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'-AGGATCCACGATGTCGTCAGC-3') contained a Bam HI site and
the antisense primer (5'-CGAATTCCAGGCCCATAGTCAGC-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
°C for 20 sec, 65 °C for 20 sec, and 72 °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-indolylβ-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 containing 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 D600 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 °C
with shaking (225 rpm) until D600 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
pepsatin, 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, MgCl2
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 4.5 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 °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 5'-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 °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
precedes the 241-residue GSTO 1-1 sequence. The theoretical mass for this
polypeptide in which a Gly-Ser-Thr peptide from the fusion construct
is 28,845 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 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 °C. This culture was diluted 1:100 into 750 mL of medium
containing 10 g/L (NH4)2SO4, 2.16 g/L NaHPO4, 1.28 g/L KH2PO4, 5 g/L
NaCl, 0.4 g/L trisodium citrate, 0.2 g/L MgSO4·7H2O, 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 CaCl2, 4 mg/L H2BO3, 1.71 mg/L MnSO4·H2O, 2 mg/L ZnSO4·7H2O,
0.373 mg/L CuSO4·5H2O, 0.4 mg/L CoCl2·6H2O, 0.2 mg/L Na2MoO4·
2H2O, pH 7.0. The culture was grown at 37 °C in a 2.8 L baffled Fernbach.
At D600 = 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 °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 (#847) requiring minimal optimization. Anoprotein (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 (NH4)2SO4.
About 10% of drops yielded one to a few large hexagonal rods that dripped
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 α = β = 90°, γ = 120°.