aminotransferase are oxidatively deaminated by crude or slightly-purified kidney preparations (15).

The halogenated tryosine aminotransferase preparation was reported to have thyroid hormone aminotransferase activity (8). This and the above evidence suggest a rather broad specificity for the enzyme that we have studied and also suggest the possibility of an interaction or role for the enzyme in thyroid hormone metabolism. This possibility is interesting to consider due to the reported increase of rat liver mitochondrial kynurenine aminotransferase activity after L-thyroxine administration (16). Such an increase might represent an adaptation to the presence of excess substrates.

Acknowledgements

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