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Crystallization and preliminary X-ray diffraction analysis of a glutathione *S*-transferase from *Xylella fastidiosa*

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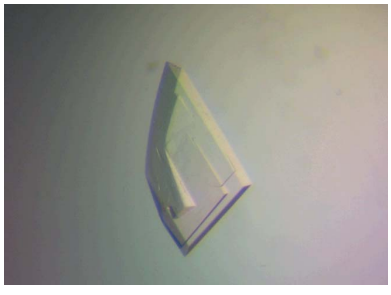

Glutathione *S*-transferases (GSTs) form a group of multifunctional isoenzymes that catalyze the glutathione-dependent conjugation and reduction reactions involved in the cellular detoxification of xenobiotic and endobiotic compounds. GST from *Xylella fastidiosa* (*xf*GST) was overexpressed in *Escherichia coli* and purified by conventional affinity chromatography. In this study, the crystallization and preliminary X-ray analysis of *xf*GST is described. The purified protein was crystallized by the vapour-diffusion method, producing crystals that belonged to the triclinic space group *P*1. The unit-cell parameters were $a = 47.73$, $b = 87.73$, $c = 90.74$ Å, $\alpha = 63.45$, $\beta = 80.66$, $\gamma = 94.55^\circ$. *xf*GST crystals diffracted to 2.23 Å resolution on a rotating-anode X-ray source.

1. Introduction

Xylella fastidiosa (Wells *et al.*, 1987) belongs to the Gram-negative bacteria and its growth, which is restricted to the xylem vessels of its host plants, has been associated with disease in various plant species of economic importance (Hopkins, 1989). Understanding the complete genome sequence of *X. fastidiosa*, together with functional studies, has resulted in a series of hypotheses related to the mechanisms of pathogenicity, antioxidant response and detoxification pathways (Van Sluys *et al.*, 2002). Detoxification is important to protect a variety of cell components against reactive molecules, including antibiotics (Dainelli *et al.*, 2002).

The XF1210 ORF from *X. fastidiosa* that encodes the glutathione *S*-transferase enzyme (GST) with 205 amino acids (22.7 kDa) was chosen for this study based on its specific role in the biodegradative metabolism, detoxification and excretion of xenobiotic substrates (Konishi *et al.*, 2005). The glutathione *S*-transferases form a group of multifunctional isoenzymes that catalyze the glutathione-dependent conjugation and reduction reactions involved in the cellular detoxification of endobiotic compounds such as hormones, haem and bilirubin, as well as xenobiotic compounds including drugs and pesticides that often impair the catalytic activity of enzymes (Salinas & Wong, 1999; Sheehan *et al.*, 2001; Deponte & Becker, 2005). For example, bacterial GSTs have been implicated in the reductive dechlorination of pentachlorophenol by *Sphingomonas paucimobilis*, in the biodegradation of dichloromethane by a dehalogenase in *Methylophylus* sp. and in the degradation pathway of biphenyls in *Pseudomonas* (Mueller *et al.*, 1990; Bader & Leisinger, 1994; Hofer *et al.*, 1994).

GSTs have been classified based on biochemical and structural properties, including their catalytic activity towards CDNB (1-chloro-2,4-dinitrobenzene), a standard substrate for almost all GSTs (Rossjohn *et al.*, 1998; Travensolo *et al.*, 2008). In eukaryotes, the soluble GSTs have been grouped into several independent classes, *i.e.* α, κ, μ, ω, π, σ, θ and ζ (mammals), δ, ε and U (insects), λ, φ and τ (plants) and ρ (fish), on the basis of similarity of the N-terminal amino-acid sequence, substrate specificity, immunological cross-reactivity and structural properties (Mannervik *et al.*, 1985; Board *et al.*, 1997, 2000; Sawicki *et al.*, 2003; Hayes *et al.*, 2004; Kosloff *et al.*,

2006). However, only β-class GSTs have been identified in prokaryotes, with members having been isolated and characterized from bacteria such as *Escherichia coli*, *Haemophilus influenzae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Streptomyces griseus* (Di Ilio *et al.*, 1988; Iizuka *et al.*, 1989; Jung *et al.*, 1996; Dainelli *et al.*, 2002; Dhar *et al.*, 2003). The majority of prokaryotic GSTs have also been shown to possess peroxidase activity, to act as epoxide thiolases and to catalyse reactions in the metabolism of some compound (Bartels *et al.*, 1999).

Comparison of aligned GST sequences has shown that less than 30% of the amino acids are strictly conserved, although crystallographic studies have indicated that the overall polypeptide folds of the various classes of soluble GSTs are very similar (Dirr *et al.*, 1994; Armstrong, 1997; Dainelli *et al.*, 2002). The GST enzymes are dimeric, with each subunit divided into two domains: the N-terminal domain (domain I) adopts a α/β topology and provides most of the contacts with glutathione (GSH; G site), whereas the C-terminal domain (domain II) is completely α-helical and contains some of the residues that form the hydrophobic binding site (H site; Armstrong, 1997; Sheehan *et al.*, 2001; Dainelli *et al.*, 2002). The G and H sites are especially important because they define the substrate repertoire for a particular GST (Rossjohn *et al.*, 1998).

In order to understand the catalytic mechanism of glutathione *S*-transferase from *X. fastidiosa* (*xf*GST), we have undertaken the resolution of its structure. Here, we report the crystallization and diffraction data for recombinant *xf*GST.

2. Materials and methods

2.1. Protein expression and purification

The *xf*GST gene was amplified by polymerase chain reaction using specific primers (forward primer 5′-CGCATATGAAGTTGTACATCATGCCAGGCGCTTGCTC-3′ and reverse primer 5′-GGAATTCTTATCAGATCAGCCCTTCCGCCTGTAATGC-3′) that were designed to generate products with vector cohesive overhangs (Travensolo *et al.*, 2008). The DNA encoding the GST was then subcloned into vector pET-28a(+) in *Nde*I and *Eco*RI sites; this was then used to transform *E. coli* DH5α. The new vector construct was named pET-GST and produced the recombinant protein in fusion with a His tag.

Recombinant glutathione *S*-transferase from *X. fastidiosa* (r*xf*GST) was expressed in *E. coli* and purified as described elsewhere (Travensolo *et al.*, 2008). Briefly, BL21(DE3) cells harbouring the plasmid containing the *xf*GST insert were grown in 500 ml LB medium supplemented with kanamycin (50 µg ml^{-1}) at 310 K until the optical density at 600 nm reached 0.6. Subsequently, the culture was induced with 0.4 m*M* IPTG and incubation was continued for an additional 4 h at the same temperature. After cell disruption by sonication at 277 K, the r*xf*GST present in the soluble fraction was purified by nickel-affinity column (Novagen) chromatography and subsequent dialysis against a solution of 25 m*M* Tris–HCl pH 8.0 buffer containing 20 m*M* NaCl. The protein purity exceeded 95% as judged by the presence of a single band on 15% SDS–PAGE (Travensolo *et al.*, 2008), with a molecular weight in close proximity to that for the predicted value for r*xf*GST (~24.5 kDa including the His tag). Finally, the r*xf*GST was concentrated to a final concentration of 15 mg ml^{-1} using Centriprep-10 (Amicon). The protein concentration was determined from the UV absorbance at 280 nm using a theoretical extinction coefficient based on the amino-acid sequence composition (Gill & von Hippel, 1989). The extinction coefficient employed was $\varepsilon_{280\,\mathrm{nm}} = 44\,920\ M^{-1}\ \mathrm{cm}^{-1}$.

2.2. Crystallization

Initial attempts to crystallize r*xf*GST were performed at 291 K by hanging-drop vapour diffusion using the sparse-matrix screening method and Crystal Screens I and II (Hampton Research). Hanging drops containing 2 µl protein solution (15 mg ml^{-1} in 25 m*M* Tris–HCl pH 8.0 buffer containing 20 m*M* NaCl and 5% glycerol) mixed with equal amounts of reservoir solution were equilibrated against 500 µl reservoir solution . Crystals grew in about 10 d from a condition containing 28% PEG 6000, 100 m*M* HEPES pH 7.6 (Fig. 1).

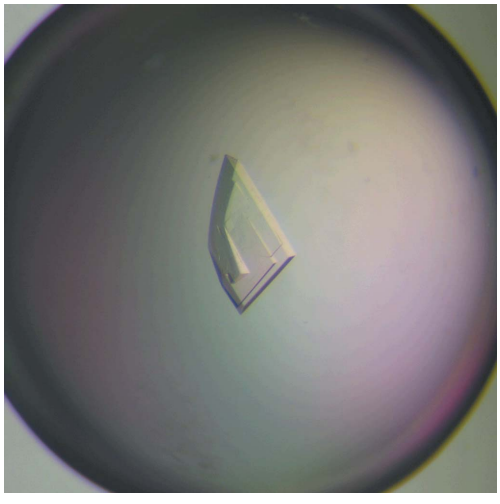

Figure 1
Crystal of recombinant *xf*GST obtained by the hanging-drop vapour-diffusion technique using 100 m*M* HEPES pH 7.6, 28%(*w*/*v*) PEG 6000 as precipitant. Typical dimensions are 0.2 × 0.2 × 0.2 mm.

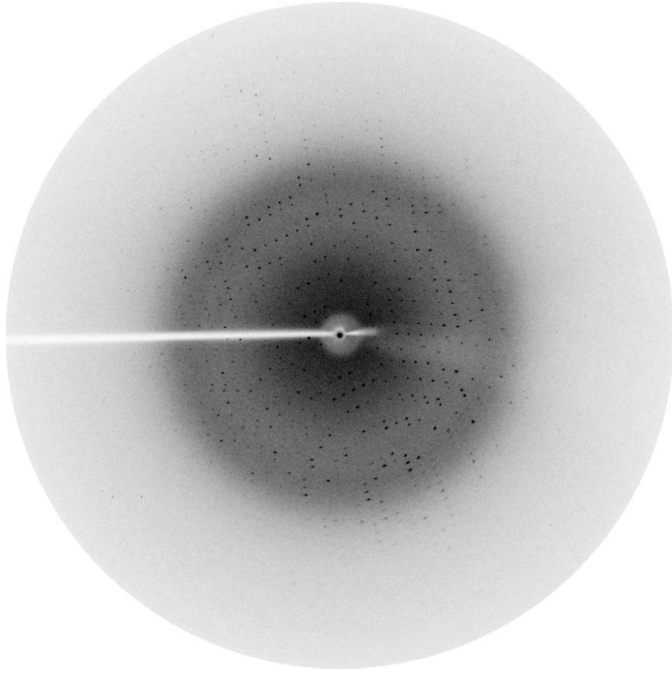

Figure 2
Typical diffraction pattern of the r*xf*GST crystal with a 1° oscillation exposure. The edge of the detector corresponds to a resolution of 2.00 Å.

Table 1
Crystal parameters and data-processing statistics.

Values in parentheses are for the highest resolution shell.

Space group	*P*1
Unit-cell parameters (Å, °)	a = 47.73, b = 87.73, c = 90.74, α = 63.45, β = 80.66, γ = 94.55
Resolution limits (Å)	30.01–2.23 (2.35–2.23)
Total no. of frames ($\Delta\varphi$ = 1°)	200
Mosaicity (°)	1.07
Total no. of reflections	117272 (15922)
Unique reflections	57152 (8104)
Multiplicity	2.1 (2.0)
$R_{\rm merge}$ (%)	9.0 (37.2)
$\langle I/\sigma(I)\rangle$	8.3 (1.9)
Completeness (%)	93.5 (89.4)

2.3. Data collection and processing

A single crystal was harvested using a nylon loop (Hampton Research) and transferred from the crystallization drop to 5 µl of a cryoprotection solution containing 5% ethylene glycol, 28% PEG 6000, 100 m*M* HEPES pH 7.6 for a few seconds. The crystal was then flash-cooled to 100 K in a nitrogen stream in order to prevent radiation damage during data collection. A diffraction data set was collected using a MAR345dtb image-plate detector mounted on a Rigaku Ultra X18 copper rotating-anode generator equipped with Osmic confocal MaxFlux optics. A total of 200 images were collected with a crystal-to-detector distance of 170.0 mm using the oscillation method with an oscillation range of 1° per image and an exposure time of 20 min. Raw data images were processed with *MOSFLM* (Leslie, 1992) and were scaled and merged with *SCALA* (Evans, 1993); amplitudes were estimated using *TRUNCATE* (French & Wilson, 1978).

3. Results and discussion

The r*xf*GST crystals belong to the triclinic system, with space group *P*1. Diffraction data extended to 2.23 Å resolution (Fig. 2) and crystal and data-processing statistics are summarized in Table 1. Using the known molecular weight of the monomer (24.5 kDa), a Matthews coefficient ($V_{\rm M}$; Matthews, 1968) of 2.33 Å^3 Da^{-1}, corresponding to a solvent content of 47.3%, was obtained assuming the presence of six molecules in the asymmetric unit. Molecular replacement using the X-ray structure of bacterial glutathione *S*-transferase from *Burkholderia xenovorans* (PDB code 2dsa; 37% amino-acid sequence identity) as a search model was carried out with *Phaser* (Storoni *et al.*, 2004). *Phaser* simulations converged to a clear solution with six molecules in the asymmetric unit and a *Z* score of 15.57 in the translation function after placement of the last molecule. The molecules are arranged as a trimer of dimers, in agreement with the known dimeric organization of GSTs in solution. Structural refinement is in progress.

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