Structural determination of GST of the silkworm, Bombyx mori.

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1 Introduction

Glutathione S-transferases (GSTs, EC 2.5.1.18) are detoxifying enzymes widely distributed in vertebrates, plants, insects, yeasts and aerobic bacteria. GSTs catalyze the conjugation of reduced glutathione (GSH) to hydrophobic substrates, for example, hormones, herbicides and insecticides [1]. Based on classification system, there have been seven classes of mammalian GSTs, alpha, mu, pi, omega, sigma, theta, and zeta [2]. In case of dipteran insects, six different GST classes, named delta, epsilon, omega, sigma, theta, and zeta [3], have been identified. We have characterized GSTs of the domesticated silkworm, Bombyx mori, a lepidopteran model insect [4,5]. In this study, we focus on a Sigma-class GST of the silkworm, Bombyx mori (bmGSTS). The physiological function of bmGSTS still remains unclear. A cDNA encoding this enzyme was sequenced and overproduced as a recombinant protein in Escherichia coli cells. To examine its biochemical and structural properties, it is important to obtain information regarding the three-dimensional crystalline structure of bmGSTS and the structure–function relationships in its catalytic reaction.

2 Experiment

(1) Crystallization and data collection
A cDNA encoding bmGSTS was inserted into expression vector, pET-11b, and transformed into BL21 (DE3) E. coli cells. Recombinant bmGSTS was purified and concentrated to approximately 10 mg/ml. Crystallization was carried out with the sitting-drop vapor diffusion method at 20 °C Data on crystal structure, obtained using NSRRC BL13B1&13C1 and PF-BL5A and SPring-8 BL44XU with crystals soaked in cryoprotectant solution containing 30% (v/v) glycerol, and cooled to 100 K in a nitrogen gas stream, were collected. The diffraction data were processed using the HKL2000 package.

(2) Structure determination
The crystal structure of bmGSTu was determined by the molecular replacement method using the program MOLREP. The structure was refined using the program REFMAC5 with diffraction data. The stereo chemical quality of the final model was evaluated by the program Coot, and the program PROCHECK.

3 Results and Discussion

The bmGSTS protein was crystallized in space group P6122. The bmGSTS crystal structure was determined at 1.9 Å. By soaking method, the crystal for GSH-bmGSTS complex was prepared. The GSH-bmGSTS complex structure was determined at 1.7 Å.

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References


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