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Aspartate:2-oxoglutarate aminotransferase from *Trichomonas vaginalis*

Identity of aspartate aminotransferase and aromatic amino acid aminotransferase

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1. Aspartate:2-oxoglutarate aminotransferase from the anaerobic protozoon *Trichomonas vaginalis* was purified to homogeneity and characterized. It is a dimeric protein of overall M_r approx. 100000. Only a single isoenzyme was found in *T. vaginalis*. The overall molecular and catalytic properties have features in common with both the vertebrate cytoplasmic and mitochondrial isoenzymes. 2. The purified aspartate aminotransferase from *T. vaginalis* showed very high rates of activity with aromatic amino acids as donors and 2-oxoglutarate as acceptor. This broad-spectrum activity was restricted to aromatic amino acids and aromatic 2-oxo acids, and no significant activity was seen with other common amino acids, other than with the substrates and products of the aspartate:2-oxoglutarate aminotransferase reaction. 3. Co-purification and co-inhibition, by the irreversible inhibitor gostatin, of the aromatic amino acid aminotransferase and aspartate aminotransferase activities, in conjunction with competitive substrate experiments, strongly suggest that a single enzyme is responsible for both activities. Such high rates of aromatic amino acid aminotransferase activity have not been reported before in eukaryotic aspartate aminotransferase.

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 protozoon *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. 1.5×10^6 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-Na_2HPO_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_{340} 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*-phthaldialdehyde.

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 2-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 N_2, 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 (10μm 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 $(NH_4)_2SO_4$. After 15 min, the mixture was centrifuged at 10000 *g* for 15 min. The saturation of $(NH_4)_2SO_4$ 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 (V_e = 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-

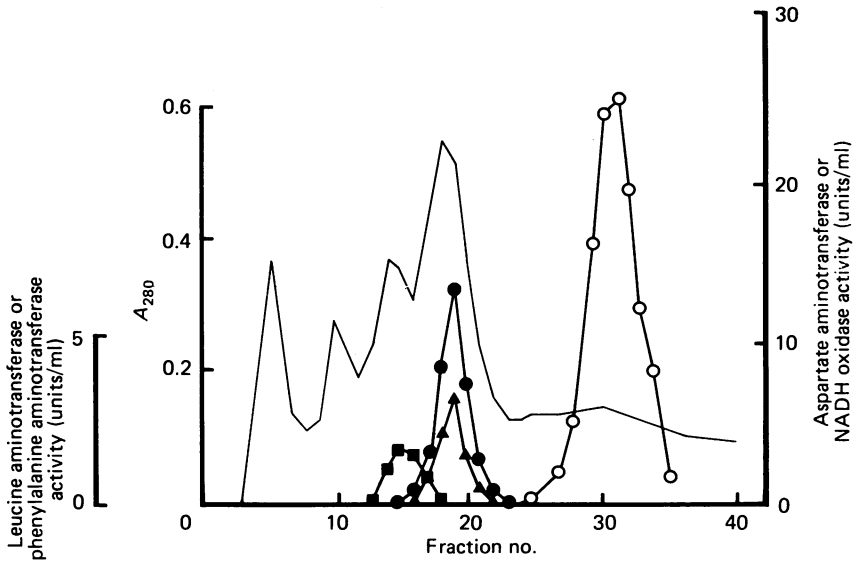

Fig. 1. Purification of *T. vaginalis* aspartate aminotransferase by chromatofocusing

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 × 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 (——, A_{280}) and for aspartate aminotransferase (●), phenylalanine aminotransferase (▲) and leucine aminotransferase (■) and NADH oxidase (○) activities.

phate buffer, pH 7, and 40 ml of 250 mM-potassium phosphate buffer, pH 7, both buffers containing 1 mM-dithiothreitol and 10 μM-pyridoxal 5'-phosphate. The aspartate aminotransferase activity was eluted as a broad peak centred at about 80 mM-potassium phosphate. The central fractions, containing the bulk of the aspartate aminotransferase activity, were pooled, then concentrated as above, and the dissolving buffer was changed to 10 mM-potassium phosphate, pH 7, containing 1 mM-dithiothreitol and 10 μM-pyridoxal 5'-phosphate, in a final volume of 0.53 ml.

The purified enzyme was stored for several weeks at 4 °C or for longer periods at −20 °C.

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_r of 44000.

The M_r 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_m (aspartate) = 0.96 mM and K_m (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_{ic} = 2.8 mM) and mixed (non-competitive) with respect to 2-oxoglutarate (K_{ic} = 6.8 mM; K_{iu} = 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_{ic} of 6 mM and K_{iu} of 70 mM, in agreement with Michuda & Martinez-Carrion (1970), who, in addition, showed that the mitochondrial isoenzyme bound adipate with $K_d \gg 100$ 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 maximum (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

Table 1. Summary of the purification of *T. vaginalis* aspartate aminotransferase

For full experimental details see the text.

Step	Volume (ml)	Protein (mg)	Total activity (units)	Specific activity (units/mg of protein)	Recovery (%)	Purification (fold)
(1) Cell-free extract*	46	2420	436	0.18	100	1
(2) Resuspended $(NH_4)_2SO_4$ pellet	10	2190	460	0.21	103	1.2
(3) Concentrated Sephacryl S-300 peak	3.8	650	367	0.56	84	3.1
(4) Concentrated gel-filtered chromatofocusing peak	1.1	106	190	1.8	44	10
(5) Concentrated Matrex Blue peak†	1.0	0.7	52	74	12	411
(6) Concentrated hydroxyapatite peak	0.6	0.17	30	172	7	955

* 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.

Substrates		Amount of transamination products (nmol/ml)	
Amino acid	2-Oxo acid	2-Oxo acid	Amino acid
Asp	2-Oxoglutarate	220	190
Phe	2-Oxoglutarate	620	506
Tyr	2-Oxoglutarate		615
Trp	2-Oxoglutarate	290	350
Cys, Ala, Ile, Leu, Val, Gln, Gly	2-Oxoglutarate	0	0
Orn, Lys, Arg	2-Oxoglutarate	N.D.	0
Glu	Oxaloacetate	524	545
Phe	Oxaloacetate	217	220
Tyr	Oxaloacetate		275
Trp	Oxaloacetate	100	73
Cys, Gln, Gly	Oxaloacetate	0	0
Asp, Glu, Phe, Tyr, Gln, Gly	Pyruvate	0	0
Asp, Glu, Ser, Ala	Glyoxylate	0	0
Asp	Phenylpyruvate	142	137
Glu	Phenylpyruvate	225	203
Tyr	Phenylpyruvate		190
Trp	Phenylpyruvate	100	84
Ala, Leu	Phenylpyruvate	0	0
Orn, Lys	Phenylpyruvate	N.D.	0

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

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

$K_m^{app.}$ 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.

Amino acid	2-Oxo acid	$K_m^{app.}$ (amino acid) (mM)	Maximum specific activity (μmol/min per mg of protein)	Relative activity
Asp	2-Oxoglutarate	1.0	60	100
Phe	2-Oxoglutarate	21	108	65
Tyr	2-Oxoglutarate	11	90	69
Trp	2-Oxoglutarate			45
Glu	Oxaloacetate			210
Asp	Phenylpyruvate			50
Glu	Phenylpyruvate			175
Tyr	Phenylpyruvate			45
Trp	Phenylpyruvate			30

for aspartate, with 2-oxoglutarate as acceptor, but the K_m for aromatic amino acids was also very much higher than for aspartate (Table 3). When measured at 1 mM-amino acid, the initial rates of transamination are highest with the glutamate–oxaloacetate and glutamate–phenylpyruvate couples. The initial rates of transamination with aromatic amino acids, with any of the 2-oxo acid acceptors, are similar to those with aspartate (Table 3).

Since the purified aspartate aminotransferase apparently showed a high aromatic amino acid aminotransferase activity, we wished to establish whether the preparation was contaminated or whether these two activities were catalysed by the same protein. We thus investigated how the two enzymes co-purified and designed experiments to test for the identity of the two enzymes.

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 M_r 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

Total activity in units and specific activity (units/mg of protein) are quoted at various stages during the purification of *T. vaginalis* aspartate aminotransferase. All activities were measured with 2-oxoglutarate as amino-group acceptor.

Step	Aspartate aminotransferase		Phenylalanine aminotransferase		Tyrosine aminotransferase		Leucine aminotransferase		Aspartate aminotransferase/ phenylalanine aminotransferase ratio
	(units)	(units/mg)	(units)	(units/mg)	(units)	(units/mg)	(units)	(units/mg)	
Cell-free extract	228	0.21	76	0.07	88	0.08	80	0.07	3.0
Resuspended $(NH_4)_2SO_4$ pellet	210	0.33	70	0.09	82	0.10	—	0.10	3.0
Concentrated Sephacryl S-300 peak	122	0.59	32	0.16	29	0.14	20	0.10	3.8
Concentrated gel-filtered chromatofocusing peak	84	3.7	22	0.99	18	0.79	1	0.05	3.9
Concentrated Matrex Blue peak	32	60	10	18	10	18	0	0	3.2

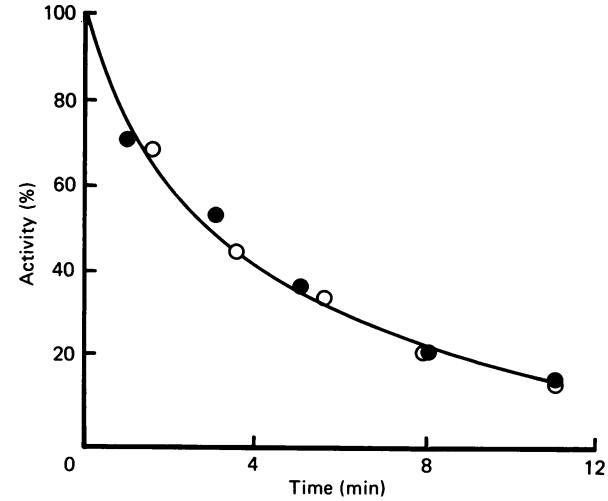

Fig. 2. Inhibition of *T. vaginalis* aspartate aminotransferase and aromatic amino acid aminotransferase activities by gostatin

T. vaginalis aspartate aminotransferase (1.4 units/ml) was incubated at 0 °C, in a medium containing 10 mM-potassium phosphate buffer, pH 7, containing 1 mM-dithiothreitol and 10 μM-pyridoxal 5′-phosphate, with 1 μM-gostatin. At intervals, 8 μl samples were removed for assay of tyrosine aminotransferase activity (○) and 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

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* (Fujioka *et al.*, 1970) and other prokaryotes (Jensen & Calhoun, 1981). There has also been a report that the protozoon *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* (Cazzulo, 1984) and a tyrosine aminotransferase from *Crithidia 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 amino acids, 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.

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