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New crystal forms of *Trypanosoma cruzi* dUTPase

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The dUTPase from *Trypanosoma cruzi* has been crystallized in two crystal forms, both belonging to space group $P6_322$, with unit-cell parameters $a = b = 134.67$, $c = 148.66$ Å (form I, two molecules per asymmetric unit) and $a = b = 136.43$, $c = 68.71$ Å (form II, one molecule per asymmetric unit). Single-wavelength data have been collected using synchrotron radiation to 3.0 Å for crystal form I and to 2.4 Å for crystal form II and structure solution is under way. *T. cruzi* dUTPase is a potential target for anti-protozoan drug design.

1. Introduction

Deoxyuridine pyrophosphatase (dUTPase; E.C. 3.6.1.23) is the enzyme responsible for the hydrolysis of dUTP to dUMP and pyrophosphate. In doing so, dUTPase not only eliminates the triphosphate form of deoxyuridine which can be erroneously incorporated in the DNA (Tye *et al.*, 1977), but also produces additional dUMP for the biosynthesis of the normal DNA equivalent dTTP (Kornberg & Baker, 1991). The presence of uracil instead of thymine in DNA is highly mutagenic and leads to the activation of repair mechanisms which nick the DNA in an attempt to excize uracil. Depending on the extent of uracil incorporation, this action gives rise to multiple breaks in the DNA molecule and can lead to its fragmentation and cell death (Richards *et al.*, 1984). Excluding uracil from DNA is one of the major cellular tasks vital to cell survival. dUTPase keeps the ratio of dTTP to dUTP in the cell sufficiently high to minimize the incorporation of uracil into the genome during replication.

Depending on the organism, dUTPases have been found to act as trimers (human, *Escherichia coli*, equine infectious anaemia virus, feline immunodeficiency virus), monomers (mammalian herpes viruses) or dimers (protozoa such as *T. cruzi* and *Leishmania major*). Structures available so far all belong to the trimeric family (Cedergren-Zeppezauer *et al.*, 1992; Mol *et al.*, 1996; Prasad *et al.*, 1996; Dauter *et al.*, 1999). Each one of the three active sites per trimer is made up of five conserved motifs contributed from all subunits (McGeoch, 1990). The mammalian herpes virus dUTPases also contain these conserved motifs within a single monomer. It can be expected, therefore, that the local structure of the enzyme around the active site will be similar to that of the trimeric dUTPases. However, the dimeric dUTPases from protozoa (Camacho *et al.*, 1997; Bernier-Villamor *et al.*, 1999) do not share any significant sequence similarity with the trimeric/monomeric families of dUTPases and lack all of the five conserved motifs. This makes them potential targets for drug-design processes.

The flagellate protozoan parasite *T. cruzi*, transmitted to humans by *Triatoma infestans* insects, is the causative agent of Chagas disease, a zoonotic disease prevalent in Central and South America. The disease affects 16–18 million people and about 100 million are estimated to be at risk of contracting it (World Health Organization, 2001).

In this note, we report the crystallization of *T. cruzi* dUTPase in two crystal forms. One of them, crystal form I, is closely similar to crystals of this enzyme reported previously (Bernier-Villamor *et al.*, 1999), but the crystallization conditions as well as the morphology are different and the crystal quality is substantially improved.

2. Experimental

2.1. Expression and purification of recombinant *T. cruzi* dUTPase

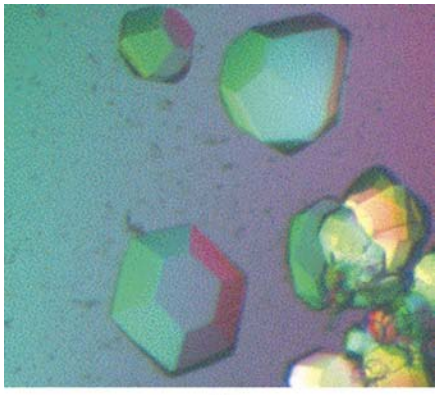
The construct encoding the *T. cruzi* dUTPase was supplied by Professor D. Gonzalez-Pacanowska and Dr V. Bernier-Villamor. The gene was inserted in the *Nde*I site of the pET-11c vector (Stratagene) and expressed in *E. coli* BL21(DE3). The transformed cells were grown in LB medium to an OD_{600} of 0.5 and induced with IPTG. After 6 h, the cells were harvested and sonicated in HEPES buffer with phenylmethylsulfonyl fluoride (PMSF). The crude extract was centrifuged at high speed and the clear lysate loaded onto Q-Sepharose resin (Pharmacia) at pH 7 and eluted with a steep NaCl gradient. After a buffer-exchange step, the fractions of interest were loaded onto a fast flow DEAE-

Sepharose column (Pharmacia) and eluted with an extended NaCl gradient. Finally, the enzyme-containing fractions were concentrated and passed through a Superdex-75 column. Prior to crystallization, the pure enzyme was stabilized in 50 m*M* HEPES pH 7, 25 m*M* $MgCl_2$, 2 m*M* dithiothreitol (DTT) and 1 m*M* PMSF. Purity was checked by SDS–PAGE.

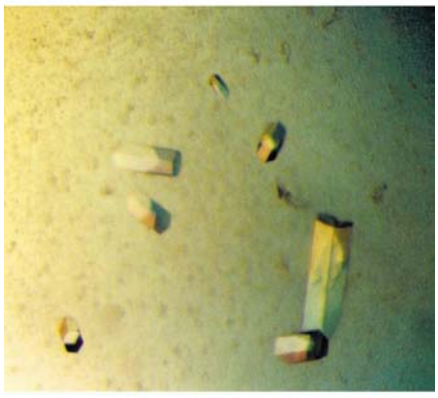
2.2. Crystallization

Experiments were performed using the hanging-drop vapour-diffusion method. Crystallization conditions were obtained through the Clear Strategy Screen (Brzozowski & Walton, 2001) at 290 K. Crystal form II appears at equilibrium conditions of 15% polyethylene glycol 2000 monomethyl ether (PEG 2000 mme), 0.10 *M* $LiSO_4$, 50 m*M* sodium cacodylate pH 6.6. Each drop initially contained 20 mg ml^{-1} dUTPase solution mixed with an equal volume of mother liquor and suspended over 500 µl of the latter. The protein concentration was determined from the absorbance at 280 nm using a calculated extinction coefficient $A_{280,0.1\%}$ of 1.605. Crystals of this form grow sporadically over periods of several weeks to months in the form of hexagonal rods of 0.5 mm length and of width varying between 0.1 and 0.3 mm (Fig. 1*a*).

Crystal form I can be easily grown through microseeding with seeds obtained from either itself or crystal form II over a range of conditions such as 15–25% PEG 2000 mme, 0.1–0.2 *M* $LiSO_4$, 50 m*M* sodium cacodylate pH 6–7. Crystals appear overnight as hexagonal bipyramids with longest dimension of up to 0.6 mm (Fig. 1*b*).



(*a*)



(*b*)

Figure 1
(*a*) Form I and (*b*) form II crystals of *T. cruzi* dUTPase.

2.3. X-ray diffraction analysis

A *T. cruzi* dUTPase form II crystal was cryoprotected through rapid transfer into 20% glycerol containing mother liquor and subsequent flash-freezing in loops in liquid nitrogen. Crystals of form I were cryoprotected by sequential transfer through solutions of the mother liquor in which the glycerol concentration was increased in 5% steps to a final concentration of 20%. X-ray data for both forms were collected at the European Synchrotron Radiation Facility at Grenoble, France and processed using the *DENZO*/*SCALEPACK* package (Otwinowski & Minor, 1997). Crystal data and X-ray data-collection statistics for both forms are given in Table 1.

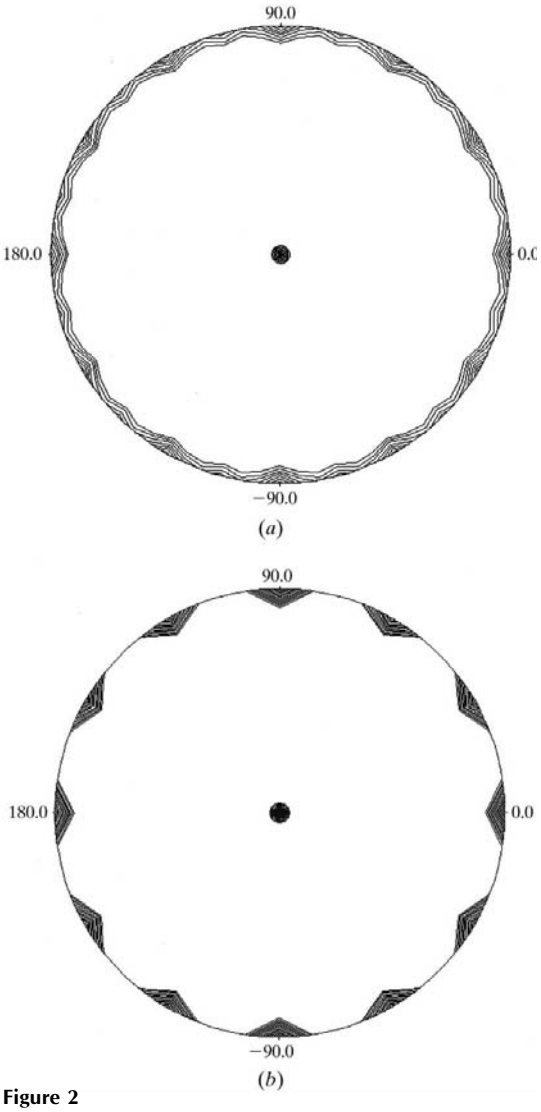


Figure 2
Self-rotation functions in section $\kappa = 180^\circ$, integration radius of 30 Å, calculated at 15–3 Å resolution for (*a*) crystal form I and (*b*) crystal form II. The presence of a non-crystallographic symmetry axis at an angle of about 15° from the crystallographic twofold axis is indicated by the extra peaks in the self-rotation pattern of crystal form I.

3. Results and discussion

Crystals of form I obtained here have larger unit-cell dimensions, *a* and *b* by 0.52 Å and *c* by 1.61 Å, than those previously reported (Bernier-Villamor *et al.*, 1999) and diffract to a better resolution (3.0 Å) than the previous form (4.3 Å). Attempts to improve the resolution limit of this form initially included experimenting with a variety of cryoprotectants and also cross-linking agents such as gluteraldehyde (Lusty, 1999). All these failed to provide better diffracting crystals. The diffraction of form I crystals was also tested at 290 K using a MAR Research image plate mounted on a Rigaku RU-200 rotating-anode X-ray generator using Cu $K\alpha$ radiation. These images showed a distinct resolution cutoff around 3.0 Å, implying a limitation in the actual crystal quality as opposed to potential damage caused by the cryoprotection manipulations.

The self-rotation function of crystal form I shows the presence of a non-crystallographic twofold axis between two monomers within the asymmetric unit. The self-rotation function of crystals of both forms in the section $\kappa = 180^\circ$ show a crystallographic twofold axis every 30° (Figs. 2*a* and 2*b*) and extra peaks at about 15° relative to these in crystal form I (Fig. 2*a*). This implies that the non-crystallographic twofold axis is at an angle of 15° relative to the crystallographic twofolds.

Because *T. cruzi* dUTPase is physiologically functional as a dimer, as in the case of the highly homologous *L. major* dUTPase

Table 1
Crystallographic data and details of data collection.

	Crystal form II	Crystal form I
Crystal data		
Space group	$P6_322$	$P6_322$
Unit-cell parameters (Å)	$a = b = 136.43$, $c = 68.71$	$a = b = 134.67$, $c = 148.66$
Monomers per asymmetric unit	1	2
Solvent content (%)	56.9	59.1
Data collection		
X-ray source	ID14-4 (ESRF)	ID14-2 (ESRF)
Image-plate system	Q4 CCD (ADSC)	Q4 CCD (ADSC)
Wavelength (Å)	0.9688	0.9326
Temperature (K)	100	100
All data		
Resolution range (Å)	12–2.4	20–3.0
Total reflections	107361	191733
Unique reflections	14827	16490
Completeness (%)	97.5	99.3
$I/\sigma(I)$	32.4	36.5
$R_{\rm merge}$† (%)	5.1	7.2
Outer shell		
Resolution (Å)	2.44–2.40	3.11–3.0
Completeness (%)	88.4	96.6
$I/\sigma(I)$	4.1	4.5
$R_{\rm merge}$† (%)	41.1	51.9

† $R_{\rm merge} = \sum_{hkl} |I - \langle I \rangle| / \sum I$.

(Camacho *et al.*, 2000), it is likely that in crystal form II the physiological dimer is positioned on a crystallographic twofold axis.

Since there are no structures homologous to *T. cruzi* dUTPase available at present, the structure cannot be solved through conventional molecular-replacement techniques and our efforts are currently focused on the acquisition of phase information through heavy-metal-atom derivatives and selenomethionine-substituted protein. Selenomethionine-substituted crystals of form I have been produced and X-ray data extending to 3.2 Å were collected at the ESRF, Grenoble (BM14). Additionally, data from a potential heavy-atom derivative of form II were collected at the ESRF, Grenoble (ID14-4).

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