

Molecular characterisation and localisation of an *Onchocerca volvulus* π -class glutathione *S*-transferase

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Abstract

Glutathione *S*-transferases (GSTs) constitute a major detoxification mechanism in helminth organisms and are regarded vaccine candidates against helminth infections. *Onchocerca volvulus* glutathione-binding proteins were purified from the aqueous soluble fraction of homogenised adult females by affinity chromatography on glutathione-agarose. The eluted proteins had a specific GST activity of $1.6 \mu\text{mol min}^{-1} \text{mg}^{-1}$. Immunohistochemical studies localised these antigens in the hypodermis, the wall of the seminal receptacle and spermatozoa of adult worms. A λgt11 clone was isolated from an expression library of *O. volvulus* by immunoscreening. Sequence analysis revealed that it encoded a π -class GST with 60% identity with *Caenorhabditis elegans* and up to 45% identity with mammalian π -class GSTs. Antibodies affinity selected with recombinant GST demonstrated cross-reactivity between *Litomosoides sigmodontis* and *O. volvulus* GSTs.

Key words: Filariasis; Glutathione *S*-transferase; Molecular cloning; *Onchocerca volvulus*; *Litomosoides sigmodontis*; L3

1. Introduction

Glutathione *S*-transferases (GSTs) are ubiquitous enzymes in eukaryotic organisms performing a series of reactions which are essential for protection of cell constituents from oxidative attack by oxygen and oxygen-associated free radicals [1,2]. GSTs catalyse

the conjugation of both endogenous and xenobiotic hydrophobic electrophiles with the major cellular nucleophile glutathione, rendering the product less reactive and water soluble, and thus facilitating excretion [2]. Most organisms contain multiple GST isoenzymes that are characterised by distinct, but often overlapping, substrate specificities which account for their capacity to metabolise structurally diverse molecules. Some GSTs also show selenium-independent glutathione peroxidase activity against lipid and nucleic acid hydroperoxides. In addition to their enzymatic function, they can act as non-covalent binding proteins for a range of hydrophobic compounds, such as bilirubin and haem [1]. They have also been implicated in the development of drug resistance of cells and organisms to elec-

Abbreviations: GST, glutathione *S*-transferase; UWGCG, University of Wisconsin Genetics Computer Group; L3, third larval stage.

Note: Nucleotide sequence data reported in this paper are available from the GenBank™ data base under the accession number L28771.

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trophilic anticancer drugs, pesticides and herbicides [2,3].

Helminths have limited detoxification enzymes and appear to lack the important cytochrome P-450 dependent detoxification reactions [4]. Thus, GSTs seem to be the major detoxification enzymes of this group of parasites [5]. The role of GSTs in detoxification may be related to their ability to scavenge secondary products of lipid peroxidation such as those derived from the host immuno-effector mechanisms and to metabolise other toxic products including chemotherapeutic drugs. These functions make GSTs attractive targets for the development of anti-helminthics. In addition to their enzymatic role, they have been identified as protective antigens in schistosomiasis [6,7] and fascioliasis [8].

This paper reports the isolation and partial characterisation of GSTs from *Onchocerca volvulus* and the molecular cloning of a cDNA derived from an adult female expression library which codes for a π -class GST.

2. Materials and methods

Isolation of *O. volvulus* glutathione-binding proteins. Female *O. volvulus* adult worms (collected from Sierra Leonean patients) were ground to a powder in liquid nitrogen using a mortar and pestle. The powder was resuspended in phosphate buffered saline (PBS: 1.8 mM KH_2PO_4 / 5 mM K_2HPO_4 / 0.15 M NaCl pH 7.4)/ 1% Triton X-100 and centrifuged at $1500 \times g$ for 10 min. The supernatant fluid was passed through a 3-ml bed-volume glutathione-agarose column (Sigma G-4510) at a flow rate of 0.5 ml min^{-1} . The column was washed with 20 column volumes of PBS, 1% Triton X-100. Glutathione-binding proteins were eluted with PBS, 1% Triton X-100 containing 5 mM glutathione.

Enzymatic assays. Glutathione S-transferase activity of the aqueous soluble fraction of homogenised *O. volvulus* adult females and purified glutathione-binding proteins was measured spectrophotometrically at 340 nm ($\epsilon = 9.6 \text{ cm}^{-1} \text{ mM}^{-1}$) [9], using 1-chloro-2,4 dinitrobenzene (CDNB) as a substrate. The reaction mixture contained 0.1 M potassium phosphate,

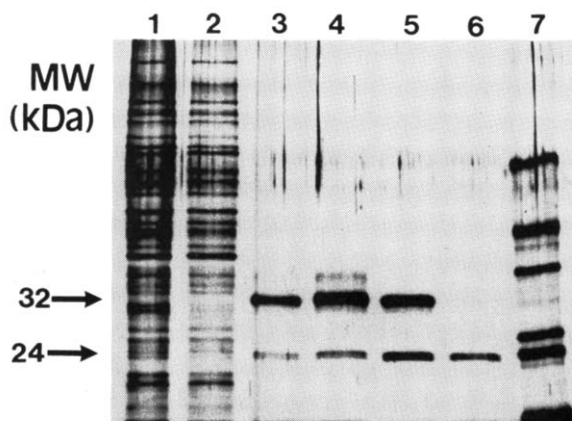


Fig. 1. SDS-PAGE analysis of *O. volvulus* adult female proteins with affinity for glutathione. Glutathione-binding proteins were purified from an aqueous extract of *O. volvulus* by affinity chromatography using a glutathione-agarose column. Samples (10 μl) of the first four eluted fractions (1.4 ml) were separated on a 13% acrylamide-SDS gel under reducing conditions and detected using a silver staining method. Lane 1, aqueous extract of homogenised adult females. Lane 2, aqueous extract depleted of GSTs. Lanes 3–6, glutathione-binding proteins eluted from the column in the first 4 sequential fractions.

pH 6.5/ 1 mM CDNB/ 5 mM glutathione in a total volume of 1 ml.

Glutathione peroxidase activity of glutathione-binding proteins was determined spectrophotometrically at 340 nm ($\epsilon = 6.22 \text{ cm}^{-1} \text{ mM}^{-1}$) using hydrogen peroxide as a substrate [10]. The 1-ml reaction mixture contained 50 mM potassium phosphate pH 7.0/ 1.0 mM glutathione/ 5 mM dithiothreitol/ 2 U of glutathione reductase (Sigma, G4759)/ 0.2 mM NADPH/ 0.25 mM hydrogen peroxide.

The protein concentration of purified glutathione-binding proteins and of the soluble fraction of homogenised adult females were determined by BCA Protein Assay ReagentTM (Pierce) on samples dialysed against 10 mM KCl. Assays were carried out in triplicate using the dialysis buffer as a negative control.

Antiserum. A rabbit was immunised with affinity-purified glutathione-binding proteins from *O. volvulus* (a pool of fractions 2 and 3 as shown in Fig. 1) using RIBITM (Immunochem Research Inc.) as adjuvant and following the manufacturer's immunisation protocol. After the first immunisation with 200 μg of the antigen, the rabbit was boosted on day 28 with

100 μg of the antigen. Serum was collected 2 weeks after the booster injection. For the immunoscreen of the cDNA library and immunohistochemical studies, the serum was extensively absorbed with *Escherichia coli* Y1090, β -galactosidase and total *O. volvulus* adult female antigen depleted of glutathione-binding proteins using standard methods [11].

In a second procedure, antibodies specific for the recombinant *O. volvulus* π -class GST were purified from the serum raised against glutathione-binding proteins by absorption on and elution from bacterial plaques expressing the recombinant antigen using the method described by Beall and Mitchell [12].

SDS-polyacrylamide gel electrophoresis and Western blot analysis. Proteins boiled in SDS sample buffer were fractionated on 13% SDS-polyacrylamide gels under reducing conditions [13]. Proteins were detected by a modified silver staining method which included a reduction step with 5 $\mu\text{g ml}^{-1}$ dithiothreitol prior to impregnation of the gels with silver nitrate [14]. The relative molecular masses of separated proteins were estimated by comparison with standard markers (Sigma MW-SDS-70L).

Western blot analyses were carried out using standard procedures [15].

Identification of cDNAs encoding *O. volvulus* glutathione S-transferases. 2×10^6 phages of an amplified λ gt11 expression library prepared from *O. volvulus* adult females (Guatemala strain, ATCC 37509) [16] were immunoscreened with the anti-serum raised against glutathione-binding proteins following standard protocols [17].

The plaque hybridisation technique [16] was used to identify clones carrying the entire cDNA coding sequence. The cDNA used as a hybridisation probe was radiolabelled by the random primer method using Klenow polymerase [11]. Hybridisations were performed at 42°C in 50% formamide. The filters were washed 3 times with $2 \times \text{SSC} / 0.1\%$ SDS and twice with $0.1 \times \text{SSC} / 0.1\%$ SDS at room temperature ($1 \times \text{SSC} = 0.15 \text{ M NaCl} / 17 \text{ mM sodium citrate, pH } 7$).

Cloning, sequencing and sequence analysis. cDNA inserts were amplified by PCR [18] using 1×10^6

phage particles as template, λ forward and reverse primers and Taq DNA polymerase (Cetus Corporation). The PCR products were purified using Clean Up™ Purification System (Promega Corporation) and cloned into pCR™ 1000 vector (Invitrogen) according to standard protocols [11]. Plasmid DNA for double strand sequencing was prepared using the Magic Miniprep™ kit (Promega Corporation) according to the manufacturer's instructions. The sequencing reactions were carried out using the dideoxy chain termination method [19] following the protocols supplied with the DNA sequencing kit (Sequenase Version 2.0, USB). Both strands of the cDNA inserts were sequenced with standard and internal oligonucleotide primers.

DNA sequences were analysed using the UWGCG computer software package [20]. DNA and protein sequence comparisons were performed using the FASTA computer programme [21].

Histological localisation of glutathione-binding proteins. *O. volvulus* nodules were fixed in 10% formal saline and processed conventionally for wax histology. Sections were dewaxed by washing twice with xylene. Endogenous peroxidase activity was removed by a 30-min incubation in methanol, 1% H_2O_2 . The sections were blocked for 1 h with PBS/5% goat serum and then incubated for 2 h at room temperature with the serum raised against glutathione-binding proteins. The serum was used at a 1:50 dilution in PBS/1% goat serum. Serum taken from the rabbit before immunisation was used as a control. After 3 washes with PBS, the sections were incubated with goat anti-rabbit IgG peroxidase conjugate diluted 1:50 in PBS/1% goat serum. Following 3 washes with PBS, the sections were incubated with 3-amino-9-ethyl-carbazole and H_2O_2 . The sections were counterstained with hematoxylin, mounted with glycerol-gelatin (Sigma GG1) and examined with a Leitz Dialux 20 EB microscope.

3. Results

Isolation of glutathione-binding proteins from *O. volvulus* adult females. Glutathione-binding proteins of *O. volvulus* adult females were purified by affin-

ity chromatography on a glutathione-agarose affinity column. The proteins eluted from the column yielded about 0.7% of the total aqueous soluble extract loaded on to the column. The eluted proteins were analysed by SDS-PAGE under reducing conditions (Fig. 1). Three major bands with molecular mass of 24, 25 and 32 kDa and a minor band of 36 kDa were detected. The higher molecular weight components (32 and 36 kDa) were eluted exclusively in the first three fractions, whereas the smaller components were also found in subsequent eluates, suggesting that they may have a higher affinity for glutathione.

Enzymatic activity of the glutathione-binding proteins. GST activity in the fractions eluted from the column was tested using CDNB as a substrate. The calculated specific enzymatic activity was $1.6 \text{ mmol min}^{-1} \text{ mg}^{-1}$ for the fraction which contains all the glutathione-binding proteins (Fig. 1, fraction 2) and $0.8 \text{ } \mu\text{mol min}^{-1} \text{ mg}^{-1}$ for those fractions which contain only the 24–25 kDa “doublet”. The specific activity of the soluble fraction of total adult female homogenate was $0.05 \text{ } \mu\text{mol min}^{-1} \text{ mg}^{-1}$.

Glutathione peroxidase activity, using hydrogen peroxide as a substrate, was not detected either in the total aqueous soluble fraction from adult females or in the affinity purified fractions. This suggests that selenium-dependent glutathione peroxidase(s) may not be present in the aqueous soluble fraction of *O. volvulus* adult females.

Isolation and characterisation of a recombinant π -class GST of *O. volvulus*. To identify cDNAs encoding GSTs of *O. volvulus*, a λ gt11 library was screened with the rabbit antiserum raised against the purified glutathione-binding proteins. The PCR products generated from the cDNA inserts of these λ clones were subcloned into the pCRTM 1000 vector. Sequence analysis revealed that one clone encoded the carboxy-terminal half of a π -class GST and contained the 3'-untranslated region.

To identify a clone encoding the entire GST molecule, a probe was generated from the previously identified clone. Two clones that hybridised strongly and consistently with that probe were purified to homogeneity. Their PCR products were subcloned into the pCRTM 1000 vector. Sequence analysis revealed that both clones encoded the same π -class

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TTGGGTAAATGAGTTATAAGCTGACATATTTCTCTATCCGAGGTTTGGCTGAACCAATTCG (60)
  M S Y K L T Y F S I R G L A E P I R
TCTATTCTCGTTGATCAAGACATAAAATTTATTGACGATCGTATTGCTAAGGATGATTT (120)
  L F L V D Q D I K F I D D R I A K D D F
TTCTTCCATAAAATCGCAATTCGAATTCGGACAACCTCCCTGTCTTTACGATGGTGATCA (180)
  S S I K S Q F Q F G Q L P C L Y D G D Q
GCAATCGTTCAGTCCGGCGCTATTCTGAGACATCTGCTCGCAAGTATAATTGTAATGG (240)
  Q I V Q S G A I L R H L A R K Y N L N G
TGAAATGAAATGGAAACGACATATATGATATGTTCTGTGAGGGGTGCTCGTATCTACA (300)
  E N E M E T T Y I D M F C E G V R D L H
CGTCAATACACACGAATGATCTATATGGCTTATGAACTGAAAGGATCCCTATATATAA (360)
  V K Y T R M I Y M A Y E T E K D P Y I K
AAGTATATTGCCAGGAGAAATGGCGAAATTTGAGAAATGCTGCGCCACAGGCAATGG (420)
  S I L P G E L A K F E K L L A T R G N G
AAGAATTTAATTTCTGGGAGACAAGATTTTCATGACAGATTACGCCCTTTTCGAAGACT (480)
  R N L I L G D K I S Y A D Y A L F E E L
TGAAGTTCATCAGATCTTGGATCCGCACTGTCTGGATAAAATTTCCGCTCTTGAAGGCATT (540)
  D V H Q I L D P H C L D K F P L L K A F
TCATCAACGAATGAAGGATAGACAAAACCTTAAGGAGTATTGTGAGAAACGTGATCGAGC (600)
  H Q R M K D R P K L K E Y C E K R D A A
AAAAGTACCAGTGAATGGAAATGGGAAACAGTCAGTGATAAAGTATAAGCAATTTAAT (660)
  K V P V N G N G K Q *
GTTTTTTTTTGCCATGTTTCCACATTTTTTCTTATTGGGAGTTGTGAAACTTTGATTTT (720)

TATTAATTTTATGATCGGATAAAAAATTATTTATTGCTaataaagTGATTTTTTTCAAAAAA (780)

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AAAAAAAAAAAAA (793)

Fig. 2. *O. volvulus* π -class glutathione S-transferase (Ov 24): nucleotide sequence and predicted amino acid sequence starting from the putative initiation methionine. The putative polyadenylation signal is indicated in lower case.

GST as the cDNA isolated by immunoscreen. One clone, designated Ov24, carried the entire coding region of a π -class GST. The nucleotide sequence of Ov24 including the deduced amino acid sequence is shown in Fig. 2.

Computer analysis revealed a single open reading frame coding for 208 amino acids, starting with the initiation codon ATG at position 8 and terminating with the stop codon TGA at nucleotide 632. It contains a short 5'-untranslated region (7 bp) and a 3'-non-coding region of 159 bp. A poly(A) tail is preceded by a putative polyadenylation signal AAT-AAA. Sequence comparison revealed that the highest identity was scored with class π -GST isoenzymes: 60% amino acid identity with *Caenorhabditis elegans* π -class GST [22] and up to 45% amino acid identity with mammalian π -class GSTs. The alignment of the predicted amino acid sequence of Ov24 with other π -class GSTs is shown in Fig. 3. Sequence comparisons with GSTs grouped in other classes showed up to 34% identity with mammalian and helminth μ -class GSTs and up to 31% identity with mammalian α -class GSTs. A 31% identity was

also shown with 28 kDa *Schistosoma mansoni*. The GST encoded by Ov24 has a predicted molecular mass of 24217 Da. No potential *N*-glycosylation sites (N-X-S/T) were detected.

Western blot analysis. *O. volvulus* π -class GST specific antibodies were used to probe total *O. volvulus* adult female antigen and total *L. sigmodontis* L3 antigen immobilised on nitrocellulose filters. The selected antibodies recognised the 24–25 kDa “doublet” in total worm antigen whereas they did not cross-react with the 32 and 36 kDa glutathione-binding proteins (Fig. 4, lane B). This result suggests that the 24–25 fractions are antigenically related and therefore may belong to the same isoenzyme family [1]. A “doublet” of the same molecular mass was recognised in total L3 *L. sigmodontis* antigen (Fig. 4, lane A). This result demonstrated that *L. sigmodontis* and *O. volvulus* GSTs cross-react and that GSTs are expressed in the infective L3 larvae of *L. sigmodontis*.

Histological localisation of glutathione-binding proteins. Immunohistochemical localisation was per-

<i>O. volvulus</i>	Ov24	MSYKL	FSI	RLA	EPILF	LVQDQ	IKFID	DRIAKDF	SS	RSQ	FOFG	(48)
<i>C. elegans</i>	pi-GST	TL	D		L	A	KQAYE	H	VITYEQ	AD	PKML	(48)
Rat	pi-GST	MPP	TIN		RC	AT	ML	A	QSNKE	EVVIT	MLQ	(51)
Human	pi-GST	MPP	TIN		RC	AL	ML	A	QSNKE	EVVIT	MLQ	(51)
Pig	pi-GST	MPP	TI		RC	AM	ML	A	QSNKE	EVVIT	MLQ	(49)
<i>O. volvulus</i>	Ov24		FLYDGO	Q		GAILR	HLARKYMLNG	ENEMETTYID	MFCEGV			(98)
<i>C. elegans</i>	pi-GST		LS		E							(98)
Rat	pi-GST		KFE		L		TLV		G	SLG	Y	(101)
Human	pi-GST		KFE		L		TLV		G	SLG	Y	(101)
Pig	pi-GST		KFE		L		TLV		G	SLG	Y	(99)
<i>O. volvulus</i>	Ov24	VKYTR	MIYMA	YETEK	DPYIK	SILP	GELAKF	EKL	LATRGNG	RNL	ILGDKIS	(148)
<i>C. elegans</i>	pi-GST	T		T		RM		DG		A		(148)
Rat	pi-GST		GTL		TN		NG		D		V	(150)
Human	pi-GST		ISL		TN		AG		D		V	(150)
Pig	pi-GST		ATL		TN		AG		EK		V	(148)
<i>O. volvulus</i>	Ov24	YADYAL	FEEL	DVH	QILDPHC	LDK	FPLLKAF	HQR	MKORPKL	KEYCEK	RDA	(198)
<i>C. elegans</i>	pi-GST		V									(198)
Rat	pi-GST		N		LDL		L		V		A	(200)
Human	pi-GST		N		LDL		L		V		A	(200)
Pig	pi-GST		N		LDL		L		V		A	(198)
<i>O. volvulus</i>	Ov24	KYPV	NGKQ									(208)
<i>C. elegans</i>	pi-GST	NP										(208)
Rat	pi-GST	NR										(218)
Human	pi-GST	NR										(218)
Pig	pi-GST	NR										(208)

Fig. 3. Comparison of the primary structure of *O. volvulus* π -class GST (GenBank accession number: L28771) with p-class GSTs of *C. elegans*, rat, man and pig (Accession Numbers: EMBL X06547, EMBL X02904, EMBL X06547 and PIR S13780 respectively). Gaps were introduced for optimal alignment at positions 39 and 40 and at position 122 and are indicated by dash. Amino acid residues which are identical to the *O. volvulus* GST are marked by dots. Boxes indicate regions of identity with respect to amino acid residues involved in the formation of the glutathione-binding site.

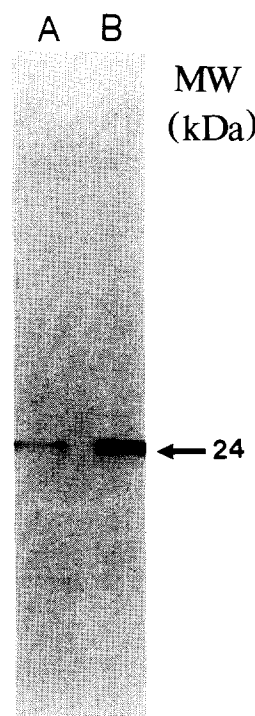


Fig. 4. Western blot of *L. sigmodontis* total L3 antigen (10 μ l of 1000 L3 boiled in 0.5 ml of sample buffer) (lane A) and total worm antigen (25 μ g) (lane B). The Western blot was probed with *O. volvulus* π -class GST specific antibodies.

formed on sections of *O. volvulus* nodules using first the rabbit serum raised to the affinity purified native glutathione-binding proteins and second, antibodies affinity selected with the recombinant π -class GST. The staining patterns were identical and localised the antigens in the hypodermis (Fig. 5) and the wall of the seminal receptacle of adult females (Fig. 6). The antigens were also identified in spermatozoa in the seminal receptacle (Fig. 6) [23]. Microfilariae, either intrauterine or free within the nodule, were not stained.

4. Discussion

Affinity purification of glutathione-binding proteins from *O. volvulus* adult females by glutathione-agarose led to the isolation of four components of 24, 25, 32 and 36 kDa. The elution profile of glutathione-binding proteins (Fig. 1) suggests that these

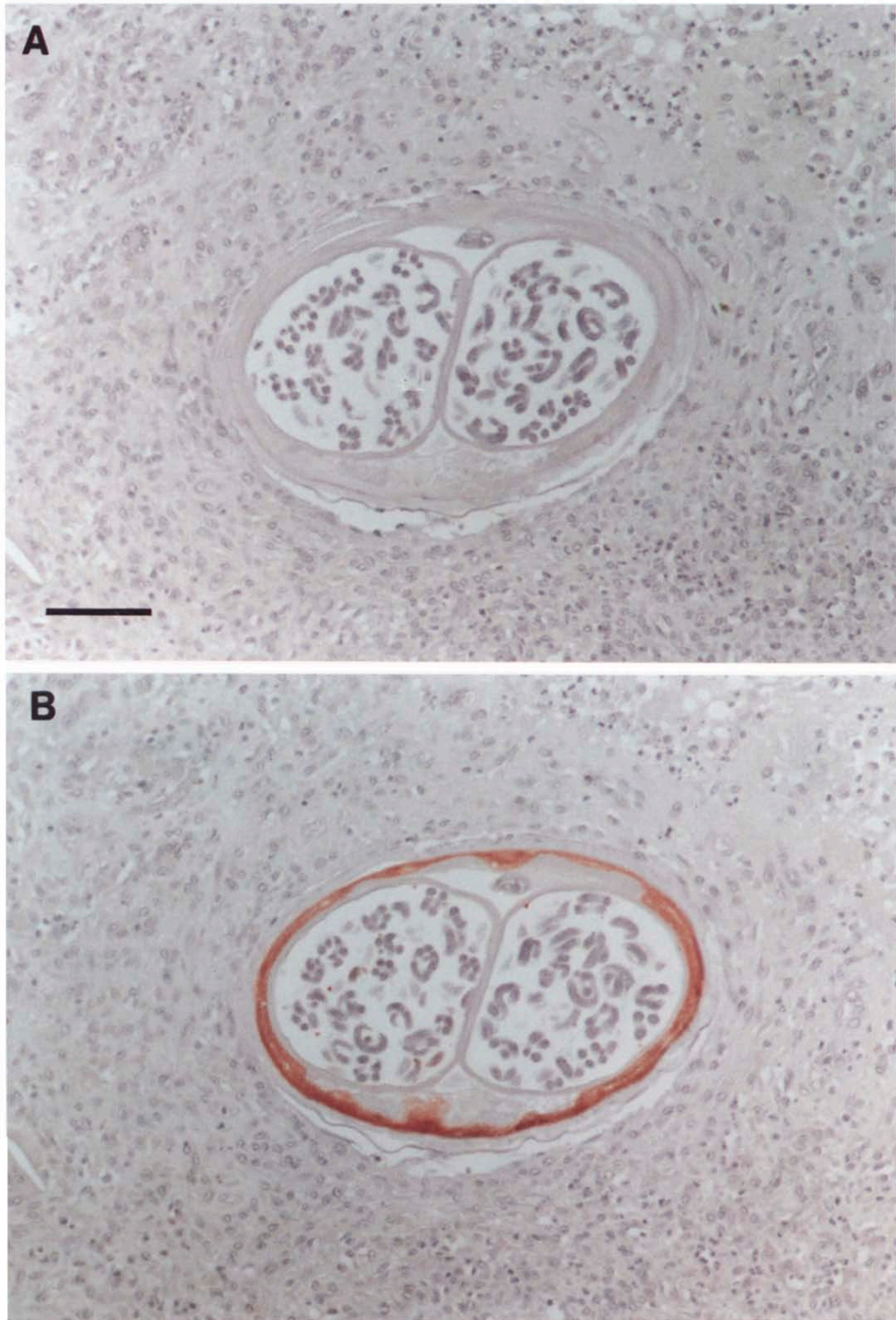


Fig. 5. Light micrograph illustrating the distribution of glutathione-binding proteins in *O. volvulus* adult females using the rabbit serum raised to affinity purified glutathione-binding proteins. This resulted in specific staining of the hypodermis (B). The serum taken from the rabbit before immunisation was used as a control (A). The sections were counterstained with hematoxylin. Calibration bar = 100 μ m.

enzymes might have different affinities for glutathione. This might be further exploited for the purification of the different components using a glutathione concentration gradient.

The specific glutathione *S*-transferase activity of the aqueous soluble fraction of homogenised adult females ($0.05 \mu\text{mol min}^{-1} \text{mg}^{-1}$) compares well with those of *Onchocerca gutturosa* [24], *Dirofilaria immitis* [10], *Brugia pahangi* [10] and *Haemonchus contortus* [25]; reported as 0.03, 0.03, 0.01 and $0.11 \mu\text{mol min}^{-1} \text{mg}^{-1}$ respectively. The specific enzymatic activity of the purified proteins is in the same range as that of purified GSTs from other filarial parasites [10]. The fraction containing all glutathione-binding proteins (Fig. 1, fraction 2) has double the specific activity of fraction 4, containing only the low molecular mass components. This implies that the high molecular mass glutathione-bind-

ing proteins exhibit a higher glutathione *S*-transferase activity.

Neither the aqueous soluble fraction of homogenised adult females nor the affinity purified enzymes possessed glutathione peroxidase activity using H_2O_2 as a substrate. This is consistent with previous observations. Glutathione peroxidase activity using H_2O_2 as a substrate has not been detected in the aqueous soluble fraction of other filarial nematodes [10]. Lack of material precluded further enzymatic characterisation of the native glutathione-binding proteins.

Clone Ov 24 described in this paper is a π -class GST. This identification is based on sequence similarities with the π -class GSTs of *C. elegans* (60% identity) and mammals (up to 45% identity). The most prominent feature of all the π -class GSTs was the marked degree of identity of the 8 carboxy-termi-

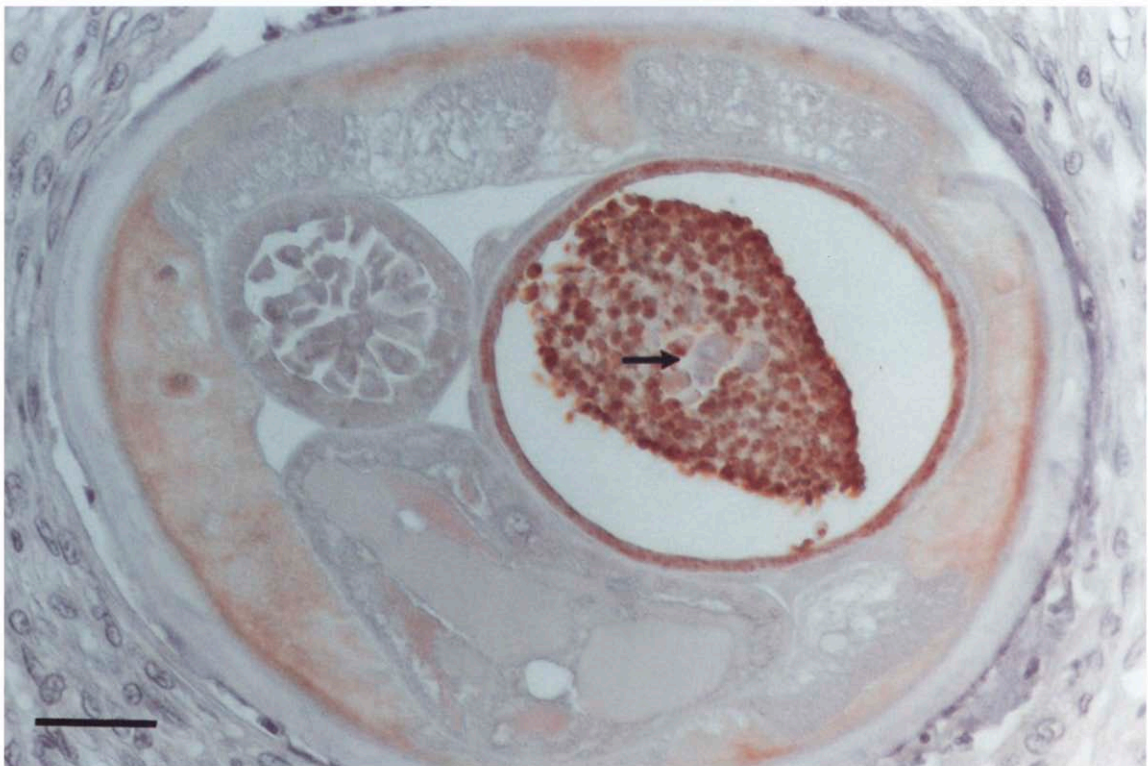


Fig. 6. Light micrograph illustrating the distribution of glutathione-binding proteins in a transverse section of adult female using the rabbit serum raised against glutathione-binding proteins. The antigens are present in the wall of the seminal receptacle and in the spermatozoa. The arrow indicates an oocyte surrounded by spermatozoa. The section was counterstained with hematoxylin. Calibration bar = $250 \mu\text{m}$.

nal amino acid residues (see Fig. 3), suggesting that this region of the molecule may be involved in specific functions common to this family. The three-dimensional structure of the pig π -class GST complexed with glutathione sulphonate has been resolved [26]. The 11 amino acid residues involved in the formation of the glutathione-binding site are all conserved in *O. volvulus* and *C. elegans* π -class GST: 8 are identical and 3 are conservatively substituted (see Fig. 3), suggesting a structural relationship with the pig isoenzyme.

Although Ov24 and the GST isolated from *C. elegans*, another nematode, have been identified as π -class enzymes [22,27], most of the cDNAs encoding GSTs isolated from trematodes have been identified as μ -class [28,29,30,31], with the exception of the 28 kDa GSTs of schistosomes, which do not conform to the classification originally adopted for mammalian GSTs [31–33]. In addition, data from *N*-terminal amino acid sequence and biochemical studies of native GSTs from trematodes and cestodes have shown that most of the GSTs from these organisms displayed μ -class features and a few, α -class features [5]. Amino acid sequence identity between Ov24 and α - and μ -class GSTs of trematodes and mammals did not exceed 34%, allowing for gaps to optimise alignments. As far as the current data permits, it is possible to speculate that π -class GSTs are a characteristic of Nematoda but not of Platyhelminthes.

Sequence comparison of Ov 24 with a novel GST isolated from rat liver and shown to exhibit macrophage migration inhibitory activity [34] did not reveal significant identity. Full alignment of the deduced amino acid sequence of Ov 24 with that of *C. elegans* suggests that the cDNA contains the entire coding region (see Fig. 3). In addition, the *O. volvulus* GST cDNA contains a purine at position –3 with regard to the putative initiation codon for methionine. This is in agreement with the consensus sequence for the initiation of translation in eukaryotic organisms [35]. Furthermore, the calculated molecular mass of the protein (24.2 kDa) is consistent with the relative molecular mass of the native proteins recognised by the π -class specific antibodies (Fig. 4).

The antigen has been localised in the hypodermis (Fig. 5) and in the wall of the seminal receptacle of

adult females (Fig. 6), but not in intrauterine or nodular microfilariae. In addition, the antigen is present in the spermatozoa. In contrast, the oocytes do not seem to express the antigen (Fig. 6). These data suggest differential regulation of GST gene expression in the gametocytes and in different stages of the life cycle of *O. volvulus*.

As demonstrated by Western blot, the antigen is also expressed in the L3 larvae of *L. sigmodontis*, a rodent parasite [36] currently being used to study the immunology of filarial infections [37]. Invading L3 larvae are thought to be the primary target of protective immunity against nematode parasites [38]. The presence of GST in L3 larvae, together with the observations that GST have been shown to elicit protective responses against trematodes [7,8] suggest that Ov 24 might be considered as a vaccine candidate against filarial infections.

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