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Preliminary X-ray Studies of a New Crystal form of 28 kDa *Clonorchis sinensis* Glutathione S-Transferase

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Abstract

A new crystal of helminth glutathione S-transferase, 28 kDa isozyme from *Clonorchis sinensis* has been grown from a 20% PEG MME 550 solution containing 50 mM $CaCl_2$ in 0.1 M bis-Tris buffer (pH 6.5) in 2~3 days. The crystals diffract to 3.0 Å resolution and belong to the orthorhombic space group $P2_12_12_1$ with cell parameters a=62.58 Å, b= 69.92 Å, and c= 339.67 Å.

1. Introduction

Glutathione S-transferases (GSTs) are multifunctional proteins encoded by a large gene family, involved in cellular detoxification of endogenous and exogenous chemicals by catalyzing the formation of glutathione (GSH) conjugates.[1] They have been implicated in a variety of resistance phenomena of cells; especially, data from cancer studies have linked aberrant expression of GST isozymes with the development and expression of resistance to a variety of chemicals, including cancer drugs.[2] GSTs have been divided on the basis of sequence identity into phi, tau, theta, zeta and lambda classes.[3] They share a common overall fold with two domains, a smaller N-terminal alpha/beta-domain and a larger C-terminal alpha-domain.

The detoxification role of GSTs may help the escape of helminth parasites from host immune attack[4] and the anthelmintic compounds.[5] Therefore, parasite GSTs have been attractive subjects as potential drug and vaccine targets. *Clonorchis sinensis* 28 kDa GST (*wCs*28GST), one of two isozymes of helminth GSTs of Mr 26,000 and 28,000 Da, was first purified and cloned by Kang *et al.*[6] It was suggested that the enzyme, localized in the tegument and underlying mesenchymal tissues, may play significant physiological roles against bioreactive molecules and be a useful reagent for serodiagnosis of clonorchiasis. The only structure of homologous protein that has been determined so far is that of a *Schistosoma haematobium* 28 kDa GST (*Sh*28GST).[7] Although *wCs*28GST and *Sh*28GST show relatively high sequence homology (42%) each other and share conserved amino acid residues in the active site, they have a different substrate specificity.

We reported the crystallization and preliminary X-ray analysis for a monoclinic crystal of the *Cs*28GST with a N-terminal flanking sequence of 20 amino acid residues (*rCs*28GST).[8] However, because of the extemely low reproducibility and poor-quality of crystals it was not possible to collect complete data for structural analysis. To obtain high-quality crystals, a truncation was made in the N-terminal flanking region of *rCs*28GST. We have successfully crystallized the truncated protein (*wCs*28GST) and report here the results.

2. Materials and Method

2-1. Cloning, expression and purification

The gene encoding full-length *wCs*28GST without a N-terminal flanking sequence of 20 amino acid residues was amplified by PCR and subcloned

into the pet23c(+) vector (Novagen). The recombinant plasmids were transformed into *E. coli* strain BL21(DE3) (Novagen). The cells were grown in broth medium containing 50 μg/ml ampicillin until the $O.D_{600}$ was about 0.5~0.8. Then, overexpression was induced with 1 mM isopropyl β-d-thiogalactopyranoside (IPTG) and cells were grown at 37°C for 5 h. Cells were harvested, resuspended in 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM PMSF, 0.2 M NaCl, and were disrupted by ultrasonication. The homogenate was centrifuged at 16500 rpm for 30 min to remove cell debris. The supernatant then was applied to a glutathione-linked Sepharose 6B column, washed with a 20 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA. The resin was prepared according to the procedure of Simons and Vander Jagt[9] using epoxy-activated Sepharose 6B). The protein was eluted with a 50 mM tris-HCl buffer (pH 9.6) containing 15 mM GSH and 1 mM EDTA. Active fractions were selected by analyzing SDS-PAGE gels visualized by Coomasie-Blue staining. Protein fractions containing *wCs*28GST were selected and applied onto Q-Sepharose chromatography column using 20 mM Tris-HCl buffer (pH 8.0) containing 1 mM PMSF. The protein was eluted with a linear gradient in NaCl. Acitve fractions were pooled, concentrated to 5 by ultrafiltration (Amicon PM10) and purified further by a size-exclusion chromatography column (Superdex 75, Pharmacia) using 20 mM HEPES buffer (pH 7.0) containing 1 mM DTT, 0.2 M NaCl. Activity of the eluted fractions was assayed by the procedure of Habig *et al.*[10] Protein concentration was determined by absorbance assay (molar absorption coefficient at 280 nm: 0.85 mg/ml).

2-2. Crystallization and data collection

The purified *wCs*28GST was dialyzed against 5 mM potassium phosphate buffer (pH 7.0), and concentrated to a final concentration of 15~20 mg/ml. Initial crystallization screening was performed using the sparse-matrix method[11] by the hanging-drop vapour diffusion method. 4 μl of hanging drop, 1 : 1 mixture of protein solution and reservoir solution, was equilibrated against 500 μl of reservoir solution at room temperature.

Prior to data collection, crystals were soaked for 30 seconds in mother liquor containing 25% polyehtylene glycol MME 550 (PEG MME 550), 5% polyehtylene glycol 400 (PEG 400), 50 mM $CaCl_2$ and frozen in liquid nitrogen. 900 frames of diffraction data were collected at 100 K using an ADSC Quantum 4R CCD detector at the Photon Factory, Japan. Each frame was measured using a 20 sec exposure with 0.2 degree oscillation. The raw data were indexed, integrated and scaled using DENZO and SCALEPACK from the HKL package.[12]

3. Results and Discussion

As mentioned already, *Cs*28GST was initially cloned with a N-terminal flanking sequence of 20 amino acid residues.[6] The enzyme with extra residues were crystallized from a variety of conditions in early stage of screening trials.[8] However, neither the reproducibility nor the diffraction quality of the crystals could be improved and further crystallization attempts were therefore focused on the truncated protein.

The flanking sequence was successfully removed from the N-terminus of *rCs*28GST. Extensive crystallization screening for the *wCs*28GST had been carried out and a few crystals were grown from under a variety of conditions. Most of the crystals tested did not diffract to better than 4 Å. However,

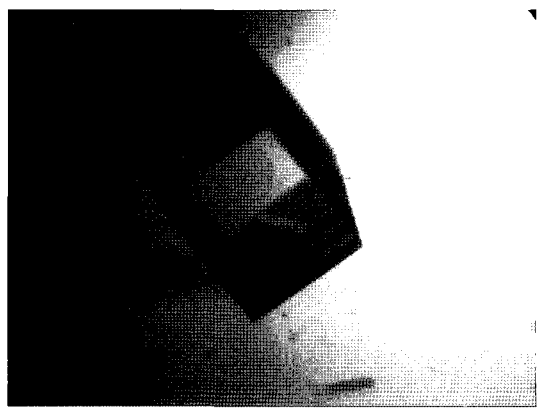

Fig. 1. *wCs*28GST crystal grown from a 0.1 M bis-Tris buffer (pH 6.5) solution containing 20% PEG MME 550 and 50 mM $CaCl_2$ (approximate dimensions 0.4×0.4×0.2 mm).

Table 1. Data-collection statistics

Space group	$P2_12_12_1$ orthorhombic
Unit cell parameters	a=62.58 Å b=69.92 Å c=339.67 Å
Resolution	3.0 Å
# of unique reflections	27,933
I/σ(I)	26.7 (5.0)
Completeness	89.4 (70.2) %
Redundancy	3.8 (3.0)
R merge	9.5 (22.9) %
Unit cell volume	1.49×10^6 Å^3
Crystal Vol./Protein Mass (Matthew constant, V_M)	2.52 Å^3/Dalton
Solvent content	51.20%

※The number in parenthesis is for the last resolution shell.

rod-shaped crystals (0.4×0.4×0.2 mm) were grown from a 20% PEG MME 550, 50 mM $CaCl_2$ in 0.1 M bis-Tris buffer (pH 6.5) in 2~3 days and diffracted to 3.0 Å resolution (Fig. 1). The crystallization condition is similar to that of the monoclinic crystal, which was obtained from a 10% polyehtylene glycol MME 550 solution in 0.05 M potassium phosphate buffer (pH 7.0) in 12 months.[8]

Data-collection statistics of all data sets collected are summarized in Table 1. Crystals diffracted to 3.0 Å resolution with an Rmerge of 9.5% and I/(I)= 26.7. The crystals belong to the orthorhombic space group $P2_12_12_1$ with cell parameters a=62.58 Å, b= 69.92 Å, and c=339.67 Å. Assuming a partial specific volume of 0.74 cm^3/g, the asymmetric unit of the crystal is estimated to contain three dimers of *Cs*28GST (molecular mass 28 kDa). That is, the crystal volume per protein mass, V_m[13] is 2.52 Å^3/dalton. This value corresponds to 51% of solvent contents. The correctness of the number of molecules in an asymmetric unit was further verified by a molecular replacement solution which is now in progress.

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