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Preliminary X-ray crystallographic studies of a newly defined human theta-class glutathione transferase

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Abstract

Human theta-class glutathione S-transferases (GST's) appear to play a critical role in the metabolism of a variety of environmental pollutants but in some cases the products of the reaction are carcinogenic. Crystals of a human theta-class GST, namely hGSTT2-2, have been grown from polyethylene glycol by the hanging-drop vapour-diffusion method. The crystals belong to the trigonal space group $P3_121$ with cell dimensions of $a = b = 94.0$ and $c = 120.5$ Å. They contain two monomers in the asymmetric unit and diffract to 3.0 Å resolution.

1. Introduction

Glutathione S-transferases (GST's, E.C. 2.5.1.18) are a supergene family of multifunctional enzymes that conjugate glutathione to a wide variety of electrophilic substrates (reviewed by Mannervik & Danielson, 1988). The conjugation increases the solubility of the target molecule thus facilitating the excretion of the molecule from the organism. Soluble GST's exist as dimers with a subunit molecular weight of about 25 kDa. They can be classified into six distinct families: alpha, kappa, mu, pi, sigma and theta based on studies of substrate specificity and primary structures (Mannervik *et al.*, 1992; Buetler & Eaton, 1992; Pemble *et al.*, 1996). The amino-acid sequence identities between any two members within a class is typically greater than 70% whereas the figure is typically less than 30% between classes. There are now representative crystal structures for five cytosolic GST classes. These include alpha-class GST's (Sinning *et al.*, 1993), mu-class GST's (Ji *et al.*, 1992; Raghunathan *et al.*, 1994; Lim *et al.*, 1994; McTigue *et al.*, 1995), pi-class GST's (Reinemer *et al.*, 1991, 1992; Dirr *et al.*, 1994; García-Sáez *et al.*, 1994), sigma-class GST (Ji *et al.*, 1995) and theta-like GST's (Wilce *et al.*, 1995; Reinemer *et al.*, 1996). The overall polypeptide fold is very similar between the crystal structures but each class exhibits unique features, particularly about the active site and at the C terminus (Wilce & Parker, 1994). GST's have been implicated in the development of the resistance of cells and organisms towards drugs, insecticides, herbicides and antibiotics and hence have been the subject of intense research over the last few years (for example, see Mannervik & Danielson, 1988; Wilce & Parker, 1994).

The mammalian theta-class family have been identified only recently in humans, rats and mice (Hiratsuka *et al.*, 1990, 1994; Meyer *et al.*, 1991; Hussey & Hayes, 1992; Mainwaring *et al.*, 1996; Whittington *et al.*, 1996). Theta-class GST's can utilize sulfate esters and dichloromethane as substrates and are suggested to be important in the prevention of hepatocarcinogenesis (Hiratsuka *et al.*, 1994). They also appear to play a critical role in the metabolism of industrial chemicals such as halogenated alkanes and aliphatic epoxides (Hiratsuka *et al.*, 1994). In some cases the resultant metabolite may become mutagenic and carcinogenic. For example, methylene chloride causes lung and liver cancer in mice *via* a glutathione metabolite (Mainwaring *et al.*, 1996). Because of their inability to bind to glutathione affinity matrices and the difficulties in their purification, the theta-class enzymes are the least studied of the GST enzyme superfamily. So far, two theta-class enzymes have been identified in human tissue: hGSTT1-1 (Pemble *et al.*, 1994) which promotes the conjugation of dihalomethanes to glutathione and hGSTT2-2 (Hussey & Hayes, 1992) which displays considerable glutathione peroxidase activity and also catalyzes the conjugation of certain arylsulfates to glutathione. The theta-class GST's have been proposed as the evolutionary forerunner of the alpha, mu, pi and sigma enzymes based on the apparent distribution of the former in a diverse range of organisms including bacteria, yeast, plants and insects (Pemble & Taylor, 1992; Buetler & Eaton, 1992).

A serine (or sometimes threonine) residue near the N-terminus probably plays an important role in the catalytic mechanism of all theta-class enzymes based on structure-based sequence alignments of theta-class GST sequences (Rossjohn *et al.*, 1996). We have targeted the conserved serine residue in hGSTT2-2 for mutagenesis and kinetic studies (Tan *et al.*, 1996). Mutating the equivalent serine (residue 11) to alanine, threonine or tyrosine abolished the enzyme's catalytic activity with the substrates cumene hydroperoxide and ethacrynic acid. However, with 1-menapthyl sulfate as substrate, the specific activity for the Ser11Ala mutant was doubled while the Ser11Thr mutant retained half the wild-type activity and the Ser11Tyr mutant had no activity. A detailed kinetic analysis suggested upon binding to substrate, the sulfate group is removed and the resultant carbonium ion reacts with the activated glutathione. Thus, hGSTT2-2 displays a novel sulfatase activity.

We are pursuing structural studies of the human theta-class enzyme for a number of reasons. It forms part of an on-going study to understand the molecular basis for catalysis of an important enzyme superfamily (Reinemer *et al.*, 1992; Wilce *et al.*, 1995). The work will shed light on the evolution of catalytic function in the family and lead to an increased understanding of the molecular basis for the remarkable range of substrates

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recognised by the enzymes. We are particularly interested in understanding the molecular basis of the sulfatase activity exhibited by hGSTT2-2. Finally, the structure will provide the basis for a structure-based design approach to inhibitors of the enzyme.

2. Methods

2.1. *Purification*

The overexpression and purification of the enzyme has been described in detail elsewhere (Tan & Board, 1996; Tan *et al.*, 1996). Briefly, wild-type protein was overexpressed in *E. coli* and purified by immobilized metal ion chromatography making use of a six-histidine-residue tag at the amino-terminal end of the expressed protein. The purity of the enzyme was checked by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Coomassie Blue staining. Although the enzyme expressed in this manner has an N-terminal extension of 16 residues (MRGSHHHHHHGSVPRG), the purified enzyme exhibits high theta-class GST activity in standard assays.

2.2. *Crystallization*

Crystallization was performed by the hanging-drop vapor-diffusion method (McPherson, 1982) using 24-well tissue-culture plates. A 2 µl droplet containing 1.76 mg ml^{-1} protein in 10 m*M* sodium phosphate buffer, pH 7.0 and 1 m*M* β-mercaptoethanol was mixed with an equal volume of reservoir solution (as described below). The protein concentration was lower than that used to crystallize other GST's (normally greater than 5 mg ml^{-1}) but attempts to further concentrate the protein led to large losses with the protein coming out of solution. Each well contained 1 ml of reservoir solution. Initial crystallization trials were performed using a screen similar to the one described by Jancarik & Kim (1991) and with Crystallization Kits I and II from Hampton Research (California, USA). The trials were carried out at a constant temperature of either 277 or 295 K.

2.3. *X-ray crystallography*

The X-ray diffraction data for wild-type protein crystals were collected on the beamline 6A2 at the synchrotron radiation source of the Photon Factory (Tsukuba, Japan). The wavelength was set to 1.0 Å and the data were measured at 11 K. The protocol for flash freezing the crystals involved transferring them to artificial mother liquor containing 10% glycerol and after 5 min transferring the crystals to 20% glycerol for a further 5 min, and then a final transfer to a solution containing 30% glycerol. The data were collected with image plates using the rotation method with 2.0° oscillations. Determination of unit-cell parameters, space group and integration of reflection intensities were performed using *DENZO* (Otwinowski, 1993) and the data were scaled with *SCALEPACK* (Otwinowski, 1993).

3. Results and discussion

Rod-shaped crystals of human GSTT2-2 appeared within 10 d using either polyethylene glycol 4000 or ammonium sulfate as a precipitant (Fig. 1). The optimal reservoir conditions consists of 15%(*v*/*v*) PEG 4000, 2 m*M* reduced glutathione, 2%(*v*/*v*) ethanol and 100 m*M* HEPES buffer, pH 7.0. The crystals reach maximum size of about 0.9 × 0.05 × 0.05 mm in two months. The optimal temperature is 295 K. Although the best crystals grow in the presence of glutathione, smaller crystals of identical habit grow in the absence of substrate. Attempts were made to improve the crystal size by changes in buffer, varying the temperature, micro- and macroseeding, the presence of various substrates and inhibitors, and the use of various additives such as alcohols, organic solvents and detergents. None of these trials led to significant increases in the size of the crystals.

Table 1. *Statistics of data collection*

Resolution range (Å)	R_{merge} (%)	$I/\sigma(I)$	Completeness (%)
200.00–6.46	3.5	21.6	77.0
6.46–5.13	8.5	10.0	89.8
5.13–4.48	7.7	11.1	91.6
4.48–4.07	9.2	8.5	92.3
4.07–3.78	12.8	6.5	91.4
3.78–3.56	19.0	4.4	88.2
3.56–3.38	30.4	2.8	87.6
3.38–3.23	40.3	2.0	87.4

The crystals diffracted too weakly and to too low a resolution (about 4 Å) on our in-house rotating-anode source and hence synchrotron radiation was essential for this project to proceed. The autoindexing procedure of *DENZO* (Otwinowski, 1993) indicated that the crystals belong to the trigonal crystal system, with unit-cell dimensions of $a = b = 94.0$ and $c = 120.5$ Å. Analysis of the various data, including a search for systematic absences, showed the data were consistent with the space group $P3_121$ (or its enantiomorph $P3_221$). The unit-cell volume is consistent with either two or three monomers in the asymmetric unit, yielding V_m values of 2.6 or 1.7 Å^3 Da^{-1}, respectively, values which fall within the normal range observed for protein crystals (Matthews, 1968). Fresh crystals diffracted to approximately 3.0 Å resolution. We were able to collect a 88.1% complete data set to 3.2 Å resolution off a single crystal with an R_{merge} of 12.4% (Table 1). We have obtained a preliminary molecular-replacement solution using a model of an insect theta-like GST (Wilce *et al.*, 1995). The solution was far from

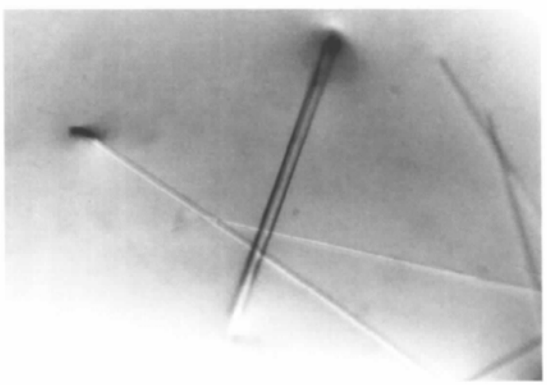

Fig. 1. Photograph of crystals of human theta-class glutathione transferase hGSTT2-2. The largest crystal is 0.9 mm in its longest dimension. See the text for detailed crystallization conditions.

trivial as the sequence identity between the human and insect enzymes was about 23% and a successful solution required the omission of all loops and conversion of the insect sequence to polyalanine sequence. The space group is confirmed as $P3_121$ and the asymmetric unit contains the physiological GST dimer. The human theta-class sequence is currently being built into electron-density maps based on the molecular-replacement phases.

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References

Buetler, T. M. & Eaton, D. L. (1992). *Environ. Carcinogen Ecotoxicol. Rev. C*, **10**, 181–203.

Dirr, H., Reinemer, P. & Huber, R. (1994). *J. Mol. Biol.* **243**, 72–92.

García-Sáez, I., Párraga, A., Phillips, M. F., Mantle, T. J. & Coll, M. (1994). *J. Mol. Biol.* **237**, 298–314.

Hiratsuka, A., Okada, T., Nishiyama, T., Fujikawa, M., Ogura, K., Okuda, H., Watabe, T. & Watabe, T. (1994). *Biochem. Biophys. Res. Commun.* **202**, 278–284.

Hiratsuka, A., Sebata, N., Kawashima, K., Okuda, H., Ogura, K., Watabe, T., Satoh, K., Hatayama, I., Tsuchida, S., Ishikawa, T. & Sato, K. (1990). *J. Biol. Chem.* **265**, 11973–11981.

Hussey, A. J. & Hayes, J. D. (1992). *Biochem J.* **286**, 929–935.

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

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

Ji, X., Zhang, P., Armstrong, R. N. & Gilliland, G. L. (1992). *Biochemistry*, **31**, 10169–10184.

Lim, K., Ho, J. X., Keeling, K., Gilliland, G. L., Ji, X., Rüker, F. & Carter, D. C. (1994). *Protein Sci.* **3**, 2233–2244.

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

McTigue, M. A., Williams, D. R. & Tainer, J. A. (1995). *J. Mol. Biol.* **246**, 21–27.

Mainwaring, G. W., Nash, J. A., Davidson, M. & Green, T. (1996). *Biochem. J.* **314**, 445–448.

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**, 305–308.

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

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

Meyer, D. J., Coles, D. J., Pemble, S., Gilmore, K. S., Fraser, G. M. & Ketterer, B. (1991). *Biochem. J.* **274**, 409–414.

Otwinowski, Z. (1993). *Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.

Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B. & Taylor, J. B. (1994). *Biochem. J.* **300**, 271–276.

Pemble, S. E. & Taylor, J. B. (1992). *Biochem. J.* **287**, 957–963.

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

Raghunathan, S., Chandross, R. J., Kretsinger, R. H., Allison, T. J., Penington, C. J. & Rule, G. S. (1994). *J. Mol. Biol.* **238**, 815–832.

Reinemer, P., Dirr, H. W., Ladenstein, R., Huber, R., Lo Bello, M., Federici, G. & Parker, M. W. (1992). *J. Mol. Biol.* **227**, 214–226.

Reinemer, P., Dirr, H. W., Ladenstein, R., Schäffer, J., Gallay, O. & Huber, R. (1991). *EMBO J.* **10**, 1997–2005.

Reinemer, P., Prade, L., Hof, P., Neuefeind, T., Huber, R., Zettl, R., Palme, K., Schell, J., Koelln, I., Bartunik, H. D. & Bieseler, B. (1996). *J. Mol. Biol.* **255**, 289–309.

Rossjohn, J., Board, P. G., Parker, M. W. & Wilce, M. C. J. (1996). *Protein Eng.* **9**, 327–332.

Sinning, I., Kleywegt, G. J., Cowan, S. W., Reinemer, P., Dirr, H. W., Huber, R., Gilliland, G. L., Armstrong, R. N., Ji, X., Board, P. G., Olin, B., Mannervik, B. & Jones, T. A. (1993). *J. Mol. Biol.* **232**, 192–212.

Tan, K.-L. & Board, P. G. (1996). *Biochem. J.* **315**, 727–732.

Tan, K.-L., Chelvanayagam, G., Parker, M. W. & Board, P. G. (1996). *Biochem. J.* **319**, 315–321.

Whittington, A. T., Webb, G. C., Baker, R. T. & Board, P. G. (1996). *Genomics*, **33**, 105–111.

Wilce, M. C. J., 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.