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# Crystal structure of Omega-class glutathione transferase of the silkworm, *Bombyx mori*

Kohji Yamamoto[1,*], Mamoru Suzuki[2], Akifumi Higashiura[2], and Atsushi Nakagawa[2]
[1]Faculty of Agriculture, Kyushu University Graduate School, Fukuoka 812-8581, Japan
[2]Institute for Protein Research, Osaka University, Suita, 565-0871, Japan

## 1 Introduction

Glutathione transferases [GSTs, EC 2.5.1.18] catalyze glutathione (GSH) conjugation reaction, which is important for the defense to xenobiotics and oxidative stress [1]. There have been various GSTs, Alpha, Mu, Pi, Omega, Sigma, Theta and Zeta in mamals [2], whereas six GSTs have been found in insect; Delta, Epsilon, Omega, Sigma, Theta and Zeta [3]. We identified one of GSTs, belonging to a previously reported Omega-class GST of the silkworm, *Bombyx mori* (bmGSTO).

In this rport, we described X-ray crystal structure of this GST. Insect GSTs have been investigated, regarding to insecticide degradation. Our result would accelerate for obtaining structural knowledge on catalysis in bmGSTO.

## 2 Experiment

For preparation of recombibant bmGSTO, expression vector, pET11b harbaring bmGSTO cDNA was used. This vector was transformed into BL21 (DE3) *Escherichia coli*. The recombinant bmGSTO was overexpressed by addition of isopropyl-1-thio- -D-galactoside and purified to near homogeneity by using ammonium sulfate fractionation, ion-exchange chromatography and gel-filtration chromatography.

Crystallization with the purified bmGSTO was done at 20˚C with the sitting-drop vapor diffusion method. Data on bmGSTO structure was collected in PF BL-5A. The crystals were soaked in cryoprotectant solution with 25% (v/v) ethylene glycol, and freezed in a stream of nitrogen gas. The diffiraction data were processed using programs *DENZO* and *SCALEPACK* in HKL2000 package [4].

The crystal structure of bmGSTO was determined by the molecular replacement method using the program *MOLREP* [5] with human Omega-class GST (hGSTO1-1, PDB ID: 1EEM). The structure was refined using the program *PHENIX* [6] and *Coot* [7]. The stereo chemical quality of the final model was assessed using the program *MolProbity* [8]. The atomic structure of bmGSTO has been deposited in the Protein Data Bank (PDB ID: 3WD3).

## 3 Results and Discussion

The bmGSTO protein was crystallized in 0.2M potasium iodide and 20% PEG3350 (w/v) [9]. The space group is $P22_12_1$ with unit cell dimensions of $a$ = 75.86 Å, $b$ = 89.89 Å, and $c$ = 182.2 Å. The structural refinement against 2.5 Å resolution showed an $R_{work}$ and $R_{free}$ values are 20.8 and 25.6%, respectively.

bmGSTO monomer includes 10 -helices and 4 -strands. The structure can be separated into two domains including the N-terminal domain and the C-terminal domain. GSH is present in the active site located in the cleft between the two domains [10-12]. The active site can contain two subsites; the G-site and the H-site. The G-site, GSH-binding site, formed by the residues Cys38, Pro39, Tyr40, Arg43, Leu62, Lys65, Lys77, Val78, Glu91 and Ser92 of bmGSTO. Our results reveal that GSH tightly binds to this site of bmGSTO. The C-terminal domain includes the H-site, substrate-binding site. Variety of H-site structures of GSTs contribute to their different substrate specificities. Comparison between bmGSTO and hGSTO1-1 reveals that, in the sequence of bmGSTO, there are 5 residues, Cys38, Pro39, Arg188, Phe234 and Tyr238 correspond to those of hGSTO1-1 [11,12]. When we compared bmGSTO to another isozyme for human Omega-class GST (hGSTO2-2), we also found that the five residues of bmGSTO corresponded in the H-site of hGSTO2-2 (Phe31, Cys32, Pro33, Arg184, Tyr188, Phe223, Phe226, Leu227 and Tyr230) [11,12].

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* yamamok@agr.kyushu-u.ac.jp