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Crystallization and preliminary X-ray analysis of tyrosine aminotransferase from *Trypanosoma cruzi* epimastigotes

CRISTINA NOWICKI,[a]† MARISA MONTEMARTINI,[a] GISELLE REYNOSO HUNTER,[a] WULF BLANKENFELDT,[b] HENRYK M. KALISZ[b] and HANS-JÜRGEN HECHT[b]*‡ *at* [a]*Instituto de Química y Fisicoquímica Biologicas (IQUIFIB), Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Junin 956, 1113 Buenos Aires, Argentina, and* [b]*GBF, Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany. E-mail: hjh@gbf.de*

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Abstract

Tyrosine aminotransferase from *Trypanosoma cruzi* has been crystallized from PEG 4000 at pH 6.8. The crystals belong to the monoclinic space group $P2_1$ and have lattice constants of a = 59.1, b = 103.0, c = 77.8 Å, β = 113.1° for a data set measured at 138 K. The presence of a non-crystallographic twofold axis together with a Matthews parameter V_m of 2.5 Å^3 Da^{-1} indicates that the asymmetric unit contains one dimeric molecule. The crystals diffract to at least 2.7 Å and are stable in the X-ray beam in a shock-frozen state. Native data sets have been collected at temperatures of 285 and 138 K using a Siemens X1000 detector on a rotating-anode generator.

1. Introduction

Aminotransferases are vitamin B_6-dependent enzymes which catalyze in a reversible two-step reaction the transfer of an amino group from an amino acid to an α-oxoacid, yielding a different amino acid and the α-oxoacid of the original amino acid as a net result. In the first step, the amino group of an L-amino acid is transferred to the pyridoxal 5′-phosphate (PLP) cofactor and the corresponding α-oxoacid plus the pyridoxamine-5′-phosphate (PMP)-enzyme are formed. In the second step, this first half reaction is reversed by the reaction of a second α-oxoacid with the PMP-enzyme to form the corresponding L-amino acid.

More than 60 different aminotransferases have been identified from both eukaryotic and prokaryotic sources varying in substrate specificity, amino-acid sequence, molecular weight and quarternary structure (Mehta, Hale & Christen, 1993). Based on sequence similarity they can be classified into four subgroups that most probably diverged from a common ancestor (Mehta *et al.*, 1993). The most thoroughly studied aminotransferases are the mitochondrial and cytosolic aspartate aminotransferases (ASAT) which belong to subgroup I. The three-dimensional structures of several ASAT's have been reported and their reaction mechanism has been established (Kirsch *et al.*, 1984). They are dimers, with two non-interacting active sites built up by residues from both subunits. In contrast to the ASAT's, subgroup I aminotransferases with other substrate specificities, such as the alanine and aromatic aminotransferases, are less well characterized and presently only a model of tyrosine aminotransferase from *E. coli* derived by homology modelling has been reported (Jäger, Solmajèr & Jansonius, 1992).

The sequence of tyrosine aminotransferase (E.C. 2.6.1.5; TAT) from *Trypanosoma cruzi*, the causative agent of American trypanosomiasis (Chagas' disease) has been deduced from the cDNA sequence (Bontempi *et al.*, 1993; Montemartini, Santome, Cazzulo & Nowicki, 1993). The enzyme is 416 residues long and shows only 30% sequence homology to ASAT and TAT from *E. coli* but approximately 77% homology (Bontempi *et al.*, 1993) to the glucocorticoid inducible rat (Grange *et al.*, 1985) and human liver (Rettenmeier, Natt, Zentgraf & Scherer, 1990) TAT's. Unlike mammalian TAT's, which transaminate only aromatic amino acids with α-ketoglutarate, the parasite enzyme is able to accept the three α-ketoacids; pyruvic, α-ketoglutaric and oxaloacetic, the three aromatic amino acids and alanine as substrate. The best substrate pairs appear to be tyrosine–pyruvic acid and alanine–α-ketoglutaric acid (Montemartini *et al.*, 1993).

2. Materials and methods

TAT from *T. cruzi* (Tulahuen strain, stock Tul 2) epimastigotes was purified to homogeneity by chromatography on DEAE celullose, gel filtration on Sephacryl S-200 and chromatography on MonoQ with a fast protein liquid chromatography system as described previously (Montemartini *et al.*, 1993). For crystallization experiments the enzyme was brought to a protein concentration of 3–4 mg ml^{-1} using Centricon concentrators. Initial crystallization conditions were identified by the sparse-matrix screening method (Jancarik & Kim, 1991). Optimal conditions for the sitting-drop vapour-diffusion method were 26%(*w*/*w*) PEG 4000 as precipitant buffered with 0.1 *M* citrate/phosphate buffer pH 6.8. The crystallization drop consisted of 2 µl of the reservoir solution together with 2 µl 5 m*M* PLP and 4 µl of the protein solution. Under these conditions light-yellow mostly plate-like crystals grew in 2–4 weeks at a temperature of 285 K to a size of approximately 0.1 × 0.2 × 0.3 mm. Similar crystals were also grown in 200 µl dialysis buttons sealed with Spectra/Por MWCO 3500 dialysis membrane by dialysis against 25%(*w*/*w*) PEG 8000, 5 m*M* PLP, buffered at pH 7.0 with 0.1 *M* citrate/phosphate.

Two native X-ray data sets have been collected on a Siemens rotating anode equipped with an X1000 area detector on a four-circle diffractometer using Cu $K\alpha$ radiation. The data sets were collected at a crystal-to-detector distance of 150 mm with a detector swing-out of 17° and a frame width of 10 min arc. The first was measured at a temperature of 285 K from four crystals mounted in glass capillaries together with a drop of mother liquor. Each crystal could only be measured for 35 to 90° in ω-rotation with an exposure time of 600 s per frame before crystal decay became unacceptable. The statistics of the data set are

† Correspondence concerning the biochemistry of TAT should be sent to C. Nowicki.

‡ Correspondence concerning crystallography should be sent to H.-J. Hecht.

given in Table 1. The second data set was collected from a single shock-frozen crystal. Prior to measurement the crystal was transferred into a cryoprotectant solution consisting of 30%(*w*/*w*) PEG 4000, 15%(*v*/*v*) glycerol, 0.1 *M* citrate/phosphate buffer pH 7.4. The crystal was washed in this solution for less than 30 s fixed in a silk loop attached to a brass pin which was then mounted on a modified goniometer head (Parkin, Rupp & Hope, 1996) for flash freezing in a nitrogen gas stream. The temperature of the gas stream was regulated to 138 K at the nozzle. The data set was collected in two settings, first a 166° rotation in φ at $\chi = 0°$ followed by a 40° rotation in ω at $\chi = 90°$. The exposure time was 600 s per frame. The statistics of the data set are given in Table 1.

Table 1. *Data-collection statistics*

	Native 1	Native 2
Precipitant	PEG 4000	PEG 8000
Temperature (K)	138	285
Space group	$P2_1$	$P2_1$
Cell dimensions (Å, °)		
a	59.1	61.6
b	103.0	102.3
c	77.8	78.0
β	113.1	110.3
Solvent (%)	51	51
V_m (Å^3 Da^{-1})	2.5	2.5
Resolution (Å)	2.7	2.7
No. of unique reflections	2054	120581
Multiplicity	1.8	1.8
$\langle I/\sigma(I)\rangle$	6.6/3.7†	11.3/8.9†
Completeness	85.7/63.0†	82.7/74.2†
$R_{\rm merge}$‡	5.8/9.9‡	5.2/7.8‡

† Data for highest resolution shell (2.85–2.7 Å). ‡ $R_{\rm merge} = (\sum|I - \langle I\rangle|/\sum I) \times 100$, with observed intensity I and average intensity $\langle I\rangle$ obtained from multiple observations of symmetry-related reflections.

3. Results and discussion

The data sets were indexed and integrated using the *X-GEN* module of the *Cerius*[2] software (Biosym/MSI, 1996). The integrated intensities were scaled and reduced using the *SCALEPACK* (Otwinowski, 1993) and *AGROVATA* programs (Collaborative Computational Project, Number 4, 1994). The indexing resulted for both data sets in the monoclinic space group $P2_1$ and gave for the data set measured at 285 K the lattice constants $a = 61.6$, $b = 102.3$, $c = 78.0$ Å, $\beta = 110.3°$. For the data set collected at 138 K the lattice constants were $a = 59.1$, $b = 103.0$, $c = 77.8$ Å, $\beta = 113.1°$. Together with the molecular weight of 45 kDa per monomer and the assumption of one dimer molecule per asymmetric unit this yields a Matthews parameter (Matthews, 1968) of $V_m = 2.5$ Å^3 Da^{-1}. A comparison of both data sets with the program *SCALIT* (Collaborative Computational Project, Number 4, 1994) gave $R_{\rm scale} = 42.7\%$ as the scaling *R* value.

In order to verify the non-crystallographic symmetry a search for the local twofold symmetry axis was carried out with the program *Replace* (Tong & Rossmann, 1990). Using 20.0–4.0 Å data and 45 Å as integration radius this revealed a maximum at $\psi = 65°$, $\varphi = 169°$ as the most probable solution (Fig. 1). Subsequent attempts to solve the structure *via* molecular replacement based on the known structures of ASAT's have yielded only unconvincing results until now. Considering the low sequence homology of *ca* 30% this was not unexpected. An additional reason for this may be that TAT, like the ASAT's, probably consists of two domains. In the ASAT's the small domain is flexibly linked to the large domain and is reoriented in relation to the large domain during substrate binding (McPhalen *et al.*, 1992). The relative orientation of the domains in TAT may, therefore, be different from the orientation in the available models. In addition to the continuing efforts to solve the structure by molecular replacement a search for heavy-atom derivatives for a structure solution *via* isomorphous replacement has been initiated.

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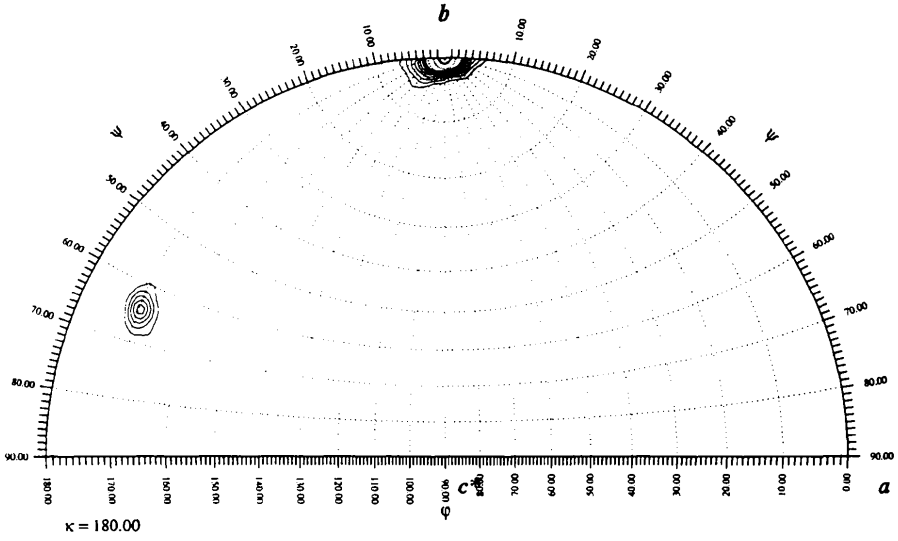

Fig. 1. Section $\kappa = 180°$ of the self-rotation function calculated for the TAT native data set collected at 285 K. Using 45° as integration radius and data in the resolution shell 20.0–4.0 Å the non-crystallographic twofold axis is indicated by a maximum at $\psi = 65°$, $\varphi = 169°$ with a height of 6σ. The section is contoured starting at 2σ in steps of 1σ.

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References

Biosym/MSI (1996). *Crystallography Workbench X-GEN Users Guide*. San Diego, California, USA.

Bontempi, E. J., Bua, J., Åslund, L., Porcel, B., Henriksson, J., Petterson, U., Segura, E. L., Orn, A. & Ruiz, A. M. (1993). *Mol. Biochem. Parasitol.* **59**, 253–262.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Grange, T., Guenet, C. J., Dietrich, J.-B., Chasserot, S., Fromont, M., Befort, N., Jami, J., Beck, G. & Pictet, R. (1985). *J. Mol. Biol.* **184**, 347–350.

Jäger, J., Solmajer, T. & Jansonius, J. N. (1992). *FEBS Lett.* **306**, 234–238.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Kirsch, J. F., Eichele, G., Ford, G. C., Vincent, M. G., Jansonius, J. N., Gehring, H. & Christen, P. (1984). *J. Mol. Biol.* **174**, 497–525.

McPhalen, C., Vincent, M. G., Picot, D., Jansonius, J. N., Lesk, A. M. & Chotia, C. (1992). *J. Mol. Biol.* **227**, 197–213.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Mehta, P. K., Hale, T. I. & Christen, P. (1993). *Eur. J. Biochem.* **214**, 549–561.

Montemartini, M., Santome, J. A., Cazzulo, J. J. & Nowicki, C. (1993). *Biochem. J.* **292**, 901–906.

Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.

Parkin, S., Rupp, B. & Hope, H. (1996). *Acta Cryst.* A**52** (Suppl.), C-10.

Rettenmeier, R., Natt, E., Zentgraf, H. & Scherer, G. (1990). *Nucleic Acids. Res.* **18**, 3853–3861.

Tong, L. & Rossmann, M. G. (1990). *Acta Cryst.* A**46**, 783–792.