Aspartate: 2-oxoglutarate aminotransferase from Trichomonas vaginalis

Identity of aspartate aminotransferase and aromatic amino acid aminotransferase

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INTRODUCTION

Aspartate aminotransferase (aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) has been characterized from a wide range of micro-organisms (Yagi et al., 1982; Kagamiyama et al., 1984; Kondo et al., 1984, and references cited therein) and vertebrate sources (Braunstein, 1973; Porter et al., 1981; Martini et al., 1983). Bacteria and fungi contain a single isoenzyme, whereas in higher organisms there are usually two isoenzymes, one cytoplasmic, the other mitochondrial (Kagamiyama et al., 1984).

Little information is available about purified aminotransferases from the protozoa. We have therefore purified the aspartate aminotransferase from the anaerobic protozoan Trichomonas vaginalis, and have examined its properties.

EXPERIMENTAL

Organism and growth of cells

Trichomonas vaginalis Bushby strain was grown in complex medium as described by Linstead (1981). Cultures were harvested in late exponential phase (approx. 1.5 x 10^8 organisms/ml) by centrifugation at 1200 g for 15 min. The cells were resuspended and washed in 85 mM-NaCl/4.6 mM-KCl/1.1 mM-MgSO_4/222 mM-NaHPO_4/HCl buffer, pH 7.4, containing 2 g of glucose/l. The pellet was resuspended in 50 mM-potassium phosphate buffer, pH 7, containing 2 mM-EDTA, centrifuged again and, after removal of the supernatant, the cell pellet was stored at -20 °C.

Enzyme assays

Aspartate aminotransferase activity was assayed with 18 mM-2-oxoglutarate as substrate at 30 °C either routinely by the coupled assay method of Bergmeyer & Bernt (1974) or in unpurified samples by measuring the formation of oxaloacetate (Morino et al., 1977). For determination of kinetic parameters the concentrations of 2-oxoglutarate and aspartate were varied in the coupled assay.

The assays for tyrosine aminotransferase, phenylalanine aminotransferase and tryptophan aminotransferase activities were based on those described by Granner & Tomkins (1970) and Mavrides & Orr (1975). Branched-chain amino acid aminotransferase activity was measured as described by Aki & Ichichara (1970).

For assessing the amino acid- and 2-oxo acid-specificities, the purified enzyme was incubated in a volume of 0.5 ml at 37 °C with 1 mM-amino acid and 1 mM-2-oxo acid for various times. The reaction was stopped by addition of 125 μl of 4 M-HClO_4. After incubation at 0 °C for 10 min, 62.5 μl of 8 M-KOH, dissolved in 80 mM-potassium phosphate buffer, pH 7.4, was added. After 10 min, the mixture was centrifuged at 10000 g for 10 min. The supernatant was removed and the concentrations of 2-oxo acids and amino acids in it were determined.

NADH oxidase activity was measured by monitoring the rate of decrease of A_{440} in 1 ml of 80 mM-potassium phosphate buffer, pH 7, containing 0.15 mg of NADH/ml.

One unit of enzyme activity was defined as that amount of enzyme which gave a rate of 1 μmol of product formed/min under the conditions specified.

Amino acid and 2-oxo acid analysis

Separation and quantitative determination of amino acids were performed on a Rank Hilger Chromospek analyser, with fluorimetric detection after post-column derivative formation with o-phthalaldehyde.
2-Oxo acids were made to react with o-phenylenediamine to form 2-quinoxalinols (Liao et al., 1977; Hayashi et al., 1982) as follows. Samples containing 2-oxo acids (0.3 ml) were mixed with 75 μl of conc. HCl and 75 μl of 50 mM-o-phenylenediamine dissolved in 2 M-HCl containing 2.5 μl of mercaptoethanol/ml. The mixture was left for 24 h at 22 °C. A saturated solution of sodium acetate in water (0.45 ml) was added, followed by 0.75 ml of ethyl acetate. The 2-quinoxalinols were extracted into the ethyl acetate by vortex-mixing for 30 s. A portion (0.5 ml) of the ethyl acetate layer was evaporated under a stream of N₂, and the residue was dissolved by addition of 100 μl of methanol, followed by 400 μl of 0.35 M-ammonium acetate.

The quinoxalinol derivatives were separated by h.p.l.c. on a LiChrosorb RP-18 column (10 μm particle size; 4 mm × 250 mm) by using the separation system of Liao et al. (1977) on an LKB liquid chromatograph equipped with an LKB 2220 recording integrator. A flow rate of 2 ml/min was used without significant loss of resolution. Quinoxalinols were monitored at 365 nm. Peaks were identified and quantified by comparison with the retention times and areas of derivatives formed from known amounts of pure 2-oxo acids. In this system, when oxaloacetate is subjected to the procedure, a single quinoxalinol derivative co-chromatographing with that formed from pyruvate (cf. Koike & Koike, 1984) is produced, probably owing to decarboxylation of oxaloacetate to pyruvate under the conditions used.

**Protein determination**

Protein was determined by the dye-binding assay method of Bradford (1976), calibrated with γ-globulin.

**Gel electrophoresis**

Sodium dodecyl sulphate/15% (w/v)-polyacrylamide-gel electrophoresis was performed on Tris/glycine-buffered slab gels (Anderson et al., 1973).

**Materials**

Glutamate dehydrogenase (type II in glycerol), malate dehydrogenase (from pig heart; in glycerol), cytoplasmic aspartate aminotransferase (from pig heart; freeze-dried), Fast Blue BB salt, antipain dihydrochloride, 2-oxo acids and o-phenylenediamine were obtained from Sigma Chemical Co. Matrex Blue A was purchased from Amicon, Stonehouse, Glos., U.K.

**Purification of aspartate aminotransferase from T. vaginalis**

All operations were at 0–4 °C. NADH oxidase activity, which interferes with the coupled assay for aspartate aminotransferase, was removed by gel filtration and at the chromatofocusing step (Fig. 1).

(1) **Preparation of cell-free extract.** T. vaginalis cells (37 g wet wt.) were thawed, and 25 ml of 50 mM-potassium phosphate buffer, pH 7, containing 2 mM-EDTA, 1 mM-dithiothreitol, 10 μM-pyridoxal 5'-phosphate and 100 μg of antipain/ml of buffer was added. The mixture was homogenized with 20 passes in a motor-driven Potter-Elvehjem homogenizer at approx. 7000 rev./min. The homogenate was centrifuged at 1500 g for 10 min. The supernatant was centrifuged at 124000 g for 1 h.

(2) **Ammonium sulphate fractionation.** The supernatant (46 ml) was brought to 45% saturation with (NH₄)₂SO₄. After 15 min, the mixture was centrifuged at 10000 g for 15 min. The saturation of (NH₄)₂SO₄ in the supernatant was increased to 80%. After 15 min, the centrifugation was repeated. The pellet was dissolved by addition of 5 ml of 10 mM-potassium phosphate buffer, pH 7, containing 0.5 mM-EDTA, 1 mM-dithiothreitol, 10 μM-pyridoxal 5'-phosphate and 10 μg of antipain/ml.

(3) **Gel filtration.** The solution was applied to a column of Sephacryl S-300 (84 cm × 1.6 cm), eluted with the same buffer at 10 ml/h. The aspartate aminotransferase activity was eluted as a symmetrical peak (Vₐ = 86 ml). The enzyme-containing fractions were pooled and concentrated to a volume of 3.8 ml in an Amicon ultrafiltration cell fitted with a Diaflo PM10 membrane. In the same cell, the buffer was then changed to 25 mM-imidazole/HCl buffer, pH 7.4, containing 1 mM-dithiothreitol.

(4) **Chromatofocusing.** The enzyme solution was then applied to a column (30 cm × 0.5 cm) of Polybuffer exchanger PBE 94, equilibrated in 25 mM-imidazole/HCl buffer, pH 7.4. The column was eluted with 112 ml of a solution of Polybuffer 74 (14 ml of Polybuffer 74, adjusted with HCl to pH 4.7, and made up to 112 ml with water), containing 1 mM-dithiothreitol, at a flow rate of 6 ml/h. Fractions of 2.5 ml volume were collected. Aspartate aminotransferase activity was eluted as a single peak at about pH 6.2 (Fig. 1). NADH oxidase activity was eluted later at about pH 5.2. The aspartate aminotransferase-containing fractions were pooled, concentrated to a volume of 4.1 ml and applied to the column of Sephacryl S-300 as described above, but antipain and EDTA were omitted. The eluted enzyme freed from ampholytes was concentrated.

(5) **Matrex Blue A chromatography.** A column (12.8 cm × 0.9 cm) of Matrex Blue A was equilibrated with 10 mM-potassium phosphate buffer, pH 7, containing 10 μM-pyridoxal 5'-phosphate and 1 mM-dithiothreitol. The enzyme was applied to the column, and the flow was stopped for 30 min. The column was then washed with 50 ml of equilibration buffer at 9.5 ml/h. A peak of 280 nm-absorbing material, which did not contain aspartate aminotransferase activity, was washed from the column. A gradient consisting of 70 ml of equilibration buffer and 70 ml of the same buffer containing 250 mM-KCl was applied at a flow rate of 6.8 ml/h. A single sharp peak containing about 50% of the aspartate aminotransferase activity was eluted at about 25 mM-KCl, followed by a tail containing the remaining activity. The sharp peak was pooled and concentrated to a volume of 1 ml in the Amicon ultrafiltration cell. In the same cell, the buffer was changed to 10 mM-potassium phosphate, pH 7, containing 1 mM-dithiothreitol and 10 μM-pyridoxal 5'-phosphate. The tailed enzyme activity could be collected, but it was less pure than the initial sharp peak.

(6) **Fractionation on hydroxyapatite.** A column (8.5 cm × 1 cm) of hydroxyapatite equilibrated with 10 mM-potassium phosphate buffer, pH 7, containing 1 mM-dithiothreitol and 10 μM-pyridoxal 5'-phosphate was loaded with the enzyme. The column was washed with 35 ml of equilibration buffer and then eluted with a gradient made from 40 ml of 10 mM-potassium phos-
**RESULTS**

**Purification and molecular properties of aspartate aminotransferase from *T. vaginalis***

The purification of aspartate aminotransferase from *T. vaginalis* is summarized in Table 1. The recovery was typically 7–30%, with a purification of 200–1000-fold. The final specific activity of homogeneous enzyme was about 120–180 units/mg of protein, similar to that of aspartate aminotransferase from vertebrate or bacterial sources. The purified enzyme gave a single protein band on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The mobility was almost identical with that of the cytoplasmic pig heart enzyme, with an apparent *M*ₐ of 44000.

The *M*ₐ of the *T. vaginalis* aspartate aminotransferase was estimated under native conditions by gel filtration on a column of Sephacryl S-300 to be 100000 ± 10000.

**Catalytic properties of aspartate aminotransferase from *T. vaginalis***

A steady-state kinetic analysis of the *T. vaginalis* enzyme showed *K*ₐ (aspartate) = 0.96 mM and *K*ₐ (2-oxoglutarate) = 0.062 mM. The enzyme obeyed Bi Bi ping-pong kinetics, except at high substrate concentrations, where substrate inhibition was observed with *K*ᵢ (2-oxoglutarate) = 30 mM.

The inhibition by the product glutamate was competitive with respect to aspartate (*K*ᵢₒ = 2.8 mM) and mixed (non-competitive) with respect to 2-oxoglutarate (*K*ᵢₐ = 6.8 mM; *K*ᵢₐ = 39 mM), as expected for an aminotransferase.

The inhibitory effect of adipate on the steady-state aspartate aminotransferase activity was measured at 0.1 mM-2-oxoglutarate. The *T. vaginalis* enzyme was not inhibited by 20 mM-adipate, and the *K*ᵢ was inferred to be greater than 100 mM. Adipate, however, inhibited the pig heart cytoplasmic enzyme with *K*ᵢₒ of 6 mM and *K*ᵢₐ of 70 mM, in agreement with Michuda & Martinez-Carrion (1970), who, in addition, showed that the mitochondrial isoenzyme bound adipate with *K*ᵢₐ ≫ 100 mM.

**Substrate specificity**

Purified aspartate aminotransferase from *T. vaginalis* was incubated with a range of amino acids, in the presence of oxaloacetate, glyoxylate, 2-oxoglutarate, pyruvate or phenylpyruvate as amino-group acceptors, as described in the legend to Table 2. Transamination products were separated and quantified by amino acid analysis and h.p.l.c. of quinoxalinol derivatives of 2-oxo acids (see the Experimental section). It should be stressed that the results in Table 2 do not show the minimum (or initial) rates of transamination, but give the average rates over 60 min incubation. During this period, with the major activities the reaction approached equilibrium with a resultant decrease in rate during the incubation period, thus causing an underestimate of the true enzyme activity. Initial transamination rates for some of the

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**Fig. 1. Purification of *T. vaginalis* aspartate aminotransferase by chromatofocusing**

Pooled and concentrated fractions from the Sephacryl S-300 column containing aspartate aminotransferase activity (122 units; 2.3 ml) were applied to a column of PBE 94 exchanger (30 cm x 0.5 cm) equilibrated with 25 mM-imidazole/HCl buffer, pH 7.4, containing 1 mM-dithiothreitol. The column was eluted with 112 ml of a solution containing 14 ml of Polybuffer 74/HCl, pH 4.7, containing 1 mM-dithiothreitol and 10 µM-pyridoxal 5'-phosphate at a flow rate of 6 ml/h. Fractions (2.5 ml) were assayed for protein (—, A₂₈₀) and for aspartate aminotransferase (●), phenylalanine aminotransferase (▲) and leucine aminotransferase (■) and NADH oxidase (○) activities.
Table 1. Summary of the purification of *T. vaginalis* aspartate aminotransferase

For full experimental details see the text.

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg of protein)</th>
<th>Recovery (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Cell-free extract*</td>
<td>46</td>
<td>2420</td>
<td>436</td>
<td>0.18</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>(2) Resuspended (NH₄)₂SO₄ pellet</td>
<td>10</td>
<td>2190</td>
<td>460</td>
<td>0.21</td>
<td>103</td>
<td>1.2</td>
</tr>
<tr>
<td>(3) Concentrated Sephacryl S-300 peak</td>
<td>3.8</td>
<td>650</td>
<td>367</td>
<td>0.56</td>
<td>84</td>
<td>3.1</td>
</tr>
<tr>
<td>(4) Concentrated gel-filtered chromatofocusing peak</td>
<td>1.1</td>
<td>106</td>
<td>190</td>
<td>1.8</td>
<td>44</td>
<td>10</td>
</tr>
<tr>
<td>(5) Concentrated Matrex Blue peak†</td>
<td>1.0</td>
<td>0.7</td>
<td>52</td>
<td>74</td>
<td>12</td>
<td>411</td>
</tr>
<tr>
<td>(6) Concentrated hydroxyapatite peak</td>
<td>0.6</td>
<td>0.17</td>
<td>30</td>
<td>172</td>
<td>7</td>
<td>955</td>
</tr>
</tbody>
</table>

* From 37 g wet wt. of *T. vaginalis* cells.
† A further 60 units of enzyme could be recovered from the tail of enzyme activity from the Matrex Blue column, with a specific activity of 30–60 units/mg of protein.

Table 2. Substrate specificity of purified *T. vaginalis* aspartate aminotransferase

*T. vaginalis* aspartate aminotransferase (0.12 unit/ml) was incubated with amino acids and 2-oxo acids for 60 min at 37 °C and the transamination products were analysed as described in the Experimental section. Hydroxyphenylpyruvate was not quantified because of degradation during derivative formation. Abbreviation: N.D., transamination products not detectable by 2-oxo acid analysis.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Amount of transamination products (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amino acid</td>
</tr>
<tr>
<td>Asp</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Phe</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Tyr</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Trp</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Cys, Ala, Ile, Leu, Val, Gln, Gly</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Orn, Lys, Arg</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Glu</td>
<td>524</td>
</tr>
<tr>
<td>Phe</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Tyr</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Trp</td>
<td>2-Oxoglutarate</td>
</tr>
<tr>
<td>Cys, Gln, Gly</td>
<td>0</td>
</tr>
<tr>
<td>Asp, Glu, Phe, Tyr, Gln, Gly</td>
<td>Pyruvate</td>
</tr>
<tr>
<td>Asp, Glu, Ser, Ala</td>
<td>Glyoxylate</td>
</tr>
<tr>
<td>Asp</td>
<td>Phenylpyruvate</td>
</tr>
<tr>
<td>Glu</td>
<td>Phenylpyruvate</td>
</tr>
<tr>
<td>Tyr</td>
<td>Phenylpyruvate</td>
</tr>
<tr>
<td>Trp</td>
<td>Phenylpyruvate</td>
</tr>
<tr>
<td>Ala, Leu</td>
<td>Phenylpyruvate</td>
</tr>
<tr>
<td>Orn, Lys</td>
<td>Phenylpyruvate</td>
</tr>
</tbody>
</table>

Activities are shown in Table 3, measured by a combination of spectrophotometric and analytical techniques.

With 2-oxoglutarate as amino-group acceptor, aspartate, tyrosine, tryptophan and phenylalanine showed high rates of transamination, whereas the other amino acids showed zero or negligible rates. With glyoxylate or pyruvate as acceptor, no significant transamination was observed. With oxaloacetate, glutamate, phenylalanine, tyrosine and tryptophan were transaminated effectively. With phenylpyruvate, aspartate, tyrosine, tryptophan and glutamate showed high rates.

The maximum specific activity for the aromatic amino acids, phenylalanine and tyrosine, was greater than that
Trichomonas vaginalis aspartate aminotransferase

Table 3. Relative transamination rates with different substrates by T. vaginalis aspartate aminotransferase

$K_m^{\text{app}}$ and the maximum specific activity of the T. vaginalis aspartate aminotransferase were obtained by varying the concentrations of amino acid, at a fixed concentration of 1 mM-2-oxoglutarate, and measuring the rates of transamination by using spectrophotometric assays. The relative activities were obtained by incubating T. vaginalis aspartate aminotransferase (0.12 unit/ml) with 1 mM-amino acid and 1 mM-2-oxo acid for 5 min at 37°C, and analysing the transamination products as described in the Experimental section. Under these conditions, this measured the initial rates of transamination.

$$
\begin{array}{cccc}
\text{Amino acid} & \text{2-Oxo acid} & K_m^{\text{app}} (\text{amino acid}) & \text{Maximum specific activity (\text{\mu mol/min per mg of protein})} \\
\text{Asp} & 2-\text{Oxoglutarate} & 1.0 & 60 (100) \\
\text{Phe} & 2-\text{Oxoglutarate} & 21 & 108 (65) \\
\text{Tyr} & 2-\text{Oxoglutarate} & 11 & 90 (69) \\
\text{Trp} & 2-\text{Oxoglutarate} & & 45 \\
\text{Glu} & \text{Oxaloacetate} & & 210 \\
\text{Asp} & \text{Phenylpyruvate} & & 50 \\
\text{Glu} & \text{Phenylpyruvate} & & 175 \\
\text{Tyr} & \text{Phenylpyruvate} & & 45 \\
\text{Trp} & \text{Phenylpyruvate} & & 30 \\
\end{array}
$$

Evidence of the identity of aspartate aminotransferase and aromatic amino acid aminotransferase in T. vaginalis

The behaviour of some of the major aminotransferases of T. vaginalis was investigated during the purification of aspartate aminotransferase. At each stage of the purification the aminotransferase activities with aspartate, tyrosine, tryptophan, phenylalanine, leucine, valine, isoleucine and lysine as amino donor and with 2-oxoglutarate as amino acceptor were assayed. Some of the results are summarized in Table 4. During a 250-fold purification of aspartate aminotransferase, the proportions of tyrosine aminotransferase, phenylalanine aminotransferase and tryptophan aminotransferase activities remained constant, whereas the lysine aminotransferase and branched-chain aminotransferase activities were removed during the purification, so that the purified enzyme catalysed only aspartate aminotransferase and aromatic amino acid aminotransferase activities. Alanine aminotransferase was also removed during the purification, in particular at the chromatofocusing step. The data for alanine aminotransferase are not shown because in crude extracts it was difficult to assay the enzyme activity reliably and the activity appeared to be unstable.

At each stage of the purification the aspartate aminotransferase and aromatic amino acid aminotransferase activities were exactly co-eluted, as is shown for the chromatofocusing step (Fig. 1), suggesting that the activities might reside in a single enzyme. The recoveries of aspartate aminotransferase and aromatic amino acid aminotransferase were identical, suggesting that no major aromatic aminotransferase activity was being lost.

Inhibition of aspartate aminotransferase activity, by the time-dependent irreversible inhibitor gostatin (Nishino et al., 1984), resulted in a simultaneous and identical inhibition of aromatic amino acid aminotransferase activity (Fig. 2), strongly suggesting the identity of active sites. The results were broadly similar when a crude extract of T. vaginalis (after step 1 of purification) was used, though after prolonged incubation a lower degree of inhibition of the aromatic amino acid aminotransferase than of the aspartate aminotransferase was seen. With 98% inhibition of aspartate activity, the aromatic amino acid aminotransferase activity was inhibited by 90%. This might suggest that there is a low activity (about 10% of total) of a second aromatic amino acid aminotransferase activity in the crude extract, though assays on crude extracts might have a systematic error.

Experiments were performed to measure the effectiveness of amino acids as inhibitors of the T. vaginalis and the cytoplasmic pig heart aspartate aminotransferase. Aspartate was a potent inhibitor of aromatic amino acid aminotransferase activity and conversely phenylalanine inhibited aspartate aminotransferase activity of T. vaginalis, demonstrating that aspartate and aromatic amino acids can compete at the same active site of the enzyme from T. vaginalis. Phenylalanine did not inhibit the pig heart aspartate aminotransferase activity, consistent with that enzyme's absence of aromatic amino acid aminotransferase activity.

DISCUSSION

The Mr and subunit number of the aspartate aminotransferase from T. vaginalis are typical of aspartate aminotransferase (Kagamiyama et al., 1984). In T. vaginalis we have found only a single cyto-
Table 4. Co-purification of T. vaginalis aromatic amino acid aminotransferase and aspartate aminotransferase activities

<table>
<thead>
<tr>
<th>Step</th>
<th>Aspartate aminotransferase (units/mg)</th>
<th>Aspartate aminotransferase/phenylalanine aminotransferase ratio</th>
<th>Lysine aminotransferase (units/mg)</th>
<th>Tyrosine aminotransferase (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free extract</td>
<td>228</td>
<td>0.21</td>
<td>36</td>
<td>52</td>
</tr>
<tr>
<td>Resuspended (NH)2SO4 pellet</td>
<td>218</td>
<td>0.33</td>
<td>122</td>
<td>122</td>
</tr>
<tr>
<td>Concentrated Sephadex G-500 peak</td>
<td>122</td>
<td>0.59</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Chromatofocusing peak</td>
<td>32</td>
<td>3.7</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Concentrated Matrix Blue peak</td>
<td>32</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Inhibition of T. vaginalis aspartate aminotransferase and aromatic amino acid aminotransferase activities by gostatin

T. vaginalis aspartate aminotransferase (1.4 units/ml) was incubated at 0 °C, in a medium containing 10 mM-phosphate buffer, pH 7, containing 1 mM-dithiothreitol and 10 mM-pyridoxal 5'-phosphate, with 1 µM-gostatin. At intervals, 0.5 µl samples were removed for assay of tyrosine aminotransferase activity (O) and 0.5 µl samples for aspartate aminotransferase activity (●). Activity is expressed relative to a control incubation, in the absence of gostatin.

plasmic isoenzyme, as in bacteria and yeast. In terms of its catalytic parameters, the T. vaginalis enzyme resembles the cytoplasmic vertebrate isoenzymes [e.g. for pig heart $K_m$ (aspartate) = 2.5 mM and $K_m$ (2-oxoglutarate) = 0.3 mM] and that from Escherichia coli [$K_m$ (aspartate) = 1.3 mM and $K_m$ (2-oxoglutarate) = 0.24 mM] more than it does the mitochondrial isoenzymes [e.g. for pig heart $K_m$ (aspartate) = 0.5 mM and $K_m$ (2-oxoglutarate) = 1 mM] or that from yeast [$K_m$ (aspartate) = 0.11 mM and $K_m$ (2-oxoglutarate) = 1.6 mM] (Kagamiyama et al., 1984) in that the $K_m$ for aspartate is much greater than for 2-oxoglutarate. Adipate inhibits the cytoplasmic, but not the mitochondrial, vertebrate isoenzyme (Michuda & Martinez-Carrion, 1970; Kagamiyama et al., 1984). The T. vaginalis (present study) and E. coli (Kagamiyama et al., 1984) aspartate aminotransferases are both insensitive to adipate.

A striking observation was that the purified T. vaginalis enzyme was also able to transaminate aromatic amino acids at high rates (Tables 2 and 3). In contrast, the vertebrate isoenzymes are almost completely specific for the amino acids aspartate and glutamate, with the mitochondrial enzyme being less specific than the cytoplasmic isoenzyme, but still only able to transaminate aromatic amino acids at rates of 0.2–1% that of aspartate (Kagamiyama et al., 1984). Although the reactivity towards aromatic amino acids is higher with lower organisms, e.g. yeast (0.5–1% of that with aspartate) and E. coli (10–30% of that with aspartate) (Kagamiyama et al., 1984), the T. vaginalis enzyme is unusual in catalysing the reaction with aromatic amino acids at similar or greater rates than with aspartate (Table 3). The evidence

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based on co-purification of enzyme activities (Table 4), co-inhibition by gostatin (Fig. 2) and inhibition by alternative substrates strongly suggests that the aromatic amino acid aminotransferase and aspartate aminotransferase activities of the T. vaginalis enzyme reside in the same protein and are not due to contamination.

The tyrosine:2-oxoglutarate aminotransferase of vertebrates (EC 2.6.1.5) catalyses the transamination of tyrosine at very much greater rates than that of phenylalanine or tryptophan, but cannot utilize aspartate (Granner & Tomkins, 1970; Braunstein, 1973; Ohisalo et al., 1982). It thus differs from the T. vaginalis enzyme, which reacts well with all three aromatic amino acids and with aspartate. However, such a broad specificity has been reported for the aromatic amino acid aminotransferase from the bacterium *Achromobacter eurydice* (Fujikura et al., 1970) and other prokaryotes (Jensen & Calhoun, 1981). There has also been a report that the protozoan *Leishmania* contains an aspartate aminotransferase that also catalyses transamination of aromatic amino acids (Le Blancq & Lanham, 1984), but the enzyme was not purified. This observation suggests that the properties of the T. vaginalis aminotransferase might be common to other protozoa. Aspartate aminotransferase and alanine aminotransferase from *Trypanosoma cruzi* (Cazzullo, 1984) and a tyrosine aminotransferase from *Candida fasciculata* (Rege, 1983) have been purified, but the substrate specificities were not described.

The *K*ₘ for aromatic amino acids of the T. vaginalis enzyme is high, and it is possible that the activity does not serve a role in vivo. As gostatin inhibited only 90% of the tyrosine aminotransferase activity of a crude extract, there might be a low activity of another aromatic amino acid aminotransferase that is of more significance at lower concentrations of aromatic amino acid.

The structure of the active site of the T. vaginalis enzyme is of great interest, as it will accept a wide range of substrates, i.e. aspartate, glutamate, tryptophan, tyrosine and phenylalanine, and of 2-oxo acids, e.g. oxaloacetate, 2-oxoglutarate and *p*-hydroxyphenylpyruvate, but will reject other amino acids, e.g. alanine, lysine, arginine, glutamine and glycine, or 2-oxo acids, e.g. glyoxylate and pyruvate.

We are most grateful to Professor S. Murao for a gift of gostatin, to Mrs. M. Clack and Dr. M. Merrett for performing the amino acid analyses, to Mr. T. Stewart and Mrs. M. A. Walters for cultivation of T. vaginalis and to Dr. C. D. Ginger for useful discussions.

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