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## Cloning and partial characterization of a *Boophilus microplus* (Acari: Ixodidae) glutathione S-transferase

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### Abstract

A cDNA of glutathione S-transferase (GST) was isolated from a cDNA library of salivary glands of *Boophilus microplus*. The recombinant protein was purified by glutathione affinity chromatography and assayed upon the chromogenic substrate CDNB. The 864 bp cloned fragment was sequenced and showed an open reading frame coding for a protein of 220 amino acids. Expression of the GST gene was tested by RT-PCR in tick tissues and larvae mRNA. Comparison of the deduced amino acid sequence with GSTs from other species revealed that the enzyme is closely related to the mammalian class mu GSTs. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Boophilus microplus*; Glutathione S-transferase; Ectoparasites; cDNA cloning

### 1. Introduction

The tick *Boophilus microplus* is a cattle ectoparasite, present in America, Asia, Africa and Oceania (Johnston et al., 1986). Conventional tick control methods are based mainly on the use of acaricides in spite of the high cost of these products and the quick appearance of resistance in the populations (Nolan, 1985). The presence of chemical residues in meat and milk emphasizes the need for other control methods (Kunz and Kemp, 1994). Thus, efforts are being made to develop new methodologies to monitor the appearance of acaricide resistance (Baxter et al., 1999) as well as alternative means of tick control, like vaccines (Willadsen et al., 1989; Riding et al., 1994; Rodriguez et al., 1994; Log-

ullo et al., 1998; Da Silva Vaz Jr. et al., 1998) and biological control (Farias et al., 1986; Kaaya et al., 1996; Frazzon et al., 2000).

Glutathione S-transferases (GSTs) belong to a multi-gene family of proteins that play a central role in detoxication of xenobiotic compounds. Increased expression levels of GST have been correlated with resistance to insecticides in various organisms (Kawalek et al., 1984; Wilce and Parker, 1994; Huang et al., 1998). A *B. microplus* GST isolated from larvae could not be correlated with tick resistance against organophosphorus compounds (He et al., 1999), however, the role of GSTs in resistance to acaricides cannot be ruled out since it is known that the functional properties of their isoforms are distinct (Jagt et al., 1985). Some GSTs may act as intracellular transporters of various non-substrate hydrophobic compounds (Mannervik et al., 1985). Also, GSTs may be involved in digestive processes (Douch and Buchanan, 1978), prostaglandin synthesis (Meyer and Thomas, 1995), and in a series of reactions that are

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essential to protect cell constituents from oxidative attack by oxygen and oxygen-associated free radicals (Sharp et al., 1991; Feng et al., 1999). Within parasites, GSTs have been studied for their potential as targets for an immunological intervention against helminthic diseases (Balloul et al., 1987; Yang et al., 1997). Because of the wide range of GST functions described (Wilce and Parker, 1994), further studies in *B. microplus* GSTs could be a valuable subject in the search for new methodologies to control tick populations.

In the present study we report the cloning, expression and partial characterization of a *B. microplus* GST.

## 2. Material and methods

The *B. microplus* GST (BmGST) cDNA sequence can be accessed at GenBank with the accession number AF366931.

### 2.1. Ticks

*B. microplus* ovipositing females, eggs and larvae (Porto Alegre isolate) were experimentally maintained in the Laboratory of Entomozooses (UFRGS, Porto Alegre, Brazil) at 28°C and 85% relative humidity and their parasitic life were completed in calves, housed in individual pens on slatted floors.

### 2.2. Immunizations

Antibodies against salivary glands extracts and the recombinant GST (rBmGST) were raised in rabbits. One bovine was also immunized with rBmGST to test its immunogenicity in this species.

### 2.3. Antigen preparation

Fully and partially engorged female ticks were washed with phosphate buffered saline, pH 7.2 (PBS) plus 500 IU penicillin ml<sup>-1</sup>. The dorsal surface was dissected with a scalped blade. Salivary gland, gut, ovary and fat body were separated with fine-tipped forceps and washed in PBS. These materials were kept frozen at -70°C until use.

The frozen tissues were thawed and solubilized in a medium containing 0.5% sodium deoxycholate, 0.1% pepstatin A, 0.1% leupeptin and 0.1 mM *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK) in 10 mM Tris buffer pH 8.2. After incubation for 15 min in an ice bath, the materials were centrifuged at 32,000g for 40 min. The supernatants were stored at -70°C. The protein concentrations of the extracts were measured using the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

### 2.4. cDNA library immunological screening

An immunological screening of a cDNA library (UNIZAP® XR Stratagene, La Jolla, CA) synthesized from salivary glands poly A<sup>+</sup> RNA of partially engorged adult females was performed using the serum of a rabbit immunized with *B. microplus* salivary glands extract. Nine thousand recombinant phages were screened on nitrocellulose membranes (Schleicher and Schüll, USA) resulting in a single positive GST cDNA. Membranes were blocked with blotto (5% skin milk in PBS) for at least 1 h at room temperature. They were then incubated with anti-salivary glands antibodies at room temperature for 18 h. After three washes in blotto, goat anti-IgG-phosphatase alkaline conjugate (Life Technologies) was incubated for 1 h at room temperature. After three washes with PBS and once with revelation buffer (5 mM MgCl<sub>2</sub>, 100 mM NaCl in 100 mM Tris pH 9.5), the reaction was revealed with 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).

The pBluescript® II (Stratagene) plasmid excision from the UNIZAP lambda vector was performed according to the instructions of the manufacturer.

### 2.5. DNA sequencing

DNA sequencing was performed on an ABI-PRISM 377 automated DNA sequencer (Perkin-Elmer, Foster City, CA) at Molecular Genetics Facility, Georgia, USA. The following oligonucleotides were used for total sequence of the GST cDNA: M13 universal forward, T7 and T3 from the vector sequence, primers 1 (5'-GCAAG AAGCACGGTCTCG-3') corresponding to nucleotides 378–395, primers 2 (5'-CCACAGTTCGCAGCCTC-3') corresponding to the inverse complement of nucleotides 413–430, and primer 3 (5'-GTCAACCGCTGAAC GCAC-3') corresponding to the inverse complement of nucleotides 795–812 in BmGST cDNA (Fig. 1).

### 2.6. Enzyme purification

Glutathione-Sepharose 4B (Pharmacia Biotech Inc.) affinity chromatography was used to purify rBmGST. Lysogens of XL1-Blue/rBmGST clones were prepared after growth in Luria-Bertani medium. Recombinant protein expression was induced with IPTG 0.1 mM. Cell pellet from 2000 ml culture were suspended in 20 ml of PBS and frozen at -70°C. Cells were thawed and disrupted in a French press. Triton X-100 was added to the supernatant to a final concentration of 1%. The supernatant was then loaded on the column that was equilibrated and washed with buffer A (140 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>) and eluted with buffer B (50 mM Tris-HCl +10 mM glutathione, pH 8.0) according to the instructions of the manufacturer (Pharmacia Biotech Inc.).

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1      ggacagagctgatatcgcataccggcgatcctctacatgctctgagagccacggttctgca
62     gaaaccgctcgcaacaacagagagaatcggtgaagagtgccatataagagccaagcata
1      M A P T P V V G Y T T A R G L A Q
122    aactccggcatggcaacctactccggtgctgtgctacaccactgaccagaggctggcccag

18     S I R N L L V Y K G V H F E D K R Y E F
182    agcatccgcaacctgctggtgtacaaggcgtgcaactcgaggacaagcgtacagagttc

38     G P A P T Y E K L G W A A D S A S L G F
242    ggaccggcgcccaagctacgagaagctgggtggcgccgacagtgctcgtcggtggcttc

58     T F P N L P Y Y I D G D V R L T Q S L A
302    acggtccccaacctgcccgtactacatcgatggcgagctacggctcaecgagtcctcagctc

78     I L R Y L G K K H G L D A R S D Q E A A
362    attctccggtaccctgggcaagaagcagcgtctcgacgcccagagtgaccagagagctgagc

98     E L W L M E Q Q A N D L L W A L V V T A
422    gaactgtggctcatggagcagcagggccaacagcctgctctgggctcctcgtgtgaccgcc
      ↓
118    M N P N A T E A R K S Q E K R L A D S L
482    atgaaccggaacgcgaccgagggcccgaagagccagagagagagctggctgactcgtctg

138    P R W Q E L L K R R W A L G N T L T Y
542    ccccgctggcagagctgctgaagaagcggcctgggcccctcgccaacagctcaactac

158    V D F L L Y E A L D W N R Q F A P D A F
602    gttgacttctgctctacagggcctcgactggaaccgccagttcgccgcccagcgcgcttc

178    A N R P E L L D Y L R R F E Q L P N L K
662    gccaaaccggaacccgactcgtggactaactcgcgctcgagcagctgccaacactgaaag

198    E Y F A S D K Y V K W P I M A P Y M F W
722    gactactcgcctccgacaaaactcgaagtgccgcatggcgccttacatgttttg

218    G H K *
782    ggacacaaataaagtgcgttcagcgggtgaccgcgctctacgaaaaaaaaaaaaaaaaaaaa

842    aaaaaaaaaaaaaaaaaaaaaaa

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Fig. 1. Nucleotide and the corresponding deduced amino acid sequence of *B. microplus* GST cDNA. The regions used for the synthesis of primers 1, 2 and 3 are underlined. The arrow indicates a potential glycosylation site. The stop codon is in bold and the polyadenylation signal is in italic. The accession number at GenBank is AF366931.

Protein purity was monitored by SDS-PAGE (Laemmli, 1970) with 5% stacking gel and 12% running gel stained with Coomassie Blue G-250.

### 2.7. RNA poly A<sup>+</sup> isolation and RT-PCR

RNA poly A<sup>+</sup> was isolated from *B. microplus* tissues and bovine blood collected with citrate, and stored at  $-70^{\circ}\text{C}$ . For RNA poly A<sup>+</sup> isolation was used the Quick Prep Micro mRNA purification kit (Pharmacia Biotech Inc.) as described by the manufacturer. Five hundred ng of RNA poly A<sup>+</sup> were submitted to reverse transcription (RT) at  $37^{\circ}\text{C}$  in the presence of oligo-dT (Pharmacia Biotech Inc.) and M-MLV Reverse Transcriptase (Gibco-BRL Life Technologies) according to the instructions of the manufacturer. PCR amplifications were performed using 1/20 of the RT reaction, 20 pmol of primers 1 and 3 (primers are described at DNA sequencing in Section 2 and in Fig. 1) and 1 U Elongase Mix (Gibco-BRL Life Technologies) in a final volume of 50  $\mu\text{l}$ . Samples were denatured for 10 min at  $94^{\circ}\text{C}$  and amplification was achieved through 35 cycles of 30 s at  $94^{\circ}\text{C}$ , 30 s at  $53^{\circ}\text{C}$  and 3 min at  $72^{\circ}\text{C}$ , with a final extension cycle of 30 s at  $53^{\circ}\text{C}$  and 10 min at  $72^{\circ}\text{C}$ . A parallel cDNA sample with tick actin-specific primers was also amplified (337 bp) as a positive control. Nega-

tive controls for the RT and PCR amplifications were always included into the assay.

### 2.8. Western Blot

For Western Blot analysis, tissue extracts (30  $\mu\text{g}$  protein lane<sup>-1</sup>), XL1-Blue/rBmGST extracts (120  $\mu\text{g}$  protein lane<sup>-1</sup>) and purified rBmGST (4  $\mu\text{g}$  protein lane<sup>-1</sup>) in sample buffer containing 2% SDS, 250 mM Tris pH 6.8, 0.025% bromophenol blue, 5% glycerol, 10%  $\beta$ -mercaptoethanol and 5 M urea were separated in SDS-PAGE 12% gel electrophoresis and transferred to nitrocellulose at 70 V for 1 h at  $4^{\circ}\text{C}$  in 12 mM carbonate buffer pH 9.9 (Dunn, 1986). The nitrocellulose sheet was blocked with blotto for 2 h at room temperature. The anti-rBmGST rabbit serum (1:500) was incubated in blotto overnight at  $4^{\circ}\text{C}$ . The revelation procedure was the same used in the immunological screening.

### 2.9. Enzyme activity assay

GST activity was measured as described by Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) (Sigma) as substrate. The reaction mixture consisted of 50 mM substrate into methanol, 5 mM glutathione into 100 mM Tris-HCl, pH 7.5 and purified enzyme was added to a final volume of 100  $\mu\text{l}$ . The activity was determined by measuring absorbance at 340 nm ( $A_{340}$ ), in a 96-well plate using a SpectraMAX 250 microplate spectrophotometer (Molecular Devices) at  $25^{\circ}\text{C}$ . Protein was assayed by the method of Bradford (1976) using BSA as standard.

## 3. Results and discussion

### 3.1. Immunological screening

A *B. microplus* GST cDNA clone was isolated from a salivary glands cDNA library by immunoscreening using a rabbit anti-salivary glands serum. This cDNA was excised from the lambda clone into the pBluescript II plasmid and the 864 bp insert was separated from the vector by digestion with *Xho*I and *Eco*RI restriction endonucleases.

### 3.2. Sequence analysis of GST cDNA

The total nucleotide sequence of BmGST cDNA and its deduced amino acid sequence are shown in Fig. 1. A putative initial ATG codon (Kozak, 1991) is found 130 nucleotides downstream of the 5' cDNA end. The putative polyadenylation signal (Proudffot and Brownlee, 1976), AATAAA (nucleotides 789–794) overlapped the translation stop codon TAA (nucleotide 790) (see Fig.

1). Feng et al. (1999) reported an overlapped stop codon and polyadenylation signal in a sequence of GST from the spruce budworm, *Choristoneura fumiferana*. Within the BmGST cDNA sequence a single main open reading frame (ORF) is found which encodes a predicted protein of 220 amino acids protein and pI 8.61. The predicted molecular weight for the deduced protein is 25,575 Da. Similar molecular sizes are found for other GSTs of some organisms (Smith et al., 1986; Balloul et al., 1987; Sharp et al., 1991; O'Neill et al., 1994; He et al., 1999). Comparing the deduced amino acid sequence of the BmGST protein with sequences in the GenBank we found that it is most similar to the class mu GSTs (BLAST 2 version Blastp 2.0.5) (Altschul et al., 1997) (Fig. 2). According to SWISS PROT/ScanProsite program (Hofmann et al., 1999), the deduced protein is composed predominantly of hydrophilic residues characteristic of cytosolic protein and a potential glycosylation site is present at residue Asn<sup>121</sup> (Fig. 1). Cysteines are absent in the sequence of BmGST like as in sequence of GST of *C. fumiferana* (Feng et al., 1999) corroborating the indication that cysteine residues are not essential to the catalytic activity of the class mu GSTs (Widersten et al., 1991).

The cytosolic GST enzymes have two active sites per dimer that behave independently from each other. The G-site binds GSH with high specificity and is located in domain I close to the N-terminal end of the sequence. Domain II is at the C-terminal end and contains the H-site that interacts with the second hydrophobic substrate in a more unspecific manner (Hansson et al., 1999). Like other GSTs, the BmGST protein contains the conserved active site motif between residues 60 and 68 where GSH binds (G-site). The BmGST protein also has the motif SLAILRYL centered into residue 78, and it was sug-

gested that it has an important structural role contributing to both the GSH binding site and to the domain interface (Wilce and Parker, 1994). The degree of similarity is much higher at the N-terminus than at the C-terminus among GSTs (Fig. 2). The crystal structures of mammalian and insect GSTs have indicated that most active-site residues are in the N-terminal region of the protein (Reinemer et al., 1991; Sinning et al., 1993)

The BmGST protein sequence analysis performed with the BLAST 2 program — version Blastp 2.0.5 (Altschul et al., 1997) within sequence databases showed a high similarity to GSTs from several organisms. The BmGST protein showed the highest similarity with 41% identity with the class mu GST isolated from *B. microplus* larvae (AAD15991) (He et al., 1999). A multiple alignment (ClustalW program — version 1.8) of the deduced BmGST protein with other GSTs is shown in Fig. 2. The sequence comparison showed 40% identity with class mu GST from *Dermatophagoides pteronissinus* (P46419) (O'Neill et al., 1994), 36% with class alpha GST from *Fasciola hepatica* (P31671) (Panaccio et al., 1992), 32% with class alpha GST from *Schistosoma japonicum* (P08515) (Smith et al., 1986) and 26% with class sigma GST from *Blattella germanica* (Arruda et al., 1997). Favaloro et al. (1998) reported that interclass sequence identity is generally lower than 30%.

Some GSTs were shown to behave as allergens, like the class mu GST of the house dust mite *D. pteronissinus* (O'Neill et al., 1994), which showed the highest identity with the BmGST sequence excluding the *B. microplus* larvae GST (BmGSTM) already described (He et al., 1999). A class sigma GST from *Blattella germanica* (Arruda et al., 1997) was also identified as a major allergen. It was reported that recombinant *B. germanica* GST causes IgE antibodies responses in approximately 70%

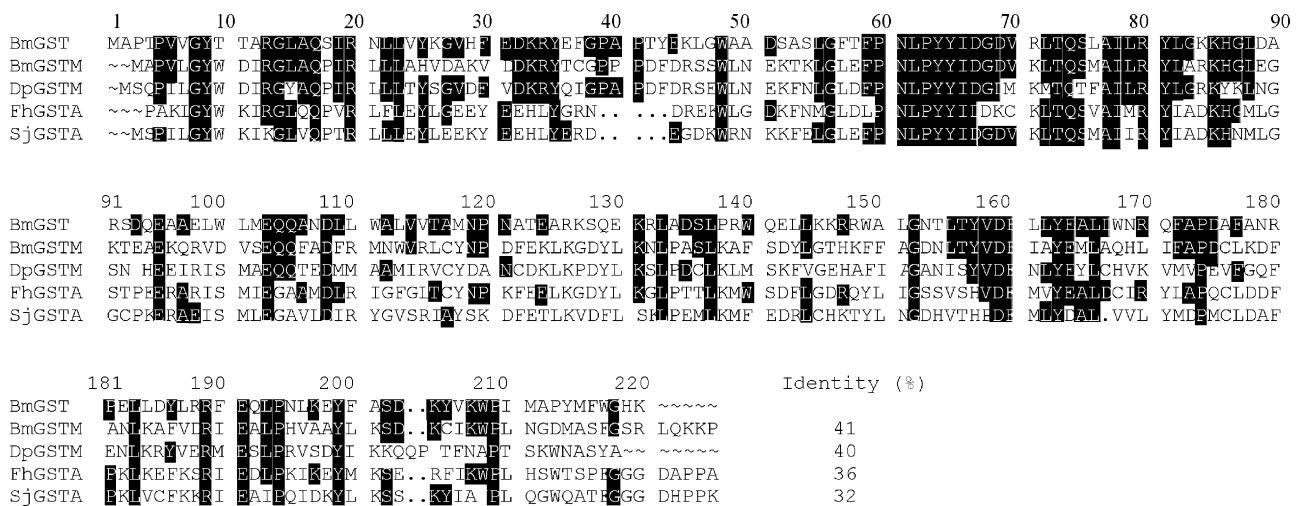


Fig. 2. Alignment of the deduced amino acid sequence of GSTs. Deduced BmGST protein sequence (BmGST); class mu GST from larvae of the *B. microplus* (BmGSTM) (AAD15991); mu-GST from mite *D. pteronissinus* (DpGSTM) (P46419); alpha-GST from *F. hepatica* (FhGSTA) (P31671); alpha-GST from *S. japonicum* (SjGSTA) (P05815). The box shade represents identity. Sequence submitted to ClustalW program version 1.8.

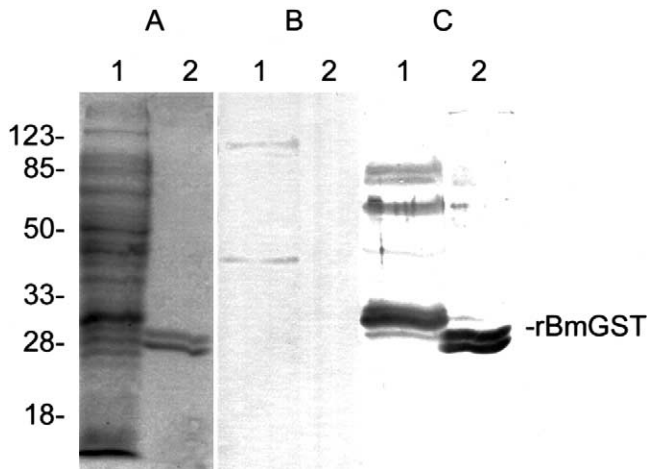


Fig. 3. SDS-PAGE (A) and Western Blot (B) and (C) of purified recombinant BmGST protein (rBmGST). (A) Stained with Coomassie Blue; (B) pre-immunized and (C) immunized rabbit serum against rBmGST. Lane 1 XLI-Blue/rBmGST extracts (120  $\mu\text{g}$  protein lane<sup>-1</sup>), lane 2 recombinant purified rBmGST (4  $\mu\text{g}$  protein lane<sup>-1</sup>). Numbers in the margins are molecular weight standards expressed in thousands of Daltons (Broad — BioRad).

of cockroach-allergic asthmatic patients. Another GST allergen was also identified in the helminth parasite *S. mansoni* (class alpha) (Capron et al., 1992), thus defining the importance of GST superfamily in causing IgE antibody responses. Hypersensitivity skin tests in bovines were used to evaluate the reactivity of rBmGST *in vivo*. An immunized bovine showed a positive skin test reaction indicating that rBmGST is able to induce a cellular immune response beyond the production of IgG. The *S. mansoni* GST, a promising candidate for a vaccine against schistosomiasis induces the production of IgE and IgA antibodies, which are thought to be beneficial in reducing parasitic infection (Arruda et al., 1997).

### 3.3. Characterization of rBmGST

SDS-PAGE analysis of affinity-purified rBmGST is shown in Fig. 3. The purified rBmGST protein appeared as two major components after Coomassie Blue staining and had an apparent molecular weight of 30,700 and 28,400 Da. rBmGST contains a fragment of the fusion protein  $\beta$ -galactosidase at the 5' end that corresponds for the difference between the purified fusion protein and the estimated GST molecular weight.

### 3.4. RT-PCR

The forward primer 1 and reverse primer 3 were used in RT-PCR experiments to analyze the expression of the gene encoded by the BmGST cDNA sequence in fully and partially engorged female guts as well as in partially engorged female salivary glands, fully engorged female ovaries, partially engorged female fat bodies and 20 days old larvae using mRNA as template (Fig. 4). The expected DNA fragments of 435 bp were amplified from RNA of partially and fully engorged female guts and from partially engorged female salivary glands. Control RT-PCR amplifications (337 bp) using actin-specific primers confirmed sample integrity as shown in Fig. 4.

The fact that BmGST mRNA and protein were not detected in *B. microplus* larvae, respectively, by RT-PCR and Western Blot and the low sequence identity shared between them suggests that the BmGST gene described in this paper is different from the already reported class mu GST (BmGSTM) isolated from *B. microplus* larvae (He et al., 1999). The BmGST cDNA was isolated from partially engorged female salivary glands and BmGST gene is also transcribed in other tick tissues, but not in 20 day old larvae.

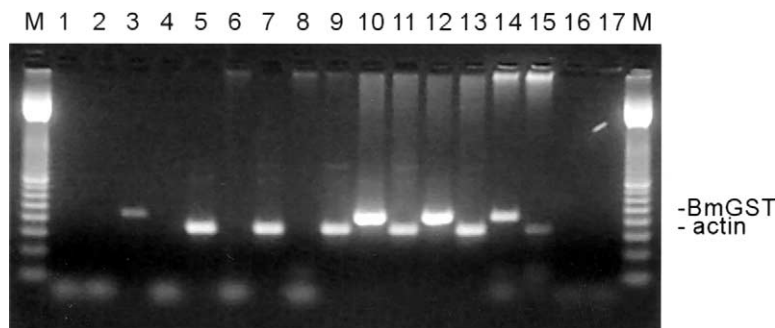


Fig. 4. PCR fragments generated from the BmGST clone and cDNAs from different tissues and larvae of *B. microplus*. In lane M are the molecular weight markers (100 bp ladder, Pharmacia). Lane 1 shows the negative control of reverse transcriptase reaction; lane 2, the negative control of PCR reaction; lane 3, the fragment amplified from the BmGST clone; lanes 4 and 5 the fragments amplified from larvae cDNA; lanes 6 and 7 the fragments amplified from fully engorged ovary cDNA; lanes 8 and 9 the fragments amplified from fat body; lanes 10 and 11 the fragments amplified from partially engorged female salivary gland cDNA; lanes 12 and 13 fragments amplified from partially engorged female gut cDNA; lanes 14 and 15 fragments amplified from fully engorged female gut cDNA; lanes 16 and 17 show the RT-PCR reaction from bovine blood cDNA. The primers used were 1 and 3 for the BmGST clone and cDNA amplifications (lanes 3, 4, 6, 8, 10, 12, 14 and 16) and primers specific for an actin gene of *B. microplus* were used as a cDNA positive control (lanes 5, 7, 9, 11, 13, 15 and 17). Samples were submitted to electrophoresis on 2% agarose gel stained with ethidium bromide.

### 3.5. Western Blot analysis

Partially engorged and fully engorged female salivary glands and fully engorged female gut extracts probed with rabbit anti-serum against rBmGST showed bands of 26,900 Da which correspond to the expression of BmGST (Fig. 5). The anti-rBmGST rabbit serum did not react with any antigen in fat body and larvae extracts, hemolymph (Fig. 5) and saliva from partially engorged female tick (data not shown) in the conditions tested.

The apparent localization of BmGST protein within the *B. microplus* body might shed some light on their physiological roles. The major functions of GSTs have long been believed to be detoxification of xenobiotics

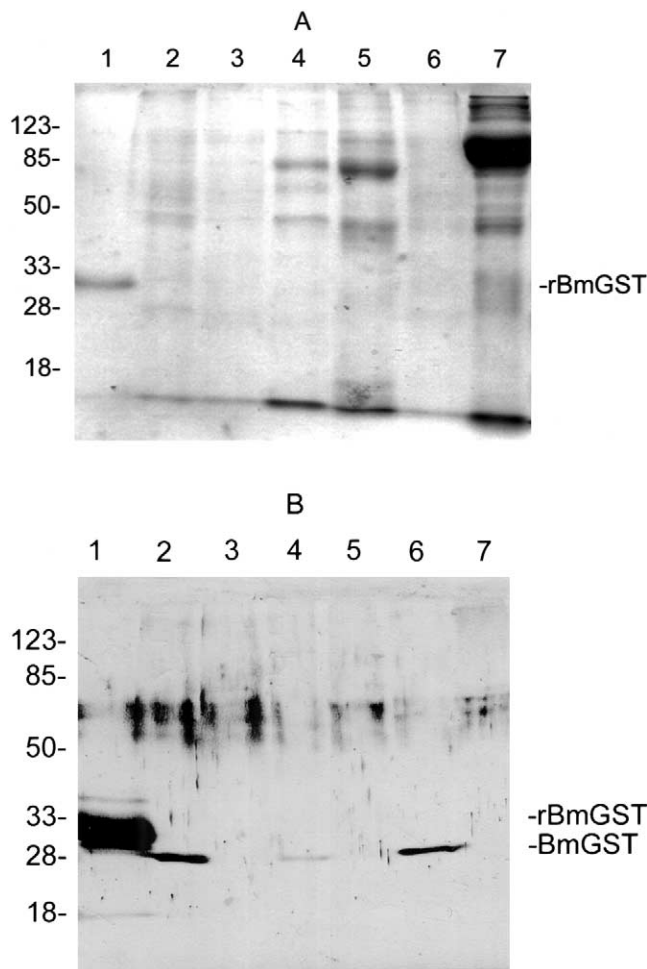


Fig. 5. Western Blot of *B. microplus* tissues extracts and purified rBmGST. Samples were run on a 12% SDS-PAGE gel and transferred to a nitrocellulose membrane. Anti-rBmGST rabbit serum was used for Western Blot analysis. (A) SDS-PAGE stained with Coomassie Blue; (B) membrane probed with anti-rBmGST serum lane 1, purified rBmGST (4 µg); lane 2, partially engorged female salivary gland; lane 3, partially engorged female fat body; lane 4, fully engorged female gut; lane 5, larvae; lane 6, fully engorged female salivary gland; lane 7, fully engorged female hemolymph. Numbers at left are molecular weight standards expressed in thousands of Daltons (Broad — BioRad).

(Stanley and Benson, 1988; Dirr et al., 1994; He et al., 1999), digestive processes (Douch and Buchanan, 1978), prostaglandin synthesis (Meyer and Thomas, 1995) and in a series of reactions which are essential to protect cell constituents from oxidative attack by oxygen and oxygen-associated free radicals (Sharp et al., 1991; Feng et al., 1999). GSTs are also implicated in the development of resistance in cells and organisms toward drugs, pesticides, herbicides and antibiotics (Mannervik and Danielson, 1988; Fournier et al., 1992; Perito et al., 1996; Neufeind et al., 1997; Spithill et al., 1997). Increased expression levels of GST have been correlated with insect resistance, particularly against the organophosphorus type (Huang et al., 1998). In *H. contortus*, Kawalek et al. (1984) demonstrated that the activity of GST was 1.5–1.8 times higher in a cambendazole-resistant strain compared with a susceptible one. Preliminary results with class mu GST of *B. microplus* larvae showed no differences in mRNA levels between untreated larvae of susceptible and organophosphorus-resistant strains (He et al., 1999).

The results obtained from RT-PCR and Western Blots are coincident regarding the presence of GST in tissues extracts of *B. microplus*. The presence of BmGST protein in salivary glands and guts of adult females suggests that it can be involved in cellular detoxification, digestive processes or protection from oxidative damage considering that adult females ingest a large content of blood from the host. GSTs may function as intracellular transporters of various non-substrate hydrophobic compounds such as heme (Jagt et al., 1985) as well as bilirubin, steroids, thyroid hormones and bile salts (Mannervik et al., 1985).

### 3.6. Enzyme activity

The fraction eluted from Glutathione Sepharose 4B (see Section 2) column containing the purified rBmGST and extracts from *B. microplus* female tissues were assayed for their activity upon the chromogenic substrate CDNB. The substrate CDNB is not class specific and can interact with class alpha, mu, pi and sigma GSTs (Takamatsu and Inaba, 1994), but not to class theta GST (Meyer et al., 1991). Substrate hydrolysis was observed in assays with purified rBmGST, fully and partially engorged female guts and salivary glands aqueous extracts (Fig. 6), being much higher the activity level detected in salivary glands than in guts. Enzyme activity was absent in hemolymph and saliva of *B. microplus* females (data not shown). Class alpha GST from *Schistosoma japonicum* (SjGST) (Pharmacia Biotech Inc.) was used as positive control (data not shown). Endogenous bacterial GST control was included in the assays and activity was not detected (data not shown). rBmGST showed GST activity even containing a fragment of  $\beta$ -galactosidase on its 5' end, but possibly with an altered

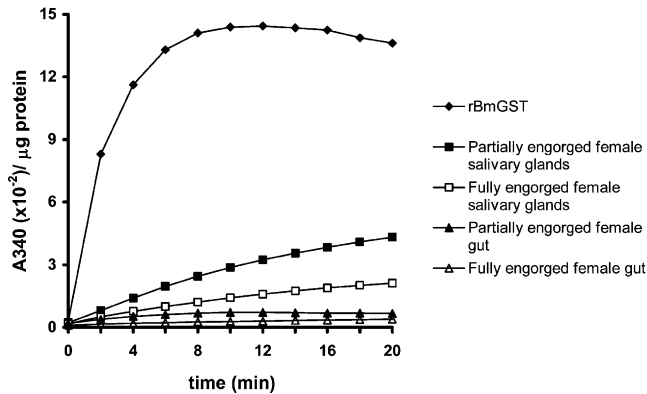


Fig. 6. Enzymatic activity of purified rBmGST and extracts from different tick tissues using CDNB as substrate.

level of activity. The results of GST enzymatic activity assays against CDNB confirmed the presence of GST in the same samples that showed positive results by RT-PCR and Western Blot.

In this paper, we described the cloning, expression and the partial characterization of a *B. microplus* glutathione S-transferase similar to mammalian class mu. We also detected its presence in adult female salivary glands and gut, but further work is needed to understand its physiological role within the tick metabolism as well as to evaluate if it performs a function in the tick–host relationship.

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