Evidence of Phenylalanine Transaminase Activity in the Isoenzymes of Aspartate Transaminase*

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SUMMARY

The transamination by pig mitochondrial and supernatant aspartate transaminase of aromatic amino acids was studied in order to determine if an analogy exists between these enzymes and a previously reported brain phenylalanine transaminase (GEORGE, H., AND GABAY, S. (1968) Biochim. Biophys. Acta, 167, 555).

Supernatant and mitochondrial aspartate transaminase from pig heart can utilize aromatic amino acids as substrates. Phenylalanine and other aromatic amino acids are competitive inhibitors of the transamination between glutamate and aspartate and their respective keto acids. The inhibition constants (K_i) are: phenylalanine = 6.8 mM, tryptophan = 4.1 mM, and tyrosine = 3.9 mM for the supernatant enzyme; and phenylalanine = 18.4 mM, tryptophan = 12 mM, and tyrosine = 3.3 mM for the mitochondrial enzyme. It can be shown that phenylalanine reacts with the pyridoxal form of both isozymes (absorbance, 350 to 360 nm) to generate the pyridoxamine form of the enzyme (absorbance, 330 nm). This form reconverts into the pyridoxal form upon addition of either phenylpyruvate or α-ketoglutarate, completing a full transamination cycle between an amino acid and its analogous keto acid or with acceptor keto acid. Other non-aromatic amino acids, with the exception of methionine, are considerably poorer substrates.

At pH 8.3 the transamination rates of isolated pig heart supernatant and mitochondrial aspartate transaminase with phenylalanine as amino donor and α-ketoglutarate as acceptor are 12 and 200 nmoles of glutamate formed per hour per μg. The substrate dissociation constants, as determined by direct spectrophotometric titrations of the isoenzyme's active site, are: phenylalanine = 18.3 mM and phenylpyruvate = 0.4 mM for the mitochondrial enzyme and phenylalanine = 25 mM and phenylpyruvate = 0.6 mM for supernatant enzyme. These values are comparable to those of purified brain phenylalanine transaminase.

Immunological and electrophoretic studies reveal identity between the isoenzymes of heart aspartate transaminase and brain phenylalanine transaminase. Antibodies against the heart enzyme inhibit the brain enzyme, with a concomitant decrease in both the aspartate transaminase and phenylalanine transaminases activities. After inhibition by heart's supernatant and mitochondrial aspartate transaminase antibodies, the ratio of aspartate transaminase activity to phenylalanine transaminase remains constant throughout the brain's enzyme purification procedure.

It appears that because of substrate specificity, substrate affinity, transamination rates, inhibition by phenylalanine, inhibition by antibodies and electrophoretic mobility, pig brain phenylalanine transaminase is aspartate transaminase. Brain contains both the cytoplasmic and mitochondrial isoenzymes. Because of a higher rate of utilization of phenylalanine by the mitochondrial enzyme, it is suggested that the production of phenylpyruvate in brain might preferentially occur, in vivo, through the participation of mitochondrial glutamate aspartate transaminase.

After glutamic aspartic transaminase (EC 2.6.1.1) was obtained in two homogeneous forms, it was necessary to determine if these were true isoenzymes of mitochondrial and supernatant origin, or if one was another transaminase with a high activity with aspartate and α-ketoglutarate as substrates. Mishuda (1) found that the mitochondrial enzyme catalyzed the transamination of aromatic amino acids, although at much reduced rates. This data agrees with earlier findings by Novogrodsky and Meister (2) for the aromatic substrate utilization of the supernatant enzyme and the more recent report of Miller and Litwack (3) on the tyrosine transaminase activity of mitochondrial aspartate transaminase.

The aspartate transaminase isoenzymes are widely distributed in the liver, heart, and brain tissues of mammals (1, 4–6). The important role of these isoenzymes in the heart and liver has been well documented (8–11). The role of aspartate transaminase in the brain has yet to be elucidated, especially in light of the significant transamination by the isoenzymes of aromatic amino acids. The aromatic amino acids are important precursors of aromatic amines (12). The metabolism of phenylalanine is important in mental disorders such as phenylketonuria (13).
In this disorder large amounts of phenylpyruvic acid are produced since the hydroxylating enzyme for conversion of phenylalanine to tyrosine is absent. Recently phenylalanine and phenylpyruvate have been shown to be inhibitors of human and rat brain hexokinase, pyruvate kinase, and 6-phosphogluconate dehydrogenase (14).

Since this laboratory is involved in the investigation of the structure and function of the aspartate transaminase isoenzymes and their role in pathological disorders, it was important to determine if the activity of the aromatic amino acid transaminases such as phenylalanine transaminase (15-19) could be attributed to one or both of the isoenzymes of glutamic aspartic transaminase.

### Experimental Procedure

#### Materials

The amino acids and pyridoxal phosphate were purchased from Sigma Chemical Co. α-[14C]Ketoglutarate was from Calbiochem and 2,5-diphenyloxazole and 1,4-[2-(5-phenyl-oxazolyl)]benzene were obtained from Packard Instrument Co.

#### Methods

**Preparation of Enzyme**—The mitochondrial and supernatant isoenzymes of aspartate transaminase were isolated as previously described (20, 21). The pig brain transaminase was prepared as reported by George and Gabay (16).

**Preparation of Antibodies**—Antisera to homogeneous preparations of mitochondrial and supernatant aspartate transaminases are prepared in separate rabbits as previously reported (22).

**Activity Studies**—Catalytic studies were performed in a 0.2-ml volume containing either phosphate buffer (pH 6.8) or pyrophosphate buffer (pH 8.0), amino acid, α-[14C]ketoglutarate, and enzyme incubated at 30°C. Aliquots of 10 μl were removed at 2-min intervals and spotted on Beckman No. 320046 paper strips, previously saturated with formic acid, which terminated the reaction. The product [14C]glutamic acid was separated and the radioactivity counted as previously described (8).

**Spectrophotometric Measurements**—Spectrophotometric titrations were performed with 1-cm light path quartz cells in a Cary model 15 recording spectrophotometer equipped with 0 to 0.1 absorbance unit slide wire. Additions were made with micropipettes, and the reference samples contained all components except the enzyme. All experimental results are the average of at least two determinations on three different enzymes preparations.

Aspartate transaminase assays were performed according to the methods of Lis (23) or by spectrophotometric monitoring of the DPNH utilization in a coupled assay containing in a total volume of 2 ml: malic dehydrogenase (4 mg), 100 mM sodium pyrophosphate buffer, pH 8.3, 3.3 mM α-ketoglutarate, and 33 mM aspartate. Phenylalanine transaminase was followed by the assay method of George and Gabay (16).

### Results

**Substrate Specificity**—The activity of the isoenzymes with various amino acids and α-ketoglutarate as the amino acceptor is presented in Table I. The natural substrates, L-glutamate and L-aspartate, were also used with each isoenzyme and as noted their transaminations are significantly higher than those observed for the aromatic substrates. Mitochondrial aspartate transaminase is less active than the supernatant enzyme when aspartate is a substrate, this is due to inhibition of the mitochondrial enzyme by the product of the reaction, oxaloacetate (8). Both isoenzymes possess great catalytic activity with phenylalanine, tryptophan, and tyrosine as substrates. The transamination rate of phenylalanine is considerably faster with the mitochondrial isoenzyme. Histidine is a poor substrate for both isoenzymes. Alanine was used mainly as a reference for nonaromatic "unnatural" amino acids as well as poor substrate. Other amino acids, with the exception of methionine, which is utilized better than histidine, show negligible activity with both isoenzymes.

**Determination of Inhibition Constant for Various Amino Acids**—The inhibition constant (Ki) of various amino acids was determined for both the mitochondrial and supernatant pig heart aspartate transaminase using aspartate and α-ketoglutarate as substrates.

From the Ki data, Table II, it can be seen that the aromatic amino acids and methionine have great affinity for the isoenzymes.

The Ki value for phenylalanine is considerably lower, in both isoenzymes of aspartate transaminase, than the Km (50 mM) reported by George and Gabay (16) for this amino acid with purified brain phenylalanine transaminase. Also their Km values for L-tyrosine and L-tryptophan are remarkably similar to the Ki values obtained for the aspartate transaminases. Thus, it would seem that the isoenzymes of aspartate transaminase show affinities for the aromatic amino acids which are very similar to those reported for brain phenylalanine transaminase.

### Table I

**Activity rates of mitochondrial and supernatant aspartate transaminase with amino acids**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration</th>
<th>Mitochondrial enzyme (μmol/min/mg)</th>
<th>Supernatant enzyme (μmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Glutamic acid</td>
<td>50</td>
<td>680</td>
<td>448</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>50</td>
<td>438</td>
<td>585</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>9</td>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>100</td>
<td>81</td>
<td>28</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>150</td>
<td>200</td>
<td>12</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>150</td>
<td>6.75 × 10⁻²</td>
<td>8.65 × 10⁻²</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>150</td>
<td>1.37 × 10⁻¹</td>
<td>8.5 × 10⁻¹</td>
</tr>
</tbody>
</table>
TABLE II
Inhibitor $K_i$ constants for mitochondrial and supernatant aspartate transaminase

The following reaction mixtures containing NADH (8 mM) and malate dehydrogenase (5 μg) were prepared as follows: mitochondrial enzyme: α-ketoglutarate (5 mM), aspartate (0.8 mM, 0.4 mM, 0.2 mM, and 0.1 mM), Tris acetate buffer, pH 8.2 (50 mM), enzyme (0.4 μg), and inhibitor amino acid in a total volume of 2.0 ml; supernatant enzyme: α-ketoglutarate (10 mM), aspartate (5.0 mM, 2.5 mM, 1.25 mM, 0.5 mM), Tris acetate, pH 8.2 (50 mM), and enzyme (0.5 μg) in a total volume of 2.0 ml. Reactions were carried out at 28° in a Gilford 2000 recording spectrophotometer at 340 nm.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mitochondrial enzyme</th>
<th>Supernatant enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Phenylalanine</td>
<td>18.4</td>
<td>7.0</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>3.3</td>
<td>3.9</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>12</td>
<td>4.1</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>100</td>
<td>78.00</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>46</td>
<td>60.50</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>73.6</td>
<td>147.0</td>
</tr>
</tbody>
</table>

Therefore, all the amino acids tested can interact with the active site chromophore and undergo a half transamination process with conversion of the pyridoxal form into the pyridoxamine form. The process is fully reversible because the addition of α-ketoglutarate, oxaloacetate, or phenylpyruvate to the pyridoxamine form resulted in an instantaneous formation of a 362 nm absorbing species characteristic of the pyridoxal form of the enzyme.

Dissociation Constants—Since the kinetically determined $K_i$ values obtained for phenylalanine were similar to the $K_m$ values of George and Gabay (16), it was of interest to know the true substrate affinities of the isoenzymes of aspartate transaminase for phenylalanine and its keto acid phenylpyruvate.

In the majority of the cases isoenzymes have been compared in terms of substrate affinities only by steady state kinetic methods. Steady state kinetic data are obtained from analyses of the overall catalytic activity, whereas spectrophotometric equilibrium data are obtained by analyzing the direct interaction of the substrate at the active site in terms of changes in absorbance which are related to enzyme-substrate complex formation (8).
Fig. 3. Determination of $K_1$ and $K_2$ for supernatant aspartate transaminase using phenylalanine and phenylpyruvate. Analysis of spectral changes at 362 nm upon the addition of phenylalanine to a solution of supernatant aspartate transaminase in 0.1 M Tris acetate buffer, pH 8.2, containing a constant concentration of phenylpyruvate. The negative abscissa intercepts are the apparent dissociation constants ($K_i$) of phenylalanine at each constant concentration of phenylpyruvate. From the ordinate intercept of the intercept $K_i$, the true dissociation constants for phenylalanine and phenylpyruvate may be calculated.

Fig. 4. Determination of $K_1$ and $K_2$ for mitochondrial aspartate transaminase using phenylalanine and phenylpyruvate. Analysis of spectral changes at 352 nm upon addition of phenylalanine to an enzyme solution in 0.1 M Tris acetate buffer, pH 8.2, containing a constant concentration of phenylpyruvate.

**Table IV**

Dissociation constants of mitochondrial and supernatant aspartate transaminase

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Phenylnaline</th>
<th>Glutamate</th>
<th>Phenylpyruvate</th>
<th>4-Ketoglutarate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial aspartate</td>
<td>25</td>
<td>13.3±</td>
<td>0.6</td>
<td>0.7±</td>
</tr>
<tr>
<td>transaminase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant aspartate</td>
<td>18.3</td>
<td>14.0±</td>
<td>0.4</td>
<td>0.4±</td>
</tr>
<tr>
<td>transaminase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine transaminase</td>
<td>50.0±</td>
<td></td>
<td></td>
<td>0.74±</td>
</tr>
</tbody>
</table>

a Results of Michuda and Martinez-Carrion (8).
b Results of George and Gabay (16).

The spectral changes due to the addition of an amino acid to a solution of either isoenzyme containing a fixed concentration of keto acid may be described as follows:

$$E_L + A_m \xrightarrow{K_1} EX \xrightarrow{K_2} E_M + O$$

where the amino acid ($A_m$) and keto acid ($O$) interact with the pyridoxal ($E_L$) or pyridoxamine ($E_M$) form of each isoenzyme to create intermediary binary complexes ($EX$) or the abortive complex between the pyridoxal form and the keto acid ($E_LO$) (7).

The apparent dissociation constants may be defined as

$$K_1 = \frac{[E_L][A_m]}{E_L}$$
$$K_2 = \frac{[E_M][O]}{E_M}$$
$$K_3 = \frac{[E_L][O]}{E_LO}$$

A plot of the ratio of the amino acid (phenylalanine) concentration to change in absorbance at 362 nm versus the amino acid (phenylalanine) concentration at fixed keto acid (phenylpyruvate) concentrations should produce a series of parallel lines (8, 24) (Figs. 3 and 4). The negative abscissa intercepts of these lines are the apparent amino acid dissociation constants (8, 24).

A secondary plot of the ratio of phenylpyruvate concentration to the apparent phenylalanine dissociation constant against the phenylpyruvate concentration produces a straight line. This line cuts the negative abscissa at a value equivalent to the phenylpyruvate dissociation constant $K_2$. The ordinate intercept of the line is $K_3/K_1$ (8, 24).

Upon interacting with their substrate keto acids, $\alpha$-keto glutarate or oxaloacetate, or other dicarboxylic acids, both isoenzymes of aspartate transaminase form catalytically inactive complexes with absorbance at 430 nm (8). However, these same isoenzymes showed no absorbance increase at 430 nm in the presence of 0.2 mM phenylpyruvate, nor was there an effect of concentrations of phenylpyruvate up to 0.2 mM on the dissociation constants of the spectroscopically detectable abortive complex(es) of these enzymes with succinate, $\alpha$-ketoglutarate, or other substrate analogues. These observations indicate that $K_3$ is very large or, in other words, the abortive complex of the type pyridoxal enzyme-phenylpyruvate which would compete with the well known pyridoxal enzyme-succinate (or other substrate analogues) is not formed. The constants for the isoenzymes are reported and compared to those of the brain phenylalanine transaminase in Table IV.

**Phenylalanine Transamination of Heart Supernatant and Mitochondrial Aspartate Transaminases as Compared to Brain Phenylalanine Transaminase**—Since the literature value (16) of the substrate affinities, for both amino and keto acids, of the brain enzyme compare with our values for the pig heart isoenzymes (Table IV) and both pig heart aspartate transaminases had activity rates with phenylalanine similar to the reported values for the so-called phenylalanine transaminase isolated from pig brain. We, therefore, isolated the brain enzyme to compare to heart aspartate transaminase.

We have repeated the purification procedure of George and...
TABLE V

Purification of brain phenylalanine transaminase

The brains were homogenized in 0.1 M potassium phosphate buffer, pH 7.0, and centrifuged at 48,000 X g for 20 min. Phenylalanine transaminase was purified according to the procedure of Gabay and George (15) except that the hydroxylapatite was eluted stepwise. The fractions are those indicated in the elution profile of Fig. 5.

<table>
<thead>
<tr>
<th>Phenylpyruvate^a per hour</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>1.6 X 10^6</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>1.3 X 10^6</td>
</tr>
<tr>
<td>Fraction I</td>
<td>3.2 X 10^5</td>
</tr>
<tr>
<td>Fraction II</td>
<td>3.6 X 10^5</td>
</tr>
<tr>
<td>Fraction III</td>
<td>4.7 X 10^5</td>
</tr>
</tbody>
</table>

^a Assay of George et al. (25).

Gabay (16), and it appears that their procedure yields aspartate transaminase rather than a separate phenylalanine transaminase. Their procedure yields mostly the supernatant isoenzyme because the mitochondrial isoenzyme, under their experimental conditions, is not completely eluted from the hydroxylapatite column. Our purification scheme is summarized in Table V and a typical elution profile from hydroxylapatite is shown in Fig. 5.

Using starch gel electrophoresis and following the enzyme location by an activity stain (1), it was possible to show that Fractions I and II contained mostly the supernatant aspartate transaminase while Fraction III contained mitochondrial aspartate transaminase. The specific activity of this latter fraction is low because it contains a large amount of endogenous protein that could be removed by chromatography in DEAE-Sephadex in 0.02 M Tris buffer, pH 7.1. By the latter treatment both the mitochondrial aspartate transaminase and phenylalanine activities eluted as a single peak. The specific activity for phenylalanine was analogous to that of purified pig heart mitochondrial aspartate transaminase. Furthermore, this brain fraction was undistinguishable from heart mitochondrial aspartate transaminase in starch gel electrophoresis. Fractions I and II had excellent aspartate transaminase activity and phenylalanine inhibited transamination between aspartate and \( \alpha \)-ketoglutarate with \( K_i = 10 \) mM for both fractions. Fraction III showed \( K_i = 20 \) mM. These values are in excellent agreement with those obtained with homogeneous preparations of supernatant and mitochondrial heart transaminase, respectively (Table VI).

Immunochromic Studies—Using the Ouchterlony immunodiffusion technique, single lines of identity were obtained between the purified heart isoenzymes and crude or purified brain preparations against antimitochondrial and antisupernatant aspartate transaminases. It was also observed that after addition of these antibodies both the aspartate and phenylalanine transaminase activities were greatly inhibited whether utilizing crude extracts or purified fractions as enzyme preparations.

If a protein fraction possesses one enzyme with two catalytic activities rather than two separate enzymes each with a distinct function, it is customary to follow the ratio of the two catalytic functions throughout a series of protein fractionation procedures. In the present situation, however, analyzing the ratio of activities of aspartate transaminase to phenylalanine transaminase is not possible. Both isoenzymes of aspartate transaminase, supernatant and mitochondrial, are present in crude extracts and possess different ratios of aspartate to phenylalanine transamination. These isoenzymes also fractionate differently. Nevertheless, we devised a procedure utilizing the ability of the supernatant and mitochondrial antibodies to inactivate the respective enzymes. Each fraction, throughout the purification procedure, was treated with both antibodies at once or separately, and the ratio of the two transaminase activities as well as the over-all inhibition was recorded. These results are presented in Table VII. These results show how specific antibodies against homogenous heart supernatant and mitochondrial aspartate transaminase inactivate the brain aspartate transaminase isoenzymes and how this inactivation is accompanied by a simultaneous and proportional decrease in ability to transaminate phenylalanine whether in the crude brain extracts or at different stages of the fractionation procedure. Of specific interest are: (a) the ratio

TABLE VI

Comparison of catalytic activities of heart and brain transaminases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Assay system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart supernatant aspartate transaminase^a</td>
<td>10</td>
</tr>
<tr>
<td>Heart mitochondrial aspartate transaminase^a</td>
<td>200</td>
</tr>
<tr>
<td>Brain Fraction I^c</td>
<td>2.3</td>
</tr>
<tr>
<td>Brain Fraction II^c</td>
<td>7.71</td>
</tr>
<tr>
<td>Brain Fraction III^c</td>
<td>5.95</td>
</tr>
<tr>
<td>Fraction III after chromatography in DEAE-Sephadex</td>
<td>220</td>
</tr>
<tr>
<td>Phenylalanine transaminase^d</td>
<td>4.8</td>
</tr>
</tbody>
</table>

^a Assay of George et al. (25).
^b Purified according to References 20 and 21.
^c Purified according to Reference 16. See Fig. 5.
^d From Table V of Reference 16.
Antibody was added to the enzyme extracts and incubated at 37°C for 15 min and then for several hours in an ice bath, after which time the mixtures were centrifuged at 10,000 × g for 15 min to remove the antigen-antibody complex. Aliquots of the supernatant liquid were taken for enzymatic assays of aspartate transaminase activity (malic dehydrogenase coupled assay) and phenylpyruvate production (16).

**Table VII**

Effects of antibodies against soluble and intramitochondrial aspartate transaminase on phenylalanine transaminase activity

Antibody was added to the enzyme extracts and incubated at 37°C for 15 min and then for several hours in an ice bath, after which time the mixtures were centrifuged at 10,000 × g for 15 min to remove the antigen-antibody complex. Aliquots of the supernatant liquid were taken for enzymatic assays of aspartate transaminase activity (malic dehydrogenase coupled assay) and phenylpyruvate production (16).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Antisupernatant</th>
<th>Antimitochondrial</th>
<th>Antisupernatant + antimitochondrial</th>
<th>Inhibition of aspartate transaminase after treatment with anti-supernatant and antimitochondrial aspartate transaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart supernatant</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>90</td>
<td>%</td>
</tr>
<tr>
<td>aspartate transaminase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart mitochondrial</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>90</td>
<td>%</td>
</tr>
<tr>
<td>aspartate transaminase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract</td>
<td>9</td>
<td>1.8</td>
<td>18</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>Brain extract</td>
<td>6</td>
<td>2.2</td>
<td>17</td>
<td>6</td>
<td>90</td>
</tr>
<tr>
<td>80% ammonium sulfate</td>
<td>6</td>
<td>2.5</td>
<td>22</td>
<td>5.5</td>
<td>85</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction I</td>
<td>8</td>
<td>2.3</td>
<td>23</td>
<td>6.5</td>
<td>90</td>
</tr>
<tr>
<td>Fraction II</td>
<td>8</td>
<td>2.5</td>
<td>25</td>
<td>7.0</td>
<td>92</td>
</tr>
<tr>
<td>Fraction III</td>
<td>5</td>
<td>4.0</td>
<td></td>
<td></td>
<td>85</td>
</tr>
</tbody>
</table>

* Per cent error ±20.
* Per cent error ±10.
* Enzyme assayed without prior addition of antibody.

**Discussion**

The isoenzymes of glutamate aspartate transaminase can utilize the aromatic amino acids and methionine as amino group donors. When assay conditions are similar to those of George and Gabay (16), the specific activities of the aspartate transaminase isoenzymes are analogous to those of brain phenylalanine transaminase.

Addition of phenylalanine to the mitochondrial or supernatant aspartate transaminases shifts the spectra from the pyridoxal form at 332 nm (mitochondrial) to the pyridoxamine form at 330 nm. This reaction is reversible upon the addition of phenylpyruvate, indicating that both phenylalanine and its analogous keto acid react with the pyridoxal and pyridoxamine form of each isoenzyme of aspartate transaminase to produce the same spectral changes observed with the "natural" substrates aspartate and oxaloacetate or glutamate and α-ketoglutarate. These interactions are, nevertheless, slower for the aromatic amino acid and this makes it possible to follow spectrophotometrically the half reaction between the pyridoxal form of the enzyme with these amino acids as a function of time. This ability of the aromatic amino acids to react with the pyridoxal form indicates that the distal carboxyl group is not essential to carry out transamination and can be replaced by an aromatic ring. Since a complex is not formed upon the addition of phenylpyruvate to the pyridoxal form of either isoenzyme of aspartate transaminase it would indicate that the formation of the well known (8, 24, 26) keto acid-enzyme complex at 430 nm (K₃) requires the existence of the distal carboxyl group of the 4- to 6-carbon dicarboxylic acid.

The low dissociation constants for phenylalanine further verify the binding of these aromatic substrates to the enzymes. These dissociation constants as well as the kinetically obtained Kᵢ constants were found to be somewhat lower than those previously reported (16), especially in the case of phenylalanine. This may be a reflection of the higher degree of homogeneity of our enzyme preparation. Our values with the heart isoenzymes, nevertheless, agree with the Kᵢ values for phenylalanine with our brain preparations.

When purifying the brain extract by the procedure that should yield phenylalanine transaminase (16), the transaminating activity can be totally accounted for by the amount of aspartate transaminase. Furthermore, upon electrophoresis of this fractionated brain extract both aspartate transaminase isoenzymes are present in very high yield with the supernatant isoenzyme in excess. The immunochemical tests show that in any given preparation antibodies against aspartate transaminase inhibited both this enzyme and phenylalanine transaminase. These tests also show that the isoenzymes present in the fractionated brain extract are identical with those found in crude heart extracts and to highly homogeneous mitochondrial and supernatant aspartate transaminase.

The fact that when using antibodies against aspartate transaminase in crude extracts and in the various enzyme fractions of the purification procedure results in a fairly constant ratio of aspartate transaminase units to phenylalanine transaminase units would locate both activities within one enzyme protein.

From the evidence presented in these investigations it is apparent that both the mitochondrial and supernatant aspartate transaminase isoenzymes possess phenylalanine-transaminating properties. Our data, therefore, indicate that the brain content of a structurally distinct phenylalanine transaminase is very...
Aspartate Transaminase as Phenylalanine Transaminase

small, or nonexistent, and that the reportedly purified phenyl-
alanine transaminase is actually glutamic aspartic transaminase.
It is also of interest that while this work was being written Miller
and Litwack (3) reported on the identity of mitochondrial as-
partate transaminase and a previously reported mitochondrial
tyrosine transaminase. Our findings on the activity of both
isoenzymes of aspartate transaminase with tyrosine and other
aromatic amino acids as substrates would substantiate the
results.

In view of these findings, the reports of other aromatic trans-
aminases (with the exception for the well documented case of
supernatant tyrosine transaminase) should be interpreted with
caution and any metabolic implications of transamination of
aromatic amino acids, especially at very low turnover numbers,
should be withheld until the respective enzymes are isolated and
shown to be distinct from other more commonly occurring trans-
aminases.

The mitochondrial enzyme has a considerable higher capacity
of utilizing phenylalanine. Thus the evidence in this paper
would favor this isoenzyme as the main catalyst in any possible
implication of transamination in the metabolism of phenylala-
nine in brain. Furthermore, since this isoenzyme system does
not show tissue specificity the enzyme would have the capability
of acting upon the aromatic amino acids in tissues as diverse as
brain, heart, liver, and kidney and may participate in the route
of control, conservation, or utilization of aromatic amino acids
in the body. In this case, mitochondrial aspartate transaminase
could be responsible for the production of phenylpyruvate in
vivo.

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