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Crystallization and preliminary X-ray analysis of tryparedoxin I from *Crithidia fasciculata*

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The thioredoxin-related protein tryparedoxin I from *Crithidia fasciculata* has been crystallized using PEG 4000 as a precipitant. The enzyme forms long needle-shaped crystals which diffract to at least 1.7 Å. A native data set has been collected at the DESY synchrotron from a flash-frozen crystal at 90 K to 1.7 Å resolution. The data set shows that the crystals belong to the orthorhombic space group $P2_12_12_1$ and have unit-cell parameters a = 37.94, b = 51.39, c = 71.46 Å. Tryparedoxin I is involved in a trypanothione-dependent peroxide metabolic pathway specific for trypanosomatids and may therefore be a suitable candidate for the design of drugs for the specific treatment of a variety of important tropical diseases caused by these parasites.

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1. Introduction

Tryparedoxin (trypanothione–peroxiredoxin oxidoreductase) is a thioredoxin-related protein which was recently demonstrated to be an essential component of the trypanothione-dependent hydroperoxide metabolism in the trypanosomatid *Crithidia fasiculata* (Nogoceke *et al.*, 1997). In the trypanosomatids, tryparedoxin specifically mediates the flux of reduction equivalents from the unique redox compound trypanothione [N^1,N^8-bis(glutathionyl)-spermidine] (Fairlamb & Cerami, 1992) to the peroxiredoxin-type protein tryparedoxin peroxidase, which readily reacts with various hydroperoxides (Gommel *et al.*, 1997; Nogoceke *et al.*, 1997). Acting in concert with trypanothione reductase, tryparedoxin and tryparedoxin peroxidase catalyse the reduction of hydroperoxides at the expense of NADPH (Nogoceke *et al.*, 1997). In the trypanosomatids this system most probably substitutes for the more common methods of hydroperoxide removal, such as dismutation by catalase (Chance *et al.*, 1979) or reduction by selenoperoxidases (Ursini *et al.*, 1995), which prevail in higher animals but are absent in the trypanosomatids (Docampo, 1990).

The tryparedoxin system uses catalytic elements homologous to those of the thioredoxin-mediated peroxide metabolism in bacteria (Cha *et al.*, 1995), yeast (Chae *et al.*, 1994) and vertebrates (Cha & Kim, 1995). However, in contrast to thioredoxin, tryparedoxin is not directly regenerated by a flavoprotein such as the thioredoxin reductase (Follmann & Häberlein, 1995/1996), but by the low molecular-weight mediator trypanothione (Gommel *et al.*, 1997; Nogoceke *et al.*, 1997). The thioredoxins are a universally distributed family of small dicysteine proteins of around 12 kDa, which exert their function mostly in regulatory and catalytic processes (Follmann & Häberlein, 1995/1996). These ubiquitous proteins have been found in all living cells from archaebacteria to humans, but not in the trypanosomatids. In the trypanosomatids, tryparedoxin possibly substitutes for thioredoxin in metabolic functions as diverse as reduction of ribonucleotides, differentiation, regulation of transcription or other regulatory processes depending on the cellular thiol/disulfide equilibrium. The possibility of multiple biological functions of tryparedoxin is suggested further by the coexistence of more than one tryparedoxin in the same species (Montemartini *et al.*, 1998) and by the recent observation that the protein serves as substrate for the ribonucleotide reductase of *Trypanosoma brucei* (Lüdemann *et al.*, 1998).

Tryparedoxin shares its catalytic mechanism (*i.e.* redox shuttling of the WCXXC sequence motif) and activity as a disulfide reductase with the classical thioredoxins (Gommel *et al.*, 1997; Nogoceke *et al.*, 1997). However, in contrast to the thioredoxins, which have the highly conserved active-site sequence WCG(A)PC, tryparedoxin contains the variant sequence motif WCPPC. The WC*x*PC motif is preceded by the sequence K*x*Vx_3FSA*x* (Montemartini *et al.*, 1998). Beyond this highly conserved sequence cluster, tryparedoxin shows little similarity to the classical thioredoxins. It contains 146 amino-acid residues and thus is substantially larger than the thioredoxins (Montemartini *et al.*, 1998). Interestingly, tryparedoxin shares the WCPPC motif with, and is more closely related to, some thio-

redoxin-like proteins to which an unequivocal biological role has not yet been attributed. These proteins include the thioredoxin-like proteins from *Arabidopsis thaliana* (Rivera-Madrid *et al.*, 1995) and *Paracoccus denitrificans* (Page *et al.*, 1995), the hypothetical protein deduced from a genomic sequence of *Caenorhabditis elegans* (Wilson *et al.*, 1994) and the nucleoredoxin of mice (Kurooka *et al.*, 1997). The pronounced similarity of tryparedoxin to the thioredoxin-like proteins as opposed to the classical thioredoxins demonstrates the tryparedoxins to be a distinct molecular clade within the thioredoxin superfamily of proteins.

The comparatively low similarity to the classical thioredoxins and its pronounced specificity for trypanothione and tryparedoxin peroxidase (Gommel *et al.*, 1997) make tryparedoxin particularly attractive as a molecular target for the design of specific inhibitors of the trypanosomal peroxide metabolism, which might render the parasites more vulnerable to physiological or drug-induced oxidative stress without adversely affecting the host organism. Elucidation of the three-dimensional structure of tryparedoxin will serve as a starting point for structure-based design of specific inhibitors.

2. Materials and methods

Tryparedoxin from *C. fasciculata* was purified to isoelectric homogeneity by ammonium sulfate precipitation and fast protein liquid chromatography on S-Sepharose, DEAE–Sepharose and Ultrogel AcA-54 as described previously (Nogoceke *et al.*, 1997). For crystallization experiments, the enzyme was occasionally purified further by anion-exchange chromatography on a Resource Q column (Pharmacia) using 10 m*M* sodium phosphate buffer pH 6.8 as starting buffer and 50 m*M* sodium phosphate buffer with 50 m*M* NaCl pH 5.0 as elution buffer. Elution was performed with a gradient of 0–80% elution buffer in 12 column volumes. The purified enzyme was concentrated to 13.5 mg ml^{-1}, determined using Coomassie Brilliant Blue-G reagent (Biorad, Germany) with bovine serum albumin as standard, and the buffer was simultaneously exchanged against 20 m*M* HEPES and 1 m*M* DTT pH 7.6 by ultrafiltration (Omegacell, Pall-Filtron, Germany). Initial crystallization conditions were identified by the sparse-matrix screening method (Jancarik & Kim, 1991). Optimal conditions for the sitting-drop vapour-diffusion method were 33%(*w*/*w*) PEG 4000 as precipitant, buffered with 0.1 *M* Tris–HCl at pH 8.7 in the presence of 50 m*M* sodium acetate. The crystallization drop consisted of 3 µl reservoir solution together with 2 µl protein solution. Under these conditions, thin needle-shaped crystals appeared after 2–3 d and grew to approximate dimensions <0.1 × 0.1 × 0.7 mm in two weeks at a temperature of 294 K (Fig. 1).

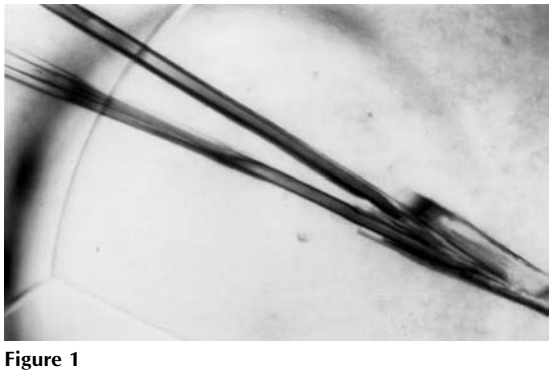

Figure 1
Crystals of tryparedoxin. The typical size of crystals grown from 33%(*w*/*w*) PEG 4000 at pH 8.7 is <0.1 × 0.1 × 0.7 mm.

A native X-ray data set has been collected at the beamline BW6 of the DESY synchrotron using a 30 cm MAR Research imaging-plate detector and monochromatic radiation of 1.1 Å wavelength. Immediately prior to the measurement, the crystal was washed for a few seconds in a cryo-protectant solution containing 15%(*v*/*v*) glycerol, 33%(*w*/*w*) PEG 4000 and 50 m*M* sodium acetate buffered with 0.1 *M* Tris–HCl at pH 8.7. The crystal was mounted in a small silk loop and flash-frozen in a nitrogen-gas stream at a temperature of 90 K. For data collection, a crystal-to-detector distance of 170 mm and a frame width of 1.5° was chosen. Typical combined exposure and transform times were 5 min per frame. The data set was indexed and integrated initially using *DENZO* (Otwinowski & Minor, 1996) and later reprocessed using *MOSFLM* (Collaborative Computational Project, Number 4, 1994). The integrated intensities were scaled and reduced using the *SCALEPACK* (Otwinowski & Minor, 1996) and *SCALA* (Collaborative Computational Project, Number 4, 1994) programs, respectively.

3. Results and discussion

The crystals belong to the orthorhombic space group $P2_12_12_1$ with lattice constants a = 37.94, b = 51.39, c = 71.46 Å. Together with a molecular weight of 16 kDa and the assumption of one monomer molecule per asymmetric unit, this yields a Matthews parameter (Matthews, 1968) of V_m = 2.2 Å^3 Da^{-1}. The statistics of the indexed X-ray data set are given in Table 1. A comparison with the program *SCALEIT* (Collaborative Computational Project, Number 4, 1994) of the integrations and scalings using *MOSFLM*/*SCALA* and *DENZO*/*SCALEPACK* gave R = 5.2%.

Table 1
Data-collection statistics.

Data using the *MOSFLM*/*SCALA* (Collaborative Computational Project, Number 4, 1994) data reduction. Data in parentheses refers to the highest resolution shell (1.79–1.7 Å).

Temperature (K)	90
Space group	$P2_12_12_1$
Cell dimensions	
a (Å)	37.94
b (Å)	51.39
c (Å)	71.46
Solvent (%)	44
V_m (Z = 4) (Å^3 Da^{-1})	2.2
Resolution (Å)	1.7
Number of unique reflections	15542
Redundancy	3.0
$\langle I/\sigma(I)\rangle$	6.1 (8.5)
Completeness	97.9 (99.4)
$R_{\rm merge}$†	5.9 (7.5)

† $R_{\rm merge} = 100(\sum|I - \langle I\rangle|/\sum I)$, with observed intensity I and average intensity $\langle I\rangle$ obtained from multiple observations of symmetry-related reflections.

A subsequent attempt to solve the structure *via* molecular replacement based on the known structures of thioredoxins has not yielded convincing results. However, this was not unexpected considering the low sequence homology to the classical thioredoxins and the large difference between the molecular weights of tryparedoxin (16 kDa) and the thioredoxins (11 kDa).

Elucidation of the crystal structure of tryparedoxin, together with the ongoing mutagenesis experiments, will provide useful information for the rational design of specific inhibitors of the enzyme, especially as tryparedoxin shows low similarity to the mammalian thioredoxins. Inhibition of tryparedoxin will probably not only affect the trypanothione-dependent hydroperoxide metabolism but should also disturb other important metabolic processes of the trypanosomatids. This could enable the specific treatment of a variety of important tropical diseases, such as Chagas' disease, African sleeping sickness and leishmaniasis, caused by mammalian parasites of the *Trypanosomatidae* family. The recent discovery of trypanothione (Ondarza *et al.*, 1997) and a peroxiredoxin-like protein (Torian *et al.*, 1990) in *Entamoeba histolitica* suggests the possible existence of a homologous trypanothione-mediated redox system comprising a tryparedoxin in the

Rhizopoda. Consequently, selective inhibitors of tryparedoxin also appear promising for the treatment of diseases caused by human pathogens distinct from trypanosomatids.

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