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Crystallization of 28 kDa *Clonochis sinesis* Glutathione S-transferase

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Abstract

A helminth glutathione S-transferase, 28 kDa isozyme from *Clonorchis sinensis* has been crystallized under several conditions. One of the crystals, grown from a 10% polyehtylene glycol MME 550 (PEG MME 5 K) solution in 0.05 M potassium phosphate buffer (pH 7.0), diffracts to 3.0 Å resolution, and belongs to monoclinic space group *C*2, with unit cell parameters $a = 95.83$ Å, $b = 63.82$ Å, $c = 235.09$ Å, and $\beta = 97.2^{\circ}$.

요 약

여러 조건 하에서 *Clonorchis sinensis*로부터 helminth glutathione S-transferase(28kDa isozyme)가 결정화되었다. 0.05 M potassium 완충 용액(pH 7.0) 내에서 10% polyehtylene glycol MME 550(PEG MME 5 K)에서 자라난 결정이 3.0 Å 분해능까지 회절하였다. 이 결정의 결정학적 자료는 다음과 같다 : 단사정계 공간 군 *C*2, $a = 95.83$ Å, $b = 63.82$ Å, $c = 235.09$ Å, $\beta = 97.2^{\circ}$.

1. Introduction

Glutathione S-transferases (GSTs), found mainly in the cytosol, are a family of enzymes involved in cellular detoxification of endogenous and exogenous chemicals by catalyzing the formation of glutathione (GSH, -Glu-Cys-Gly) conjugates.[1] They have also been implicated in a variety of resistance phenomena of cells to cancer chemotherapy agents, insecticides, herbicides, and microbial antibiotics (reviewed in reference #2).

GSTs have been grouped into at least seven classes, alpha, mu, phi, theta, sigma, kappa,[3] and zeta[4] based on a variety of criteria, including amino acid/nucleotide sequence, and immunological, kinetic and tertiary/quaternary structural properties.[2] Representative crystal structures are available for most classes and found to maintain typical overall fold, with two domains, a smaller N-terminal alpha/beta-domain and a larger C-terminal alpha-domain. Although GSTs share such common folding pattern, each structure has characteristic features, especially around the active site and at the inter-subunit interface.

It is believed that the detoxification role of the GSTs helps protect helminth parasites from host immune attack[5] and the anthelmintic compounds.[6] Consequently, parasite GSTs have long been regarded as attractive vaccine and drug targets due to the proposed role in detoxification. *Clonorchis sinensis* 28 kDa GST (*Cs*28GST), one of two distinct classes of helminth GSTs of Mr 26,000 and 28,000, was purified and cloned by Kang *et al.*[7] 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.[7] Recently, the three-dimensional structure of a *Schistosoma haematobium* 28 kDa GST (*Sh*28GST) had been determined and showed characteristic features of Tyr10 side chain that occupies two alternative positions.[8] The results might prove to be relevant for the design of specific antischistosomal drugs. *Cs*28GST and *Sh*28GST show relatively high sequence homology (42%) each other and share conserved amino acid residues in the

active site.

Here, we report a crystallization and preliminary X-ray analysis for the *Cs*28GST as a fist step towards determining the X-ray structure of the enzyme. The three-dimensional structure of *Cs*28GST might confirm the functional role of the Tyr residue of the active site as observed in the *Sh*28GST structure.

2. Materials and Method

2-1. Purification

*Cs*28GST was purified by using the procedure of Kang *et al.*[7] *E. coli* BL21 [DE3] cells with the pET23c(+) vector containing the coding region of *Cs*28GST with N-terminal flanking sequence of 20 amino acids were provided by Dr. Hong. The cells were grown in the broth medium containing 50 μg/m*l* ampicillin until the $O.D_{600}$ was about 0.7. Then, cells were induced with 1 mM IPTG and cultured at 37°C for 5 h. Harvested cells were suspended in 10 m*l* of 20 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 mM PMSF, and were disrupted by ultrasonication. Cell debris was removed by centrifugation at 14000 rpm for 30 min. The supernatant 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] by 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. Activity of the eluted fractions was assayed by the procedure of Habig *et al.* (1974). Active fractions were selected by analyzing SDS-PAGE gels visualized by Coomasie-Blue staining, and pooled. Protein concentration was determined by the method of Lowry *et al.*[10]

2-2. Crystallization

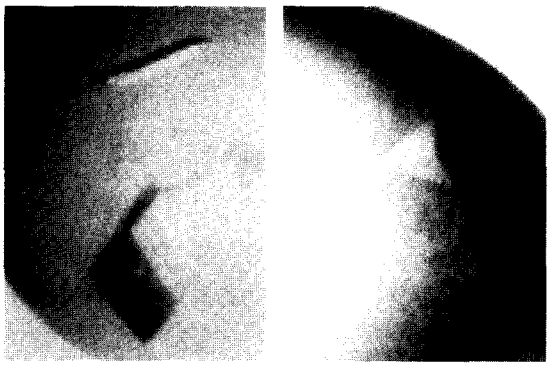
The pooled sample was dialyzed against 5 mM potassium phosphate buffer (pH 7.0), and then concentrated with a Centricon concentrator (Amicon corporation) to a final concentration of 15~20 mg/m*l*. Initial crystallization screening was performed by the sparse-matrix method[11] by the hanging-drop vapor diffusion method. 4 μ*l* of hanging drop, a 1 : 1 mixture of protein solution and reservoir solution, was equilibrated against 700 μ*l* of the reservoir solution at room temperature. Extensive attempts, including seeding techniques, were made to optimize conditions that yielded microcrystals. In order to confirm that protein in crystal retains the intact molecular size, crystals were carefully washed with the reservoir solution and dissolved in a phosphate buffer. The dissolved crystals were then analyzed by SDS-PAGE.

2-3. Preliminary X-ray diffraction analysis

X-ray diffraction experiments were carried out using CuK radiation on a DIP-2000 imaging plate detector (MAC Science Co.) mounted on a MAC Science M06XHF rotating anode X-ray generator running at 50 kV and 90 mA. Each frame of the data was measured using a 15-min exposure with 1.0 degree oscillation. Crystal-to-detector distance was set to 150 mm. The raw data were indexed, integrated, and scaled by using DENZO and SCALEPACK from the HKL package.[12]

3. Results and Discussion

Crystals of *Cs*28GST were obtained from a vari-



(a) (b)

Fig. 1. *Cs*28GST crystals grown from (a) a 0.05 M potassium phosphate buffer (pH 7.0) solution containing 12% PEG MME 550 (approximate dimensions 0.4 × 0.4 × 0.5 mm); (b) a 0.1 M Tris buffer (pH 8.5) solution containing 1.6 M ammonium sulfate and 12% glycerol (approximate dimensions 0.3 × 0.3 × 0.5 mm).

ety of conditions in early stages of screening trials. However, the crystals were too small for X-ray analysis or severely twinned. By seeding and refining the crystallization conditions, large single crystals were grown under several conditions as shown in Fig. 1. SDS-PAGE analysis of the protein sample used for crystallization and the dissolved crystals showed a strong band on the SDS-PAGE gel at approximately 28 kDa, indicating that crystals contain fully intact *Cs*GST.

Although the crystals grew to a relatively large size and had a clean morphology, diffraction quality was often poor. Bragg reflections were smeared out, often only in one region of the diffraction pattern. In addition, diffraction was often anisotropic with respect to resolution and mosaicity. To improve diffraction quality of the crystals, we have varied a variety of conditions, including equilibration of the crystal over a high or low salt-content reservoir, soaking in different precipitants and/or with solutions of high or low salt content, as well as varying the temperature, the pH of protein solution, and the protein concentration. After the extensive efforts exerted, we were able to determine cell parameters for crystals (0.4 × 0.4 × 0.5 mm) grown from a 10% polyehtylene glycol MME 550 (PEG MME 5 K) solution in 0.05 M potassium phosphate buffer (pH 7.0) in 12 months.

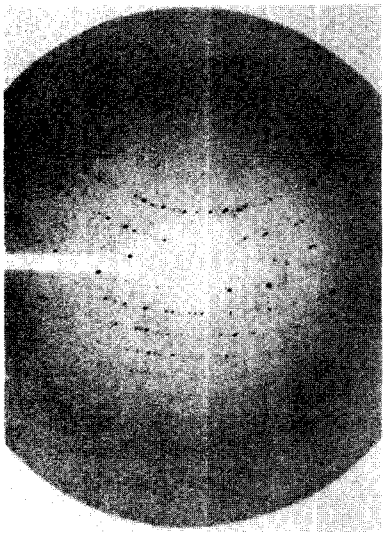


Fig. 2. Diffraction image from a DIP2000 imaging plate detector for a *Cs*28GST crystal.

The crystals diffracted to 3.0 Å resolution as observed in 15-min oscillation images recorded on a DIP2000 imaging plate system with CuK radiation (4.5 kW) (Fig. 2). X-ray diffraction data showed that the crystals belong to the monoclinic space group $C2$ with cell parameters $a = 95.83$ Å, $b = 63.82$ Å, $c = 235.09$ Å, and $\beta = 97.2°$. Assuming a partial specific volume of 0.74 cm^3/g, an asymmetric unit of the crystal is estimated to contain two or three dimers of *Cs*28GST (molecular mass 28 kDa): the crystal volume per protein mass, V_m,[13] is 3.2 $Å^3$/dalton for two dimers and 2.1 $Å^3$/dalton for three dimers. These values correspond to 62% and 42% of solvent contents, respectively.

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