Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction studies of the glutathione *S*-transferase from *Plasmodium falciparum*

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Received 2 April 2003
Accepted 19 May 2003

Glutathione *S*-transferases (GSTs) belong to a family of detoxification enzymes that conjugate glutathione to various xenobiotics, thus facilitating their expulsion from the cells. For high-resolution crystallographic investigations, GST from the human malarial parasite *Plasmodium falciparum* was overexpressed in bacterial cells and crystallized using hanging-drop vapour diffusion. X-ray intensity data to 2.8 Å resolution were collected from an orthorhombic crystal form with unit-cell parameters $a = 62.2$, $b = 88.3$, $c = 75.3$ Å. A search for heavy-atom derivatives has been initiated, along with phase-determination efforts by molecular replacement.

1. Introduction

The human malarial parasite *Plasmodium falciparum* is the causative agent of malaria tropica, the most prevalent parasitic disease worldwide, with 300–500 million infections and 1.5–2.7 million deaths per year. The rapidly developing resistance to drugs used for prophylaxis and treatment makes the identification of novel drug targets necessary (Butler *et al.*, 1997; Olliaro, 2001).

Glutathione *S*-transferases (GSTs; EC 2.5.1.18) are a major family of detoxification enzymes that are found in organisms ranging from prokaryotes to mammals. They catalyse the nucleophilic addition of glutathione to a large variety of electrophilic substrates, thereby detoxifying both endobiotic and xenobiotic compounds. Besides catalyzing conjugation reactions, GSTs can also reduce organic hydroperoxides of phospholipids, fatty acids and DNA before they become engaged in free-radical propagation reactions, ultimately leading to the destruction of macromolecules during oxidative stress (Hayes & Strange, 1995). In addition to their enzymatic functions, GSTs have been shown to serve in structural roles (S-crystallins) or act as regulatory proteins. For example, GSTs are involved in the sequestering and transport of exogenous potentially toxic compounds such as pesticides, herbicides and antibiotics and have been shown to bind a large variety of endogenous compounds such as steroids, bilirubin, bile acids and ferriprotoporphyrin IX with high to moderate affinities (Salinas & Wong, 1999; Sheehan *et al.*, 2001). Within a parasitic context, it is important to additionally consider their role in drug resistance (Hemingway *et al.*, 1998). Mammalian cytosolic GSTs comprise seven major gene classes that are distinguishable on the basis of structure and substrate specificity (Mannervik, 1985; Board *et al.*, 1997). Despite the overall low level of sequence identity across the different classes, all structures have a similar canonical fold, with each subunit consisting of two distinct domains. The highly conserved N-terminal domain binds glutathione and is similar to the thioredoxin fold, consisting of four β-strands with three flanking α-helices. This domain is connected to the C-terminal domain by a short linker sequence. The second domain is all-helical and contributes most of the residues that interact with the hydrophobic substrate (Wilce & Parker, 1994; Sheehan *et al.*, 2001). Including mutant and complex structures, $\sim$70 representative GST crystal structures are presently available and deposited in the Protein Data Bank (Berman *et al.*, 2000).

The GST gene of *P. falciparum* (Pf-GST) is localized on chromosome 14. The enzyme was cloned and expressed in *Escherichia coli*, yielding a homodimeric active enzyme. According to primary structure and substrate specificity, the protein can be placed into the vicinity of the μ or π subclass of GSTs (Liebau *et al.*, 2002). Pf-GST has the highest sequence identity (of about 30%) to GSTs from two other parasites, namely those from *Dirofilaria immitis* and *Onchocerca volvulus*. However, the structures of these GSTs are not yet known. Pf-GST was shown to interact with ferriprotoporphyrin IX, which is the major toxic degradation product released during the parasite's digestion of haemoglobin, its source of amino acids for growth and maturation. It has been postulated that one function of the highly abundant Pf-GST1 (0.1–1% of total parasite protein) is to sequester non-polymerized haem that diffuses from the food vacuole into the cytosol, thus preventing its toxic effects within the parasite (Harwaldt *et al.*, 2002). In this context, structural investiga-

tion of the interaction with Pf-GST is an interesting aspect for drug-design studies, because it may be possible to intensify the effect of already existing drugs such as chloroquine, which act by disturbing haem polymerization in the parasite's food vacuole (Platel *et al.*, 1999).

2. Material and methods

2.1. Recombinant expression and purification of the Pf-GST

The construction of the expression vector and the expression and purification of Pf-GST were carried out as described previously (Liebau *et al.*, 2002). After transformation into *E. coli* BL21(DE3) pLys, expression was carried out in a 2 l high-density fermenter. The overnight culture (40 ml) was added to 1.5 l of Terrific Broth supplemented with 50 µg ml^{-1} ampicillin. The cultures were grown at 310 K to an OD_{600} of 1.0; 500 ml of Terrific Broth medium, 50 µg ml^{-1} ampicillin and 1 m*M* isopropyl-β-D-thiogalactoside were then added. After harvesting of the cells by centrifugation, the bacterial pellet was resuspended in phosphate-buffered saline (PBS) and sonicated. The cell lysate was centrifuged at 100 000*g* and 277 K for 1 h. In a batch purification, the supernatant was incubated with 5 ml glutathione-Sepharose (Amersham Pharmacia) overnight, washed with 10 bed volumes of PBS and eluted with 5 ml of 15 m*M* glutathione in 50 m*M* Tris–HCl pH 8.0. The protein concentration was adjusted to 7 mg ml^{-1} and the solution was dialysed against 100 m*M* Tris–HCl pH 8.5 with 2 m*M* glutathione. Prior to crystallization experiments, the solution was analysed by dynamic light scattering (DLS) using a laser-light system manufactured by RiNA GmbH (Berlin, Germany).

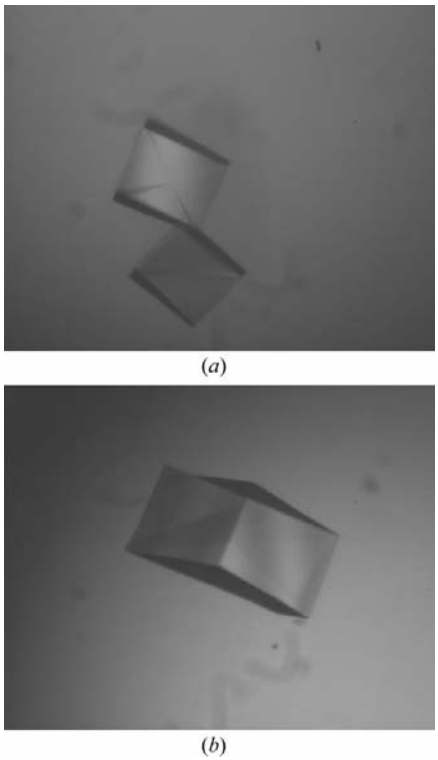

Figure 1
(*a*) Initial small crystals of Pf-GST. (*b*) Crystals after optimization with dimensions of up to 0.4 × 0.4 × 0.3 mm.

2.2. Crystallization

Initial screening was performed at room temperature (291 K) by hanging-drop vapour diffusion (McPherson, 1982) using sparse-matrix kits from Hampton Research (Jancarik & Kim, 1991; Hampton Research, Laguna Niguel, CA, USA). Drops were prepared on siliconized cover slips and equilibrated against 0.5 ml of reservoir solution. The volume of the drops was 4 µl in total. Unless otherwise noted, screens were prepared by combining equal volumes of reservoir solution with protein at 7 mg ml^{-1}. Crystals obtained from the initial screens were further optimized to obtain crystals suitable for X-ray data collection.

2.3. Data collection

After transferring the crystals to the reservoir solution, a single crystal was mounted in a glass capillary. Diffraction data were collected at room temperature on a MAR Research 300 mm image-plate detector using Cu $K\alpha$ radiation from a Rigaku RU-200 rotating-anode X-ray generator operating at 50 kV and 100 mA. The programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) were used for data processing and analysis.

3. Results and discussion

DLS analysis of the protein solution revealed a perfect narrow monomodal distribution with a monomeric dynamic radius (R_h) of 2.92 nm and a polydisperity factor of about 20%. Crystals from the initial screen (Fig. 1*a*) were obtained from a reservoir of 2 *M* $(NH_4)_2SO_4$ and 0.1 *M* Tris–HCl pH 8.5. The optimized crystals were grown by simultaneous variation of the $(NH_4)_2SO_4$ concentration and of the pH value. These crystals (Fig. 1*b*) had approximate dimensions of 0.4 × 0.4 × 0.3 mm and were grown from a reservoir consisting of 2.1 *M* $(NH_4)_2SO_4$, 0.1 *M* sodium cacodylate pH 6.0 and 2 m*M* glutathione. The crystals diffracted X-rays to about 2.5 Å resolution.

Table 1
X-ray diffraction data.

Values in parentheses are for the last resolution shell (2.9–2.8 Å).

Space group	$P2_12_12$
Unit-cell parameters	a = 62.2, b = 88.3, c = 75.3
V_M† (Å^3 Da^{-1})	2.1
Resolution range (Å)	25–2.8
No. of observed reflections	108412
No. of unique reflections	10529
R_{sym} (%)	11.6 (37.5)
$I/\sigma(I)$	10.8 (3.8)
Completeness (%)	98.1 (97)

† One dimer in the asymmetric unit.

A complete data set to 2.8 Å was collected (Table 1) at a crystal-to-detector distance of 150 mm, an oscillation range covering 120° with $\Delta\varphi$ = 1° and 20 min exposure per frame.

Autoindexing yielded unit-cell parameters a = 62.2, b = 88.3, c = 75.3 Å, $\alpha = \beta = \gamma = 90°$ (Table 1). This crystal lattice is orthorhombic and belongs to space group $P2_12_12$. The data completeness and $I/\sigma(I)$ were 98.1% and 10.8, respectively, overall and 97.0% and 3.8, respectively, for the 2.9–2.8 Å resolution shell. The overall R_{sym} of the data set was 11.6% and R_{sym} was 37.5% for the 2.9–2.8 Å resolution shell.

Packing-parameter calculations based on a molecular weight of 50 kDa indicate the presence of a homodimer in the asymmetric unit. This corresponds to a typical Matthews coefficient of 2.1 Å^3 Da^{-1} (Matthews, 1968) and a solvent content of 40%. Initial calculations to solve the phase problem by molecular-replacement methods using homologous GSTs as search models are in progress. Because of the relative low sequence homology of the model structures (<35%), a search for heavy-atom derivatives of this protein has also been initiated in parallel.

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