Purification and partial structural and kinetic characterization of tyrosine aminotransferase from epimastigotes of *Trypanosoma cruzi*

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Tyrosine aminotransferase was purified to homogeneity from epimastigotes of *Trypanosoma cruzi* by a method involving chromatography on DEAE-cellulose, gel filtration on Sephacryl S-200 and chromatography on Mono Q in an f.p.l.c. system. The purified enzyme showed a single band in SDS/PAGE, with an apparent molecular mass of 45 kDa. Since the apparent molecular mass of the native enzyme, determined by gel filtration, is 91 kDa, the native enzyme is a dimer of similar subunits. The amino-acid composition was determined, as well as the sequences of three internal peptides obtained by CNBr cleavage at Met residues. Both criteria suggest considerable similarity with the tyrosine aminotransferases from rat and from human liver. The enzyme contains nine $\frac{1}{2}$ Cys residues, three free and the others forming three disulphide bridges. The enzyme is not N-glycosylated. The isoelectric point is 4.6–4.8. The optimal pH for the reaction of the enzyme with tyrosine as a substrate is 7.0. The apparent K_m values for tyrosine, phenylalanine and tryptophan, with pyruvate as a co-substrate, were 6.8, 17.9 and 21.4 mM, respectively, whereas those for pyruvate, α-oxoglutarate and oxaloacetate, with tyrosine as a substrate, were 0.5, 38 and 16 mM respectively. The purified tyrosine aminotransferase acts as an alanine aminotransferase as well and the activity seems to reside in the same enzyme molecule. The results suggest that the enzyme is a general aromatic-amino-acid transaminase, with high sequence similarity to tyrosine aminotransferases from rat and human liver.

INTRODUCTION

Tyrosine aminotransferase (EC 2.6.1.5, TAT), the first enzyme involved in tyrosine catabolism, differs from other transaminases in some respects. The full sequence of the glucocorticoid-inducible rat-liver enzyme has been determined, both from direct peptide sequencing by fast-atom-bombardment mass spectrometry [1] and by inference from cDNA sequences [2]. It differs from aspartate aminotransferase (ASAT) from various sources, being longer at both the N- and the C-termini. The overall sequence similarity between TAT and ASATs is only about 15%, while sequence similarity among ASATs reaches 50–80%. Despite the low sequence similarity between TAT and the ASATs, they must belong to a superfamily [1].

The metabolism of aromatic amino acids in parasitic protozoa has received little attention so far. The presence of enzymes turning phenylalanine into phenylpyruvate [3], tyrosine into *p*-hydroxyphenyl-lactate [3] and tryptophan into indole lactate, indole acetate and tryptophol [4] has been reported in *Trypanosoma brucei*. This metabolism may be important for the development of the pathology of sleeping sickness, both because of the depletion of important metabolites of the amino acids and because of the toxicity of some of the products [5]. In addition, since this metabolism is quite active and the aromatic 2-oxo acids formed are reduced by NAD^+-linked dehydrogenase(s), transamination of aromatic amino acids might be linked to the re-oxidation of glycolytic NADH [3]. The presence of TAT has been reported in *Crithidia fasciculata* [6] and in the ciliate *Tetrahymena pyriformis* [7], and transamination of aromatic amino acids has been detected in *Leishmania donovani* [8]. The enzyme from *C. fasciculata* has been purified and some of its properties have been determined [9]. At least in some *Leishmania* strains, the activity might be the result of ASAT, which may have a broad specificity [10]. The latter enzyme is known to be able to use tyrosine as a substrate in higher animals [11]. We have recently shown that *T. cruzi*, the causative agent of American trypanosomiasis (Chagas' disease), contains TAT activity, which can be separated from ASAT and *p*-hydroxyphenyl-lactate dehydrogenase (pHPLDH) activities. Both enzymes are located mainly in the cytosol, although they are present to a small extent in the mitochondrial fraction as well [12].

We have now purified TAT to electrophoretic homogeneity from homogenates of cultured epimastigotes (the parasite forms similar to those found in the gut of the insect vector of Chagas' disease) and have performed some structural and kinetic studies on the enzyme. These studies suggest that TAT is a general aromatic-amino-acid transaminase that seems to have an associated alanine aminotransferase (ALAT) activity, and that it has substantial sequence similarity to the inducible TAT from rat and human liver.

MATERIALS AND METHODS

Organism and culture

Epimastigotes of *T. cruzi*, strain Tul 0, were grown and harvested as described previously [13]. The cells were washed twice in a solution containing 25 mM potassium-phosphate buffer, pH 7.8, 120 mM KCl, 1 mM dithiothreitol (DTT), 2.5 mM 2-oxoglutaric

Abbreviations used: TAT, tyrosine aminotransferase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; DTT, dithiothreitol; endo H, endo-*N*-acetylglucosaminidase H; buffer 1, 25 mM potassium-phosphate buffer, pH 7.8, 120 mM KCl, 1 mM DTT, 2.5 mM 2-oxoglutaric acid, 0.2 mM pyridoxal 5-phosphate, 1 mM phenylmethanesulphonyl fluoride, 0.5 mM tosyl-lysylchloromethylketone, 5 μM leupeptin and 2 μM pepstatin; buffer 2, 20 mM potassium-phosphate buffer, pH 7.8, 1 mM DTT, 1 mM EDTA and 2.5 mM 2-oxoglutaric acid; buffer 3, 20 mM triethanolamine, pH 7.6, 1 mM DTT and 1 mM EDTA.

Table 1 Apparent K_m and $V_{max.}$ values for the TAT from *T. cruzi*

Apparent K_m and $V_{max.}$ values, which are the means of 2–4 determinations, were obtained using the coupled dehydrogenase assay described in the Materials and methods section, at the co-substrate concentrations stated.

Co-substrate (concentration, mM)	Substrate	Apparent K_m (mM)	Apparent $V_{max.}$ (units · mg^{-1})
Aromatic aminotransferase			
Pyruvate (6)	Tyrosine	7.1 ± 0.4	129.3 ± 5.3
Pyruvate (6)	Phenylalanine	20.1 ± 5.8	157.7 ± 41.4
Pyruvate (6)	Tryptophan	26.7 ± 1.4	73.3 ± 3.6
Tyrosine (5)	Pyruvate	0.7 ± 0.1	181.4 ± 18.7
Tyrosine (5)	Oxaloacetate	19.2 ± 4.0	134.7 ± 17.3
Tyrosine (5)	2-Oxoglutarate	38.7 ± 2.1	33.4 ± 1.2
Alanine aminotransferase			
L-Alanine (5)	2-Oxoglutarate	2.8 ± 0.4	85.8 ± 0.4
L-Alanine (50)	2-Oxoglutarate	6.7 ± 0.5	155.1 ± 5.1
2-Oxoglutarate (20)	L-Alanine	5.7 ± 0.4	132.9 ± 2.7

acid, 0.2 mM pyridoxal 5-phosphate, 1 mM phenylmethanesulphonyl fluoride (PMSF), 0.5 mM tosyl-lysylchloromethylketone (TLCK), 5 μM leupeptin and 2 μM pepstatin (buffer 1). Crude extract from rat liver was obtained by the method of Hargrove and Granner [14].

Extraction and purification of the enzyme

The cells were suspended (0.5–0.6 g wet weight per ml) in buffer 1 and were disrupted by three cycles of 15 s of sonic disintegration, at 4 °C, at maximum power, in a Branson sonifier. The homogenate was centrifuged at 27000 *g* for 45 min, at 4 °C. The pellet was discarded and the supernatant, after dialysis at 4 °C against 20 mM potassium-phosphate buffer, pH 7.8, containing 1 mM DTT, 1 mM EDTA and 2.5 mM 2-oxoglutaric acid (buffer 2), was applied to the top of a DEAE-cellulose column (13.5 cm × 2.0 cm) equilibrated with the same buffer. The column was washed with 40 ml of buffer 2 and eluted with a linear KCl gradient made up of 50 ml of each of buffer 2 alone and buffer 2 containing 50 mM KCl. The fractions with the higher specific activity were pooled and lyophilized. The dry material was dissolved in water and was applied to the top of a column (85 cm × 1.6 cm) of Sephacryl S-200, equilibrated with 20 mM triethanolamine, pH 7.6, containing 1 mM DTT and 1 mM EDTA (buffer 3). The column was eluted with buffer 3 at a rate of 12 ml · h^{-1}. The active fractions were pooled and were applied to a Mono Q column in an f.p.l.c. system (Pharmacia LKB, Uppsala, Sweden) and eluted with a linear NaCl gradient (0–300 mM) in buffer 3, at a rate of 1 ml · min^{-1}.

Determination of enzyme activities

The activity of TAT was assayed by the method of Diamondstone [15], without the addition of diethyldithiocarbamate. One unit of enzyme activity is defined as the amount catalysing the formation of 1 μmol of *p*-hydroxyphenylpyruvic acid (measured as *p*-hydroxybenzaldehyde, molar absorbance 19900 M^{-1} · cm^{-1} at 331 nm) per min at 37 °C. For kinetic studies, the TAT activity was assayed at 340 nm in a reaction mixture containing 150 mM Hepes buffer, pH 7, 0.6 mM DTT, 1.2 mM EDTA, 0.12 mM NADH and 0.75 μg of *p*-hydroxyphenyl-lactate dehydrogenase, purified to homogeneity from *T. cruzi* epimastigotes (M. Montemartini, J. A. Santomé, J. J. Cazzulo and C. Nowicki, unpublished work). The dehydrogenase was also used when phenylalanine or tryptophan were used as substrates instead of tyrosine (Table 1), since this enzyme can reduce the three aromatic 2-oxoacids (M. Montemartini, J. A. Santomé, J. J. Cazzulo and C. Nowicki, unpublished work). The apparent K_m values for the amino acids were determined in the presence of 6 mM pyruvate and those for the 2-oxoacids were determined in the presence of 5 mM tyrosine.

ASAT was assayed spectrophotometrically at 340 nm, in a reaction mixture containing 50 mM Tris/HCl buffer, pH 7.6, 12.5 mM L-aspartate, 1 mM 2-oxoglutarate, 0.12 mM NADH and 1 μg of pig-heart malate dehydrogenase [16]. ALAT was assayed in a similar reaction mixture, in which L-alanine and lactate dehydrogenase were substituted for L-aspartate and malate dehydrogenase, respectively.

Protein determination

The protein content was determined in cell-free extracts using the method of Bradford [17].

SDS/PAGE

Enzyme samples were subjected to SDS/PAGE at room temperature in slab gels under reducing conditions, as described by Laemmli [18]. The gels were stained for protein with Coomassie Brilliant Blue R-250.

Determination of the molecular mass by gel filtration

The approximate molecular mass of TAT was estimated by gel filtration on a Sephacryl S-200 column (120 cm × 0.8 cm) equilibrated with 20 mM potassium-phosphate buffer, pH 7.4, containing 1 mM DTT, 1 mM EDTA, 2.5 mM 2-oxoglutarate and 100 mM KCl. The proteins were eluted with the same buffer solution. The column was calibrated with the following molecular-mass standards: β-amylase from sweet potato (200 kDa), yeast alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa) and cytochrome c (12.4 kDa).

Determination of the thermostability

Cell-free extracts from rat liver and partially purified TAT from *T. cruzi* epimastigotes were diluted in buffer 1 and incubated for 5 min at different temperatures. The samples were then cooled on ice and the activity of TAT was assayed as described above.

Amino-acid analysis

The amino-acid composition was determined in quintuplicate using an Applied Biosystems amino-acid analyser model 420A, which performs hydrolysis, derivatization and h.p.l.c. of the phenylthiohydantoin-amino acids automatically. Tryptophan was determined spectrophotometrically, using the method of Edelhoch [19].

Pyridylethylation of Cys-SH residues and the determination of the number of disulphide bridges

Approx. 100 μg of desalted, purified TAT was pyridylethylated, with or without reduction first, using the method described

in [20]. The samples were then desalted by h.p.l.c. in a 30 mm × 2.1 mm C4 column, using a gradient of 0–80% (v/v) acetonitrile in 0.1% (w/v) trifluoroacetic acid. The modified protein was eluted at a concentration of acetonitrile of about 55% (v/v). The amino-acid composition was determined as described above.

CNBr cleavage

Approximately 100 μg of purified TAT was precipitated with trichloroacetic acid [final concentration 20% (w/v)]. The precipitate was washed with a 5% (w/v) trichloroacetic acid solution, then with cold acetone, and was dissolved in 100 μl of 70% (w/v) formic acid. CNBr (0.2 mg) was added, also as a solution in 70% (w/v) formic acid, and the mixture was incubated at room temperature for 24 h. Then the sample was diluted with water and dried in a Speed Vac rotatory dessicator (Savant Instruments). The fragmented protein was reduced and pyridylethylated as described above and the peptides were desalted and separated by h.p.l.c. on a 25 mm × 4.6 mm Vydac C4 column, using a 0–80% (v/v) acetonitrile gradient in 0.1% (w/v) trifluoroacetic acid, at a flow rate of 1 ml·min^{-1}.

Peptide sequencing

The intact purified TAT, as well as selected CNBr peptides, were applied to a polybrene-coated glass filter and sequenced in an Applied Biosystems model 477 automatic sequencer (Applied Biosystems, Foster City, CA, U.S.A.), run according to the manufacturer's instructions.

Determination of the isoelectric point

The isoelectric point was determined in the Phast system (Pharmacia LKB, Uppsala, Sweden) using a Phast gel pH 4–6.5 pre-formed pH-gradient gel, with Methyl Red dye, soybean trypsin inhibitor, β-lactoglobulin A and human carbonic anhydrase B as markers (pI values of 3.75, 4.55, 5.20 and 6.55 respectively).

Endo-*N*-acetylglucosaminidase H (endo H) treatment

Endo H treatment was performed at pH 5.5 in the presence of 0.1% SDS, as described previously [21].

Chemicals

Sephacryl S-200, DEAE-cellulose, molecular-mass markers and all the enzyme substrates were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Reagents for amino-acid analysis and peptide sequencing were obtained from Applied Biosystems, Foster City, CA, U.S.A. All other chemicals were analytical grade reagents.

RESULTS

Purification of TAT from *T. cruzi* epimastigotes

Table 2 shows the results of a typical purification. TAT was purified 36-fold with a yield of 10%. The results obtained at the DEAE-cellulose step were similar to those reported previously [12], and the bulk of the ASAT activity was not adsorbed onto the column. When ALAT activity was assayed, about 75% of the activity recovered was found in the non-adsorbed fraction. However, the remainder of the ALAT activity recovered, amounting to about 25%, co-purified with TAT. Figure 1 shows the elution profiles obtained in Sephacryl S-200 (Figure 1a) and Mono Q chromatography (Figure 1b). The enzyme preparations obtained were homogeneous in SDS/PAGE, giving a single band with an apparent molecular mass of 45 kDa (Figure 2). The purified enzyme was completely free of ASAT activity, when assayed as described in the Materials and methods section, using three-fold more protein than in the standard TAT assays. However, the purified TAT still had considerable ALAT activity. This is probably an instrinsic activity of the enzyme and not due to a contaminating ALAT, since the peaks of TAT and ALAT activity superimposed exactly, both in the Sephacryl S-200 and in the Mono Q purification steps (results not shown).

The molecular mass of the native enzyme, as determined by gel filtration on Sephacryl S-200, was 91 kDa, which suggests that the native enzyme is a dimer made up of two similar subunits. The electrophoretic behaviour of the purified TAT was influenced by reduction, the non-reduced enzyme having a slightly higher electrophoretic mobility than the fully reduced samples (results not shown). This suggests that the trypanosomal TAT contains intra-chain disulphide bridges. When the isoelectric point was determined by isoelectrofocusing in a pre-packed pH gradient gel, two very close bands were observed, with pI values of 4.6 and 4.8 (results not shown).

Amino-acid composition and peptide sequences of *T. cruzi* TAT

The amino-acid composition of the purified TAT is shown in Table 3. The ½Cys content was nine residues when the enzyme was pyridylethylated after full reduction, but when pyridylethylation was performed without previous reduction only three ½ Cys residues were found. This indicates the presence in the native enzyme of three disulphide bridges and three free Cys-SH residues.

Digestion of the purified trypanosomal TAT with endo H did not result in any increase in the electrophoretic mobility, whereas an ovalbumin control did increase in mobility under identical conditions (results not shown). This result suggests that the enzyme is not N-glycosylated.

When the purified TAT was applied directly to the automatic sequencer, no phenylthiohydantoin-amino-acid peaks were detected, indicating that the N-terminus is blocked. The peptide mixture obtained by cleavage at Met residues was desalted and

Table 2 Purification of TAT from *T. cruzi*

The enzyme was purified from 6 g (wet weight) of epimastigotes and the fractions were assayed for protein concentration and for TAT activity, as described in the Materials and methods section.

Step	Protein product (mg)	TAT activity		Yield (%)	Purification (fold)
		Total (units)	Specific (units·mg^{-1})		
Crude extract	258.40	45.05	0.17	100	1
DEAE-cellulose	22.55	22.22	0.98	49	5.8
Sephacryl S-200	7.87	21.87	2.78	48	16.3
Mono Q	0.77	4.66	6.06	10.3	35.6

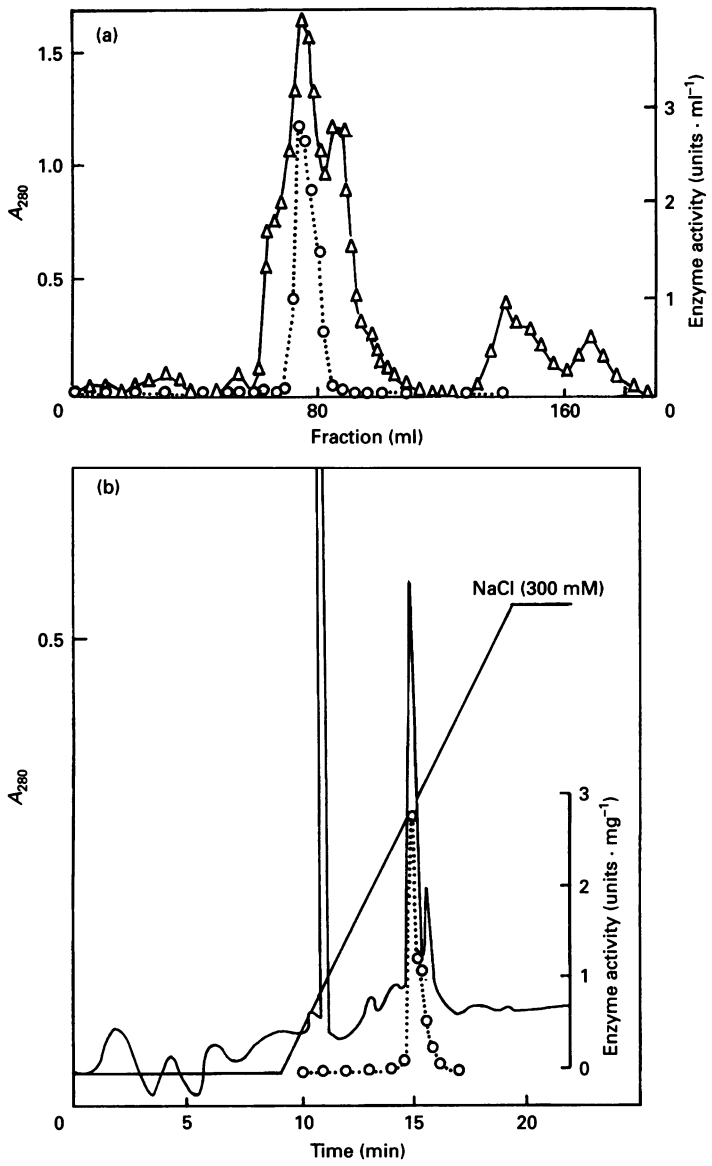

Figure 1 Purification of TAT from *T. cruzi*

(**a**) Gel filtration through Sephacryl S-200 and (**b**) chromatography on a Mono Q column. Both purification steps were performed as described in the Materials and methods section. ○, Enzymic activity; △ (in **a**) or solid line (in **b**), absorbance at 280 nm.

purified by h.p.l.c. Two very close peaks gave the sequence YLYNHIGECIGLAPT, a further two very close peaks gave the sequence AITAICDAGDYALVPQPGFPXYETVCKAY and a fifth peak gave the sequence DFYNCRPENDWEAD. Figure 3 shows a comparison of the sequenced peptides of the trypanosomal enzyme with the corresponding sequences of the rat- and human-liver TATs, as well as with the aromatic-amino-acid transaminase and ASAT from *Escherichia coli*.

Stability and kinetic characteristics of *T. cruzi* TAT

The glucocorticoid-inducible TAT activity in rat liver is known to be stable for at least 5 min at 70 °C in the presence of 2-oxoglutarate and pyridoxal 5-phosphate as protecting agents [22]. Coincidentally the rat liver enzyme (a crude extract) was completely thermostable up to the highest temperature tested (65 °C), whereas the *T. cruzi* TAT activity (a partially purified preparation at the DEAE-cellulose step) was stable up to 60 °C, above which the activity decreased, the residual activity being zero after 5 min at 65 °C.

The optimum pH for the enzyme reaction, with tyrosine and 2-oxoglutarate as substrates, was 7.0. Table 1 shows the apparent K_m and $V_{max.}$ values obtained for the substrates tyrosine, phenylalanine and tryptophan, using pyruvate as the co-substrate, and for pyruvate, 2-oxoglutarate and oxaloacetate, using tyrosine as the co-substrate. The best $V_{max.}/K_m$ ratio was obtained with pyruvate/tyrosine, which may be the physiological pair of substrates in the living cell. Table 1 shows the apparent K_m and $V_{max.}$ values for the ALAT activity present in the purified TAT preparations as well. For comparison, the apparent K_m values for the ALAT activity that was non-adsorbed to the DEAE-cellulose column were 22.8 ± 2.5 mM for L-alanine (in the

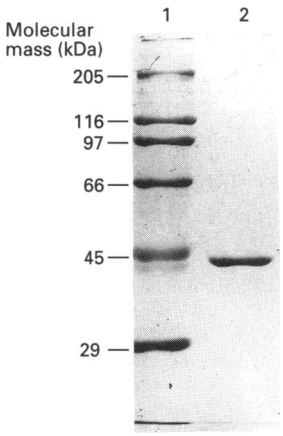

Figure 2 SDS/PAGE of purified TAT from *T. cruzi*

Electrophoresis was carried out on a 10% acrylamide gel, as described in the Materials and methods section, under reducing conditions (lane 2). Lane 1 contains the molecular-mass markers carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa), phosphorylase *b* (97 kDa), β-galactosidase (116 kDa) and myosin (205 kDa).

Table 3 Amino-acid composition of the TAT from *T. cruzi*

Values were determined in quintuplicate. Values in parentheses indicate the value to the nearest integer. Cys residues were modified with 4-vinylpyridine, as described in the Materials and methods section.

Amino acid	Residues per subunit (45 kDa)
Asx	45.5 (46)
Glx	37.5 (38)
Ser	23.7 (24)
Thr	23.1 (23)
Pro	28.3 (28)
Gly	34.3 (34)
Ala	35.2 (35)
Cys	8.8 (9)
Val	31.3 (31)
Ile	17.6 (18)
Leu	38.1 (38)
Met	6.7 (7)
Tyr	11.8 (12)
Phe	15.6 (16)
His	10.9 (11)
Lys	21.8 (22)
Arg	20.1 (20)
Trp	5.9 (6)
Total residues	418

presence of 20 mM 2-oxoglutarate) and 0.34±0.11 mM for 2-oxoglutarate (in the presence of 100 mM L-alanine).

DISCUSSION

We have purified the TAT from *T. cruzi* epimastigotes to electrophoretic homogeneity and to a specific activity of 6 units·mg of protein^{-1}. This value is very much lower than those reported by Rege [9] for the enzyme from *C. fasciculata*

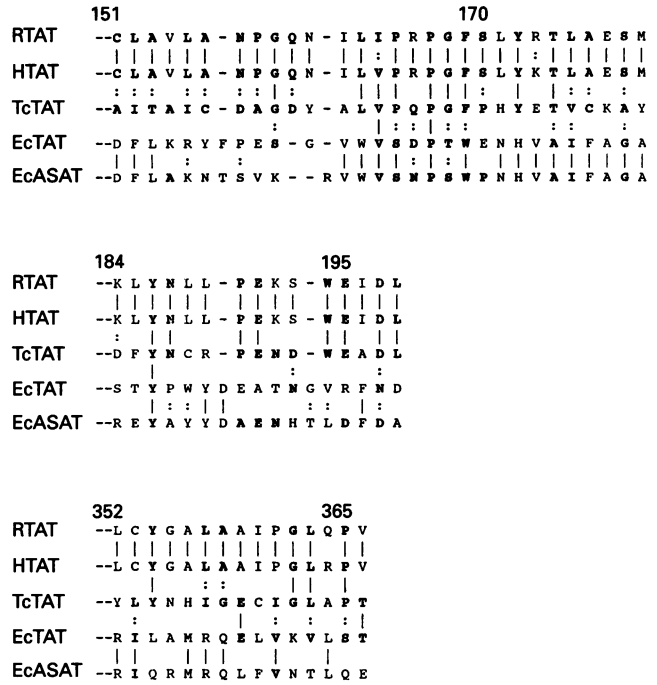

Figure 3 Comparison of the amino-acid sequences of isolated peptides from TAT from *T. cruzi* (TcTAT) with different aminotransferases

The aligned amino-acid sequences are: TAT from rat liver (RTAT) [1], TAT from human liver (HTAT) [25], TAT from *E. coli* (EcTAT) [27] and ASAT from *E. coli* (EcASAT) [28]. Residue numbering corresponds to the RTAT sequence [1]. Gaps, represented by dashes, were introduced according to the optimized alignment described by Mehta et al. [26]. Bold type is used for identical residues and conservative substitutions with respect to the TcTAT sequence; for the latter, the classification of similar amino acids proposed by Barker and Dayhoff [29] was used. Identical amino acids are indicated by a vertical line, and conserved amino acids by a double dot.

(97.4 units·mg of protein^{-1}) and by Hargrove and Granner [14] for the enzyme from rat liver (616 units·mg of protein^{-1}). The considerably greater mass of TAT in *T. cruzi* epimastigotes, which can be calculated as about 3% of the total soluble protein, in comparison with TATs from other sources, may be a consequence of this low specific activity.

The *T. cruzi* TAT seems not to be N-glycosylated, as has been shown for the *C. fasciculata* enzyme as well [9].

The TAT from *T. cruzi* could transaminate tryptophan and phenylalanine as well as tyrosine; this was also the case for the *C. fasciculata* enzyme. The latter, however, had only poor activity when pyruvate was substituted for 2-oxoglutarate [9]. The ability to transaminate different 2-oxo acids with aromatic amino acids differentiates the *T. cruzi* TAT clearly from the similar enzymes from mammals and from *E. coli* [23]. Another important difference is the ALAT activity that is shown by the trypanosomal enzyme, but not by the other similar enzymes described so far. The fact that both activities behaved identically throughout the purification procedure makes it likely that the ALAT activity is intrinsic to the *T. cruzi* TAT. However, since TAT seems to be encoded by a large number of genes in *T. cruzi* (see below), the possibility exists that the two activities might reside in two very closely related proteins coded for by members of the same gene family, with the different substrate specificities arising from a limited number of amino-acid substitutions. We shall be able to evaluate this possibility only when *T. cruzi* TAT expressed from a single cloned gene becomes available. It is noteworthy that the

apparent K_m values for the ALAT activity in the TAT preparations are quite different from those determined for the ALAT activity that was non-adsorbed to the DEAE-cellulose column, which are similar to those reported previously by Barros and Caldas for a partially purified ALAT preparation from the same parasite [24].

The apparent subunit molecular mass of the *C. fasciculata* enzyme was 48–50 kDa (a double band was obtained), slightly higher than the value we report for the enzyme from *T. cruzi*. The approximate molecular mass of the latter, as determined by gel filtration with Sephacryl S-200, was 91 kDa, coincident with that of ASAT. Therefore it seems likely that in the parasite both enzymes have a similar size, unlike the TATs from rat and from human liver, which have a slightly higher molecular mass, due to the presence both of N-terminal and of C-terminal extensions [1,25]. The calculated number of amino-acid residues in the *T. cruzi* TAT (418; Table 3) is intermediate between that of the rat- [1] and human-liver [25] enzymes (454), and that of the aromatic aminotransferase from *E. coli* [27] (410).

The partial sequences determined suggest that the trypanosomal enzyme has sequence similarity with the rat- and human-liver enzymes, the only ones that have been sequenced so far in mammals [1,25]. However, the three sequenced peptides from the *T. cruzi* enzyme have lower sequence similarity with the aromatic-amino-acid transaminase from *E. coli*. The latter enzyme has a higher sequence similarity with the ASATs from the same bacterium and from mammals [26].

Recently, another research group, whilst screening a λgt11 expression library with a polyclonal antiserum raised against an antigenic fraction of *T. cruzi*, has cloned and sequenced a gene (GenBank accession number LOO673) that may be the one coding for the TAT of the parasite, since the predicted amino-acid sequence has a high similarity with the rat-liver TAT (E. J. Bontempi, J. Búa, L. Åslund, B. Porcel, E. L. Segura, J. Henriksson, U. Pettersson, A. Orn and A. M. Ruiz, personal communication). The three peptides that we have sequenced and have shown to have similarity with the rat- and human-liver enzymes are identical to the corresponding parts of the predicted amino-acid sequence. The amino-acid composition predicted from the DNA sequence is very similar to the one that we have determined, which is shown in Table 3. Since the cloned gene is present in a high copy number in the parasite genome, it remains to be determined whether TAT and the cloned gene product are indeed the same molecule or whether they are encoded by two members of the same gene family, perhaps with different functions and localizations in the parasite cell.

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