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Crystal structures of 26 kDa *Clonorchis sinensis* glutathione S-transferase reveal zinc binding and putative metal binding

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ABSTRACT

The crystal structures of *Cs*GST in two different space groups revealed that Asp26 and His79 coordinate a zinc ion. In one space group, His46 of an adjacent molecule participates in the coordination within 2.0 Å. In the other space group, Asp26, His79 and a water molecule coordinate a zinc ion. The *Cs*GST–D26H structure showed that four histidine residues – His26 and His79 from one molecule and the same residues from a symmetry-related neighboring molecule – coordinate a zinc ion. The coordinated zinc ions are located between two molecules and mediate molecular contacts within the crystal.

1. Introduction

Glutathione S-transferases (GSTs) are well-known enzymes that play a key role in enzymatic detoxification. They catalyze the nucleophilic addition of the thiol group of glutathione to a wide variety of electrophilic endo- and xeno-substrates [1,2]. The glutathione-conjugated substrates are more water-soluble and can be easily discharged from cells. GSTs are ubiquitously found in wide range of bio-organisms from mammals to bacteria. Based on their cellular localization, GSTs can be divided into the following three subgroups: mitochondrial, microsomal, and cytosolic [3]. Cytosolic GSTs are classified into several classes on the basis of substrate specificity, sequence and structural similarity as follows: alpha, mu, pi, kappa, omega, and theta classes in mammals and sigma, zeta, beta classes, fungi, plants, insects, and helminths in non-mammals [4]. The structural and functional characteristics of a number of GSTs have been well studied. The three-dimensional structures of GSTs have been determined and revealed that GSTs exist as homo or hetero-dimers, and each monomer of GSTs is composed of two domains, a smaller N-terminal α/β domain and a larger C-terminal α-domain [1,5,6]. The N-terminal domain, in which GSH is bound, adopts a thioredoxin-like fold that consists of four β-strands and three α-helices. The C-terminal domain shares all α-helices and serves as the binding site for a range of substrates [5,6]. Although GSTs share this common folding pattern, the structures of each class have characteristic features, particularly concerning the substrate-binding site.

The crystal structures of some GSTs have been determined from the helminths *Schistosoma japonicum* (*Sj*GST), *Fasciola hepatica* (*Fh*GST) and *Necator americanus* (Na-GST) [7–11]. The *Sj*GST and the *Fh*GST are classified as mu class, but they lack the mu loop between the β2 strand and α2 helix that is characteristic of mammalian mu class structures. Enzymatic detoxification has been classified into three distinct phases in mammals. The cytochrome P-450 system is primarily responsible for phase I and turns xenobiotics into epoxide-containing compounds. GSTs play a major role in phase II detoxification by catalyzing the conjugation of endogenous GSH to the xenobiotics activated by phase I [4]. In helminths, because cytochrome P-450-dependent detoxification is absent, GSTs carry out most of the detoxification process [12–14]. Detoxifying enzymes have been considered as potential chemo- and immuno-therapeutic targets [15,16]. Inhibitors of *Sj*GST have been developed as anti-parasite agents [17].

*Sj*GST is widely used as a fusion partner in mammalian and bacterial expression systems [18]. It is reported that the human GST M1-1 and A1-1 isozymes have been altered to induce nickel-binding affinity by introducing adjacent histidine residues, and a glutamate to histidine mutation of *Sj*GST resulted in increased nickel-binding affinity [19–21].

In this study, we determined the crystal structures of *Clonorchis sinensis* 26 kDa GST (*Cs*GST) in complex with GSH. The structures are very similar to that of *Sj*GST and harbor a zinc ion in one mono-

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mer. Mutation of Asp26 to histidine alters zinc ion coordination and crystal packing. The substituted histidines participate in zinc coordination.

2. Material and methods

2.1. Cloning and site-directed mutagenesis

The coding gene for *Cs*GST was amplified by PCR using the forward primer 5′-GTCGAATTCCATGGCTCCCGTATTGGGCTAC-3′ and the reverse primer 5′-AACTCG AGGTTATTTCGGAGGAGCATCGC-CAC-3′ from the template described previously [23,22]. The PCR products were ligated into pET25b plasmids from which the pelB leader sequence had been removed and that were treated with the endonuclease *BamHI* and *XhoI*.

In order to mutate *Cs*GST Asp26 to His, the above construct was modified by site-directed mutagenesis with the forward primer 5′-CTGGAGTACGTCGGTCATAGTTACGAAGAAC AT-3′ and the reverse primer 5′-ATGTTCTTCGTAACTATGACCGACGTACTCCAG-3′ using the QuikChange site-directed mutagenesis kit (Stratagene) following the manufacturer's instructions.

2.2. Protein expression and purification

The *Escherichia coli* BL21(DE3) expression strain was transformed by the plasmids harboring recombinant *Cs*GST and the *Cs*GST–D26H mutant gene and cultured at 37 °C in LB media. Production of the recombinant protein was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to the culture medium at an A_{600} of 0.7 to a final concentration of 1 mM, and the cultures were held at 20 °C overnight. After harvesting by centrifugation, the cells were resuspended in 20 mM potassium phosphate buffer pH 7.0 and 5 mM EDTA and lysed by sonication. The supernatants were loaded onto a GSH affinity column and eluted with 15 mM GSH [22]. The eluted *Cs*GST and *Cs*GST–D26H mutant were applied to a Superdex 75 16/60 gel-filtration column (GE Healthcare) pre-equilibrated with 20 mM potassium phosphate buffer pH 7.0 and 100 mM NaCl. Fractions containing the *Cs*GST and *Cs*GST–D26H mutant were concentrated to 14 mg/ml for crystallization.

2.3. Crystallization, data collection and structure determination

One native crystal was obtained in the described method [22]. The other crystals and the *Cs*GST–D26H crystals were grown in 0.1 M Tris pH 8.5 solution containing 5 mM zinc sulfate and 2.0 M ammonium sulfate. These crystals were soaked in cryoprotectant solution containing glycerol at a final concentration of 18% (v/v) and flash cooled in the nitrogen stream at 100 K. X-ray diffraction data of *Cs*GST and *Cs*GST–D26H were collected using the ADSC Quantum 315r and Quantum210 CCD detector with synchrotron radiation at Pohang Accelerator Laboratory (PAL) beamline 4A and beamline 6C (Pohang, Korea). All data sets were integrated and scaled using HKL2000 [23] and iMosflm [24]. The structure of *Cs*GST was calculated by molecular replacement using MOLREP [25] and *Sj*GST (PDB ID: 1M9A) as the search model. The initial model was built with COOT [26] and refined with REFMAC5 [27]. A strong peak in the electron density was determined to be a zinc ion based on the metal ion geometry.

X-ray data collection and refinement statistics are provided in Table 1. The coordinates of two structures of *Cs*GST and the *Cs*GST–D26H mutant have been deposited in the RCSB Protein Data Bank with accession codes 3ISO, 4L5L and 4L5O, respectively.

3. Results

3.1. Structural features

*Cs*GST crystals were obtained from solutions containing 0.1 M MES pH 6.5, 5 mM zinc sulfate, and 10% PEG MME 550 and 0.1 M Tris pH 8.5, 5 mM zinc sulfate, and 2.0 M ammonium sulfate. The

Table 1
Data collection and refinement statistics.

	Native 1	Native 2	D26H
	Data collection		
Space group	$P2_12_12_1$	$P3_221$	$P3_121$
Unit cell parameters			
a, *b*, *c* (Å)	66.27, 67.45, 120.70	96.39, 96.39, 115.42	98.37, 98.37, 178.50
α, β, γ (°)	90, 90, 90	90, 90, 120	90, 90, 120
Wavelength	1.23985	1.00000	1.00000
Resolution (Å)	50.0–2.2	50.0–1.9	59.5–2.09
R_{merge}	0.052 (0.172)[a]	0.058 (0.130)	0.193 (1.928)
Completeness (%)	99.0 (98.5)	99.9 (100)	100 (99.9)
Redundancy	5.4 (4.4)	10.5 (10.2)	5.9 (5.7)
No. of unique reflections	28,185	49,367	60,028
$I/\sigma(I)$	19.2	20.0	7.2
	Refinement		
Resolution (Å)	50.0–2.2	50.0–1.9	50.5–2.09
R_{work}/R_{free}	0.188/0.253	0.179/0.226	0.201/0.249
No. of atoms			
Protein	3524	3540	5296
Ligand	40	40	60
Ion	26	42	32
Water	187	253	255
B-factors			
Protein	26.66 (A), 38.46 (B)	18.61 (A), 16.71 (B)	41.00 (A), 41.88 (B), 35.41 (C)
Ligand	36.89	19.50	44.15
Ion	41.05 (Zn)	22.77 (Zn)	53.19 (Zn)
	51.33 (MES)	46.14 (SO_4)	63.73 (SO_4)
Water	36.157	24.71	40.19
R.M.S. deviations			
Bonds (Å)	0.021	0.030	0.016
Bond angles (°)	2.019	2.187	1.771

[a] Values in parentheses refer to the highest-resolution shell.