Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization of two glutathione S-transferases from an unusual gene family

Aaron J. Oakley,[a] Kanya Jirajaroenrat,[b] Thasaneeya Harnnoi,[b] Albert J. Ketterman[b] and Matthew C. J. Wilce[a]*

[a]Department of Pharmacology/Crystallography Centre, University of Western Australia and the Western Australian Institute for Medical Research, Nedlands WA 6907, Australia, and [b]Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakorn Pathom 73170, Thailand

Correspondence e-mail: mwilce@receptor.pharm.uwa.edu.au

Received 18 December 2000
Accepted 19 March 2001

Two glutathione S-transferase isozymes from the mosquito *Anopheles dirus* (AdGST1-3 and AdGST1-4) from an alternately spliced gene family have been expressed, purified and crystallized. The isozymes share an N-terminal domain derived from a single exon and C-terminal domains from unique exons. Despite the high level of sequence identity (64% overall), the two isozymes crystallize in different space groups, the 1-3 isozyme in $P3_121$ or $P3_221$ (unit-cell parameters $a = 49.9$, $c = 271.8$ Å at 100 K) and the 1-4 isozyme in $P4_1$ or $P4_3$ (unit-cell parameters $a = 87.8$, $c = 166.1$ at 100 K). Determination of these structures will advance our understanding of how these enzymes inactivate pesticides and the structural consequences of alternate splicing.

1. Introduction

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) are widely distributed in nature. Found in many bacteria and eukarya (Wilce & Parker, 1994), they play an important role in cellular detoxification. The most important reaction catalysed by GSTs is the nucleophilic addition of the thiol group of glutathione (γ-Glu-Cys-Gly; GSH) to compounds with electrophilic centres (Mannervik & Danielson, 1988). Glutathione conjugates are more water-soluble and act as a molecular marker, being selectively removed from the cells by specific pumps in the cell membrane (Hayes & McLellan, 1999).

Traditionally, GSTs have been divided into pi, alpha, mu and theta classes (Mannervik *et al.*, 1992). However, it has become apparent that this enzyme family is far more structurally and functionally diverse than previously thought. Recently, a number of extra classes has been proposed, including beta (Rossjohn *et al.*, 1998), delta (Toung *et al.*, 1993; Ketterman *et al.*, 2001), zeta (Board *et al.*, 1997), phi (*e.g.* Jepson *et al.*, 1994), sigma (Ji *et al.*, 1995), kappa (Pemble *et al.*, 1996) and Omega (Board *et al.*, 2000).

While mammalian GSTs have been well characterized structurally and biochemically, insect GSTs have not been comprehensively studied. Insect GSTs have been identified and reported in multiple forms from the house fly (Clark & Dauterman, 1982; Clark *et al.*, 1984; Motoyama & Dauterman, 1978), *Drosophila melanogaster* (Toung *et al.*, 1990, 1993), grass grub (Clark *et al.*, 1985) and mosquito (Grant & Matsumura, 1989; Prapanthadara *et al.*, 1993). Elevated GST levels have been detected in pesticide-resistant strains of insects and insect GSTs have been shown to break down pesticides (Prapanthadara *et al.*, 2000). Thus, the understanding of the structure and function of these enzymes is of considerable importance. Only one insect GST structure has been determined to date (Wilce *et al.*, 1995) in the absence of any pesticide or xenobiotic. AdGST1-3 and AdGST1-4 have 71 and 60% sequence identity with *Lucilia* GST, repectively (Fig. 1). The recombinant enzymes have high activities with 1-chloro-2,4-dinitrobenzene (CDNB) and detectable activity with 1,2-dichloro-4-nitrobenzene, but markedly low activity with ethacrynic acid and *p*-nitrophenethyl bromide (Jirajaroenrat *et al.*, 2001). DDT-dehydrochlorination performed by the alternatively spliced GSTs showed 2.80 and 3.72 nmol DDE formation per milligram of protein for AdGST1-3 and AdGST1-4, respectively (Jirajaroenrat *et al.*, 2001). We hope to utilize biochemical and structural information to understand how these GST attack such pesticides. Our interest also lies in understanding the effects of the unusual gene arrangement on structure and the mechanism of pesticide detoxification by these GSTs.

2. Materials and methods

2.1. Recombinant protein expression and purification

The recombinant proteins of AdGST1-3 and AdGST1-4 were cloned, overexpressed and purified as previously described (Jirajaroenrat *et al.*, 2001).

2.2. Crystallization

Using the hanging-drop vapour-diffusion method, initial screens of crystallization

Table 1
X-ray data-collection statistics for GST1-3.

Resolution shell	No. of reflections	Completeness (%)	R_{sym}	$I/\sigma(I)$
15.00–3.75	10024	79.0	0.042	26.4
3.75–2.99	8330	66.1	0.062	20.1
2.99–2.61	7447	59.1	0.068	16.2
2.61–2.37	8078	64.5	0.078	10.3
2.37–2.20	9928	79.0	0.120	5.1
2.20–2.07	10757	85.8	0.134	4.4
2.07–1.97	10652	84.7	0.155	4.3
1.97–1.88	10397	82.9	0.192	3.8
1.88–1.81	10555	83.9	0.208	3.3
1.81–1.75	10613	85.1	0.303	2.2
Overall	96781	77.0	0.073	9.4

conditions were carried out employing a sparse-matrix kit (Hampton Research, CA, USA).

Crystals of GST1-3 were observed in about 50% of the Crystal Screen I drops. The largest crystals grew in condition 10 (0.2 *M* ammonium acetate, 0.1 *M* sodium acetate pH 4.6, 30% PEG 4000 at room temperature). The optimized conditions consisted of 0.2 *M* ammonium acetate, 0.1 *M* sodium acetate pH 4.4, 25–40% PEG 4000. The final drops were made up of 2 µl GST1-3 at 12 mg ml^{-1} (preincubated for 2 h on ice with an equal volume of 10 m*M* GSH and 10 m*M* DDT) and 2 µl well solution. All crystals were grown at room temperature. The largest measured 0.5 × 0.1 × 0.1 mm, appearing as a thick rod. These crystals could be flash-frozen without the need for an additional cryoprotectant.

Crystals of GST1-4 were obtained using condition 28 of Crystal Screen I (0.2 *M* sodium acetate, 30% PEG 8000, 0.1 *M* sodium cacodylate pH 6.5 at room temperature). Optimum conditions for crystal growth were subsequently found with a solution containing 10–20% PEG 8000, 0.1 *M* sodium cacodylate pH 6.2 and 1% glycerol. The crystals were also improved when reduced glutathione was added to the drop. The final drops were made up of 2 µl GST1-4 at 14 mg ml^{-1}, 2 µl 10 m*M* GSH and 2 µl well solution; crystals were grown at room temperature. The presence of PEG 8000 in the crystallization buffer allowed the crystals to be cryofrozen without the need for any additional reagents. Crystals appeared after 4 d. The largest was 0.2 × 0.05 × 0.05 mm and appeared as fine rods.

2.3. Collection of crystallographic diffraction data

Diffraction data from crystals of GST1-3 and GST1-4 were collected at 100 K using a MAR Research image-plate detector and Cu $K\alpha$ radiation from a Rigaku rotating-anode generator operating at 40 kV and 100 mA equipped with focusing mirrors. The crystals were cooled using an Oxford cryocooler. 0.25° oscillations were used.

The X-ray data were processed with the *HKL* package (Otwinowski & Minor, 1997). Space-group identification utilized the programs *XDISPLAYF* and *SCALEPACK*. Data were reduced and merged using *DENZO* and *SCALEPACK*. The quality of the data are shown in Tables 1 and 2.

3. Results and discussion

Two closely related GSTs from *A. dirus* have been successfully crystallized under different conditions utilizing PEG as the main precipitant (PEG 4000 and 8000). The different space groups indicate that the crystal packing is different for the two enzymes.

Table 2
X-ray data-collection statistics for GST1-4.

Resolution shell	No. of reflections	Completeness (%)	R_{sym}	$I/\sigma(I)$
20.0–5.25	1640	97.3	0.034	29.5
5.25–4.18	1542	98.9	0.049	26.4
4.18–3.65	1522	99.0	0.063	21.3
3.65–3.32	1466	98.2	0.085	14.9
3.32–3.08	1448	97.2	0.119	10.3
3.08–2.90	1415	95.0	0.178	6.6
2.90–2.76	1366	92.3	0.230	4.5
2.76–2.64	1362	90.4	0.337	3.2
2.64–2.54	1261	85.7	0.410	2.4
2.45–2.45	1215	83.7	0.444	2.2
Overall	14237	93.9	0.077	13.1

For AdGST1-3, diffraction spots were observed to 2.4 Å. Processing in *DENZO* revealed the Bravis lattice to be hexagonal, with unit-cell parameters $a = b = 49.9$ Å, $c = 271.8$ Å. Attempts to reduce the data in *SCALEPACK* revealed the overall Patterson symmetry to be $P\bar{3}m1$. Using observations of systematic absences, the range of possible space groups was reduced to $P3_121$ or its enantiomorph $P3_221$. The resolution dependence and overall strength and completeness of the data are shown in Table 1.

For AdGST1-4, diffraction spots were observed to 1.6 Å. Processing with *DENZO* showed that the Bravis lattice was tetragonal, with unit-cell parameters $a = 87.8$, $c = 166.1$ Å at 100 K. *SCALEPACK* was used to demonstrate that the Patterson symmetry was $P4/m$. Analysis of systematic absences revealed the space group to be $P4_1$ or its enantiomorph $P4_3$. The resolution dependence and overall strength and completeness of the data are shown in Table 2.

It is anticipated that these GSTs will be solvable using the *Lucilia* GST structure (Wilce *et al.*, 1995) as a molecular-replacement model. These crystals will be used in soaking experiments with DDT and other pesticides. Together with enzymatic data, it is hoped that an understanding of how these important enzymes confer resistance to pesticides upon insects such as the mosquito *A. dirus*.

The structures of the GST isozymes will reveal information on how alternate gene splicing affects structure. The first 45 amino-acid residues, which comprise most of the glutathione-binding domain, are identical between the two isozymes, with all of the differences occurring at the C-terminus (Fig. 1). The structures of these enzymes will

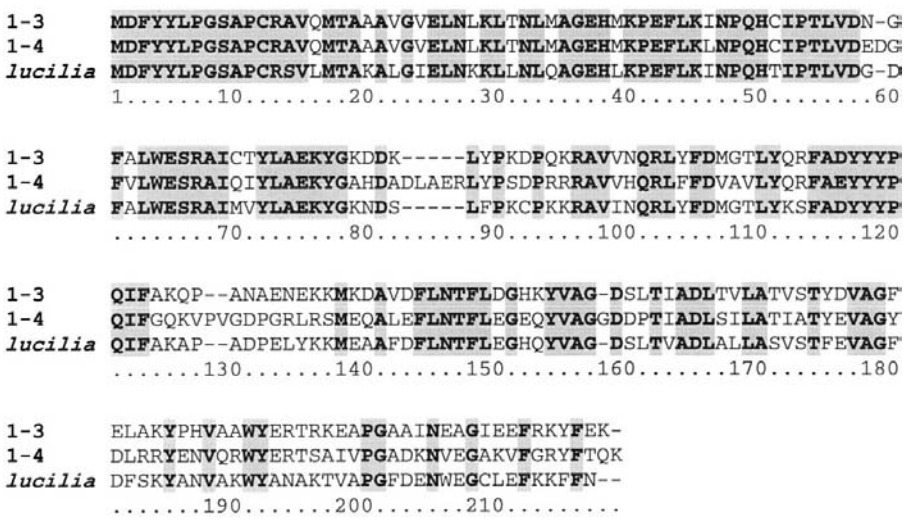

Figure 1
BLAST comparison of GST1-3 and GST1-4 from *A. dirus* with GST from *L. cuprina*. Regions with identical residues are shaded.

also aid in the identification of catalytic residues involved in pesticide degradation and the explanation of differences in catalytic activities of the isozymes.

This research is supported by a grant from the Thailand Research Fund (AJK) and a UWA/ARC Small Research Grant (AJO). AJO is a recipient of a UWA postdoctoral research fellowship. MCJW is supported by a Raine Medical Research Foundation Fellowship.

References

Board, P. G., Baker, R. T., Chelvanayagam, G. & Jermiin, L. S. (1997). *Biochem J.* **15**, 929–935.

Board, P. G., Coggan, M., Chelvanayagam, G., Easteal, S., Jermiin, L. S., Schulte, G. K., Danley, D. E., Hoth, L. R., Griffor, M. C., Kamath, A. V., Rosner, M. H., Chrunyk, B. A., Perregaux, D. E., Gabel, C. A., Geoghegan, K. F. & Pandit, J. (2000). *J. Biol. Chem.* **275**, 24798–24806.

Clark, A. G. & Dauterman, W. C. (1982). *Pestic. Biochem. Physiol.* **17**, 307–314.

Clark, A. G., Dick, G. L., Martindale, S. M. & Smith, J. N. (1985). *Insect Biochem.* **15**, 35–44.

Clark, A. G., Shamaan, N. A., Dauterman, W. C. & Hayaoka, T. (1984). *Pestic. Biochem. Physiol.* **22**, 51–59.

Grant, D. F. & Matsumura, F. (1989). *Pestic. Biochem. Physiol.* **33**, 132–143.

Hayes, J. D. & McLellan, L. I. (1999). *Free Radic. Res.* **31**, 273–300.

Jepson, I., Lay, V. J., Holt, D. C., Bright, S. W. & Greenland, A. J. (1994). *Plant Mol. Biol.* **26**(6), 1855–1866.

Ji, X., von Rosenvinge, E. C., Johnson, W. W., Tomarev, S. I., Piatigorsky, J., Armstrong, R. N. & Gilliland, G. L. (1995). *Biochemistry*, **34**(16), 5317–5328.

Jirajaroenrat, K., Pongjaroenkit, S., Krittanai, C., Prapanthadara, L. & Ketterman, A. J. (2001). In the press.

Ketterman, A. J., Prommeenate, P., Boonchauy, C., Chanama, U., Leetachewa, S., Promtet, N. & Prapanthadara, L. (2001). *Insect Biochem. Mol. Biol.* **31**, 65–74.

Mannervik, B., Awasthi, Y. C., Board, P. G., Hayes, J. D., Di Ilio, C., Ketterer, B., Listowsky, I., Morgenstern, R., Muramatsu, M., Pearson, W. R., Pickett, C. B., Sato, K., Widersten, M. & Wolf, C. R. (1992). *Biochem. J.* **282**, 3 05–306.

Mannervik, B. & Danielson, U. H. (1988). *Crit. Rev. Biochem. Mol. Biol.* **23**, 283–337.

Motoyama, N. & Dauterman, W. C. (1978). *Insect Biochem.* **8**, 337–348.

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

Pemble, S. E., Wardle, A. F. & Taylor, J. B. (1996). *Biochem. J.* **319**, 749–754.

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

Prapanthadara, L., Promtet, N., Koottathep, S., Somboon, P. & Ketterman, A. J. (2000). *Insect Biochem. Mol. Biol.* **30**, 395–403.

Rossjohn, J., Polekhina, G., Feil, S. C., Allocati, N., Masulli, M., Di Ilio, C. & Parker, M. W. (1998). *Structure*, **6**, 721–734.

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

Toung, Y. P., Hsieh, T. S. & Tu, C. P. (1993). *J. Biol. Chem.* **268**, 9737–46.

Wilce, M. C., Board, P. G., Feil, S. C. & Parker, M. W. (1995). *EMBO J.* **14**, 2133–2143.

Wilce, M. C. J. & Parker, M. W. (1994). *Biochim. Biophys. Acta*, **1205**, 1–18.