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Crystallization of agGST1-6, a recombinant glutathione *S*-transferase from a DDT-resistant strain of *Anopheles gambiae*

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Glutathione *S*-transferases (GSTs) belong to a family of detoxification enzymes that conjugate glutathione to various xenobiotics, thus facilitating their expulsion from the cell. GST activity is elevated in many insecticide-resistant insects, including the DDT-resistant malaria vector *Anopheles gambiae*. Crystals of the recombinant form of a GST from *A. gambiae*, agGST1-6, have been grown in at least five different crystal forms, with a broad range of diffraction resolution limits. A complete 2.0 Å data set has been collected on a *C*-centered orthorhombic crystal form with unit-cell parameters $a = 99.0$, $b = 199.4$, $c = 89.6$ Å. A search for heavy-atom derivatives has been initiated, along with phase-determination efforts by molecular replacement.

1. Introduction

Glutathione *S*-transferases (GSTs; E.C. 2.5.1.18) are a multigenic family of multifunctional proteins that, among other functions, conjugate glutathione to various drugs or pesticides, converting them from reactive lipophilic molecules into water-soluble non-reactive conjugates which may be easily excreted (Hayes & Pulford, 1995). Elevated levels of GST activity are associated with organochlorine and organophosphorus insecticide resistance in *Drosophila* and a number of important insect pests (Wang *et al.*, 1991; Grant *et al.*, 1991; Tang & Tu, 1994). In the malaria vector *A. gambiae* (Ranson *et al.*, 1997), elevated GSTs are associated with resistance to the organochlorine insecticide DDT. The mechanism of GST elevation in *A. gambiae* is upregulation of the expression of several different GST genes. This mosquito is the source of a new form of GST, agGST1-6, which has been cloned into an *Escherichia coli* expression system; pure homodimeric preparations of the protein have been shown to metabolize DDT and interact with a number of pyrethroid insecticides (Ranson *et al.*, 1997). Although the crystal structures of a number of GSTs and GST complexes have been determined, structures of any GSTs showing high amino-acid sequence homology to agGST1-6, including GSTD1 (67.5%) from *D. melanogaster* (Toung *et al.*, 1990) and mdGST-1 (68.8%) from *Musca domestica* (Fournier *et al.*, 1992), have yet to be published. The role of agGST1-6 in pesticide detoxification makes it an attractive molecule for structural analysis by X-ray diffraction. Here, we report on the crystallization of agGST1-6. A complete 2.0 Å data set has been collected on one of the crystal forms.

2. Materials and methods

2.1. Expression and purification

E. coli BL21(DE3)pLysS transformed with pXaggst1-6 (Ranson *et al.*, 1997) were grown at 310 K to an A_{600} of 0.6 and then induced with 0.4 m*M* isopropyl-β-D-thiogalactoside. After a further 3 h incubation, cells were harvested by centrifuging at 5000*g* for 5 min at 277 K. Pelleted cells were stored at 203 K until needed. After thawing at 277 K, the cells were resuspended in 50 m*M* Tris–HC1 pH 7.4, 1 m*M* EDTA, 10 m*M* β-mercaptoethanol and sonicated (Branson Sonifier 250) on ice. The sonicate was centrifuged for 80 min at 75 000*g* at 277 K (Beckman Avanti J25). The supernatant was dialyzed overnight against 50 m*M* Tris–HCl pH 7.4, 1 m*M* EDTA, 10 m*M* β-mercaptoethanol, 0.25 *M* sucrose, followed by a second overnight dialysis against 25 m*M* bis-tris methane pH 6.5, 0.2 *M* NaCl, 15 m*M* dithiothreitol. The enzyme was purified by affinity chromatography on an *S*-hexylglutathione Sepharose column as previously described (Prapanthadara *et al.*, 1993; Reddy *et al.*, 1983), eluting with 5 m*M* *S*-hexylglutathione in 25 m*M* bis-tris methane pH 6.5, 0.2 *M* NaCl, 15 m*M* dithiothreitol. This was followed by anion-exchange chromatography (BioRad Macro High Support Q Media) with a linear elution gradient of 0–1 *M* NaCl in 50 m*M* Tris–HCl pH 7.5, 10 m*M* dithiothreitol, 0.1 m*M* phenylmethylsulfonyl fluoride. The approximately 25 kDa recombinant agGST1-6 was purified to high homogeneity as demon-

strated by the presence of a single protein band on both SDS–PAGE and IEF gels (pI $\simeq$ 5.85; Amersham–Pharmacia Phast System) and activity was assayed with 1-chloro-2,4-dinitrobenzene as previously described (Habig *et al.*, 1974; Ranson *et al.*, 1997). The protein was concentrated using Amicon Centriprep 10 and Centricon 10 filters. Protein concentrations were estimated by absorbance at 280 nm using a calculated A_{280} of 0.763 for 1 mg ml^{-1} (http://paris.chem.yale.edu/extinct.html).

2.2. Crystallization

Initial screening was performed at room temperature (295.5 K) by hanging-drop vapor diffusion (McPherson, 1982) using sparse-matrix kits from Hampton Research (Jancarik & Kim, 1991; Hampton Research, Laguna Niguel, CA). Drops were prepared on siliconized cover slips and equilibrated against a 1 ml reservoir solution. Unless otherwise noted, screens were prepared by combining equal volumes of reservoir solution with protein at 10 mg ml^{-1}. Conditions producing crystals from the initial screens were refined to produce crystals suitable for analysis by X-ray diffraction. Specific details of these procedures are described further in §3.

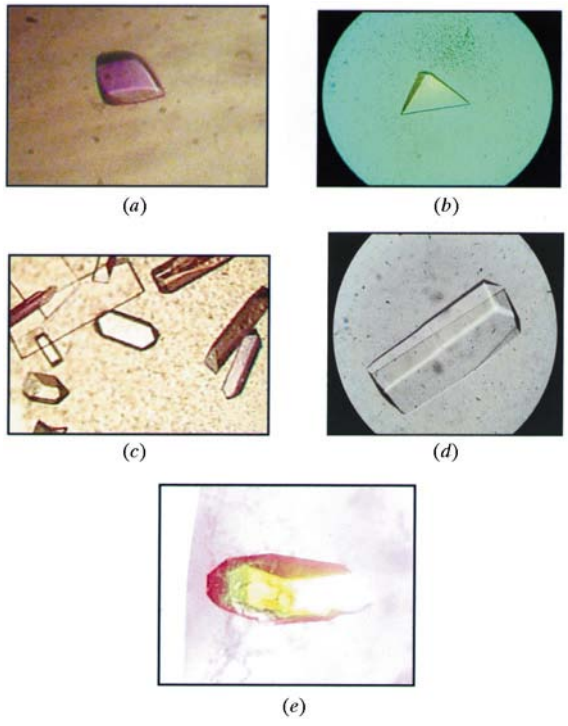

Figure 1
Photomicrographs of crystals of agGST1-6. See text for descriptions.

2.3. Data collection

After transferring to a cryoprotectant solution (either 10 µl of reservoir solution plus 2 µl glycerol or 25% PEG 3350, 50% saturated dextrose), crystals were picked up using a fiber loop and flash-frozen at 100 K in a stream of cold nitrogen gas. Diffraction data were collected on a Rigaku R-AXIS IV image-plate detector using Cu $K\alpha$ radiation from a Rigaku RU-300 rotating-anode X-ray generator operating at 50 kV and 100 mA. The programs *DENZO*, *SCALEPACK* (Otwinowski & Minor, 1997) and *CNS* (Brunger *et al.*, 1998) were used for data processing and analysis.

Table 1
X-ray diffraction data.

Values in parentheses refer to the highest resolution shell.

Space group	$C222$ or $C222_1$
Unit-cell parameters (Å,°)	a = 99.0, b = 199.4, c = 89.6, $\alpha = \beta = \gamma$ = 90
No. of observed reflections	1675261
No. of unique reflections	60199
Resolution range (Å)	25.00–2.00 (2.05–2.00)
$I/\sigma(I)$	22.4 (5.39)
Completeness (%)	97.3 (85.3)
$R_{\rm merge}$ (%)	8.4 (27.5)

3. Results and discussion

The number of conditions producing crystals from the initial crystallization screens were as follows: 13 conditions from Hampton Crystal Screen 1, ten conditions from Hampton Crystal Screen 2 and 16 conditions from an abbreviated version of the Hampton Low Ionic Strength Screen. Most of these crystals grew within 1 d to two weeks and were too small for diffraction studies. These conditions were refined to produce crystals in at least five different crystal forms.

The first crystal form (Fig. 1*a*), grown against a reservoir solution of 90% saturated $(NH_4)_2SO_4$, 0.05 *M* sodium citrate pH 4.4, belonged to the tetragonal system. These crystals had unit-cell parameters $a = b$ = 87, c = 105 Å and a maximum resolution of 8.0 Å.

Two different primitive orthorhombic crystal forms were produced. One (Fig. 1*b*) had unit-cell parameters a = 106, b = 116, c = 131 Å and a resolution limit of 2.8 Å. This crystal was grown over a reservoir of 40% saturated $(NH_4)_2SO_4$, 0.05 *M* citrate buffer pH 5.0. The second primitive orthorhombic crystal (Fig. 1*c*), with unit-cell parameters a = 50, b = 90, c = 100 Å, was grown over 30% PEG 4000, 10% 2-propanol, 0.1 *M* Tris–HCl pH 7.5. This crystal diffracted to 3.5 Å resolution.

Two *C*-centered orthorhombic crystal forms of agGST1-6 were grown. The first (Fig. 1*d*) was grown over a reservoir of 2% PEG 400, 2.0 *M* $(NH_4)_2SO_4$, 0.1 *M* HEPES pH 7.5 and had unit-cell parameters a = 55, b = 141, c = 155 Å. Although this form diffracted to 1.9 Å, the crystals were twinned and proved unsuitable for further analysis.

Another crystal form (Fig. 1*e*) was grown for 22 d in a hanging drop composed of 2 µl protein (9 mg ml^{-1}), 1 µl 0.05 *M* bis-tris pH 6.5, 2.5 µl 22% PEG 3350 equilibrated against a 1 ml reservoir of 24% PEG 3350. This crystal had approximate dimensions of 1.0 × 0.4 × 0.3 mm and diffracted X-rays to at least 1.8 Å. Diffraction data were collected at cryogenic temperature in a cryosolution of 25% PEG 3350 and 50% saturated dextrose. A complete data set to 2.0 Å was collected (Table 1) at a crystal-to-detector distance of 170 mm, an oscillation range of 0.5° covering 228° in φ and 20 min exposure per frame.

Autoindexing yielded unit-cell parameters of a = 99.01, b = 199.40, c = 89.57 Å, $\alpha = \beta = \gamma = 90°$ (Table 1). This crystal is *C*-centered orthorhombic and belongs to either space group $C222$ or $C222_1$. The $00l$ (l = even) zone reflections are markedly stronger than the l = odd reflections, but the presence of seven out of 20 $00l$ (l = odd) zone reflections with $I/\sigma(I) > 3.0$ suggests the proper space group may be $C222$. Data completeness and $I/\sigma(I)$ were 97.3% and 22.4, respectively, overall and 85.3% and 5.39, respectively, for the 2.05–2.00 Å shell. Overall $R_{\rm merge}$ of the data set was 8.4% and 27.5% for the 2.05–2.00 Å shell. Using the molecular weight of 49 476 Da for a homodimer and a crystal volume per asymmetric unit of 221 094 Å^3, assuming two dimers per asymmetic unit gives a typical protein Matthews coefficient $V_{\rm M}$ of 2.2 Å^3 Da^{-1} (Matthews, 1968) and a solvent content of 45% (Westbrook, 1985).

Structural analysis of agGST1-6 by molecular replacement using a number of low-

homology proteins (<35% identical) as search models is in progress. A search for heavy-atom derivatives of this protein has also been initiated.

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References

Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L, Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, N., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.

Fournier, D., Bride, J. M., Poire, M., Berge, J. B. & Plapp, F. W. (1992). *J. Biol Chem.* **267**, 1840–1845.

Grant, D. F., Dietze, E. C. & Hammock, B. D. (1991). *Insect Biochem.* **4**, 421–433.

Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974). *J. Biol. Chem.* **249**, 7130–7139.

Hayes, J. D. & Pulford, D. J. (1995). *Crit. Rev. Biochem. Mol. Biol.* **30**, 445–600.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

McPherson, A. (1982). *Preparation and Analysis of Protein Crystals.* New York: John Wiley.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Prapanthadara, L. Hemingway, J. & Ketterman, A. J. (1993). *Pest Biochem. Physiol.* **47**, 119–133.

Ranson, H., Prapanthadara, L. & Hemingway, J. (1997). *Biochem. J.* **324**, 97–102.

Reddy, C. C., Burgess, J. R., Gong, Z. Z., Massaro, E. J. & Tu, C. P. D. (1983). *Arch. Biochem. Biophys.* **224**, 87–101.

Tang, A. H. & Tu, C. P. D. (1994). *J. Biol. Chem.* **269**, 27876–27884.

Toung, Y.-P. S., Hsieh, T.-S. & Tu, C. P. D. (1990). *Proc. Natl Acad. Sci. USA*, **87,** 31–35.

Wang, J.-Y., McCommas, S. & Syvanen, M. (1991). *Mol. Gen. Genet.* **227**, 250–266.

Westbrook, E. M. (1985). *Methods Enzymol.* **114**, 187–196.