**SUPPLEMENTARY INFORMATION**

# Identification, characterization and crystal structure of the Omega class of glutathione transferases.

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## Cloning, Expression and Purification of GSTO 1-1.

cDNA encoding GSTO 1-1 was cloned by PCR. The sense primer (5'-AGGATCCACGATGTCCGGGGAGTCAG-3') contained a *Bam* HI site and the antisense primer (5'-CGAATTCAGAGCCCATAGTCACAG-3') contained an *Eco* RI site, both to allow insertion into the pGEX2T expression vector (AP Biotech, Piscataway, NJ). After 14 cycles of PCR (94 $^{o}$C for 20 sec, 65 $^{o}$C for 20 sec, and 72 $^{o}$C for 60 sec), double-stranded DNA products were analyzed by agarose gel electrophoresis. Fragments of the expected size (740 bp) were excised with a QIAquick gel extraction kit (Qiagen, Valencia, CA), ligated into a pGEMT cloning vector (Promega, Madison, WI), and transformed into competent *E. coli* DH5α subcloning efficiency cells (Gibco BRL, Rockville, MD). After overnight growth on LB plates containing 100 μg/ml ampicillin, 0.5 mM IPTG and 40 μg/ml Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactoside), white colonies were selected and expanded by growth in liquid culture. Plasmid DNA from these cultures was digested with *Bam* HI and *Eco* RI, and the restriction fragments were analyzed by agarose gel electrophoresis. Sequencing of plasmids with a 740 bp insert confirmed that the correct sequence was obtained.

For expression, GSTO 1-1 DNA was cloned into pGEX2T (AP Biotech) at the *Bam* HI/*Eco* RI sites and *E. coli* DH5α cells were transformed and plated on LB with 100 μg/ml ampicillin. Positive colonies were confirmed by restriction digests, and plasmid DNA was isolated. BL21 [DE3] cells (Novagen, Milwaukee, WI) were transformed with plasmid DNA and plated on LB containing 100 μg/ml ampicillin. Single colonies were selected for expansion. 100 mL of culture medium (LB broth with 50 μg/mL ampicillin) was inoculated with 10-20 μL of BL21 [DE3] *E. coli* transformed with pGEX-2T carrying the GSTO 1-1 insert. This culture was grown at 37 °C with shaking (225 rpm) until $D_{600}$ reached 0.4 to 0.6 (about 90 min). To scale up, the seed culture was diluted to 1 L with fresh LB, and the resulting culture was maintained at 37 $^{o}$C with shaking (225 rpm) until $D_{600}$ reached 0.4-0.6 (about 90 min). To induce expression of the fusion protein, IPTG was added to 50 μM, and incubation was continued for 2 h at 37 °C. The cells were then harvested by centrifugation.

The 4 g pellet was resuspended in 12 mL of 0.02 M Tris HCl, 0.1 M NaCl, 0.1% Triton X-100, 5 mM DTT (reduced form), 1 mM PMSF, 1 μg/mL pepstatin, 1 mM EDTA, 5 μg/mL aprotinin, and 25 μg/mL leupeptin, pH 8.0. Hen egg lysozyme (Boehringer Mannheim, Indianapolis, IN) was added to 1 mg/mL, and the suspension was incubated at 0 °C for 30 min. Next, $MgCl_2$ was added to 0.01 M with bovine deoxyribonuclease I (Sigma, St Louis, MO) (10 μg per g of cells), and the suspension was again incubated for 30 min at 0 °C. It was then sonicated on ice for 4 x 45 s with 45 s intervals, using a 40% duty cycle and power setting 4 on a Branson Sonifier 450. The lysate was centrifuged at 18,000 x *g* for 30 min, the pellet was discarded, and centrifugation was repeated.

Following affinity chromatography on a 2 mL bed of Glutathione Sepharose (AP Biotech), the 1 mg/mL protein sample eluted with 0.1 M Tris HCl, 0.02 M glutathione (reduced), pH 7.5, was exchanged into PBS (Fast Desalting Column HR 10/10, AP Biotech), and made 5 mM in DTT (reduced). The 20 mL sample was treated with 200 μg of bovine thrombin (Calbiochem), and incubated at 25 $^{o}$C to cleave the fusion protein. Digestion required overnight incubation, but was limited to the desired cleavage. The rate was improved by concentrating the fusion protein to 2 mg/mL. With the fusion protein >90% cleaved, the sample was passed over Glutathione Sepharose to remove free *Sj*-GST (GST of *S. japonicum*, the fusion partner of GSTO 1-1; GSTO 1-1 does not bind to Glutathione Sepharose) and uncleaved fusion protein, and benzamidine-agarose was used to remove thrombin. Protein purified to this stage was suitable for crystal growth, but routinely was further purified by passage over a MonoQ HR 10/10 column (AP Biotech) in 0.05 M Bis-tris HCl, 0.05 M NaCl, pH 7.0. Under these conditions, GSTO 1-1 passed through the column without binding, while impurities detected by Coomassie Blue-stained SDS-PAGE were retained.

For enzymology, further purification was conducted to remove minor impurities visible by silver-stained SDS-PAGE. This was important to remove any residual traces of *Sj*-GST. The sample was dialyzed against 0.02 M Tris HCl, 5 mM DTT (reduced form), pH 8.0, and fractionated on Q Sepharose Fast Flow (AP Biotech). GSTO 1-1 bound to the column and was eluted by a NaCl gradient. It was dialyzed into 0.05 M Bis-tris HCl, 0.05 M NaCl, pH 7.0, and passed without binding through MonoQ HR 10/10 as above. The product was stored frozen at -80 $^{o}$C.

Purified recombinant GSTO 1-1 was predicted to be a 244-residue polypeptide in which a Gly-Ser-Thr peptide from the fusion construct preceded the 241-residue GSTO 1-1 sequence. The theoretical mass for this product with all cysteines in the reduced state was 27,811.1 Da. Numerous ES-MS spectra from successive preparations gave experimental mass values within 0.01% of the predicted value (not shown). Automated Edman degradation confirmed the N-terminal sequence was as expected.

## Expression and Purification of SeMet-Substituted GSTO 1-1.

For crystallography by the multiwavelength anomalous dispersion method, GSTO 1-1 was expressed as a selenomethionine-substituted (SeMet-substituted) protein. pGEX-2T with the GSTO 1-1 insert was transformed into the methionine auxotroph *E. coli* B834 (Novagen) and the resulting cells were plated on LB with 100 μg/mL ampicillin. A single colony was inoculated into 10 mL of LB with 50 μg/mL carbenicillin and grown overnight at 37 $^{o}$C. This culture was diluted 1:100 into 750 mL of medium containing 10 g/L $(NH_4)_2SO_4$, 2.16 g/L $Na_2HPO_4$, 1.28 g/L $KH_2PO_4$, 5 g/L NaCl, 0.4 g/L trisodium citrate, 0.2 g/L $MgSO_4$-$7H_2O$, 0.1 g/L thiamine, 2 mg/L biotin, 10 g/L glycerol, 0.1 g/L carbenicillin, 40 mg/L of all L-amino acids except methionine, 100 mg/L D, L-selenomethionine (Bachem), 10 mg/L $CaCl_2$, 4 mg/L $H_3BO_3$, 1.71 mg/L $MnSO_4$-$H_2O$, 2 mg/L $ZnSO_4$-$7H_2O$, 0.373 mg/L $CuSO_4$-$5H_2O$, 0.4 mg/L $CoCl_2$-$6H_2O$, 0.2 mg/L $Na_2MoO_4$-$2H_2O$, pH 7.0. The culture was grown at 37 $^{o}$C in a 2.8 L baffled Fernbach. At $D_{600}$ = 0.75, fusion protein expression was induced by adding IPTG to 0.1 mM and growth was continued at room temperature overnight. Cells were harvested and stored at -80 $^{o}$C.

The *Sj*-GST fusion protein of GSTO 1-1 was purified as above, except that biotinylated thrombin (Novagen) and streptavidin-agarose supplied with it were used in cleavage, and the final MonoQ step was omitted. Mass spectrometry was used to gauge SeMet-for-Met substitution. In the protein first used to solve the structure, 70% of the product was substituted in all seven Met residues, 25% in six out of seven, and smaller percentages at lower levels. Later runs gave nearly 100% substitution.

## Crystal Growth.

Purified GSTO 1-1 was exchanged into fresh 0.025 M Tris HCl, 0.06 M NaCl, 5 mM DTT, pH 7.5 and brought to 20-25 mg/mL in Centricon units (Millipore, Bedford, MA). When the intent was to crystallize the covalent glutathione adduct of GSTO 1-1, DTT was used at 1 mM and GSH:GSSG (5:1) was added to 2 mM. Screening by hanging drop/vapor diffusion using Crystal Screen I (Hampton Research, Laguna Hills, CA) identified a condition (#47) requiring minimal optimization. Apoprotein (not glutathione-complexed) crystals grew in drops containing 8-12 mg/mL protein with 50 mM sodium acetate, pH 4.6, and 1.0-1.2 M $(NH_4)_2SO_4$. About 10% of drops yielded one to a few large hexagonal rods that diffracted to at least 2.0 A. Glutathione-complexed protein crystallized in the same conditions, yielding several smaller crystals in about 80% of drops. Crystals were in the trigonal space group P321 with a=b=56.931 A, c=140.314 A, and $\alpha = ß = 90^{o}$, $\gamma = 120^{o}$.