Preliminary X-ray crystallographic analysis of
glutathione transferase zeta 1 (GSTZ1a-1a)

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Glutathione transferase zeta 1 (GSTZ1-1) is a homodimeric enzyme found in the cytosol and mitochondrial matrix of the liver and other tissues. It catalyzes the glutathione-dependent isomerization of maleylacetoacetate to fumarylacetoacetate in the tyrosine catabolic pathway and can metabolize small halogenated carboxylic acids. GSTZ1a-1a crystals diffracted to a resolution of 3.1 Å and belonged to space group *P*1, with unit-cell parameters $a = 42.0$, $b = 49.6$, $c = 54.6$ Å, $\alpha = 82.9$, $\beta = 69.9$, $\gamma = 73.4^\circ$, with a calculated Matthews coefficient of $2.1 \text{ Å}^3 \text{ Da}^{-1}$ assuming a dimer in the crystallographic asymmetric unit. Refinement of the structure is currently in progress.

1. Introduction

Glutathione transferase zeta 1 (GSTZ1-1) is a homodimeric enzyme found in the cytosol and mitochondrial matrix of the liver and other tissues (Board & Anders, 2011; Li, James *et al.*, 2011). GSTZ1-1 catalyzes the glutathione-dependent isomerization of maleylacetoacetate to fumarylacetoacetate in the tyrosine catabolic pathway (Board & Anders, 2005) and can metabolize small halogenated carboxylic acids (Tong *et al.*, 1998). Of particular interest is the ability of GSTZ1-1 to convert dichloroacetate (DCA) to glyoxylate: GSTZ1-1 is the only enzyme known to metabolize DCA and therefore its activity determines the elimination half-life of this investigational drug (Stacpoole, 2011). During its GSTZ1-1-catalyzed conversion to glyoxylate, DCA destroys some of the enzyme, resulting in reduced elimination of DCA upon chronic administration (Cornett *et al.*, 1999; Anderson *et al.*, 1999; Guo *et al.*, 2006; Shroads *et al.*, 2008, 2012). Although DCA has beneficial effects on mitochondrial metabolism, prolonged exposure to high concentrations can lead to peripheral neuropathy in humans (Stacpoole, 2011); thus, tailoring the dose to minimize toxicity requires an understanding of the factors that affect the function of GSTZ1-1.

Three non-synonymous single-nucleotide polymorphisms in the coding region of the *gstz1* gene give rise to four common haplotypes of the GSTZ1-1 protein (Board & Anders, 2011); studies in human volunteers have shown that DCA pharmacokinetics vary with haplotype (Shroads *et al.*, 2012). Ongoing *in vitro* studies have shown that chloride and other anions modulate the extent of inactivation of GSTZ1-1 by DCA (unpublished studies) and that the half-life of inactivation of hepatic cytosolic GSTZ1-1 varies with haplotype in the presence of physiological concentrations of chloride (Li, Gu *et al.*, 2011). Enzymatic activity with DCA in liver cytosol from individuals that were heterozygous for the *gstz1a* gene was less well protected by chloride from DCA-induced inactivation compared with results from individuals that possess the *gstz1b* or *gstz1c* genes. We sought further information on the crystal structure of the GSTZ1a-1a protein (24.1 kDa; 216 amino acids) compared with that of GSTZ1c-1c (the most commonly found haplotype) in order to understand the interaction of chloride and other anions with this protein, and how this may reduce the formation of adducts that lead to destruction of the GSTZ1-1 protein.

Table 1

Data-collection statistics and processing parameters for GSTZ1a-1a.

Values in parentheses are for the outermost resolution shell.

Beamline	Rotating Cu anode
Detector	R-Axis IV ⁺⁺
Crystal-to-detector distance (mm)	150
Wavelength (Å)	1.5418
Temperature (K)	100
Resolution range (Å)	20.00–3.10 (3.21–3.10)
Space group	<i>P</i> 1
Unit-cell parameters (Å, °)	<i>a</i> = 42.0, <i>b</i> = 49.6, <i>c</i> = 54.6, <i>α</i> = 82.9, <i>β</i> = 69.9, <i>γ</i> = 73.4
No. of measured reflections	26271
No. of unique reflections	7068
Multiplicity	3.7 (3.7)
Completeness (%)	97.7 (96.6)
Mean <i>I</i> / <i>σ</i> (<i>I</i>)	7.4 (2.4)
<i>R</i> _{merge} [†] (%)	11.3 (34.6)
<i>R</i> _{p.i.m.} [‡] (%)	10.4 (32.3)
<i>R</i> _{meas} [§] (%)	12.6 (39.8)
<i>V</i> _M (Å ³ Da ^{−1})	2.1

[†] *R*_{merge} is defined as $\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl) \times 100$, where *I*_{*i*}(*hkl*) is the intensity of an individual reflection and $\langle I(hkl) \rangle$ is the average intensity for this reflection; the summation is over all intensities. [‡] *R*_{p.i.m.} is defined as $\sum_{hkl} [1/[N(hkl) - 1]]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl) \times 100$, with the parameters defined as above and where *N*(*hkl*) is the number of reflections. [§] *R*_{meas} is defined as $\sum_{hkl} [N(hkl)/[N(hkl) - 1]]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl) \times 100$, with the parameters defined as for *R*_{p.i.m.}

2. Materials and methods

Cloning, protein expression and purification of human GSTZ1a-1a with an N-terminal histidine tag were carried out exactly as described previously (Guo *et al.*, 2006). The purity was assessed by SDS–PAGE and Coomassie Blue staining and a single band at 24 500 Da was observed. The protein was then dialyzed against buffer consisting of 20 mM HEPES pH 7.5, 2 mM DTT, 2 mM GSH, 40 mM NaCl. Using the Bio-Rad bicinchoninic acid protein assay (Thermo Scientific, Rockford, Illinois, USA), the expressed GSTZ1a-1a was determined to have a concentration of about 20 mg ml^{−1}.

Crystallization screens were performed utilizing a Gryphon system (Art Robbins Instruments, Sunnyvale, California, USA) to dispense a

96-well Intelli-Plate sitting-drop vapour-diffusion crystallization plate using Crystal Screen (Hampton Research). Drops consisted of protein solution (20 mg ml^{−1}) in 20 mM HEPES pH 7.5, 2 mM DTT, 2 mM GSH, 40 mM NaCl and precipitant solution in 1:1 and 2:3 ratios (drop sizes of 1.0 and 0.8 µl, respectively) equilibrated against 60 µl precipitant solution. The crystallization plate was equilibrated at 290 K for around three weeks until crystal detection in 1:1 drops with a reservoir consisting of 0.2 M Li₂SO₄, 0.1 M Tris–HCl pH 8.5, 30% (w/v) PEG 4000. The crystal was cryoprotected in reservoir solution containing 20% (v/v) glycerol prior to data collection.

Diffraction data were collected at 100 K on an in-house Rigaku R-Axis IV⁺⁺ image-plate detector using an RU-H3R rotating Cu anode generator (Cu Kα, 1.5418 Å) operating at 50 kV and 22 mA with a crystal-to-detector distance of 150 mm. The X-rays were focused via Osmic optics, followed by a helium-purged beam path. Diffraction data were collected in 1° oscillations with an exposure time of 10 min per image. *HKL*-2000 (Otwinowski & Minor, 1997) was used to integrate, merge and scale the data in the triclinic space group *P*1 (with unit-cell parameters *a* = 42.0, *b* = 49.6, *c* = 54.6 Å, *α* = 82.9, *β* = 69.9, *γ* = 73.4°) to a final resolution of 3.1 Å. A summary of the data statistics is provided in Table 1. *R*_{p.i.m.} and *R*_{meas} were calculated *via* *SCALA* (Evans, 2006). An initial molecular-replacement model has been solved and will be reported elsewhere.

3. Results and discussion

Crystals of GSTZ1a-1a were observed after around three weeks in a 1:1 mixture of protein solution and precipitant solution equilibrated against 0.2 M Li₂SO₄, 0.1 M Tris–HCl pH 8.5, 30% (w/v) PEG 4000. Diffraction data were collected on an in-house detector and indexed to a final resolution of 3.1 Å, with an *R*_{merge} of 11.3%, an *R*_{p.i.m.} of 10.4%, an *R*_{meas} of 12.6% and an overall completeness of 97.6% (Table 1). The crystals were shown to belong to the triclinic space group *P*1, with unit-cell parameters *a* = 42.0, *b* = 49.6, *c* = 54.6 Å, *α* = 82.9, *β* = 69.9, *γ* = 73.4° (Fig. 1).

Figure 1

Crystal packing diagram of GSTZ1a-1a in (a) the *xy* plane and (b) the *yz* plane. The two molecules in the asymmetric unit are colored green and cyan. The unit-cell axes are shown as white lines.

Higher quality crystals that diffract to higher resolution may be obtained by optimization *via* screening around the crystallization condition, varying salt concentrations, PEG content and pH values, in an automated fashion utilizing the Alchemist DT (Rigaku). Upon determination of the optimal conditions, hanging and sitting drops utilizing vapour diffusion in 24-well macro plates (0.5–1.0 ml precipitant well capacity) will be implemented for large crystal growth.

Visualization of chloride in the vicinity of the Ser14/15 glutathione-binding site (Board & Anders, 2011) could ultimately provide an explanation of the observed concentration-dependent effect of chloride in preventing adduct formation during the conversion of DCA to glyoxylate (studies in progress). This is important because it should lead to a better understanding of individual differences in the extent of destruction of GSTZ1-1 upon chronic treatment with DCA as a therapeutic agent (Shroads *et al.*, 2012).

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