Preliminary X-Ray Diffraction Study of Glutathione S-Transferase from *Pseudomonas* sp. DJ77

Heungsoo Choi, Jurang Woo, Junghee Lee, An-Sik Chung[1],
Seong-Eon Ryu[2], Youngchang Kim[3] and Yong Je Chung*

*Department of Biochemistry and [3]Department of Microbiology, Chungbuk National University, Cheongju 360-763,
[1]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon 305-701,
[2]Protein Engineering Research Group, Korea Research Institute of Bioscience and Biotechnology,
KIST, Taejon 305-600, Korea*

Abstract : A bacterial glutathione S-transferase from *Pseudomonas* sp. DJ77 has been crystallized. The crystals diffract to at least 2.3 Å resolution, and belong to the orthorhombic space group $P2_12_12_1$, with cell parameters a=97.4 Å, b=100.3 Å, and c=46.0 Å. There is one dimer molecule of *p*GST per crystallographic asymmetric unit, with the crystal volume per protein mass of 2.34 Å^3/dalton and a solvent content of about 47% (v/v).

Key words : crystal, glutathione S-transferase, X-ray diffraction

Glutathione S-transferases (GSTs; EC 2.5.1.18) are a group of enzymes that catalyze the conjugation of glutathione to a wide variety of electrophilic substrates. The resultant glutathione-conjugates are eventually eliminated from the organism indicating that GSTs are involved in cellular detoxification of xenobiotics and reactive endogenous compounds. As a consequence of such multifunctional properties, the GSTs are believed to be responsible for the development of the resistance of cells and organisms towards drugs, herbicides, antibiotics and pesticides (Timmerman, 1989; reviewed by Wilce and Parker, 1994). Cytosolic GSTs have been grouped into five classes, referred to as alpha, mu, phi, theta, and sigma (Mannervick *et al.*, 1985; Hiratsuka *et al.*, 1990; Ji *et al.*, 1995). Functional cytosolic GSTs are homo- or hetero-dimers composed of subunits with two domains, the smaller N-terminal domain and the larger C-terminal domain. Although they share a common structural feature, amino acid sequence identities between the GSTs in the different classes are considerably low (25-35%) (Wilce *et al.*, 1995). So far, a number of three dimensional structures of GSTs have been determined by X-ray crystallography including those of non-mammalian isozymes from *Schistosoma japonicum* (McTigue *et al.*, 1995), squid (Ji *et al.*, 1995), *Lucilia cuprina* (Wilce et

al., 1995), and *Arabidopsis thaliana* (Reinemer *et al.*, 1996). However, no crystal structures of bacterial GSTs have been reported. Recently, a bacterial GST from *Pseudomonas* (*p*GST) was purified and characterized by Jung *et al.* (1996). The *p*GST, a homodimer composed of 23 kDa subunits, is quite different in primary structure from cytosolic GSTs of which crystal structures have been determined; sequence identities are less than 30% (Wilce *et al.*, 1995). Therefore, *p*GST may be the first structure of a new class of GSTs. As the first step to determine a three-dimensional structure, *p*GST has been crystallized. We report here the results of the crystallization experiments.

Materials and Methods

Reagents

Glutathione-linked column matrix was prepared according to the procedure of Simons and Vander Jagt (1977) using epoxy-activated Sepharose 6B purchased from Pharmacia Co. Ltd.. Polyethylene glycol 400 (PEG 400) was purchased from Fluka. Reagent grade chemicals and buffers were purchased from Sigma.

Purification of *p*GST

*p*GST was purified by affinity chromatography using a glutathione-linked Sepharose 6B matrix (Jung *et al.*, 1996) from *E. Coli* strain XL1-BLUE which contains pSP13 plasmid carrying a GST gene from *Pseudomonas*

*To whom correspondence should be addressed.
Tel : 82-431-61-2311, Fax : 82-431-67-2306
E-mail : Chungyj@cbucc.chungbuk.ac.kr

sp. DJ77 (Shin, 1992). In brief, the *E. Coli* cells were grown in LB broth containing ampicillin (50 μg/ml) and tetracycline (10 μg/ml) at 37°C for 4-5 hours, and then isopropyl-β-D-thiogalactopyranoside (40 μg/ml) was added. After 8 h of induction, the cells were harvested by centrifugation and disrupted by sonication. The supernatant obtained from centrifugation at 12000 rpm for 20 min was loaded onto a glutathione-linked Sepharose 6B column without further treatment. The pGST was eluted with 15 mM glutathione solution (50 mM Tris-HCl, pH 9.6, 1 mM EDTA). Active fractions from the affinity column were identified by assaying enzyme activity of pGST by the procedure of Habig et al. (1974). Homogeneous fractions were selected by analyzing SDS-PAGE gels for the active fractions and were pooled. The pooled sample was dialyzed against 5 mM potassium phosphate buffer (pH 7.0), and then concentrated in a centricon tube (Amicon corporation) until the protein concentration reached 15-20 mg/ml. Protein purity was assessed by densitometry of SDS-PAGE gels loaded with dilutions of the concentrated sample and visualized by silver staining. Protein concentration was determined by the method of Lowry et al. (1951).

Crystallization

Initial crystallization conditions were found by the sparse matrix sampling method (Jancarik and Kim, 1991) using a hanging drop experiment with 24-well tissue culture plates (Linbro Division, Flow Laboratories Inc.). Hanging droplets, 4 μl of 1:1 mixture of protein solution (15 mg protein/ml in 5 mM potassium phosphate (pH 7.0)) and reservoir solutions, were equilibrated against 700 μl of reservoir solution in an incubator (20°C).

X-ray diffraction study

Crystals were mounted in thin-walled capillaries (φ=0.5 mm) containing a small amount of mother liquor and sealed with mineral oil to maintain appropriate humidity. Prior to mounting crystals, they were soaked in an artificial mother liquor which contained 58-60% saturated ammonium sulfate, 0.1 M HEPES (pH 7.0), 2% PEG-400, and 2.5 mM potassium phosphate. Diffraction data were collected at room temperature on an R-AXIS IIC phophoimaging plate system (Rigaku Corporation) using CuKα radiation from a Rigaku rotating anode X-ray generator operated at 40 kV and 80 mA. Each frame of the oscillation data was collected using a 15-minute exposure with a 1.2 degree oscillation. The data were indexed and processed using R-AXIS software. A self-rotation map was calculated using the program PO-LARRFN (Collaborative Computational Project No. 4, 1994) with data from 15-5 Å and a Patterson radius of 24 Å.

Results and Discussion

pGST was purified by affinity chromatography using a glutathione-linked Sepharose 6B matrix. About 600 mg of pGST has been prepared from 75 g of cells from 20 L of culture during the course of these studies. Silver stained SDS-PAGE gel for the purified enzyme showed a strong single band at approximately 23 kDa (data not shown), indicating that the protein sample is pure enough and suitable for subsequent crystallization experiments.

Initially, long rod-shaped small crystals of pGST were grown from 2.5 M ammonium sulfate solution (10 mM tris buffer, pH 8.0) for a week. However, these crystals were highly twinned and too small to be used for X-ray analysis. Seeding of the microcrystals did not give large single crystals. Therefore, we carried out further screening experiments to search for new crystallization conditions, and were able to prepare large crystals reproducibly (Fig. 1) at 20°C from an ammonium sulfate solution containing a low concentration of PEG 400. i.e. 50-55% saturated ammonium sulfate and 2% PEG-400 in 0.1 M HEPES buffered at pH 7.5. Under the condition, crystals appeared within 2 days, and grew up to 0.2×0.2×0.7 mm in about 5-7 days. PEG-400 as an additive significantly increased the reproducibility of nucleation process and decreased the degree of twinning. The addition of PEG-400 also greatly improved the size of the crystals. It is interesting to note that similar results were reported in the case of phosphoglucomutase crystallization (Ray and Puvathingal, 1986), i.e. that PEG-400 plays a catalytic role in the nucleation, and an indispensable role in long-term growth of large crystals. Crystals of pGST seemed to be relatively unstable in
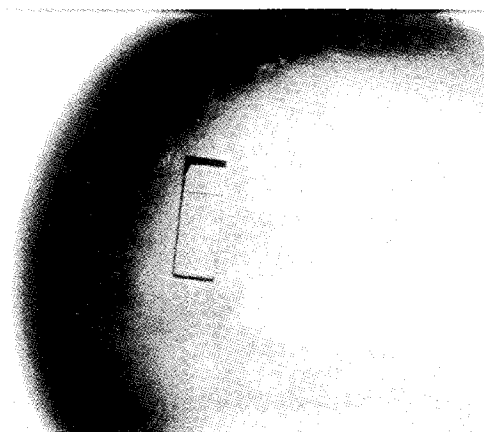

Fig. 1. Crystals of pGST, photographed using a Polaloid MicroCam SLR camera with X40 magnification. were grown from 50-55% saturated ammonium sulfate solution containing 2% PEG-400 in 0.1 M HEPES buffered at pH 7.5. The maximum dimensions of the crystals were 0.2×0.2×0.7 mm.

the mother liquor because they were often dissolved during observation. Therefore, it was necessary to have an appropriate artificial mother liquor for convenient handling and subsequent soaking of the crystals. We found that crystals were stable in the 58-60% saturated ammonium sulfate solution containing 0.1 M HEPES (pH 7.0), 2% PEG-400, and 2.5 mM potassium phosphate. In this case, the range of allowed concentration of ammonium sulfate was extremely narrow. Crystals were immediately cracked in higher concentrations of ammonium sulfate and dissolved in lower concentrations.

pGST crystals diffracted to at least 2.3 Å resolution as observed in 15 minute oscillation images recorded on a Rigaku image plate system using CuKα radiation (3 kW). Crystals soaked in the artificial mother liquor were stable enough to collect a complete data set from one crystal. X-ray diffraction data showed that the crystals belong to the orthorhombic space group $P2_12_12_1$, with cell parameters a=97.4 Å, b=100.3 Å, and c=46.0 Å. There is one dimer molecule of pGST per crystallographic asymmetric unit, with a crystal volume per protein mass (V_m) of 2.34 Å^3/dalton (Mathews, 1968). The solvent content of the crystal was estimated to be about 47% (v/v). In order to gain insight on the orientation of homodimers in the crystal, self-rotation function studies were carried out. However, the rotation map showed a few weak densities with a peak height of about 30% of the crystallographic 2-fold, indicating that they might not be significant enough to represent molecular orientation. Several data sets for heavy atom-soaked crystals were collected and crystallographic analysis is in progress.

Acknowledgement

References

Collaborative Computational Project No. 4 (1994) *Acta Crystallogr. D.* **50**, 760.

Habig, W. H., Pabst, M. J. and Jacoby, W. B. (1974) *J. Biol. Chem.*, **249**, 7130.

Hiratsuka, A., Sebata, N., Kawashima, K., Okuda, H., Ogura,. K., Watabe, T., Satoh, K., Hatayama, I., Tsuchida, S., Ishikawa, T. and Sato, K. (1990) *J. Biol. Chem.* **265**, 11973.

Jancarik, J. and Kim, S.-H. (1991) *J. Appl. Crystallogr.* **24**, 409.

Ji, X., von Rosenvinge, E. C., Johnson, W. W., Tomarev, S. 1., Piatigorsky, J., Amstron, R. N. and Gilliland, G. L. (1995) *Biochemistry* **34**, 5317.

Jung, U., Cho, Y.-S., Seong, H.-M., Kim, S.-J., Kim, Y.-C. and Chung, A.-S. (1996) *J. Biochem. Mol. Biol. (formerly Korean Biochem. J)* **29**, 1111.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265.

Mannervik, B., Alin, P., Guthenberg, C., Jensson, H., Tahr, M. K., Warholm, M. and Jornvall, H. (1985) *Proc. Natl., Acad. Sci. USA* **82**, 7203.

Marhews, B. W. (1968) *J. Mol. Biol.* **33**, 491.

McTigue, M. A., Williams, D. R. and Tainer, J. A (1995) *J. Mol. Biol.*, **246**, 21.

Ray, W. J. and Puvanthingal, J. M. (1986) *J. Biol. Chem.* **261**, 11544.

Reinemer, P., Prade, L., Hof, P., Neuefeind, T., Huber, R., Zettl, R., Palme, K., Schell, J., Koelln, I., Bartunik, H. D. and Bieseler, B. (1996) *J. Mol. Biol.* **255**, 289.

Shin, M.-S. (1992) M. S. Thesis, Chungbuk National University, Cheongju.

Simons, P. C. and vander Jagt, D. L. (1977) *Anal. Biochem.* **82**, 334.

Timmerman, K. P. (1989) *Physiol. Plant.* **77**, 465.

Wilce, M. C. J, Board, P. G., Feil, S. C. and Parker, M. W. (1995) *EMBO J.* **14**, 2133.

Wilce, M. C. J. and Parker, M. W. (1994) *Biochim. Biophys. Acta.*, **1205**, 1.