

## RESEARCH BRIEF

*Onchocerca volvulus*: Isolation and Sequence of a Second Glutathione S-Transferase cDNA

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Glutathione S-transferases (GSTs) are currently among the leading candidates for a vaccine against parasitic helminths. Vaccination of mice with recombinant *Schistosoma japonicum* GST (Sj26) resulted in up to 50% protection from infection (Mitchell 1989), and immunization using native as well as recombinant *Schistosoma mansoni* GST (Sm28) provided 40–70% protection against infection in rats, mice, and hamsters (Balloul *et al.* 1987a,b). Immunization of baboons with recombinant Sm28 GST affected worm viability (worm burden reduction of 38%) and fecundity (Boulanger *et al.* 1991). Other groups have also reported success using GST in the vaccination of sheep against *Fasciola hepatica*, with a reduction of 57% worm burden (Sexton *et al.* 1990). Such studies indicate the importance of investigating the potential of GSTs as components of a molecularly defined vaccine against onchocerciasis as well. Our laboratory has previously described the isolation and sequence of a cDNA encoding a 27-kDa GST polypeptide of *Onchocerca volvulus* (OvGST1) (Liebau *et al.* 1994a). In this research brief we report the full-length nucleotide sequence encoding a second, distinct 24-kDa *O. volvulus* GST (OvGST2).

Glutathione-binding proteins were purified from *O. volvulus* as previously described (Liebau *et al.* 1994a). Rabbits were immunized with the three isolated proteins to produce antisera. An *O. volvulus* adult worm  $\lambda$ gt11 cDNA library (J. Donelson) was screened using one of the antisera (Ovtrans), and nine immunoreactive clones were identified. Using the polymerase chain reaction (PCR) and  $\lambda$ gt11 forward and reverse primers, the cDNA inserts were amplified, the fragments were subcloned into pBluescript (Stratagene), and the nucleotide sequences were determined. One of

these fragments (clone 9) encodes a polypeptide that possesses some homology with GSTs, whereas three other shorter inserts display overlapping sequences identical to those of clone 9. The longest fragment (Fig. 1, nucleotides 384–835) was subsequently used to screen an *O. volvulus* adult worm Lambda ZAPII cDNA library (K. Henkle-Dührsen) using a standard DNA hybridization protocol. Thirty-two phage clones were identified and purified. Following the *in vivo* excision protocol, the nucleotide sequences of the cDNA inserts in pBluescript were determined. The longest fragment possessed most of the missing OvGST2 5' end, starting at nucleotide 54 (Fig. 1), but was still incomplete. To obtain the complete 5' end of the mRNA and the sequence of the N-terminal portion of OvGST2, the RACE (rapid amplification of cDNA ends), or "anchored" PCR, procedure was used. The first-strand cDNA synthesis was primed using the gene-specific anti-sense oligonucleotide 692 (bp 215–231, Fig. 1). Following cDNA synthesis and subsequent purification, a homopolymeric dC tail was attached to the 3' end of the cDNA. Tailed cDNA was amplified using the nested gene-specific primer 508 (bp 141–156, Fig. 1) and a complementary homopolymer-containing anchor primer permitting amplification from the homopolymeric tail. To increase the specificity, a second nested PCR was performed using oligonucleotide 495 (bp 101–116, Fig. 1) and the anchor primer. The 75-bp PCR fragment obtained was ligated into pCRII using the TA Cloning system (Invitrogen) and the nucleotide sequence was determined.

The entire nucleotide sequence encoding the OvGST2 polypeptide, as well as complete 3' (bp 672–835) and 5' (bp 1–44) nontranslated regions, is illustrated in Fig. 1. The message possesses a 5' spliced leader (SL1) sequence, which is followed by a stretch of 24 nucleotides before the initiation codon AUG at nucleotide 45. The termination codon UGA lies at nucleotide 669. The cDNA possesses a 208-amino-acid

Sequence data from this article have been deposited with the EMBL data library under Accession No. X77393.

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          ttttaattacccaagtttgagggtgaagtagtggcatcattgtgta 44
M   S   Y   K   L   T   Y   F   S   I   R   G   L   A   E   P   16
ATG AGT TAT AAG CTG ACA TAT TTC TCT ATC CGA GGT TTG GCT GAA CCA 92
I   R   L   F   L   V   D   Q   D   I   K   F   I   D   D   R   32
ATT CGT CTA TTT CTG GTT GAT CAA GAC ATA AAA TTT ATT GAC GAT CGT 140
I   A   K   D   D   F   S   S   I   K   S   Q   F   Q   F   G   48
ATT GCT AAG GAT GAT TTT TCT TCC ATA AAA TCG CAA TTC CAA TTC GGA 188
Q   L   P   C   L   Y   D   G   D   Q   Q   I   V   Q   S   G   64
CAA CTT CCT TGT CTT TAC GAT GGT GAT CAG CAA ATC GTT CAG TCC GGC 236
A   I   L   R   H   L   A   R   K   Y   N   L   N   G   E   N   80
GCT ATT CTG AGA CAT CTT GCT CGC AAG TAT AAT TTG AAT GGT GAA AAT 284
E   M   E   T   T   Y   I   D   M   F   C   E   G   V   R   D   96
GAA ATG GAA ACG ACA TAT ATT GAT ATG TTC TGT GAG GGT GTC CGT GAT 332
L   H   V   K   Y   T   R   M   I   Y   M   A   Y   E   T   E   112
CTA CAC GTC AAA TAC ACA CGA ATG ATC TAT ATG GCT TAT GAA TAA 380
K   D   P   Y   I   K   S   I   L   P   G   E   L   A   K   F   128
AAG GAT CCC TAT ATA AAA AGT ATA TTG CCA GGA GAA TTG GCG AAA TTT 428
E   K   L   L   A   T   R   G   N   G   R   N   L   I   L   G   144
GAG AAA TTG CTC GCC ACA CGA GGC AAT GGA AGA AAT TTA ATT CTG GGA 476
D   K   I   S   Y   A   D   Y   A   L   F   E   E   L   D   V   160
GAC AAG ATT TCA TAT GCA GAT TAC GCC CTT TTC GAA GAA CTT GAC GTT 524
H   Q   I   L   D   P   H   C   L   D   K   F   P   L   L   K   176
CAT CAG ATC TTG GAT CCG CAC TGT CTG GAT AAA TTT CCG CTC TTG AAG 572
A   F   H   Q   R   M   K   D   R   P   K   L   K   E   Y   C   192
GCA TTT CAT CAA CGA ATG AAG GAT AGA CCA AAA CTT AAG GAG TAT TGT 620
E   K   R   D   A   A   K   V   P   V   N   G   N   G   K   Q   208
GAG AAA CGT GAT GCA GCA AAA GTA CCA GTG AAT GGA AAT GGG AAA CAG 668

*
TGA gtgataaagtgataaagcaatttaagtgttttttttttgcctatgtttccacttttttc 730
ttattgggatgttgtgaaactttgattttttattataattatgatgcgataaaaattattttt 793
gctaataaagtgatttttttcaaaaaaaaaaaaaaaaaaaaaa 835

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FIG. 1. Nucleotide and deduced amino acid sequences of the OvGST2. Both strands of the cDNA were completely sequenced. Initiation and termination codons are in boldface type and underlined. The asterisk indicates the termination codon. The spliced leader sequence is indicated in boldface type. The anti-sense primers 495, 508, and 692 used in the "anchored" PCR and the potential polyadenylation signal are underlined.

open reading frame with a calculated molecular weight of 24,291. There are no potential N-glycosylation sites and no signal peptide is apparent, indicating that OvGST2 is not membrane associated. Seven of the conserved amino acid residues lining the glutathione-binding region, as described for the glutathione sulfonate-bound Pi-class GST (Reinemer *et al.* 1991), are present in the polypeptide OvGST2 (Y7, G12, K42, Q49, P51, Q62, and S63; Fig. 2).

The amino acid sequence encoded by OvGST2 was compared with other sequences using the NBRF protein sequence database. The most similarity was scored with *Caenorhabditis elegans* GST (Weston *et al.* 1989). When the OvGST2 was compared with mammalian GST polypeptide sequences, the most homology was scored with Pi-class GSTs, including the

human myocardial fatty acid ethyl ester synthetase III (Bora *et al.* 1991). A comparison of OvGST2 with the other known nematode GST sequences and the most homologous mammalian sequence of the Pi class (Sugawara *et al.* 1985) is illustrated in Fig. 2. The OvGST2 has 60.1% sequence identity to the *C. elegans* GST and 44.7% to the rat Pi-class GST, clearly indicating a structural relationship with these enzymes. The *Ascaris suum* GST (Liebau *et al.* 1994b) is less similar, 27.9%, and the previously described GST from *O. volvulus* (OvGST1) (Liebau *et al.* 1994a) has 23.1% sequence identity. The structural differences between OvGST1 and OvGST2 could convey different substrate specificities, inhibitor sensitivities, and intracellular binding and transport capacities, which will be assessed in future studies.

|               |                                                           |            |
|---------------|-----------------------------------------------------------|------------|
| OvGST1        | MNFVVEAASSNANQAITSSENSIKPKGKLQPQ                          | 31         |
| <b>OvGST2</b> | <b>MS-YKLTYFSIRGLAEPRLFLVDQDIKFIDDRIAKDDFSS--IKSQFQF</b>  | <b>47</b>  |
| OvGST1        | *EK*T***NG**R**V***LFALANVSYE*N*TR*EWKY--L*PRT*P*         | 79         |
| CeGST         | *T-L*****D*H*****L*A*KQVAYE*H*VTYEQWAD---*PKMI*           | 47         |
| Ratpi         | *PP*TIV**PV**RC*AT*ML*A**GQSWKEEVVTI*VWLQGS*SL**TCLY      | 50         |
| AsGST1        | *PQ*****D***G*GA**IFHQAGV**E*N*LKRE*WPA--L*PKTP*          | 48         |
| <b>OvGST2</b> | <b>GQLPCLYDGDQIQVQSGAILRHLARKYNLNGENEMETTYIDMFCEGVRDL</b> | <b>97</b>  |
| OvGST1        | *HV*M*NVSIGNVLGE*H**ELL*GGRFG*L*T*DW*EA-KI*AVVLNI*E       | 128        |
| CeGST         | **V***LS**EE*****I*****LNG***S**T***F***Y**L***           | 97         |
| Ratpi         | ***KFE***LTLY**N*****G*SLG*Y*KDQK*AALV**VND**E**          | 100        |
| AsGST1        | ***L*EVDGEVLA**A**Y*Y*G*QFG*A*KTP**EAQV*SIFDQFK*F         | 98         |
| <b>OvGST2</b> | <b>HVKYTRMIYM--AYETEKDPYIKSILPGELAKFEKLLA--TRNGRNL</b>    | <b>142</b> |
| OvGST1        | LFQKLIPWTH--EKNT*K*AELFRNLSESDVMP*LGRYEKFLKESTG*H*        | 176        |
| CeGST         | *T***T***R--N**DG*A***DV*****RL***FH--*YK**EHYV           | 142        |
| Ratpi         | RC**GTL**T--N**NG**D*V*A-***H*KP**T**S--QNQG*KAF*         | 144        |
| AsGST1        | MAELRPCFRVLAGE*GD*EKVL*EVAVPARD*HLP**EKFLAKS*SEYM         | 148        |
| <b>OvGST2</b> | <b>LGDKISYADYALFEELDVHQILDPHCLDKFPLLKAFHQRMKDRPKLKEYC</b> | <b>192</b> |
| OvGST1        | V*N*V*V**LTV*NM*MTLDDEVKLEEYPQLASFVNK--IGQM*GI**WI        | 224        |
| CeGST         | I***E*****V*****I*L**T*NA**GV*A**K**E*FAE**NI*A*L         | 192        |
| Ratpi         | V*NQ**F***N*LDL*L***V*A*G***N***S*YVA*LSA***I*AFL         | 194        |
| AsGST1        | V*KSVTW**LVITDS*ASWES*I*DF*SGHLQ**KYIEHVREL*NI*KWI        | 198        |
| <b>OvGST2</b> | <b>EKRDAAKVPVNGNGKQ</b>                                   | <b>208</b> |
| OvGST1        | K**PKTYF                                                  | 224        |
| CeGST         | N**A*INP*****                                             | 208        |
| Ratpi         | SSP*HLNR*I*****                                           | 210        |
| AsGST1        | AE*PKTPY                                                  | 206        |

FIG. 2. Comparison of the deduced amino acid sequences of the cDNAs from *O. volvulus* GST2 (OvGST2, this study), *O. volvulus* GST1 (OvGST1; Liebau *et al.* 1994a), *C. elegans* GST (CeGST; Weston *et al.* 1989), rat Pi-class GST (Suguoka *et al.* 1985), and *A. suum* GST1 (AsGST1; Liebau *et al.* 1994b). Gaps indicated by a dash were introduced into the sequence to optimize the alignment. An asterisk indicates identities between these different GST sequences. The conserved amino acids lining the glutathione-binding site are underlined.

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