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Crystallization of the major cytosolic glutathione *S*-transferase from *Onchocerca volvulus*

J. Höppner,[a] M. Perbandt,[b] Ch. Betzel,[c] R. D. Walter[a] and E. Liebau[a]*

[a]Department of Biochemistry, Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany, [b]Department of Chemistry, Devision of Biochemistry and Molecular Biology, University of Hamburg, Martin Luther King Platz 6, 20146 Hamburg, Germany, and [c]Institute of Biochemistry and Molecular Biology I, Center for Experimental Medicine, University Hospital Eppendorf, c/o DESY, Building 22a, Notkestrasse 85, 22603 Hamburg, Germany

Correspondence e-mail: liebau@bni.uni-hamburg.de

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Glutathione *S*-transferases (GSTs) are a family of detoxification enzymes that catalyse the conjugation of glutathione to xenobiotic and endogenous electrophilic compounds, thus facilitating their elimination from cells. The recombinant *Onchocerca volvulus* GST2 has been expressed in *Escherichia coli*, purified and crystallized by the hanging-drop vapour-diffusion technique. Two different crystal forms were grown under identical conditions. They belong to space groups $P2_12_12$ and $P2_1$, respectively. The unit-cell parameters obtained are $a = 112.6$, $b = 84.3$, $c = 45.1$ Å for the $P2_12_12$ crystal form and $a = 51.6$, $b = 82.3$, $c = 56.7$ Å, $\beta = 95.89^\circ$ for the $P2_1$ form. Complete data sets to 2.6 and 1.5 Å, respectively, have been collected at 100 K with synchrotron radiation.

1. Introduction

The filarial parasite *Onchocerca volvulus* is the causative agent of onchocerciasis, a disease affecting about 18 million people in Africa, the Arabian Peninsula and Central and South America. It is the world's second leading infectious cause of blindness. Even though the disease is rarely life-threatening, it causes chronic suffering and severe disability (http://www.who.int/tdr/index.html).

GSTs (EC 2.5.1.18) are a superfamily of multifunctional proteins represented by a number of species-independent gene classes. They play a key role in enzymatic detoxification by catalysing the nucleophilic addition of reduced glutathione (GSH) to numerous endobiotic and xenobiotic electrophilic substrates. Furthermore, GSTs are involved in protecting tissue against oxidative damage by reducing organic hydroperoxides of phospholipids, fatty acids and DNA before they become engaged in free-radical propagation reactions that ultimately lead to the destruction of macromolecules (Hayes & Strange, 1995). In addition to their enzymatic functions, they also serve as non-catalytic carrier proteins in the intracellular transport of hydrophobic ligands or act as components of stress-related cellular signalling pathways that lead to apoptosis (Wilce & Parker, 1994; Salinas & Wong, 1999; Sheehan *et al.*, 2001). In the parasitic context, it is especially important to consider their function in the regulation of oxidative stress response, in drug resistance and possibly in the modulation of host immune-defence mechanisms (Hemingway *et al.*, 1998).

All cytosolic GSTs are dimeric. Each monomer consists of two distinct domains. The smaller N-terminal domain has a core structure consisting of a central four-stranded β-pleated sheet flanked on one side by two α-helices and on the other by a single helix. Glutathione recognition is attributable to the residues of this highly conserved N-terminal domain. It is connected to the C-terminal domain by a short linker sequence. This domain is all-helical, consisting of five amphipathic α-helices arranged in a right-handed spiral. It contributes most of the residues that interact with the hydrophobic substrate.

In previous studies, we identified three different *O. volvulus* GSTs (Ov-GST1, Ov-GST2 and Ov-GST3) with varying *in vitro* biochemical properties, indicating different physiological functions within the parasite. On the basis of gene structure, amino-acid sequence, immunological and kinetic properties, it was possible to place Ov-GST1 in close vicinity to the σ class (Liebau *et al.*, 1994), Ov-GST2 into the periphery of the π class and Ov-GST3 into the ω class. Ov-GST2 is the major cytosolic GST in *O. volvulus* and has highest sequence identity with the human π-class GST (42%) and with the GST from the malarial parasite *Plasmodium falciparum* (32%). The intracellular amount of Ov-GST2 was estimated to constitute about 0.1% of the total protein content in adult filarial worms. It has limited glutathione peroxidase activity, but the toxic secondary products of lipid peroxidation are readily neutralized. The general distribution of the highly abundant enzyme in all tissues and life stages of the parasite indicates its essential function as a housekeeping enzyme (Liebau *et al.*, 1996; Wildenburg *et al.*, 1998).

2. Material and methods

2.1. Recombinant expression and purification of Ov-GST2

The construction of the expression vector, expression and purification of the recombinant Ov-GST2 were carried out as previously described (Liebau *et al.*, 2000). The expression was performed in a 2 l high-density fermenter. Recombinant Ov-GST2 was expressed in *Escherichia coli* BL21 DE3 pLysS. Briefly, 40 ml of an overnight culture was added to 1.5 l of Terrific Broth supplemented with 50 µg ml^{-1} ampicillin. The cultures were grown to an OD_{600} of 1.0 and subsequently 500 ml of Terrific Broth medium was added. The expression of the GST cDNA was induced by the addition of isopropyl-β-D-thiogalactoside to a final concentration of 0.5 m*M*. The culture was incubated for a further 4 h. Bacteria were precipitated and resuspended in phosphate-buffered saline (PBS; 150 m*M* NaCl, 16 m*M* Na_2HPO_4, 4 m*M* NaH_2PO_4 pH 7.3). Finally, the cells were lysed by sonication and the debris was precipitated by centrifugation at 100 000*g* and 277 K for 1 h. The supernatant was mixed with GSH-Sepharose affinity matrix (Amersham Pharmacia). After an overnight incubation at 277 K followed by washing with ten bed volumes of PBS, the recombinant protein was eluted with 15 m*M* GSH in 50 m*M* Tris–HCl pH 8.0. The enzyme, which was pure according to SDS–PAGE, was concentrated to 10 mg ml^{-1}. Finally, the integrity of every new preparation of the Ov-GST2 in solution was analysed by dynamic light scattering (RiNA GmbH, Germany).

2.2. Crystallization and X-ray diffraction analysis

Crystallization of Ov-GST2 was performed at room temperature (293 K) using the hanging-drop vapour-diffusion method (McPherson, 1982). The initial crystal screening was carried out using Hampton Research sparse-matrix kits (Jancarik & Kim, 1991; Hampton Research, Laguna Niguel, CA, USA). 2 µl protein solution (6 mg ml^{-1} Ov-GST2 in 50 m*M* Tris–HCl pH 8.0, 15 m*M* GSH) was mixed with an equal volume of reservoir solution and equilibrated against 500 µl reservoir solution (10% 2-propanol, 0.1 *M* HEPES pH 7.0, 20% PEG 4000) to produce crystals suitable for X-ray analysis. The optimized crystals were grown from a reservoir consisting of 10% 2-propanol, 0.1 *M* HEPES pH 7.0, 24% PEG 3350. No further cryoprotectant was required for data collection.

Table 1
Data-collection statistics for two different crystal forms of Ov-GST2.

Values in parentheses are for the highest resolution shell.

	Form I	Form II
Synchrotron-radiation source	DESY/HASYLAB, Germany (Consortium Beamline X13)	DESY/HASYLAB, Germany (Consortium Beamline X13)
Detector	MAR CCD, 165 mm	MAR CCD, 165 mm
Wavelength (Å)	0.8030	0.8046
Space group	$P2_12_12$	$P2_1$
Unit-cell parameters (Å, °)	a = 112.6, b = 84.3, c = 45.1	a = 51.6, b = 82.3, c = 56.7, β = 95.89
V_M (Å^3 Da^{-1})	2.1, one dimer per AU	2.4, one dimer per AU
Solvent content (%)	44.4	50.3
Resolution range (Å)	50–2.6 (2.65–2.6)	50–1.5 (1.53–1.5)
No. observed reflections	209776	603865
No. unique reflections	13970	76005
R_{sym} (%)	8.4 (37.1)	12.8 (38.3)
$I/\sigma(I)$	16.2 (3.2)	10.8 (4.3)
Completeness (%)	99.6 (99.1)	98.2 (96.3)

Diffraction data were collected at DESY/HASYLAB Hamburg, Germany (Consortium beamline X13 equipped with a MAR Research CCD detector, 100 K). The data were indexed, processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Data-collection statistics are given in Table 1.

3. Results and discussion

Crystals were observed after 3 d and grew to full size within one week. Most of the droplets showed two different crystal morphologies: crystal form I, which grew in small plates with dimensions of up to 200 × 50 × 10 µm and diffracted to 2.6 Å resolution, and crystal form II with an elongated prismatic shape, typical dimensions of 400 × 20 × 20 µm and which diffracted to 1.5 Å (Fig. 1).

Analysis of the diffraction pattern shows that the two crystal forms belong to different space groups. Crystal form I was assigned to space group $P2_12_12$, with unit-cell parameters a = 112.6, b = 84.3, c = 45.1 Å. Given the Ov-GST2 molecular weight of 24 kDa, V_M is 2.1 Å^3 Da^{-1} for one dimer in the asymmetric unit (Matthews, 1968). However, crystal form II was assigned to space group $P2_1$, with unit-cell parameters a = 51.6, b = 82.3, c = 56.7 Å, β = 95.89°. The V_M value of 2.4 Å^3 Da^{-1} is slighly higher than for crystal form I, but crystal from II also has one dimer in the asymmetric unit. Although the solvent content in crystal form II is slightly higher, the diffraction resolution is noticeably improved in comparison with crystal from I.

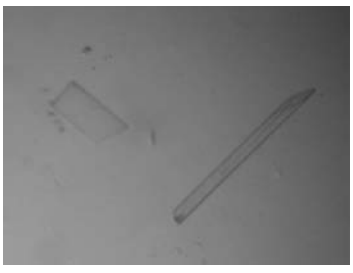

Figure 1
The two crystal forms of Ov-GST2. The rectangular crystal was assigned to space group $P2_12_12$ and the elongated prismatic crystal was assigned to space group $P2_1$.

Currently, structure determination and model building are in progress. Comparing the intermolecular interactions of neighbouring molecules in the two crystal forms may help to explain the differences in the resolution limits on the molecular level and the high-resolution structure will serve as a basis for rational drug design by detailed comparison of the parasitic enzyme with the human host enzyme.

References

Hayes, J. D. & Strange, R. C. (1995). *Free Radic. Res.* **22**, 193–207.

Hemingway, J., Hawkes, N., Prapanthadara, L., Jayawardenal, K. G. & Ranson, H. (1998). *Philos. Trans. R. Soc. London Ser. B*, **353**, 1695–1699.

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

Liebau, E., Eschbach, M., Tawe, W., Sommer, A., Fischer, P., Walter, R. D. & Henkle-Dührsen, K. (2000). *Mol. Biochem. Parasitol.* **109**, 101–110.

Liebau, E., Wildenburg, G., Brophy, P. M., Walter, R. D. & Henkle-Dührsen, K. (1996). *Mol. Biochem. Parasitol.* **80**, 27–39.

Liebau, E., Wildenburg, G., Walter, R. D. & Henkle-Dührsen, K. (1994). *Infect. Immun.* **62**, 4762–4767.

McPherson, A. (1982). *Preparation and Analysis of Protein Crystals.* New York: John Wiley.

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

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Salinas, A. E. & Wong, M. G. (1999). *Curr. Med. Chem.* **6**, 279–309.

Sheehan, D., Meade, G., Foley, V. M. & Dowd, A. (2001). *Biochem. J.* **360**, 1–16.

Wilce, M. C. J. & Parker, M. W. (1994). *Biochim. Biophys. Acta*, **1205**, 1–18.

Wildenburg, G., Liebau, E. & Henkle-Dührsen, K. (1998). *Exp. Parasitol.* **88**, 34–42.