SHORT COMMUNICATIONS

Acta Cryst. (1996). D52, 189–191

Crystallization and preliminary X-ray analysis of a bacterial glutathione transferase

SUSANNE C. FEIL,[a] MATTHEW C. J. WILCE,[a]† JAMIE ROSSJOHN,[a] NERINO ALLOCATI,[b] ANTONIO ACETO,[c] CARMINE DI ILIO[c] AND MICHAEL W. PARKER[a] *at* [a]*The Ian Potter Foundation Protein Crystallography Laboratory, St Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia,* [b]*Istituto di Medicina Sperimentale, and* [c]*Istituto di Scienze Biochimiche, Facoltà di Medicina e Chirurgia, Università 'G. D'Annunzio', 66100 Chieti, Italy*

(Received 12 June 1995; accepted 12 July 1995)

Abstract

Crystals of a bacterial glutathione S-transferase from *Proteus mirabilis* have been grown from polyethylene glycol by the hanging-drop vapour-diffusion method. Successful crystallization required the presence of the substrate glutathione. The crystals belong to the tetragonal space group $P4$ with cell dimensions $a = b = 90.9$ and $c = 117.3$ Å. They contain between three and six monomers in the asymmetric unit and diffract to beyond 2.3 Å resolution.

1. Introduction

Glutathione S-transferases (GST's, E.C. 2.5.1.18) are a ubiquitous family of multifunctional enzymes that conjugate electrophilic substrates to the tripeptide glutathione (GSH) (reviewed by Mannervik & Danielson, 1988). Their substrates include a wide number of exogenous and endogenous hydrophobic electrophiles. The conjugation increases the solubility of the target molecule thus facilitating the excretion of the molecule from the organism. Cytosolic GST's exist either as homo- or heterodimers with a subunit molecular weight of about 25 kDa. They can be classified into five distinct families: alpha, mu, pi, sigma and theta based on studies of substrate specificity and primary structures (Mannervik *et al.*, 1992; Buetler & Eaton, 1992). 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. 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).

There are now representative crystal structures for all five cytosolic GST classes. These include alpha-class GST's (Sinning *et al.*, 1993), mu-class GST's (Ji, Zhang, Armstrong & Gilliland, 1992; Raghunathan *et al.*, 1994; Lim *et al.*, 1994; McTigue, Williams & Tainer, 1995), pi-class GST's (Reinemer *et al.*, 1991; Reinemer *et al.*, 1992; García-Sáez, Párraga, Phillips, Mantle & Coll, 1994), sigma-class GST (Ji *et al.*, 1995) and theta-class GST (Wilce, Board, Feil & Parker, 1995). 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).

The presence of GST's in bacteria has only recently been discovered and hence little is known about their properties. They have been detected in *Escherichia, Proteus, Pseudomonas, Klebsiella, Enterobacter* and *Serratia* species (Di Ilio *et al.*, 1988; Iizuka, Inoue, Murata & Kimura, 1989; Arca, García, Hardisson & Surez, 1990). The best characterized bacterial GST is one from *Proteus mirabilis* which is a homodimer of 23 kDa subunit molecular weight (Di Ilio *et al.*, 1988). It displays distinct kinetic and immunological properties and exhibits activity towards a range of compounds that are not substrates for the mammalian enzymes. It shares high sequence identities with a number of other bacterial GST's but possesses less than 20% sequence identity with the mammalian GST's (Mignogna *et al.*, 1993; Wilce & Parker, 1994). It has been classified as a theta-class GST based on limited sequence homology with its mammalian counterpart (Hiratsuka *et al.*, 1990; Meyer *et al.*, 1991; Pemble & Taylor, 1992). The theta-class GST's have been proposed as the evolutionary forerunner of the alpha, mu and pi 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). The presence of GST's in bacteria is thought to reflect the evolution of a number of glutathione-dependent enzymes which evolved in response to the toxic by-products of oxygen metabolism (Pemble & Taylor, 1992). In addition to detoxification reactions, bacterial GST's appear to have been recruited for other roles including the degradation of lignin (Masai *et al.*, 1993) and dichloromethane utilization (La Roche & Leisinger, 1990).

Mutagenesis studies have shown that a conserved tyrosine near the N-terminus is responsible for activation of glutathione by promoting thiolate formation (Wilce & Parker, 1994, and references therein). This residue is in close proximity to the S atom of glutathione in all the crystal structures of mammalian GST's (Reinemer *et al.*, 1991; Reinemer *et al.*, 1992; Ji *et al.*, 1992; Sinning *et al.*, 1993; Raghunathan *et al.*, 1994; García-Sáez *et al.*, 1994). Surprisingly, the equivalent residue in the theta-class insect enzyme was found not to be in the active site but its role appears to have been replaced by a serine residue (Wilce, Board, Feil & Parker, 1995). Such a role for the serine residue has now been confirmed by mutagenesis (Board, Coggan, Wilce & Parker, 1996). Recently, the conserved tyrosine near the N-terminus in the *E. coli* GST was shown by mutagenesis not to be essential for catalysis (Nishida, Kong, Inoue & Takahashi, 1994).

We are pursuing structural studies of the bacterial enzyme for a number of reasons. Although the sequence identities between the GST's from bacteria and from other organisms

† Present address: c/o Larapinta, Pacific Ocean.

are very low, it is expected that the three-dimensional structures will be similar based on the structural results of mammalian GST isoenzymes of equally low sequence identity. The availability of crystal structures from such diverse organisms will aid in pinpointing those residues responsible for determining the GST polypeptide fold and help explain the enzyme's remarkable tolerance towards substrates of diverse structure. We wish to clarify the role of the conserved tyrosine and serine residues near the N-terminus of the bacterial enzyme. It has been shown that bacterial GST's can bind to a range of antibiotics (Piccolomini *et al.*, 1989) and can reduce the antimicrobial activity of β-lactam drugs (C. Di Ilio, unpublished results). Hence, bacterial GST's may be a novel target for the design of inhibitors that could increase the effectiveness of antibiotic treatment.

2. Experimental

The purification of this enzyme has been described in detail elsewhere (Di Ilio *et al.*, 1988). Briefly, washed cells of *P. mirabilis* are grown aerobically for 18 h at 310 K in Trypticase Soy Broth followed with disruption by sonication. After centrifugation, the supernatant is applied to a glutathione–Sepharose affinity column. Fractions showing GST activity are pooled, followed by preparative isoelectric focusing and a final dialysis step against phosphate buffer. The purified enzyme is active towards a number of known eukaryotic GST substrates and is homogeneous as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Di Ilio *et al.*, 1988).

Crystallization was performed by the hanging-drop vapor-diffusion method (McPherson, 1982) using 24-well tissue-culture plates. A 2 µl droplet containing 5 mg ml^{-1} protein in double-distilled water was mixed with an equal volume of reservoir solution (as described below). 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). The trials were carried out at a constant temperature of 295 K.

The X-ray diffraction data were collected on the X-11 beamline at the European Molecular Biology Laboratory Outstation, Deutsche Electronen Synchrotron (DESY), Hamburg, Germany. The wavelength was set to 0.93 Å and the data were measured at room temperature. The data were collected with a MAR Research imaging-plate scanner using the rotation method with 0.5° oscillations. Determination of unit-cell parameters, space group and integration of reflection intensities were performed using *DENZO* (Otwinowski, 1993) and the data scaled with *SCALEPACK* (Otwinowski, 1993).

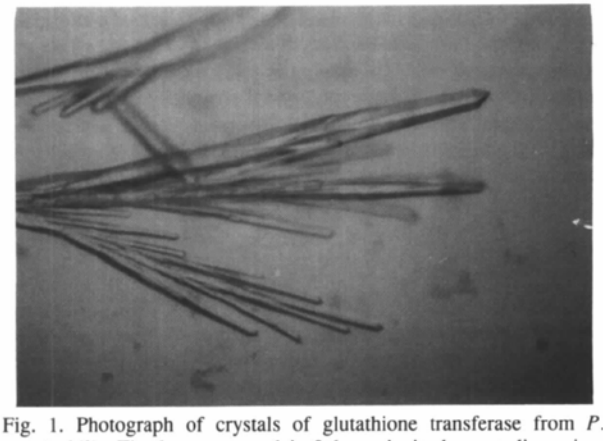

Fig. 1. Photograph of crystals of glutathione transferase from *P. mirabilis*. The largest crystal is 0.6 mm in its longest dimension. See the text for detailed crystallization conditions.

3. Results and discussion

Rod-shaped crystals appeared within 6 d using polyethylene glycol as a precipitant (Fig. 1). Two criteria had to be met for successful crystallization. Firstly, the phosphate buffer present in the protein solution from the purification procedure had to be removed by dialysis prior to the crystallization trials. Secondly, the presence of reduced glutathione (one of the enzyme's substrates) was found essential. The optimal reservoir conditions consists of 22–28%(*w*/*v*) PEG 8000, 10 m*M* reduced glutathione and 100 m*M* HEPES buffer, pH 6.5 to 7.3. The crystals grow with maximal dimensions 0.2 × 0.2 × 0.6 mm.

The autoindexing procedure of *DENZO* (Otwinowski, 1993) indicated that the crystals belong to the tetragonal crystal system, with unit-cell dimensions of $a = b = 90.9$ and $c = 117.3$ Å. Analysis of the various data, including a search for systematic absences, showed the data were consistent with the space group $P4$. The unit-cell volume is consistent with between three and six monomers in the asymmetric unit, yielding V_m values of between 1.8 and 3.5 Å^3 Da^{-1}, values which fall within the normal range observed for protein crystals (Matthews, 1968). Fresh crystals diffracted to approximately 2.3 Å resolution. We were able to collect an 86% complete data set to 2.7 Å resolution off a single crystal with an R_{sym} of 14.7%. We are planning to determine the structure of the *P. mirabilis* GST by the method of molecular replacement using our model of the insect theta-class GST (Wilce, Board, Feil & Parker, 1995). Should the bacterial GST prove to be too dissimilar to the insect model (and to the other available GST models) we will solve the structure by the method of multiple isomorphous replacement.

We appreciate the help given to us by Dr Zbigniew Dauter during our visit to the Hamburg synchrotron facilities. Financial support from the Australian National Beamline Facility (ANBF) for our visit is gratefully acknowledged. The ANBF is funded by a consortium comprising the ARC, DITARD, ANSTO, CSIRO, ANU and UNSW. MWP is a Wellcome Australian Senior Research Fellow and acknowledges support from the Australian Research Council. JR is a recipient of a Royal Society Fellowship.

References

Arca, P., García, P., Hardisson, C. & Surez, J. E. (1990). *FEBS Lett.* **263**, 77–79.

Board, P. G., Coggan, M., Wilce, M. C. J. & Parker, M. W. (1996). *Biochem. J.* In the press.

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

Di Ilio, C., Aceto, A., Piccolomini, R., Allocati, N., Faraone, A., Cellini, L., Ravagnan, G. & Federici, G. (1988). *Biochem J.* **255**, 971–975.

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

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.

Iizuka, M., Inoue, Y., Murata, K. & Kimura, A. (1989). *J. Bacteriol.* **171**, 6039–6042.

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

Ji, X., von Rosenvinge, E. C., Johnson, W. W., Toamrev, S. I., Pitigorsky, 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.

La Roche, S. D. & Leisinger, T. (1990). *J. Bacteriol.* **172**, 164–171.

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.

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., Widerstern, M. & Wolf, C. D. (1992). *Biochem. J.* **282**, 305–308.

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

Masai, E., Katayama, Y., Kawai, S., Nishikawa, S., Yamasaki, M. & Morohoshi, N. (1993). *J. Bacteriol.* **173**, 7950–7955.

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

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

Mignogna, G., Allocati, N., Aceto, A., Piccolomini, R., Di Ilio, C., Barra, D. & Martini, F. (1993). *Eur. J. Biochem.* **211**, 421–425.

Nishida, M., Kong, K.-H., Inoue, H. & Takahashi, K. (1994). *J. Biol. Chem.* **269**, 32536–32541.

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

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

Piccolomini, R., Di Ilio, C., Aceto, A., Allocati, N., Faraone, A., Cellini, L., Ravagnan, G. & Federici, G. (1989). *J. Gen. Microbiol.* **135**, 3119–3125.

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.

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.

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.