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Crystallization and preliminary X-ray crystallographic studies of the ρ-class glutathione *S*-transferase from the Antarctic clam *Laternula elliptica*

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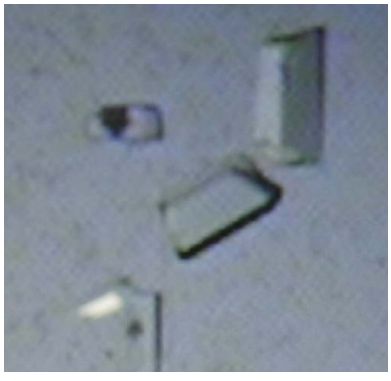
Glutathione *S*-transferases are involved in phase II detoxification processes and catalyze the nucleophilic attack of the tripeptide glutathione on a wide range of endobiotic and xenobiotic electrophilic substrates. The ρ-class glutathione *S*-transferase from *Laternula elliptica* was overexpressed in *Escherichia coli*, purified and crystallized with two substrates: glutathione and 1-chloro-2,4-dinitrobenzene (CDNB). Diffraction data were collected to 2.20 Å resolution for the glutathione-complex crystals and to 2.00 Å resolution for the CDNB-complex crystals using a synchrotron-radiation source. Both crystals belonged to the *C*-centred monoclinic space group *C*2. The unit-cell parameters for the CDNB-complex crystals were a = 89.66, b = 59.27, c = 55.45 Å, β = 124.52°. The asymmetric unit contained one molecule, with a corresponding V_M of 2.36 Å^3 Da^{-1} and a solvent content of 47.8%.

1. Introduction

Glutathione *S*-transferases (GSTs; EC 2.5.1.18) are essential enzymes that are found in all kingdoms of life. They participate in phase II detoxification processes and catalyze the nucleophilic attack of glutathione on many types of endobiotic and xenobiotic nucleophilic substrates (Armstrong, 1997; Remmerie *et al.*, 2008). The increased solubility of the conjugated products renders them more readily eliminated by the cell (Cardoso *et al.*, 2003). GSTs have been implicated in the development of cellular and organismal resistance towards insecticides, herbicides, antibiotics and other drugs (Coles & Ketterer, 1990; Mannervik & Danielson, 1988). GSTs have been classified based on their biochemical and structural properties, such as type of substrate and inhibitor specificity, as well as by primary and tertiary structure. There are more than 15 classes of soluble GSTs in eukaryotes and prokaryotes (Fan *et al.*, 2007; Garcia *et al.*, 2008). Alignments of members of each GST class have shown that less than 30% of the amino acids are strictly conserved; however, crystallographic studies have indicated that the overall polypeptide folds of the various classes of soluble GSTs are very similar (Blanchette *et al.*, 2007; Oakley *et al.*, 1999; Oakley, 2005). The recently classified ρ class of GSTs show particular specificity for CDNB and little activity with other substrates. Additionally, these ρ-class GSTs show extraordinary heat instability. This suggests that the ρ class of GSTs may be adapted to cold temperatures (Konishi *et al.*, 2005).

Laternula elliptica, also known as the Antarctic soft-shelled clam, is a member of the Laternulidae family. They are ubiquitous in the Antarctic region and contribute to coastal water ecosystems by filtering seawater pollutants (Park *et al.*, 2007). The temperature of the Antarctic is extremely low, so animals living there require enzymes that remain active at these low temperatures. The cold-adapted ρ class of GSTs may therefore play an important role in low-temperature survival. The ρ-class glutathione *S*-transferase (ρ-GST) from *L. elliptica* was chosen for structural studies because of its narrow substrate specificity and extreme temperature sensitivity. There is no protein structure available for a ρ-class GST to date. In



order to understand its structure–function relationship, we have crystallized and performed preliminary X-ray crystallographic experiments on ρ-GST from *L. elliptica*.

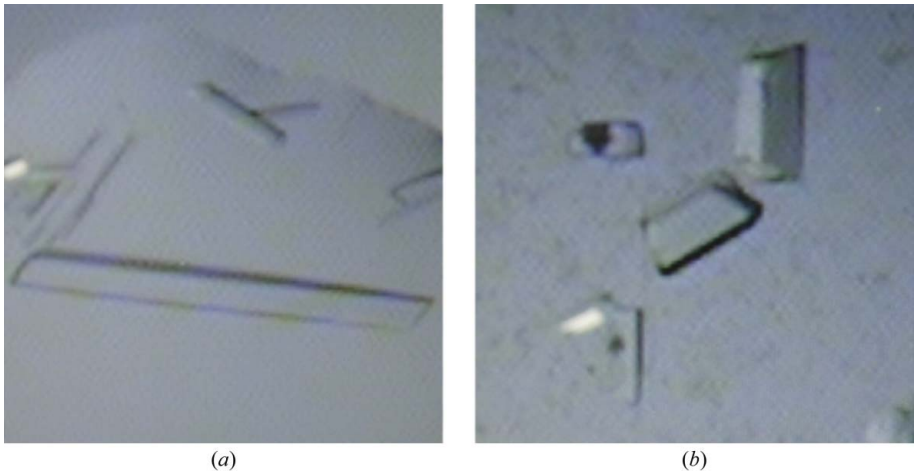
2. Materials and methods

2.1. Cloning, protein expression and purification

The gene encoding the ρ-class GST was amplified from an *L. elliptica* complementary DNA library by the polymerase chain reaction (PCR) using specific primers. The forward primer contained an *Nde*I restriction site (bold) and had the sequence 5′-**CATATG**GCCACCACCAGCAAACCGTT-3′, while the reverse primer contained an *Xho*I restriction site (bold) and had the sequence 5′-**CTCGAG**CTAGCAGAGGTCAAGAAGATTTC-3′. The PCR product was then subcloned between the *Nde*I and *Xho*I sites of the pET-28a vector (Novagen, USA). This construct contains a hexahistidine tag at the N-terminus for purification purposes. The ρ-GST/pET28a plasmid was transformed into *Escherichia coli* BL21 (DE3) pLysE strain (Novagen) and the cells were grown in a shaking incubator at 310 K in Luria–Bertani (LB) broth medium supplemented with 50 µg ml^{-1} kanamycin. Protein expression was induced by adding 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) when the cells reached an optical density at 600 nm of about 0.6; the cells were then grown for an additional 4 h at 310 K. After this treatment, the cells were harvested by centrifugation at 3000*g* for 30 min at 277 K. The cell pellet was resuspended in binding buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl and 50 m*M* imidazole) and disrupted by sonication at 277 K. The crude lysate was centrifuged at 25 000*g* for 1 h at 277 K. The supernatant was then loaded onto an Ni^{2+}-chelated HiTrap chelating HP column (GE Healthcare, USA) which had been pre-equilibrated with binding buffer. The protein was eluted with a linear gradient of elution buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 1 *M* imidazole). The protein was subsequently purified to its final state by gel-filtration chromatography on a HiLoad 16/60 Superdex 200 column (GE Healthcare, USA) which had been pre-equilibrated with gel buffer (20 m*M* Tris–HCl pH 7.9, 200 m*M* NaCl, 2 m*M* DTT). The final concentration of 10 mg ml^{-1} was obtained with the use of an Amicon Ultra-15 centrifugal filter device (Millipore, USA). This procedure yielded approximately 80 mg of GST protein from a 1 l culture. The protein concentration was determined using a Bradford assay and the protein purity was examined by 15% SDS–PAGE and determined to be >95%.

2.2. Crystallization and X-ray analysis

Preliminary crystallization screens for the ρ-GST enzyme were performed using the sitting-drop vapour-diffusion method (0.2 µl protein solution and 0.2 µl reservoir solution equilibrated against 100 µl reservoir solution) using a Hydra II Plus One crystallization robot (Matrix Technologies Ltd, UK) set up with 96-well Intelli-Plates (Art Robbins Instruments, USA) at 295 K. Commercial screening kits from Hampton Research were used for the preliminary screens. Initial crystals were obtained under the following conditions: 8% Tacsimate pH 8.0 and 20%(*w*/*v*) polyethylene glycol 3350. Crystal growth was optimized using the sitting-drop vapour-diffusion method in 24-well VDX plates (Hampton Research, USA) with Micro-Bridges (Hampton Research, USA); each drop was a mixture of 4 µl protein solution and 4 µl reservoir solution [6% Tacsimate pH 8.0 and 26%(*w*/*v*) polyethylene glycol 3350] and was equilibrated over 500 µl reservoir solution. For cocrystallization, the protein solution was mixed with glutathione at a molar ratio of 1:1 and with 1-chloro-2,4-dinitrobenzene (CDNB) at a molar ratio of 1:5. The glutathione-complex crystals were obtained in 7% Tacsimate pH 8.0, 24%(*w*/*v*) polyethylene glycol 3350 and the CDNB-complex crystals were obtained in 8.5% Tacsimate pH 8.0, 25%(*w*/*v*) polyethylene glycol 3350. The crystals of both complexes were obtained at 295 K using the sitting-drop vapour-diffusion method as for the native protein. Suitable-sized crystals were obtained within 3 d and were used for X-ray diffraction. The crystal dimensions of the native, glutathione-complex and CDNB-complex crystals were 0.1 × 0.05 × 0.4, 0.1 × 0.05 × 0.4 and 0.1 × 0.1 × 0.1 mm, respectively. For X-ray data collection, the crystals were transferred from drops to cryoprotection solution [native, 4.8% Tacsimate pH 8.0, 20.8%(*w*/*v*) polyethylene glycol 3350 and 20%(*v*/*v*) ethylene glycol; glutathione complex, 5.04% Tacsimate pH 8.0, 17.3%(*w*/*v*) polyethylene glycol 3350 and 28% sucrose; CDNB complex, 6.8% Tacsimate pH 8.0, 20%(*w*/*v*) polyethylene glycol 3350 and 20% glycerol]. The native crystals were very weak and fragile and the diffraction pattern showed very high mosaicity. For this reason, it was not possible to collect X-ray data from the native crystals. Data sets for the glutathione and CDNB complexes were collected on beamline 4A at the Pohang Light



(*a*) (*b*)

Figure 1
Crystals of the ρ-GST protein from *L. elliptica*. (*a*) Glutathione-complex crystals; (*b*) CDNB-complex crystals. The crystal dimensions for the glutathione-complex and the CDNB-complex crystals were 0.1 × 0.05 × 0.4 and 0.1 × 0.1 × 0.1 mm, respectively.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shells.

	Glutathione complex	CDNB complex
Space group	*C*2	*C*2
Unit-cell parameters (Å, °)	a = 90.39, b = 57.62, c = 55.45, β = 123.83	a = 89.66, b = 59.27, c = 55.45, β = 124.52
Resolution range (Å)	50–2.2 (2.20–2.28)	50–2.0 (2.00–2.07)
Total reflections	57369	81229
Unique reflections	11607	15882
Redundancy	4.9 (3.9)	5.1 (3.0)
Completeness (%)	95.9 (86.6)	96.0 (83.7)
$R_{\rm merge}$† (%)	13.3 (24.3)	7.3 (19.3)
$\langle I/\sigma(I)\rangle$	19.8 (4.2)	35.6 (5.1)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ represents the observed intensity, $\langle I(hkl)\rangle$ represents the average intensity and i counts through all symmetry-related reflections.

Source (Pohang, South Korea) using an ADSC Quantum 210 CCD detector. A total range of 360° was covered with 1.0° oscillations and 10 s exposure per frame. The wavelength of the synchrotron X-ray beam was 1.00000 Å. The crystal-to-detector distance was set to 180 mm. X-ray diffraction data were collected to 2.2 Å resolution for glutathione-complex crystals and to 2.0 Å resolution for CDNB-complex crystals. All data sets were indexed, integrated and scaled using the *HKL*-2000 software package (Otwinowski & Minor, 1997).

3. Results and discussion

The gene encoding the ρ-GST protein from *L. elliptica* was cloned, expressed in *E. coli* and purified for structural studies. Crystals suitable for X-ray diffraction were obtained using the following optimized crystallization conditions: 7% Tacsimate pH 8.0 and 24%(*w*/*v*) polyethylene glycol 3350 for the glutathione complex and 8.5% Tacsimate pH 8.0 and 25%(*w*/*v*) polyethylene glycol 3350 for the CDNB complex (Fig. 1). The glutathione-complex crystals belonged to space group *C*2, with unit-cell parameters a = 90.39, b = 57.62, c = 55.45 Å, β = 123.83°. The CDNB-complex crystals also belonged to space group *C*2, with unit-cell parameters a = 89.66, b = 59.27, c = 55.45 Å, β = 124.52°. X-ray diffraction data were collected to 2.20 Å resolution for the glutathione-complex crystals and to 2.00 Å resolution for the CDNB-complex crystals. The asymmetric unit contained one molecule, yielding a crystal volume per protein weight ($V_{\rm M}$) of 2.33 Å^3 Da^{-1} with a solvent content of 47.2% for the glutathione-complex crystals and of 2.36 Å^3 Da^{-1} with a solvent content of 47.8% for the CDNB-complex crystals (Matthews, 1968). The data-collection statistics are summarized in Table 1. The coordinates of other class GSTs [δ-class GST from *Anopheles dirus* (PDB code 1r5a; Udomsinprasert *et al.*, 2005) and ζ-class GST from *Arabidopsis thaliana* (PDB code 1f6b; Huang *et al.*, 2001)] were used as a search model to solve the phase problem by molecular replacement (MR) using the program *CNS* (Brünger *et al.*, 1998). All attempts to solve the structure by the MR method were unsuccessful. Therefore, selenomethione-labelled protein has been expressed and purified and crystallization experiments are in progress.

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