Purification, Properties, and Identity of Liver Mitochondrial Tyrosine Aminotransferase*

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SUMMARY

Mitochondrial tyrosine aminotransferase (L-tyrosine + 2-oxoglutarate = p-hydroxyphenylpyruvate + L-glutamate, EC 2.6.1.5) has been purified about 900-fold to homogeneity from rat liver and was crystallized. The enzyme is cationic and has a molecular weight of approximately 90,000, determined by amino acid composition and molecular sieve chromatography and of 114,000 by sedimentation analysis. It catalyzes the conversion of tyrosine to p-hydroxyphenylpyruvate and the reverse reaction. From the reverse direction with p-hydroxyphenylpyruvate, the reaction has a 3-fold greater rate with L-glutamate as cosubstrate than with aspartate. In the forward direction with L-tyrosine and α-ketoglutarate or oxaloacetate as cosubstrate, L-aspartate, α-methyl-L-aspartate, and β-methyl-L-aspartate are potent inhibitors. With α-ketoglutarate, amino acids were effective as cosubstrates in the following order of activity: L-aspartate > L-phenylalanine > L-cysteine > L-tyrosine > monooiodo-L-tyrosine = dihydroxy-L-phenylalanine > L-tryptophan > L-methionine = L-asparagine = p-chloro-L-phenylalanine. Evidence based on substrate specificity, inhibition by common substrates, inhibition by specific antibodies upon enzyme activity, and physical chemical properties indicate that the mitochondrial tyrosine aminotransferase is identical with mitochondrial aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1). This activity relationship would then distinguish this enzyme from the tyrosine aminotransferase and aspartate amiotransferase found in cytosol on the basis of properties and function.

Hepatic tyrosine aminotransferase activity is found in cytosol and in mitochondria (1–6), but this activity is the result of the action of different enzymes (6). The mitochondrial form of the enzyme seems to have a broader tissue distribution than the cytosol form (9, 17).

The finding of very low levels of the hepatic cytosol enzyme in a patient who had increased levels of urinary p-hydroxyphenylpyruvate suggested that, at least under some physiological conditions, the liver mitochondrial enzyme can function metabolically (5). This study was undertaken to isolate and investigate the properties of the mitochondrial tyrosine aminotransferase from rat liver. Evidence is presented to suggest that the mitochondrial tyrosine aminotransferase reaction is catalyzed by the same protein that catalyzes aspartate amination. Tyrosine transamination, perhaps, is also a property of a single amino transferase (L-alanine: 2-oxoglutarate aminotransferase EC 2.6.1.2) and the action of all of these enzymes can result in the utilization or formation of tyrosine.

EXPERIMENTAL PROCEDURE

Materials—Carboxymethyl-Sepharose C-50 and Sephadex G-100 were purchased from Pharmacia. Electrophoresis equipment and reagents were obtained from LKB Instruments, Rockville, Maryland. Aspartate aminotransferase from porcine heart, alanine aminotransferase (EC 2.6.1.2) from heart muscle, pyruvate, L-arginine, L-glutamic acid, L-leucine, L-tryptophan, tyrosine, 3,5-dimethoxytyrosine, and dihydrothreitol were purchased from Calbiochem. L-Phenylalanine, α-methyl-dL-aspartic acid, β-methyl-dL-aspartic acid, and N-hexiloxycarbonyl-dL-aspartic acid were purchased from Cyclo Chemical Corporation, Los Angeles, California. L-Tyrosine, L-alanine, L-ethionine, L-dihydroxyphenylalanine, L-dihydroxyphenylalanine, and L-histidine were purchased from Sigma. Oxalacetic acid, L-valine, and p-hydroxyphenylpyruvic acid were obtained from Sigma. L-Aspartic acid, α-ketoglutaric acid, pyridoxal-5'-P, DL-asparagine, L-cysteine, L-3,5-diodotyrosine, L-isoleucine, L-lysine, L-methionine, L-proline, and L-serine were purchased from Nutritional Biochemicals Corporation. L-Glycine and DL-amino-n-butyric acid were obtained from Eastern. L-α-Methyl tyrosine hydrochloride was purchased from Regis. SA-2 anion exchange resin-loaded paper was purchased from Reeve Angel Company, New York, New York. Sodium 2-ketoglutarate-5-14C (specific activity 19.2 mCi per mmole) was purchased from Amersham/Searle.

Preparation of Liver Mitochondria and Extraction of Tyrosine Aminotransferase—Groups of normal or adrenalectomized male Fisher rats (Charles River Breeding Laboratories) were killed by decapitation; the livers were removed, rinsed, and weighed by

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difference in 0.25 M sucrose. A 10% homogenate of the liver was prepared with a Potter-Elvehjem homogenizer fitted with a Teflon pestle. Cellular debris and nuclei were removed by centrifugation at 600 x g for 10 min at 0-4°. Mitochondria were prepared from the supernatant solution by centrifugation at 15,000 x g for 10 min at 0-4°. The mitochondrial pellet was then suspended in the desired medium for further study. For purification purposes the medium was 0.05 M potassium phosphate, pH 7.2, containing 0.01 M pyridoxal-5'-P and 0.1 M α-ketoglutarate. The mitochondrial suspension was homogenized, adjusted to a final concentration of 5 volumes of buffer per g of original tissue, and frozen for at least 48 hours. The preparation was allowed to thaw at 37° in a shaking water bath and was removed just as the last ice was disappearing. The preparation was homogenized again gently with a Potter-Elvehjem homogenizer and centrifuged at 15,000 rpm in a Servall centrifuge for 30 min to sediment the membranous material and other particulate material which might be present.

Purification of Enzyme from Mitochondrial Extracts—Tyrosine aminotransferase activity was purified from the crude extract by addition of solid ammonium sulfate to final concentrations of 30%, 50%, 60%, and 90% of saturation. When possible, the 50 to 60% fraction was collected by allowing the preparation to stand overnight to improve the yield of enzyme activity utilizing pyruvate as amino group acceptor. If the yield of enzyme was incomplete in the 60 to 90% fraction, more ammonium sulfate was added to bring the concentration to 95% saturation. The active fraction, 60 to 90%, was suspended in 0.01 M potassium phosphate, pH 7.2, containing 0.01 M pyridoxal-5'-P and 0.1 M α-ketoglutarate and dialyzed overnight against 200 volumes of the same buffer. Occasionally, protein precipitation occurred during dialysis and this was not prevented by adding 1 mM EDTA and 1 mM dithiothreitol to the buffer. This could be overcome partially by increasing the buffer concentration to 0.05 M or 0.1 M and then diluting the preparation to 0.01 M just prior to addition of the enzyme to the column for the next step of the purification. The preparation was added to a column of carboxymethyl-Sephadex C-50 (3 x 28 cm) which had been equilibrated with 0.01 M Medium A (0.01 M potassium phosphate, pH 7.2, containing 0.01 M pyridoxal-5'-P and 0.1 M α-ketoglutarate) and then equilibrated with 0.01 M Medium A in a concentration range of 5 to 10 mg of protein per ml.

Analytical ultracentrifugation was performed with a Beckman model E instrument. Sedimentation coefficients were determined by standard procedures using a double sector cell at 60,000 rpm at 20°. Photographs were taken at 8- or 16-min intervals. Diffusion constants were determined using an artificial boundary cell at 22,000 rpm and 20° by determining the slope of a plot of a² in square centimeters versus 2t in seconds (8, 9). Photographs were taken at 8- or 16-min intervals.

Measurements of distances moved by the maximum ordinate or of a were made with a Nikon two dimensional microcompparator. Molecular weight values were calculated from the s° and the Dmₜ values at 10 mg protein per ml determined experimentally, according to the Svedberg equation

\[ M = \frac{RTs^2_{s,0}}{D_{m,t}(1 - e^2)} \]

For this equation the partial specific volume (e) was calculated from the amino acid composition to be 0.75 according to the method of Cohn and Edsall (10).

Enzyme Assays—Tyrosine aminotransferase was measured by the modified Briggs assay (12, 13), the Diamondstone method (14), and the method of Gabay and George (15). A unit of enzymatic activity is defined as 1 nmole of product formed per min at 37°. The specific activity is given as nanomoles of p-hydroxyphenylpyruvic acid formed per min per mg of protein. The aspartate aminotransferase reaction was followed by the procedure described by Banks et al. (16).

Protein Measurements—Protein was measured by the biuret method (17), the method of Lowry et al. (18), the ultraviolet method of Warburg and Christian (19), and qualitatively by measuring absorbance at 280 nm.

Interaction of Tyrosine Aminotransferase with Antibodies to Rat Liver Anionic and Cationic Aspartate Aminotransferase Isozymes—Antibodies to soluble (anionic) and mitochondrial (cationic) aspartate aminotransferase (kindly provided by Dr. H. Wada) were incubated for 60 min at 37° in various ratios with soluble aspartate aminotransferase or to our enzyme preparation from 1:1 to 2000:1 and then the enzymes were assayed for the respective activities. The preparations were allowed to stand for 24 hours at 4° and assayed again.

RESULTS

Release of Tyrosine Aminotransferase from Rat Liver Mitochondria—In order to purify and characterize this mitochondrial enzyme, it had to be released from the mitochondria in a reproducible and quantitative but simple fashion with minimal loss of activity. Detergents such as sodium dodecyl sulfate and sodium deoxycholate were tried but did not improve the yield and in some cases resulted in lower activity. Sonic treatment of fresh mitochondria resulted in about 70% release, but recovery varied considerably (40 to 80%), with most of the remainder of the activity being recoverable in the pellet after two 1-min treatments at
Table I
Release of mitochondrial tyrosine aminotransferase (effect of buffer and dilution on release from rat liver mitochondria)

Tyrosine aminotransferase activity was determined by measuring p-hydroxyphenylpyruvic acid formation (12, 13). All assays were performed at 37°C for 10 min or 20 min with a 5-min preliminary incubation in the presence of all components of the system except a-ketoglutarate. Control tubes contained no tyrosine. The assay mixture contained the following components in a total volume of 3 ml (final concentration): 0.2 M potassium phosphate buffer, pH 8.1; 10 mM a-ketoglutarate (or oxaloacetate); 9 mM tyrosine; 4 mM diethyldithiocarbamate (used only to assay homogenates and subcellular fractions); 40 µM pyridoxal-5'-P; and a source of enzyme (1 to 8 mg of protein). The reaction was linear for at least 45 min and over a protein concentration range of 0.5 to 20 mg for the homogenate through crude extracts. With highly purified enzyme, linearity began at 4 µg of protein.

Since freeze-thawing did not result in loss of activity, but sometimes resulted in slightly higher activity, results are expressed as percentage of activity found released after centrifugation at 15,000 rpm for 15 min in a Sorvall centrifuge at 3°C. The number of animals tested is given in parentheses. Similar results were obtained with oxaloacetate.

| Medium               | Concentration | Released activity | % total |
|----------------------|---------------|-------------------|---------|
| 0.25 M sucrose       | 2 (10)        | 7                 |         |
| 0.05 M potassium phosphate, pH 6.8 | 2 (2) | 25                |         |
| 0.05 M potassium phosphate, pH 8.1 | 2 (2) | 50                |         |
| 0.14 M KCl           | 4 (6)         | 68                | 78      |
| 0.14 M KCl           | 6 (6)         | 110               |         |

Table II
Purification of mitochondrial tyrosine aminotransferase

Details of this purification are given in the text. Enzymatic activity is expressed as nanomoles p-hydroxyphenylpyruvate formed per min at 37°C. Details of the assay are given with Table I.

| Fraction       | Protein | Enzyme | Specific activity | Recovery |
|----------------|---------|--------|-------------------|----------|
| Homogenate     | mg      | units  | units/mg          | %        |
| Mitochondria   | 0.17-0.18a | 0.9a | 100               |
| Extract        | 5388    | 3891   | 1.87              | 63       |
| (NH₄)₂SO₄ 60%   | 3391    | 19.27  | 100               |
| Carboxymethyl-Sephadex chromatography | 51 | 2679 | 52.53 | 50 |
| Isoelectrofocusing and Sephadex G-100 chromatography | 10.8 | 1695 | 155 | 31 |

a There are three enzymes which catalyze tyrosine transamination in liver homogenate and two enzymes which catalyze tyrosine transamination in mitochondria. The values for the rest of the purification data refer essentially to one enzyme, therefore, the fold purification from the homogenate is probably an underestimation; see text for details.

Table III
Evidence for existence of two proteins in mitochondria possessing tyrosine aminotransferase activity

Tyrosine aminotransferase was solubilized by freeze-thawing rat liver mitochondria suspended in 0.05 M potassium phosphate, pH 8.1 (5 ml of buffer per g of tissue, wet wt). Tyrosine was 9 mM. Concentrations of a-ketoglutarate (A), pyruvate (C), and oxaloacetate (B) were varied from 1 to 50 mM to find the saturating level of keto acid. The results given are for 50 mM keto acid.

| KETO ACIDS | ENZYME ACTIVITY | ADDITIVE |
|------------|-----------------|---------|
| A          | 4.1             | No      |
| B          | 3.0             | Yes     |
| C          | 1.6             | Yes     |
| A + C      | 5.9             | Yes     |
| B + C      | 4.2             | Yes     |
Fig. 1. Sedimentation velocity of purified mitochondrial tyrosine aminotransferase. A double sector filled epon centerpiece was used. The protein concentration was 12.4 mg per ml. The solvent was 0.1 M phosphate buffer, pH 7.5, containing 0.1 M chondrial alanine aminotransferase. Each of these factors was considered when the final yield was calculated. The final specific activity was 0.18 (0.145 to 0.180) moles of p-hydroxyphenylpyruvate formed per min per mg of protein at 37°C in 0.2 M potassium phosphate buffer, pH 7.1, with 9 mM tyrosine as amino donor. Activity with pyruvate was reduced to less than 0.05% of activity with α-ketoglutarate.

A comparison was made of the different fractions from (NH₄)₂SO₄ using α-ketoglutarate, oxaloacetate, and pyruvate as amino group acceptor. Most of the pyruvate activity could be recovered between 50 and 60% saturation and most of the oxaloacetate activity between 60 and 90% saturation. These results are consistent with the existence of two mitochondrial enzymes utilizing tyrosine as a substrate. An additional study was made on the crude extract from mitochondria using the three keto acids as given in Table III. At 9 mM tyrosine, negligible inhibition was observed with oxaloacetate at 50 mM final concentration. When pyruvate was incubated in the presence of one of the other two keto acids, an additive effect was seen when both keto acids were at saturating levels. Similar results were obtained with monooiodotyrosine instead of tyrosine. These data also suggest that perhaps 30% of the mitochondrial activity could be the result of an alanine aminotransferase.

Properties of Purified Enzyme—In the analytical ultracentrifuge the enzyme moved as a single component as can be seen in Fig. 1. Dependence of the sedimentation coefficient upon protein concentration is shown in Fig. 2 where it can be seen that the value of 4.5 x 10⁻¹³ sec⁻¹ is invariable over a substantial concentration range of the enzyme. The diffusion coefficient has been measured at a protein concentration of 10 mg per ml as shown in Fig. 3. Using these values in the molecular weight equation (see “Experimental Procedure”), a molecular weight of 114,000 was calculated. Sephadex G-100 chromatography yielded a symmetrical curve with respect to protein and activity and removed contaminants. The presence of amphotiles could be qualitatively determined by adding colorimetric reagents used in the Briggs assay (12, 13) and observing the white precipitate that formed. The molecular weight for mitochondrial tyrosine aminotransferase as determined on Sephadex G-100 was approximately 90,000. The purified enzyme was subjected to isoelectric focusing on both pH 7 to 10 and pH 8 to 10 gradients and one peak occurred at pH 9.3 (Fig. 4). Similar results were observed at various steps in the purification process. The elution volume for the enzyme before and after isoelectric focusing from Sephadex G-100 was essentially unchanged, suggesting that no major alteration in molecular weight had resulted from this step. Disc gel electrophoresis (11) in sodium dodecyl sulfate disc gel-urea yielded two major bands (Rₚ = 0.18 and 0.22) and one minor band which was barely visible (0.26). Tiselius free boundary electrophoresis at pH 7.6 in 0.1 M potassium phosphate yielded two closely traveling peaks. These data suggest that there may be binding of the enzyme to some substance which alters its mobility, that there is still some minor protein contaminant with a molecular weight very similar and a pHₚ of around 9.3 on isoelectric focusing, or that fractionation of the enzyme into its subunit occurred. The enzyme was found to elute from CaPO₄ gel over a wide range of buffer concentration. In an attempt to find several enzymes the ratio of activity in fractions eluted with 0.05 M, 0.1 M, and 0.3 M were compared for α-ketoglutarate and oxaloacetate. No ratio difference occurred suggesting that the same protein was eluting from the gel. Repeated
FIG. 4. Isoelectrofocusing of mitochondrial tyrosine aminotransferase showing homogeneity. pH range of ampholines was 8 to 10. The run was for 45 hours using 3 mg of mitochondrial tyrosine aminotransferase. The enzymatic assay system contained 9 mM l-tyrosine, 10 mM a-ketoglutarate, 0.1 ml of eluate at pH 8.1 with a 20-min incubation period. The specific activity prior to isolectric focusing was 155 nmoles of product per min per mg of protein. Due to interference from ampholines, specific activities cannot be calculated directly on column eluates. Inhibition is variable, ranging usually from 30 to 70% if these ampholines are added to enzyme of known activity. Absorbance readings at 280 nm were corrected for absorbance due to ampholines which ranged from 0.17 at pH 8.0 to 0.06 at pH 10.

The crystals were blocks the conversion of tyrosine to p-hydroxyphenylpyruvate. In contrast, aspartate has no effect under these conditions on the activity of the cytosol tyrosine aminotransferase.

| Amino acid residue | Liver mitochondrial tyrosine aminotransferase | Liver cytosol tyrosine aminotransferase | Heart mitochondrial alanine aminotransferase |
|------------------|--------------------------------------------|----------------------------------------|--------------------------------------------|
| Aspartic acid    | 105                                        | 65                                     | 90                                         |
| Threonine<sup>a</sup> | 20                                    | 34                                     | 37                                         |
| Serine<sup>b</sup> | 5                                        | 68                                     | 53                                         |
| Glutamic acid    | 109                                       | 115                                    | 97                                         |
| Proline          | 49                                        | 6                                      | 44                                         |
| Glycine          | 104                                       | 62                                     | 80                                         |
| Alanine          | 111                                       | 70                                     | 80                                         |
| Valine           | 100                                       | 60                                     | 62                                         |
| Methionine       | 18                                        | 25                                     | 25                                         |
| Isoleucine       | 65                                        | 55                                     | 55                                         |
| Leucine          | 135                                       | 94                                     | 69                                         |
| Tyrosine         | 27                                        | 28                                     | 28                                         |
| Phenylalanine    | 65                                        | 33                                     | 46                                         |
| Lysine           | 146                                       | 49                                     | 68                                         |
| Histidine        | 10                                        | 18                                     | 25                                         |
| Arginine         | 46                                        | 46                                     | 46                                         |
| Tryptophan       | ND<sup>c</sup>                           | 9                                      | 9                                          |
| Cysteine         | ND<sup>d</sup>                           | 28                                     | 12                                         |

<sup>a</sup> Data from Reference 20.
<sup>b</sup> Data from Reference 21.
<sup>c</sup> Not extrapolated to zero time hydrolysis.
<sup>d</sup> Not determined.

The apparent Michaelis constants for the enzyme are as follows: tyrosine, 12 mM; a-ketoglutarate, 0.4 mM; and oxaloacetate, 2 mM.

When the purified enzyme was concentrated to about 10 mg of protein per ml or more, spontaneous crystallization occurred when dilute buffers were employed (0.01 M). The crystals were usually shaped in the form of a triangle. This "crystallization" may represent the condition where the protein is insoluble with low salt concentration. This may explain why protein precipitated under certain conditions during dialysis. Precipitation during dialysis and crystallization may be identical processes and prolonged standing out of solution results in loss of activity such that it is difficult to resolubilize the protein.

**Amino Acid Analysis of Mitochondrial Tyrosine Aminotransferase**—Samples of the final preparation were analyzed for amino acid content. The results are presented in Table IV.

**Substrate Specificity**—The relative initial velocity of mitochondrial tyrosine aminotransferase with various amino acids was determined using C-14-ketoglutaric acid as the amino group acceptor. Activity of the enzyme was followed measuring C-14-glutamate formation. The results are presented in Table V showing a comparison of each amino acid with tyrosine which has been adjusted arbitrarily equal to 1. Several amino acids, other than tyrosine, are good amino group donors. The ratio data indicate that the optimum couple would be aspartate-glutamate or the reaction catalyzed by mitochondrial aspartate aminotransferase. Further evidence that mitochondrial aspartate aminotransferase might be catalyzing the reaction for mitochondrial tyrosine transamination was obtained with the addition of aspartate to the complete system for the tyrosine aminotransferase reaction since, as can be seen in Table VI, aspartate effectively blocks the conversion of tyrosine to p-hydroxyphenylpyruvate. In contrast, aspartate has no effect under these conditions on the activity of the cytosol tyrosine aminotransferase.

**Table IV**

| Amino acid composition of mitochondrial tyrosine aminotransferase from rat liver |
|-----------------------------------------------|
| Protein was hydrolyzed for 21 or 48 hours in 0.1 N HCl at 110° (average of three determinations). Correction for water of hydrolysis was made. Tryptophan and cysteine content was not determined. The molecular weight based on amino acid analysis is 87,000. |

| Amino Acid | Liver mitochondrial tyrosine aminotransferase | Liver cytosol tyrosine aminotransferase | Heart mitochondrial alanine aminotransferase |
|------------|--------------------------------------------|----------------------------------------|--------------------------------------------|
| Aspartic acid | 105                                        | 65                                     | 90                                         |
| Threonine   | 20                                        | 34                                     | 37                                         |
| Serine      | 5                                         | 68                                     | 53                                         |
| Glutamic acid | 109                                       | 115                                    | 97                                         |
| Proline     | 49                                        | 6                                      | 44                                         |
| Glycine     | 104                                       | 62                                     | 80                                         |
| Alanine     | 111                                       | 70                                     | 80                                         |
| Valine      | 100                                       | 60                                     | 62                                         |
| Methionine  | 18                                        | 25                                     | 25                                         |
| Isoleucine  | 65                                        | 55                                     | 55                                         |
| Leucine     | 135                                       | 94                                     | 69                                         |
| Tyrosine    | 27                                        | 28                                     | 28                                         |
| Phenylalanine | 65                                       | 33                                     | 46                                         |
| Lysine      | 146                                       | 49                                     | 68                                         |
| Histidine   | 10                                        | 18                                     | 25                                         |
| Arginine    | 46                                        | 46                                     | 46                                         |
| Tryptophan  | ND                                        | 9                                      | 9                                          |
| Cysteine    | ND                                        | 28                                     | 12                                         |

<sup>a</sup> Data from Reference 20.
<sup>b</sup> Data from Reference 21.
<sup>c</sup> Not extrapolated to zero time hydrolysis.
<sup>d</sup> Not determined.
TABLE V
Specificity of mitochondrial tyrosine aminotransferase for various L-amino acids

L-Amino acids were 9 mM final concentration and the assay was as described by Galay and George (15) and by Rosenberg and Litwack (22). α-Ketoglutarate-14C was 0.12 mM with approximately 200,000 dpm. Pyridoxal-5'-P (30 μM) was added. Incubation at 37° in 0.2 M potassium phosphate, pH 8.1, was for 10, 20, and 30 min at two levels of protein in the range of 1 to 40 μg of protein (750-fold purification). Results are expressed as a ratio of radioactivity of glutamate formation compared with tyrosine taken as equal to 1. No activity was observed for d-tyrosine, L-lysine, L-proline, L-serine, L-leucine, L-valine, α-methyl-L-tyrosine, glycine, and L-alanine.

| L-Amino acid                        | Relative activity |
|-------------------------------------|------------------|
| Aspartic acid                       | 0.29             |
| Phenylalanine                       | 1.65             |
| Cysteine                            | 1.25             |
| Tyrosine                            | 1.00             |
| Monoiodo-L-tyrosine                 | 0.81             |
| Dihydroxyphenylalanine              | 0.85             |
| Tryptophan                          | 0.62             |
| Methionine                          | 0.20             |
| Ethionine                           | 0.17             |
| p-Chlorophenylalanine               | 0.17             |
| Asparagine                          | 0.15             |
| Histidine                           | 0.07             |
| Isoleucine                          | 0.04             |
| Diiodotyrosine                      | 0.01             |

TABLE VI
Inhibition of mitochondrial tyrosine aminotransferase by aspartate and related compounds

The enzyme was purified approximately 900-fold over the starting homogenate. L-Tyrosine concentration was 9 mM. L-Aspartate and related compounds were 5 mM in concentration and were added to the preincubation medium along with the enzyme for 5 min before the reaction was initiated with L-tyrosine. p-Hydroxyphenylpyruvate disappearance by the modified Briggs reaction (12, 13) was determined. Additional details of this assay are given in Table I. Duplicate determinations were made.

| Addition                        | Inhibition |
|--------------------------------|------------|
|                                | α-Ketoglutaric acid | Oxalosuccinic acid |
| L-Aspartate                    | 95          | 86          |
| d-Aspartate                    | 8           | 10          |
| α-Methyl-L-α-aspartate         | 90          | 75          |
| β-Methyl-L-α-aspartate         | 68          | 68          |
| N-Benzyl-L-aspartate           | 0           | 0           |
| DL-Asparagine                  | 6           | 0           |
| DL-α-aminoacetic acid          | 6           | 0           |
| L-Alanine                      | 4           | 0           |

DISCUSSION

Tyrosine is a precursor for a number of biologically important compounds and can be metabolized as a source of carbon during gluconeogenesis although this may not be an important role for tyrosine. The cytosol tyrosine aminotransferase found in liver is inducible (for example, see Reference 23), has diurnal variations in activity levels (24), has a short half-life (see Reference 25), and an adequate apparent Michaelis-Menten constant with respect to tyrosine and thus seems well suited to controlling the levels of tyrosine. Consequently, it would not seem essential for the cell...
to have a second enzyme which is not subject to large changes in activity (5, 6) and has an unfavorable apparent Michaelis-Menten constant for tyrosine of 12 nM. In this paper we have presented evidence suggesting that mitochondrial tyrosine aminotransferase activity is identical with mitochondrial aspartate aminotransferase and also with alanine aminotransferase. These proteins could thus use various amino acids, including tyrosine, to regulate the important keto acids, oxaloacetate and α-ketoglutarate for oxidative metabolism. This could explain the role of these enzymes within liver mitochondria. The existence of the mitochondrial form in other tissues such as brain (7), heart, and kidney (26) could aid in the conservation of tyrosine at the expense again of aspartate and glutamate which would then be available to the Krebs cycle. Recent studies with p-hydroxyphenylpyruvate in vivo support the concept that the level of blood tyrosine could, in part, be controlled in such a manner at least under certain conditions.

The association of mitochondrial tyrosine aminotransferase activity with mitochondrial aspartate aminotransferase suggests the need to investigate the role of mitochondrial aspartate aminotransferase in the metabolic control of these amino acids and keto acids.

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