Cytosolic glutathione S-transferases of *Oesophagostomum dentatum*

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SUMMARY

Oesophagostomum dentatum stages were investigated for glutathione S-transferase (GST) expression at the protein and mRNA levels. GST activity was detected in all stages (infectious and parasitic stages including third- and fourth-stage larvae of different ages as well as males and females) and could be dose-dependently inhibited with sulfobromophthalein (SBP). Addition of SBP to *in vitro* larval cultures reversibly inhibited development from third- to fourth-stage larvae. Two glutathione-affinity purified proteins (23 and 25 kDa) were detected in lysates of exsheathed third-stage larvae by SDS-PAGE. PCR-primers were designed based on peptide sequences and conserved GST sequences of other nematodes for complete cDNA sequences (621 and 624 nt) of 2 isoforms, Od-GST1 and Od-GST2, with 72% nucleotide similarity and 75% for the deduced proteins. Genomic sequences consisted of 7 exons and 6 introns spanning 1296 bp for Od-GST1 and 1579 and 1606 bp for Od-GST2. Quantitative real-time-PCR revealed considerably elevated levels of Od-GST1 in the early parasitic stages and slightly reduced levels of Od-GST2 in male worms. Both Od-GSTs were most similar to GST of *Ancylostoma caninum* (nucleotides: 73 and 70%; amino acids: 80 and 73%). The first three exons (75 amino acids) corresponded to a synthetic prostaglandin D_2 synthase (53% similarity). *O. dentatum* GSTs might be involved in intrinsic metabolic pathways which could play a role both in nematode physiology and in host-parasite interactions.

Key words: *Oesophagostomum dentatum*, glutathione S-transferase, genomic sequence, stage-specific expression.

INTRODUCTION

Glutathione S-transferases (GSTs) were originally described as a group of enzymes involved in the conjugation of glutathione to various metabolites in mammalian cells. They are common in the animal kingdom and are divided into different classes according to their substrate specificity (for review, see Armstrong, 1991; Sheehan *et al*. 2001; Pearson, 2005). In helminths (trematodes, cestodes and nematodes) GSTs have attracted attention due to their high-level constitutive expression and antigenicity and their potential role as vaccine targets (see Mitchell, 1989; Sexton *et al*. 1990; Brophy and Pritchard, 1994; Barrett, 1995; Campbell *et al*. 2001*a*). Consequently, both native and recombinant enzymes have been characterized and developed for anthelmintic vaccination. GST of helminth origin are implicated in a variety of metabolic pathways by conjugation of glutathione to different agents, including detoxification of anthelmintic substances and intracellular transport (Precious and Barrett, 1989; Brophy and Barrett, 1990*a*; Sharp *et al*. 1991; O'Leary and Tracy, 1992; Miller *et al*. 1994; Kampkötter *et al*. 2003; Leiers *et al*. 2003). The majority of helminth GSTs characterized to date are of cytosolic origin and comprise the sigma (or alpha) class. While trematodes (first of all, *Schistosoma mansoni* and *Schistosoma japonicum* as well as the liver fluke *Fasciola hepatica*) were in the focus of GST-related research, nematodes have been less well characterized, with the exception of *Onchocerca volvulus*, a filarial pathogen of humans (for review see Gupta and Srivastava, 2006).

The advent of large-scale sequencing projects of invertebrate expressed sequence tags and whole genomes has brought about additional information regarding the GST variations of *Caenorhabditis elegans*, a free-living model nematode belonging to the systematic clade of the Rhabditida, and other related organisms, including members of the Strongylida (Blaxter *et al*. 1998). The Strongylida are the sister clade of the Rhabitida and include *Oesophagostomum dentatum* as well as other species of which native or recombinant GSTs have been characterized, such as *Haemonchus contortus* (Sharp *et al*. 1991; van Rossum *et al*. 2004) and the hookworms *Heligmosomoides polygyrus* (syn. *Nematospiroides dubius*; Brophy *et al*. 1994*a*) and *Ancylostoma caninum* (Zhan *et al*. 2005).

The aim of this work was to detect native GST and characterize the corresponding cDNA and genomic

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DNA sequences of *O. dentatum* and to investigate its expression during parasite development.

MATERIALS AND METHODS

Parasite material

A monospecific strain of *Oesophagostomum dentatum*, OD-Hann (Joachim *et al*. 1997), is routinely maintained by infection of parasite-free pigs kept at the animal facilities of the Institute of Parasitology and Zoology, University of Veterinary Medicine Vienna. Third-stage larvae (L3) were harvested from cocultures and purified as described (Talvik *et al*. 1997). L3 were used as ensheathed (L3s) or exsheathed (L3x) larvae (exsheathment was performed with NaOCl as described by Joachim *et al*. 1997). L3x were further maintained in culture (2000 L3/ml) as described by Daugschies and Watzel (1999) except that the standard broth was replaced by a simplified medium consisting of 90% LB-medium (20 g/l as recommended by the manufacturer (Roth, Lactan, Graz, Austria) plus 10% inactivated pig serum with antibiotics and antimycotics (i.e., 10 0000 U penicillin, 10 mg streptomycin and 2·5 μg amphotericin B per 50 ml of medium; Sigma Aldrich, Vienna, Austria). The development to fourth-stage larvae peaked after 12–14 days of cultivation when the larvae were harvested and separated by sedimentation into cultured L3 (L3c) and L4 (L4c). Post-histotropic L4 (L4p) were derived from the intestines of pigs 12 days post-infection (p.i.) and adult females (F) and males (M) were derived from the intestines of pigs during the patent stage of infection. All stages were snap-frozen in liquid nitrogen and stored at −80 °C until required (for detailed methodology, see Joachim *et al*. 1998).

Assays of GST activity and inhibition

Homogenates of water-soluble proteins were prepared from the different stages as described by Joachim *et al*. (1998) and the protein content was measured using the DC Protein Assay (Bio-Rad, Munich, Germany). These preparations were used for GST activity measurements and for GST isolation. GST activity was measured using the Glutathione S-Transferase Assay Kit (Cayman Chemical Company, Ann Arbour, USA) at 25 °C for parasite homogenates from L3s, L3x, L4c, M, F, and mixed adults, and for isolated GST (supplied by the kit manufacturer). Statistical analysis was performed in Microsoft Excel (2002) using an F-test for comparison of stages.

Inhibition studies were conducted with sulfobromophthalein (SBP; dissolved in distilled water in concentrations of 0·015–1·5 mM) (SigmaAldrich, Vienna, Austria) determining the level of 50% inhibition (IC_{50}) in L3x in relation to uninhibited controls. For bioassays (*in vitro* cultivation of L3x to L4c; approximately 150 L3x/well in a 24-well plate in quadruplicates) the IC_{50} concentrations as well as 0·1 × and 10 × this concentration were added to the medium during cultivation. Reversibility of the effect was evaluated in cultures which were treated for 7 days followed by a period of 7 days in SBP-free medium.

Isolation and electrophoresis of GST

GSTrap® HP columns with a volume 1 ml (Amersham Biosciences, Vienna, Austria) were loaded with whole protein preparations of L3x and eluted with reduced glutathione (Merck, Vienna, Austria) as recommended by the manufacturer. Eluates were electrophoretically separated on 12% SDS-PAGE gels and silver stained according to Blum *et al*. (1987). From the SDS-PAGE gels the separated proteins were excised and analysed by peptide digestion and mass spectrometry analysis (Max F. Perutz Laboratories, University of Vienna, Vienna, Austria).

Isolation and sequence analysis of genomic and cDNA

Genomic DNA was isolated from L3x using the PeqGold® TISSUE DNA Mini kit (PeqLab, Erlangen, Germany) as recommended by the manufacturer. Total RNA from all stages was extracted with the PeqGold® Total RNA kit (PeqLab, Erlangen, Germany) and reverse transcribed using TaqMan® Reverse Transcription Reagents (Applied Biosystems, Brunn am Gebirge, Austria) as recommended by the manufacturers.

The information obtained from peptide sequencing was compared with sequences in GenBank (using BLASTp) and partially redundant primers were designed in the conserved domains of the first and the last Exon (GST_common_FOR: 5′-CACTA(CT)AAGCT(GT)ACCTACTTC-3′ corresponding to nt 7-28 and GST_common_REV: 5′-C(CT)GG(AGT)CG(AG)GTCTC(AG)ATCC-ATT-3′ corresponding to nt 587-608 in Od-GST1 and nt 590-611 in Od-GST2; see below) to amplify cDNA products of 601 and 604 base pairs, respectively.

Both complementary DNA (cDNA) and genomic DNA from L3 were amplified by PCR in a volume of 50 μl using PeqGold® Taq-DNA-Polymerase (PeqLab, Erlangen, Germany) as described by the manufacturer (initially 5 min/94 °C, followed by 39 cycles of 30 sec/94 °C, 45 sec/56 °C, 45 sec/72 °C; finally 3 min/72 °C). Amplicons were cloned using the TOPO® TA Cloning Kit for Sequencing (Invitrogen, Vienna, Austria) and recombinant DNA from single clones was prepared (PeqGold Plasmid MiniprepKit II; PeqLab, Erlangen, Germany) and sequenced (AGOWA, Berlin, Germany).

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Based on the finding that 2 isotypes could be differentiated (see Results section), partially redundant primers were designed to amplify the 5′ ends of each isotype (FOR_GST1: 5′-ATG(GC)(CT)(AGCT)-CACTA(CT)AAGCTGACC-3′; FOR_GST2: 5′-ATG(GC)(AG)(AGCT)CACTATAAGCTGAC-CTA-3′) in combination with a commercial anchor primer for 3′-RACE-PCR as recommended by the manufacturer (5′/3′RACE Kit, 2nd Generation, Roche Applied Science, Vienna, Austria).

Sequence analyses were either performed using the Basic Local Alignment Search Tool (BLAST; www.ncbi.nlm.nih.gov/BLAST/) or with the AlignPlus® Version of the SciEd Central Software Package for Windows (Scientific and Educational Software, Cary, NC, USA, 1999).

Expression analysis of GST isotypes

Real-time PCR of cDNA from L3s, L3x, L3c, L4c, L4p, F and M was carried out on an Mx3000p Cycler (Stratagene, La Jolla, CA, USA) using isotype-specific forward primers (5′-TAC(CT)TGGCTA-GGAAATTCGG-3′ for Od-GST1 and 5′-CCTT-GCGAGACAGTATGGTC-3′ for Od-GST2) and the reverse primer GST_common_REV (see above). The primers amplified a product of 399 bp in the variable region in exons 3 to 7 (see Results section) for both isotypes. As Normalizer the housekeeping gene ubiquitin-conjugating enzyme 2 (OD-ubc-2; product size: 444 bp) was amplified as described (Cottee *et al*. 2006) in 20 μl of pre-manufactured master mixes (Power SYBR® Green PCR Master Mix; Applied Biosystems, Brunamgebirge, Austria) using the same cycling conditions as above. For all stages 2 cDNA preparations were tested except L3c (1 preparation); each sample was tested in 2 independent PCR runs, each in duplicate (i.e. 6–8 replicates/stage).

RESULTS

GST activity and inhibition

The mean activity ranged from 2·27 μmol/min/mg of total protein in the L4c to 4·95 μmol/min/mg in the L3s (Table 1). The difference between these two stages was significant ($P=0·0159$) while the other comparisons were not ($P>0·05$). Concentrations of SBP >25 μM inhibited GST activity in a dose-dependent manner as shown for L3x homogenates treated with SBP in concentrations from 25 to 670 μM in 3-fold dilution steps (Fig. 1). The inhibition by 150 μM SBP was 51·8% in the isolated GST control and 62·9–68·5% in the parasite homogenates of different stages (Table 1).

The development of L4 *in vitro* reached 18% after 14 days in the uninhibited controls. The addition of SBP in concentrations of 150 mM or 1·5 mM resulted in partial or complete inhibition of L4 development, while 15 μM SBP had no marked effect on development. When SPB was removed from the culture after 7 days, larvae resumed growth and the percentage of L4 after 14 days was comparable to the controls (Fig. 2). The larvae remained motile throughout the culture period in all groups despite growth inhibition by SBP.

Table 1. Activity of GST in homogenates of different stages (μmol/min/mg protein)

(Each stage was tested in 2 different batches each at least 3 times except for the separate measurements of male and female worms which were done only once and therefore not included in the statistical analysis. Inhibition was calculated in % reduction of the activity of the respective untreated control at a concentration of 150 μM SBP. *Control*: GST supplied by the manufacturer of the Glutathione S-Transferase Assay Kit (Cayman Chemical Company, Ann Arbour, USA).)

Stage	Mean GST activity [μmol/min/mg of total protein] (minimum–maximum)	Inhibition (%) by 150 μM SBP
L3s	4·95 (2·65–7·96)	64·5
L3x	4·12 (2·58–6·64)	62·9
L4c	2·27 (1·54–3·02)	68·5
Mixed Adults	3·75 (1·74–5·16)	65·8
M	3·31	n.d.
F	3·87	n.d.
Average	3·97 (1·54–7·96)	65·5
Control	*3·91*	*51·8*

Protein analysis

SDS-PAGE, under reducing conditions, revealed 2 bands of approximately 23 and 25 kDa. Each band was excised and peptides were analysed. One peptide (KFGFAGK) was detected in both bands and analysis showed extensive homology with various other GSTs of nematode origin [mostly Chain B of *C. elegans* GST (GenBank Accession no. 1YQ1_B) and gst-17 and gst-37 of *C. elegans* (NM_064464 and NM_071131)]. Another peptide (KWIETR-PASDW) was found in the 23 kDa band that had significant similarity with the *C. elegans* gst-38 (NM_074582).

cDNA sequence analysis

The clones derived from the amplification of the partial cDNA sequence were 73–96% similar to each other and clustered in 2 groups, Od-GST1 (601 bp) and Od-GST2 (604 bp).

Full length cDNAs were obtained using redundant isotype-specific forward primers deduced from comparisons of the obtained sequences with the 5′ ends of other nematode GSTs and 3′-RACE-PCR. The complete sequences including start and stop

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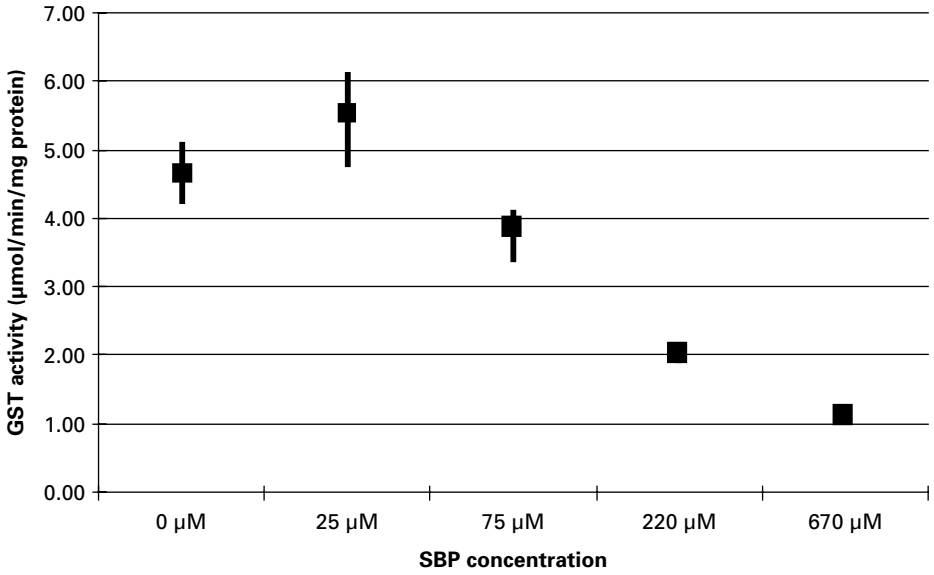

Fig. 1. Dose-dependent inhibition of GST in homogenates of L3x by SBP ($n=3$ replicates for each dilution). Bars indicate the minimum and maximum values, squares mark the mean values.

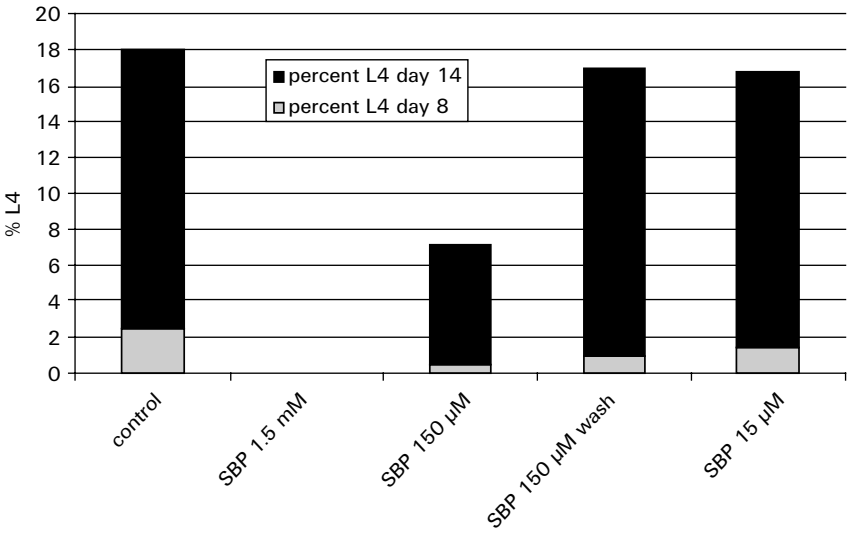

Fig. 2. Development of L4 *in vitro* after addition of SBP in different concentrations compared to an untreated control group (in %, average from quadruplicates). Wash: SBP was removed after 7 days by washing the larvae in SBP-free medium.

codon were 621 (Od-GST1) and 624 bp (Od-GST2) long with 72% overall similarity[1]. The sequences also showed high similarities (73% for Od-GST1 and 70% for Od-GST2) to GST of *A. caninum* (GenBank Accession no. AY605283), followed by *H. polygyrus* GST2 (GenBank Accession no. AF128959) with 69 and 68%, and *C. elegans* gst-5 (GenBank Accession no. NM_063956), *H. contortus* GST (GenBank Accession no. AF281663), *H. polygyrus* GST1 (GenBank Accession no. S76129), and *Ascaris suum* GST (GenBank Accession no. X75502) with similarities between 57 and 63% for both isotypes (Table 2).

Both cDNAs consisted of 7 exons (deduced from genomic sequencing, see below) of 60–126 bp for Od-GST1 and 60–129 bp for Od-GST2, with similarities between 65 and 83% (Fig. 3).

The proteins translated from the predicted open reading frames had 75% similarity between the two (62–95% for the different exons; Fig. 3). The similarities between Od-GST1/GST2 and other nematodes with regard to the predicted amino acids (aa) were 36–80% (Table 2; Fig. 4).

[1] Genbank Accession nos:

cDNA Od-GST1: EU418977
cDNA Od-GST2: EU418978

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Table 2. Pairwise comparison of nucleotide (top) and amino acid (bottom) sequence similarities (in %) between GSTs of different nematodes

(Od: *Oeosophagostmum dentatum*, Ac: *Ancylostoma caninum*, Hp: *Heligmosomoides polygyrus*, Hc: *Haemonchus contortus*, Ce: *Caenorhabditis elegans*, As: *Ascaris suum*. Data were matched over the cDNA sequences available for all GSTs. For GenBank Accession numbers see text.)

	Od-GST1	Od-GST2	Ac-GST	Hp-GST1	Hp-GST2	Hc-GST	Ce-GST5	As-GST
Od-GST1	100	72	73	58	69	63	60	58
Od-GST2	*75*	100	70	57	68	62	61	57
Ac-GST	*80*	*73*	100	53	65	62	61	57
Hp-GST1	*36*	*40*	*36*	100	74	50	52	52
Hp-GST2	*68*	*70*	*64*	*44*	100	57	60	54
Hc-GST	*61*	*60*	*58*	*30*	*53*	100	60	55
Ce-GST5	*60*	*60*	*59*	*31*	*55*	*54*	100	53
As-GST	*54*	*52*	*49*	*29*	*44*	*47*	*50*	100

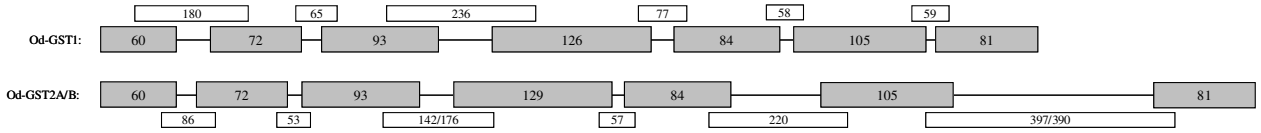

Fig. 3. Length and structure of the 7 exons (grey bars) and 6 introns (white bars) of Od-GST1 and Od-GST2A/B. Genomic DNA of Od-GST2 was considerably longer than that of GST2 although cDNA sequences varied only by 3 nucleotides. Within Od-GST2 two genomic structures were detected.

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Od-GST1   MVHYKLTYFNGRGLGECARQIFALADQKYEDVRMTQET--FPALKPKL--PFGQVPLLEEDGKELAQSNAINRYLARKFG   228
Od-GST2   .............N.........V.G.Q.....L.H.Q--.APM..N.--......V..V...Q....A..A.....QY.   228
Ac-GST    ....................L......Q...I.V.H.D--..EI..N.--....L...N.....................   262
Hp-GST1   --............A.......LRWPTR---NT..SSH.RNICTAETNV--.......V..V..QQ....Q..L....KTPD   219
Hp-GST2   --............A...................L....--.AP..ATF--.......V..V..QQ....Q..C....KT..   222
Hc-GST    .............AA.II..V.V..G.D.....L.H.E--W.KH.ASM--....L.V..V...Q.P..V..V........   228
Ce-GST5   ..S..........A..VS.....Y.G.Q...N.V...Q--W....ETCAA....L.F..V...K....H..A.F...E.K   297
As-GST    .PQ......DI.....G..L..HQ.GV.F..N.LKR.D--W......T-....L....V..EV....A..Y...G.Q..   243

Od-GST1   FAGKTPFEEALVDSLADQFTDYRLEIKPYSMVAYGFQK-G--DVEKLKKELVLPARDKFLGFITKFLKNNKSGFL--VGD   453
Od-GST2   L..AD.............YA...V.V...VYTLL..M.A.--.LD.....VMM.G.............KS.....--...   456
Ac-GST    ..................M....V....FVYT...H..F.--.L.T...DVM..................P....--...   490
Hp-GST1   ...A....S..I.....AY....A.M.T.YYTGPSLSH-.TG.LD...TDVC..PGSYQVLDLHESRDSDLV.TIRSA..   456
Hp-GST2   ...A....S..I..P..AY....A.M.T.YYM.L..MT-.--..D...TDVL....T.........P.K.S....--...   447
Hc-GST    Y...SAW...V...I....K.FLN.VR..FK.LL.MDQ-.--.LKA.E.DVFE...Q..FTIV..I..E..T.Y.--...   453
Ce-GST5   LN...AW...Q.N.....YK..SS.AR..FYAVM..GP-.--...T...DIF...FE..Y..LVN...ASG....--...   522
As-GST    L.....M...Q...IF...K.FMA.LR.CFR.LA..EE-.--.K..VL..VAV.....H.PLLE...AKSG.EYM--..K   468

Od-GST1   SVTWADLLIAEHSSDMSHRIPEFLNGFPEVKAHMEKVRSIPKLKKWIESRPASVF*------------    621
Od-GST2   K...V....S..MA...A......D..............A......T..DTP.*------------    624
Ac-GST    ....I...L...A..IQSKV..Y.E.....................TT.DTH.*------------    658
Hp-GST1   VCC.T-.PTVHR..TSMGPMSQP.DCITDTRSRCD--.TVM.-QLYCV.Q.VL.MDYVQARRSIDAYT*    651
Hp-GST2   KIS.V...V...VA..TN.V..YIE..........RIQQT.RI.....T..ETP.*------------    615
Hc-GST    .L.F...YV..MTTFTE.-Y.KLYD........A.....N........T....K.*------------    618
Ce-GST5   .L..I..A..Q..A.LIAKGGD.-SK...L...A..IQA..QI.....T..VTP.*------------    687
As-GST    .......V.TDSLASWESL..D..S.HLQL.KYI.H..EL.NI....AE..KTPY*------------    636
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Fig. 4. Amino acid alignments of GSTs from different nematodes deduced from the respective cDNA sequences. Od: *Oeosophagostmum dentatum*, Ac: *Ancylostoma caninum*, Hp: *Heligmosomoides polygyrus*, Hc: *Haemonchus contortus*, Ce: *Caenorhabditis elegans*, As: *Ascaris suum*. For GenBank Accession numbers of cDNA sequences see text.

The predicted sizes were around 23·57 kDa for Od-GST1 and 23·39 kDa for Od-GST-2, the calculated isoelectric points were 9·37 (Od-GST1) and 8·44 (Od-GST2). BLAST analysis (www.ncbi.nlm.nih.gov/blast/Blast.cgi) of the translated proteins revealed significant similarity to the N- and C-terminal ends of sigma-class like GSTs Conserved Domains (GenBank Accession no. cd03039 and cd03192).

Besides homologies with other nematode GSTs, protein analysis also revealed high similarity with the protein sequence of a 200 aa synthetic prostaglandin D_2-synthase (GenBank Accession no. AAX37080) with 36 and 35% matching aa over the complete

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Table 3. Differences in threshold cycle values for quantitative RT-PCR for the two GST isoforms in different developmental stages using ubc-2 as Normalizer

(In parentheses: range of the ΔCt values between different runs. $2^{-\Delta Ct2\text{-}1}$: $\Delta Ct2\text{-}1 = Ct_{Od\text{-}GST2} - Ct_{Od\text{-}GST1}$.)

ΔCt values	Average Od-GST1 (min–max)	Average Od-GST2 (min–max)	$2^{-\Delta Ct2\text{-}1}$
L3s	−2·14 (1·79–2·50)	5·32 (5·15–5·77)	176·44
L3x	−3·35 (2·75–4·53)	4·17 (3·08–4·89)	183·55
L3c	3·78 (4·42–4·10)	4·75 (4·24–5·24)	1·96
L4c	4·14 (3·70–4·67)	6·19 (5·90–6·59)	4·15
L4p	2·00 (1·52–2·56)	3·58 (3·38–3·78)	2·98
M	1·73 (1·55–1·87)	2·42 (2·07–2·69)	1·61
F	0·69 (0·47–0·85)	4·70 (4·11–5·15)	16·07

predicted sequences and 53% within exons 1–3 corresponding to the first 75 aa (see Fig. 3 for exon positions).

Genomic DNA sequence analysis

Primers spanning the cDNA exons 1–7 produced a product of 1296 bp for Od-GST1 and 2 sequences for Od-GST2, namely Od-GST2A with 1579 bp and Od-GST2B with 1606 bp[2]. They consisted of 7 exons separated by 6 introns of varying sizes (see Fig. 3).

Expression analysis

After normalization against OD-ubc-2 ($Ct_{ubc\text{-}2} - Ct_{GST}$) the ΔCt values of the different stages varied from −3·35 to 4·14 for Od-GST1 and 2·42 and 6·19 for Od-GST2 (Table 3).

When L3s were used as Calibrator (as the free-living stage, in contrast to the other, parasitic, stages), the $2^{-\Delta\Delta Ct}$ values indicated differential expression of the two isotypes during development; Od-GST1 was up-regulated in the parasitic stages, especially during early development of cultured L3 and L4 – corresponding to the histotropic stages – while Od-GST2 remained on lower levels with similar expression throughout development (Fig. 5).

In L3 the ratio Od-GST1 : Od-GST2 (calculated as the $2^{-\Delta Ct2\text{-}1}$) was highest (1 : 176 for the L3s and 1 : 184 for the L3x), while in the advanced parasitic stages it was only 1 : 2 to 1 : 16 (Table 3).

DISCUSSION

Cytosolic GST activity, characterized by glutathione-binding and substrate (CDNB) metabolism, was demonstrated in the pre-parasitic and parasitic stages of *O. dentatum*. Based on the information from peptide sequencing of two proteins isolated by glutathione affinity chromatography and database comparison, full-length cDNA sequences for two isoforms of GST and the corresponding genomic sequences were obtained. Both consisted of 7 exons interrupted by variable introns and were expressed in increased levels in the histotropic stages of *O. dentatum* as determined by quantitative real-time PCR of the corresponding cDNAs. They were most similar to the GST of the canine hookworm *A. caninum* and belonged to the sigma-class of GSTs. They also showed significant similarity to mammalian prostaglandin D_2-synthases in the conserved protein regions.

Cytosolic GSTs can be involved in a variety of cellular functions, including uptake of steroids, regulation of signal transduction pathways and anti-oxidative stress responses (as reviewed by Sheehan *et al.* 2001 and Mahajan and Atkins, 2005). The conjugation and subsequent inactivation of carbonyls and other metabolites of lipid peroxidation by (excretory-secretory) GSTs has been implicated as an internal and external detoxification mechanism of nematodes in the absence of other detoxification systems (Brophy and Barrett, 1990*b*; Brophy *et al.* 1995*a*; Pritchard, 1995) primarily as a housekeeping gene function (Campbell *et al.* 2001*b*; van Rossum *et al.* 2001, 2004). Activities at the host-parasite interface may also include binding of haematin and detoxification in blood-feeding *H. contortus* (Zhan *et al.* 2005), the inactivation of reactive oxygen species produced by pro-inflammatory activities of the host, as suggested for the excretory-secretory GSTs of hookworms (Brophy *et al.* 1995*a*; Pritchard, 1995), or differentially expressed stress-inducible GST3 from *O. volvolus* (Liebau *et al.* 2000; Kampkötter *et al.* 2003). The ability to detoxify a range of xenobiotics has been implicated in the development of anthelmintic resistance (Kawalek *et al.* 1984). Since GST isotypes (see below) have also been identified in the free-living *C. elegans*, an intrinsic function of this enzyme group is evident. However, the RNA interference experiments conducted with the *C. elegans* model so far have not revealed specific functions. In fact, RNAi effects could only be attributed to gst-5 which inhibits germ line apoptosis (www.wormbase.org) and, like gst-30 and gst-38, seems to be involved in oxidative stress and ageing (Ayyadevara *et al.* 2006). The finding that addition of the GST inhibitor SBP impaired the development of L4 *in vitro* indicates that this enzyme plays a role for nematode viability, although the underlying mechanism is yet to be elucidated.

There is growing evidence that helminth GSTs are also involved in the production of bioactive lipids (for review, see Sheehan *et al.* 2001). GSTs of nematode species from various clades as well as from

[2] GenBank Accession nos.:

genomic DNA Od-GST1: EU418979
genomic DNA Od-GST2A: EU670238
genomic DNA Od-GST2B: EU670239

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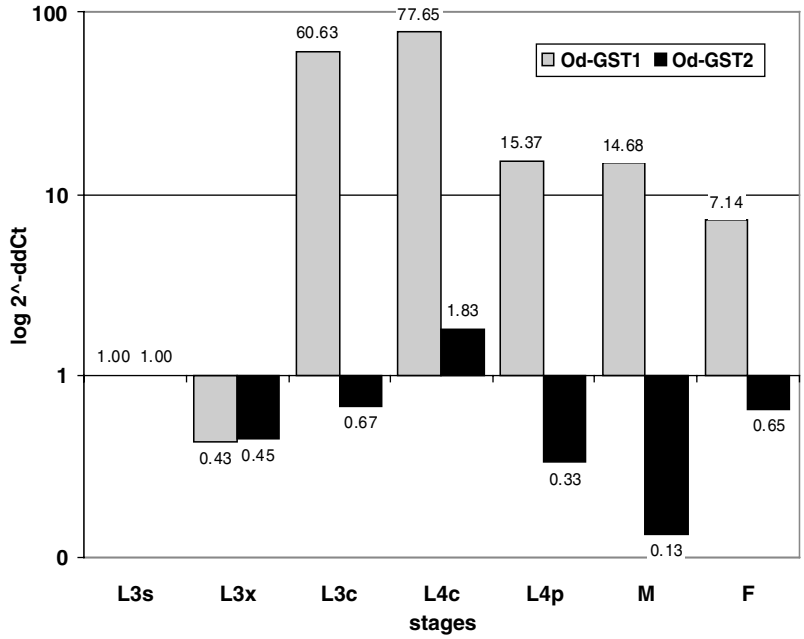

Fig. 5. Differences in the expression of Od-GST1 and Od-GST2 (given as $2^{-\Delta\Delta Ct}$ values) in different developmental stages using ubc-2 as Normalizer and L3s as Calibrator. Values were calculated as averages from 2 independent PCR runs, each in duplicate. For all stages 2 cDNA preparations were tested except L3c (1 preparation).

the blood flukes *S. haematobium* and *S. mansoni* were isolated and characterized and a role in the metabolism of the anti-inflammatory prostaglandins was implicated (Liebau *et al.* 1994; Meyer *et al.* 1996; Hervé *et al.* 2003; Johnson *et al.* 2003; Sommer *et al.* 2003).

In *O. dentatum*, prostaglandins have been detected and their production could be inhibited by non-steroidal anti-inflammatory compounds (Daugschies, 1995), and prostaglandins were implicated in intrinsic functions of worm biology (Daugschies and Ruttkowski, 1998; Daugschies and Joachim, 2000). Although Western blots of *O. dentatum* homogenates showed antigenic reactivity with antibodies directed against human lipoxygenase and cyclooxygenase-2 (Joachim *et al.* 2001), no correlating cDNAs could be isolated (Joachim, unpublished observations). If these enzymes are absent in nematodes it is possible that, given the sequence homology between the first 75 aa of the GSTs reported here to prostaglandin D_2 synthase, the GSTs may take over functions in eicosanoid metabolism. This hypothesis could explain why the GST inhibitor SBP had a negative influence on larval development of *O. dentatum*.

For the free-living *C. elegans*, 44 isoforms of GST are listed in Wormbase (www.wormbase.org). Different GST isoforms have also been characterized for *A. suum* (Liebau *et al.* 1994), *H. polygyrus* (Brophy *et al.* 1994*b*; 1995*b*) and *O. volvulus* (Liebau *et al.* 2000; Sommer *et al.* 2003). Differential expression of GST isotypes has been described for *H. polygyrus* in relation to the immune response to the host (Brophy *et al.* 1995*a*) and in *O. volvulus* in response to oxidative stress (Liebau *et al.* 2000). In the present work two different isotypes of GST with considerable sequence differences could be identified, and Od-GST1 was strongly upregulated in the parasitic stages, especially in the histotropic larvae. Od-GST2, on the other hand, was downregulated in male worms. Possible explanations for the change in expression levels could be host-induced stress, changes in growth rate and metabolism or changes in the utilization of energy sources with subsequent production of endotoxic metabolites. The ratio GST2: GST1 was higher in females than in males, although not as high as in the early L3 stages. Since both males and females were derived from the same animals and treated identically, this difference may be related either to specific functions of GST in this stage or to expression of this enzyme in the maturating egg since the majority of the females was fertilized at harvesting. Changes in oxidative stress levels between the free-living and the parasitic stages can be assumed although this has not been investigated. On the other hand, the production of prostaglandins and leukotrienes in *O. dentatum* also occurs in a stage-specific manner (Daugschies, 1995; Daugschies and Ruttkowski, 1998), and a possible role of GST in this context is still to be investigated. Although a role for putative GST metabolites has been proposed in homoeostasis in lower eukaryotes (Noverr *et al.* 2003), expression profiles during nematode development have not been investigated for parasitic nematodes. To date, specific functions cannot be attributed to the respective isotypes of *O. dentatum* GSTs; and binding kinetics to various substrates need to be investigated either for the

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native and the recombinant proteins, as well as expression sites and possible induction of elevated or reduced expression.

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