

Short communication

Molecular cloning and expression of a cDNA encoding glutathione *S*-transferase from *Ascaris suum*

Eva Liebau^{a*}, Öyvind L. Schönberger^b, Rolf D. Walter^a, Kimberly J. Henkle-Dührsen^a

Bernhard Nocht Institute for Tropical Medicine, Departments of ^aBiochemistry and ^bMolecular Biology, Hamburg, Germany

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1. Introduction

Glutathione *S*-transferases (GSTs), widespread among vertebrates and invertebrates, are a family of multifunctional detoxification enzymes that react with diverse, potentially damaging compounds. They catalyse the conjugation of electrophilic groups of a variety of substances with the thiol group of the tripeptide glutathione (GSH), as well as binding to hydrophobic molecules [1]. GSTs potentially aid in parasite survival by playing a role at the interface between the parasite and the host and may repair host-induced damage [2]. As one of the major detoxification enzymes in parasitic helminths, their substrates include the toxic secondary products of lipid peroxidation and anthelmintics [3,4]. The success observed with GSTs as candidate protective antigens in schistosomiasis [5–7] underlines the importance of investigating the GSTs in parasitic nematodes. In this

report we describe the partial purification of two GSTs from *Ascaris suum*, which differ in their molecular weight and possess distinct N-terminal sequences (AsGST1 and AsGST2). The full-length nucleotide sequence encoding one of these GSTs was isolated and the nucleotide sequence determined. The cloned cDNA allowed us to examine the predicted amino acid sequence and the homology of AsGST1 with other GSTs of parasitic helminths. To provide a more definitive structure-function relationship between the cDNA and its protein and to confirm that this cDNA encodes a functional GST, this cDNA was also expressed in *Escherichia coli*.

The partial purification of GSH-binding proteins from adult female *A. suum* uterus and ovary using GSH affinity chromatography is shown in Fig. 1A. Two closely migrating species of roughly 26 kDa (AsGST1) and 25 kDa (AsGST2) were shown to possess enzymatic activity. These two enzymes were electrotransferred onto Problot and the N-terminal sequences were determined using an ABI 473 A protein sequencer. The automated

* Corresponding author. Tel: (49)-40-31182-415; Fax: (49)-40-31182-400.

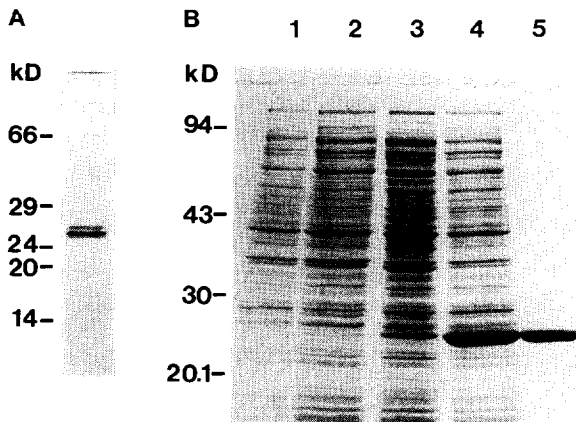


Fig. 1. (A) Partial purification of the native AsGST1 and AsGST2. The $100\,000 \times g$ supernatant of *A. suum* homogenate was incubated with GSH-Sepharose overnight, washed with PBS (150 mM NaCl/ 16 mM Na_2HPO_4 / 4 mM NaH_2PO_4 , pH 7.3), eluted with 15 mM GSH in 50 mM Tris-HCl, pH 8.0 and dialysed against 1 l PBS overnight. Triton X-100 was added to a final concentration of 1% to the sample, which was then loaded onto a GSH-sepharose column. After washing with PBS, specifically bound material was eluted with a GSH-gradient (0–20 mM). The GSH-binding proteins were separated on a 12.5% SDS-PAGE gel and silver stained. (B) Production and purification of rAsGST1. *E. coli* BL21 containing the expression vector pJC20 were grown to $\text{OD}_{600} = 0.6$, and the expression of rAsGST1 was induced with 0.5 mM isopropyl- β -D-thiogalacto-pyranoside. The cells were harvested, sonicated, and the lysate was resuspended in PBS + 1% Triton X 100 and analysed on a Coomassie blue-stained 12.5% SDS-PAGE gel. Lanes 1 and 2 show lysates of *E. coli* containing the vector only (pJC20) with and without induction, respectively; Lane 3 and 4 show lysates of *E. coli* containing the pJC20-AsGST1 plasmid with and without induction, respectively. In lane 4, the 24-kDa rAsGST1 is observed; lane 5 shows the rAsGST1 purified by affinity chromatography.

Edman degradation revealed the first 40 amino acids for AsGST1 and 20 amino acids for AsGST2. The sequences were PQYKLTYF-DIRGLGEGAXLIFXQAGVKFEDNRLXXED-XPA and GYKVITYFAIRGLAEPIXLLL, respectively (X marks the amino acids which were ambiguous). The oligonucleotide 5'-TAGAATT-CATA(G)TCA(G) AAA(G)TAAGTAAGC(T)T-T-3' was designed based upon the amino acid sequence underlined in the AsGST1 N-terminal peptide, where the bases in brackets represent degenerate positions in the sequence. This oligonucleotide was used in RNA-polymerase chain reaction experiments with the spliced leader 1 oligonucleo-

tide (5'-GGTTTAATTACCCAAGTTTGAG-3') to obtain a 61-bp fragment representing the 5' end of the AsGST1 mRNA. From this sequence a new oligonucleotide was designed and used in polymerase chain reaction amplification along with an oligo d(T) primer to obtain the complete AsGST1 coding sequence. This amplified fragment was used to screen an adult *A. suum* λ ZapII cDNA library, using a standard plaque hybridization assay. Following purification of 25 positive clones, one of the isolated phage clones was found to contain the complete coding region, and the 5' and 3' nontranslated regions. The 710-bp cDNA possesses a 205-amino acid open reading frame which encodes a protein of 23 453 Da omitting the initiation methionine.

The NBRF protein sequence database was screened for sequences related to the predicted polypeptide sequence of AsGST1. Comparison with GST sequences from trematodes and the one available nematode sequence (Fig. 2) revealed that, with the exception of highly conserved residues likely to be required for activity, there is relatively little overall homology, even when allowing gaps in the sequence to produce better alignment. The highest level of homology was scored with the *Caenorhabditis elegans* GST [8]. Alignment of these two sequences indicated 33.0% identity, with major differences in the central region (positions 84–113). The AsGST1 sequence possessed 24.8% and 18.9% identity with *Schistosoma mansoni* Sm28 and Sm26 [5,9,10] sequences, respectively. When comparing the AsGST1 with mammalian GST polypeptide sequences, the best homology (31.6%) was scored with the pig π subunit [11]. Residues lining the GSH-binding site of pig π GST have been identified from the three-dimensional structure [11]. As shown in Fig. 2, 8 of the 10 residues lining the GSH-binding site of pig π GST are conserved in the AsGST1 sequence. A high level of homology (29.6%) was also scored with the lens crystallin protein of *Octopus dofleini* [12], raising the question whether the AsGST1 is a true GST or an interesting intermediate between a metabolic enzyme and a structural protein. On the basis of the N-terminal 20 amino acids, the AsGST2 is more similar to the *C. elegans* GST (75%) than the AsGST1 is. The AsGST2 partial

AsGST1	MF---	QYKLT	YFDIRGLGEGARLIFHQAGVVFEDNRLKRE-----DW	39
CeGST	*T---	L*****H**A*PI*	LLADKQ*AY**H*V*Y*-----Q*	38
Sm28	*A-GEHI*VI**G**RA*SI*MTLVA**DY**E*ISFQDWP	KIKPTI-		48
Sm26	*A----	P*FG*WKVK**VQPT*	LLEHLEETY*ERAYD*NEI-----DA*	41
PigGST	*----P*TI***PV**RC*AM*MLADQDQSWKEEVVTM*	-----T*		38
SC3	**---S*AT*H**NH**RA*IC*ML*AA***QYN*R*IESS-----E*			39
AsGST1	PA--LKP	KTPFQGLLEVDGEV-LAQSA	AIYRYLGRQFGLAGKTPMEEA	86
CeGST	AD--I**MI***V*CLSGD*E-IV**G**I*H*A*LN**N*SNET*TT			85
Sm28	~PGGR---	L*AVKVTDDHGHVKWMLE--L**A**MAKKHHMM*E*DE*YY		93
Sm26	SNDKF*LGLEFPN**YIDGDFK--T**M**I**IADKHNL*AC*K*R*			90
PigGST	*P--***SCL*R***KFQ--*DLT*Y**N**L*H***S***Y**DQK*A*			86
SC3	NG--MRNQMN*CNMM*M**L*NRTQIP*M*MA***A**E**YH**SN**M*			87
AsGST1	QVDSIFDQKDFMAELR	PCFRLVLAGFEGDKEKVLKEVAVFARDK-----		131
CeGST	FI*MFYEGLR*LHTKYTTMIYR---	NY*DG*APYI*D*LPGELAR-----		127
Sm28	S*EKLIG*AE*VEH*YHKTLMKPQEEK*KITKEI*NGKVPVLLNM-----			138
Sm26	EISMLEGAVL*IRMGVLRIAYNK--EY*TL*VDF*NK-LPGR	LKM-----		132
PigGST	L**MVN*GVE*LRCKYATLIYT--NY*AG**YV**LPEHLKP-----			127
SC3	R**F*S*CA*Y*I*DDYMRMYQDGNCRMMFQSRDMNSSSESRRMFQETCR			137
AsGST1	-HLP	LLEKFLA--KSGSEYHVGKSVTWADLVITDLSASWESLIPDPLSGH		178
CeGST	-LEK*FH-----TY*N*EH*VI*DKESY**Y*LFEE*DIHLI*T*NA*D*V			172
Sm28	-ICES*-----KG*TGKLA**DK*L****LIAVIDHVTD*DKG*AT*K			181
Sm26	-FEDR*SN-----KTYLN*NC**HP*FMLY*A*DVVLYMDSQC*NEF			173
PigGST	-FET**SQ-----NQG*QAFV**SQISF**YNLL*L*RIHQV*N*SC*DAF			171
SC3	RI**FM*RT*DMHSG**KFFM*DQM*M**MCMYCA*ENPLMEESSM**SY			187
AsGST1	--LQLK	KYIEHVRELPNIKKIAERPKTFY		206
CeGST	--PA***FH*RFAR****AYLNK*AAINP	VPVNGNGKQ		208
Sm28	YPEIH*HRENLLASS*RLA*YLSN*A**F			208
Sm26	-PKL-VSFKKCIED**Q**NYLNSSRYIKWPLQGW	DTFGGDTPPK		218
PigGST	PL--SA*VARLSAR*K**AFL*SFEHVNRPNG			203
SC3	--PK*MSLRNR*MSH*KMCNLYKK*CR*DF			215

Fig. 2. Comparison of the deduced amino acid sequence from the *A. suum* GST1, *C. elegans* GST (CeGST) [8], *S. mansoni* 28-kDa GST (Sm28) [5], 26-kDa GST (Sm26) [9,10], pig π GST (pigGST) [11] and the *O. doylei* S-3 crystallin (SC3) [12] cDNA sequences. Gaps indicated by (—) were introduced into the sequence to optimise the alignment. Residues that CeGST, Sm28, Sm26, pigGST and SC3 have in common with AsGST1 are indicated by (*). Conserved residues lining the glutathione-binding site in AsGST1 are underlined.

sequence is most related to mammalian GSTs in the μ .

To confirm that the encoded enzyme from the AsGST1 cDNA is a glutathione S-transferase, the cDNA was expressed in pJC20 (Clos, J. and Brandau, S., manuscript submitted) in *E. coli* (Fig. 1B, lanes 1–4). The recombinant AsGST1 (rAsGST1) was purified using GSH affinity chromatography. The estimated molecular weight was 24 000 (Fig. 1B, lane 5). Routinely, 20–30 mg of purified enzyme were obtained from a 500 ml *E. coli* culture. The enzyme activity of this purified rAsGST1 was determined spectrophotometrically by measuring

the formation of the conjugate of GSH and 1-chloro-2,4-dinitrobenzene [13] and was found to be $38.2 \mu\text{mol min}^{-1} \text{mg}^{-1}$. In addition, the ability of the enzyme to catalyse the GSH-mediated reduction of cumene hydroperoxide was also determined [14]. The specific activity was calculated to be $0.145 \mu\text{mol min}^{-1} \text{mg}^{-1}$, indicating that this enzyme is also capable of functioning as a glutathione peroxidase.

The sequence analysis of the AsGST1 indicated that, although the overall homology to other GSTs from nematodes and trematodes was low, most of the residues likely to be required for GSH binding and enzymatic activity were present. The ability of the expressed rAsGST to bind GSH and the enzyme activity observed confirm that the AsGST1 clone described here encodes a functional GST.

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