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Expression and characterisation of a *Psoroptes ovis* glutathione S-transferase

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Abstract

The astigmatid mite *Psoroptes ovis* is the causative agent of sheep scab, a highly contagious parasitic disease of sheep. Infection causes severe allergic dermatitis, resulting in damage to the fleece and hide, loss of condition and occasional mortality. Interest in the *P. ovis* allergens led us to characterise a glutathione S-transferase (GST) which displays homology to GST allergens isolated from the house dust mite, *Dermatophagoides pteronyssinus* and the cockroach, *Blattella germanica*. A cDNA encoding a mu-class GST from *P. ovis* was expressed in *Escherichia coli* and the recombinant protein purified for biochemical analysis. SDS-PAGE analysis indicated that the purified product was homogeneous and had an apparent molecular weight of 30 kDa. The recombinant GST (rGST) is active towards the substrate 1-chloro-2,4-dinitrobenzene (CDNB), whereas 1,2-dichloro-4-nitrobenzene (DCNB) is a poor substrate. The recombinant protein was also tested for recognition by IgE and IgG antibodies in serum from *P. ovis* naïve and *P. ovis* infested sheep. Neither IgE nor IgG antibodies were detected to the rGST. Prausnitz–Küstner testing with rGST did not provoke a characteristic weal and flare response. Biopsies collected at the PK test sites were stained for eosinophils, neutrophils, mast cells and basophils. Neutrophil, mast cell and basophil counts were not significantly different to the controls. Eosinophil numbers were significantly higher than controls, but were not due to an IgE response. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Glutathione S-transferase; GST; *Psoroptes ovis*; Sheep-arthropoda; Sheep scab; Allergen; Antigen

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1. Introduction

In a study to define ectoparasite allergens, we cloned a glutathione S-transferase (GST) cDNA (clone Pso o2) from the acarine ectoparasite *Psoroptes ovis*, commonly known as the sheep scab mite (Lee et al., 1999). GSTs are a multi-functional family of enzymes found throughout the plant and animal kingdoms (Mannervik, 1985; Wilce and Parker, 1994; Salinas and Wong, 1999). They catalyse the conjugation of the tripeptide glutathione to an extensive range of electrophilic substrates. Their many functions include the metabolism of toxic substances and the protection of cells from oxidative damage. GSTs are also non-enzymatic carrier proteins (ligandins) and participate in the intracellular transport of many hydrophobic compounds (for review see Pickett and Lu, 1989; Armstrong, 1991; Salinas and Wong, 1999).

GSTs are mainly found in the cytosol, although membrane bound and mitochondrial forms have been isolated (DeJong et al., 1988; Walländer et al., 1979; Jocelyn and Cronshaw, 1985). The cytosolic GSTs are grouped, independent of species, into nine classes: alpha, beta, kappa, mu, omega, pi, sigma, theta and zeta, based on their sequence homology, structure, substrate specificity, immunological studies and phylogenetic analyses (Meyer et al., 1991; Meyer and Thomas, 1995; Hayes and Pulford, 1995; Pemble et al., 1996; Board et al., 1997; Rossjohn et al., 1998; Board et al., 2000).

Infestation of sheep with *P. ovis* provokes an allergic dermatitis, characterised by an intense pruritis and weeping sores, resulting in fleece loss and severe debilitation (Bates, 1997). There is considerable concern that the current methods to control sheep scab, which include the use of organophosphate and synthetic pyrethroid formulations, pose risks to the health of farmers (Bartle, 1991; Murray et al., 1992; Stephens et al., 1995) and the environment (Littlejohn and Melvin, 1991; Virtue and Church, 1993; Gasser et al., 1997). Moreover, the occurrence of acaricide-resistant *P. ovis* isolates (Synge et al., 1995; Clark et al., 1996) may have serious consequences for current chemical control strategies.

Studies of primary and re-challenge *P. ovis* infestations of sheep have demonstrated a reduced mite burden (Bates, 2000) and diminished lesion growth on re-challenged animals (Bates, 2000; Van den Broek et al., 2000). This apparent resistance to reinfestation suggests that sheep develop a protective immunity to *P. ovis*. Therefore, an alternative and safer way to control sheep scab may be to vaccinate with mite-derived antigens. However, to develop such a strategy the immunology of *P. ovis* infections must be understood and potential vaccine antigens identified.

The *P. ovis* GST is of interest to us because it displays homology to a GST cloned from the house dust mite, *Dermatophagoides pteronyssinus*, which promotes an IgE antibody response in mite-allergic patients (O'Neill et al., 1994). It has also been reported that a cockroach (*Blattella germanica*) GST reacts with IgE in sera from approximately 70% of cockroach-allergic patients (Arruda et al., 1997). This suggests that this group of enzymes has a role in allergic disease associated with insect and mite infestations. Secondly, there have been numerous studies demonstrating that GSTs isolated from helminths are effective vaccine antigens (reviewed in Spithill et al., 1997; Riveau et al., 1998; Shuxian et al., 1998).

In this paper we have conducted a phylogenetic analysis of the *P. ovis* GST protein sequence and describe the expression of the *P. ovis* GST in *E. coli* and biochemical characterisation of the recombinant protein (rGST). In addition, we have assessed whether rGST

induces a cutaneous inflammatory response in sheep and whether IgE or IgG antibodies to *P. ovis* GST are generated during a natural infection of sheep scab.

2. Materials and methods

2.1. Synthesis of *P. ovis* GST expression construct

The *P. ovis* GST cDNA (clone Pso o2) was isolated from a *P. ovis* cDNA library as previously described in Lee et al. (1999). The open reading frame of Pso o2 was amplified by PCR using the sense primer 5' <AATTTAACACCATGGGCAAACCAACC>3' and anti-sense primer 5' <CATTTCCGACAGATCTACAATGAATTC>3' to introduce an *Nco*I site at the initiation codon and a *Bgl*II site 11 bases downstream from the stop codon (see Fig. 1). This cloning strategy removed the bases encoding the histidine purification tag from the plasmid expression vector. Creation of the *Nco*I site required the mutation of base 240 from an A to G and hence Ser² was modified to a glycine. The amplification reaction mixture (100 µl) contained 20 mM Tris–HCl buffer, pH 8.4, 50 mM KCl, 5 mM MgCl₂, 0.2 mM dNTPs, 20 pmol of each primer, 2 units *Taq* DNA polymerase (Life Technologies, UK) and 20 ng template DNA. PCR was performed in a Hybaid Omnigene thermal cycler with an initial denaturation step of 95 °C for 5 min, followed by 25 cycles of amplification, each consisting of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. A final extension cycle was performed at 72 °C for 10 min. The resulting PCR product was purified with a GeneClean spin kit (BIO 101, USA) and ligated into the shuttle vector pCR-script Amp SK (+) (Stratagene, UK) according to the manufacturer's instructions. Recombinant clones were screened by restriction enzyme analysis and the cDNA inserts were sequenced to confirm the fidelity of the PCR reaction. Positive clones were digested with *Nco*I and *Bgl*II (Life Technologies, UK) and the modified GST cDNA subcloned into *Nco*I–*Bgl*II digested expression vector pBAD/His(A) (Invitrogen, USA). Ligation products were transformed into TOP10 *E. coli* by standard methods. Recombinant clones were screened by restriction digestion and sequenced to confirm an in-frame fusion at the 5' end.

2.2. Nucleotide sequencing

Plasmid mini-preps. (Promega, UK) were used as template for sequencing. pCR-script SK (+) clones were sequenced using M13For and M13-20 primers and pBAD/His(A) clones were sequenced using the pBAD Forward primer (Invitrogen BV, The Netherlands). Sequencing reactions were performed in an LEP scientific PREM thermal cycler using Amplitaq DNA polymerase FS dye terminator cycle sequencing (Applied Biosystems, USA). Reaction products were analysed on an ABI-prism 373A automated DNA sequencer (Applied Biosystems, USA).

2.3. Expression and purification of recombinant *P. ovis* GST

An overnight culture of 2.5 ml was used to inoculate 250 ml Luria-Bertani media containing 50 µg ml⁻¹ ampicillin and grown at 37 °C to an OD₆₀₀ of 0.5. Expression of the

GGCTGCGAGAAGACGACAGAAGGGGACACACGTTGGCTTTTGAAGCATCAACTATTAA 60

TTCAAATTGAATTTTGTATTTCAAATATTTCTTTTGTAGTTTGTGAAATTTTAATTGCAA 120

Forward primer

5'<aatttaacacccatgggcaaccaacc>3'

ATTTAAATTTAACAAAATGAGCAAACCAACCGTTGGCTATTGGGATATCCGTGGTTTAGG 180

M S K P T V G Y W D I R G L G 15

TCAACCAATCCGTTTGTATGTTGACTTATGCTGGTCAAGATTCCATGATAAACGTTACAA 240

Q P I R L M L T Y A G Q D F H D K R Y N 35

TATTGGTCCAGCACCAACATTCGATCGTTCAGAATGGTTGAATGAAAAATTCAATTTAGG 300

I G P A P T F D R S E W L N E K F N L G 55

CTTAGATTTTCCGAATTTACCATATTACATTGATGGTGATTGAAAATGACACAAACATT 360

L D F P N L P Y Y I D G D L K M T Q T L 75

GGCAATTCTCCGATACTTGGGTCAAAACACAACATGGAAGGTAAAACCGAACATGAACG 420

A I L R Y L G H K H N M E G K T E H E R 95

TGTTCTGTTTGCAATGTTGGAACAACACACATGATATGTTTTGGCAATTTGCACGTAT 480

V R V A M L E Q Q T H D M F W Q F A R M 115

GTGTTATGATGAAAATATGAAAAATTACGCGCTGATTGATGGCAAATTACCGGAAAC 540

C Y D E N M E K L R A D L M A K L P E T 135

ATTCAAATTATTATCGAAATTCATGGGTGATCATTTTTGTTGCTGGAACATATGTGAC 600

F K L L S K F M G D H H F V A G T Y V T 155

CTATGTGGATTCTTTTCTACGAATATTACTTCGTATCAAAGTTTTGATGCCAGAAAT 660

Y V D F F F Y E Y L L R I K V L M P E I 175

ATTTAATCAATTTGATAATTGGAACAATATGTGGAAGGCTTGGATTCAATGCTGCGTGT 720

F N Q F D N W K Q Y V E G L D S L L R V 195

ATCGGGATACATTAAACAACAACAACCGAAAACATTCAATGCACCAATGGCCAAATTGAA 780

S G Y I K Q Q Q P K T F N A P M A K L N 215

TGGTTCATATGCTTGAATTCATTGTGAATCAGTCGGAAATGATTGAATTATTGACTAATA 840

G S Y A * 219

3'<cttaagtaacatctagacagcctttac>5'

Reverse primer

GCATCATTC CCTTAAACTAAAATAAAATTC CAAAATCCTTAAAATTTAGCTTTTAATTC 900

AAAAATTGAAAATTTTCCAGAAAACCAATTTTTC CAAATAAAATTTTAAAAA AAAAAA 960

AAAAAAAAAAAAAAAAA 977

Fig. 1. Complete nucleotide and deduced amino acid sequence of clone Pso o2. The nucleotide and amino acid residues are numbered at the end of each line. The stop codon is represented as by * and the PCR primers used to create *Nco*I and *Bgl*II restriction enzyme sites are shown. This sequence data is available from GenBank under accession number AF078684.

recombinant enzyme was induced by the addition of 0.2% arabinose and the culture incubated at 30 °C for a further 4 h. Cells were harvested by centrifugation and the pellet resuspended in 25 ml phosphate-buffered saline (PBS: 8.4 mM Na₂HPO₄, 1.9 mM NaH₂PO₄, pH 7.4, 150 mM NaCl). The cell suspension was sonicated on ice using a 1 cm diameter probe (three separate bursts of 30 s with an interval of 30 s for cooling the samples on ice) and insoluble bacterial debris removed by centrifugation (2500 rpm, 30 min, Jouann BR 3.11 centrifuge). The supernatant was applied to a 1 ml 50% (v/v) glutathione sepharose-4B suspension (Sigma-Aldrich, UK) equilibrated with ice-cold PBS. The eluate was collected and re-applied to the column before washing with 4 × 10 bed volumes of ice-cold PBS. rGST was eluted in 8 × 0.3 ml fractions with 50 mM Tris-HCl, pH 8.0, 10 mM reduced glutathione (GSH). Eluted fractions were stored in aliquots at –20 °C.

2.4. Protein assay

Protein concentration of purified GST was determined using the BioRad Protein Assay Kit (BioRad, UK), based on the method of Bradford (1976), using bovine serum albumin as the standard.

2.5. SDS-PAGE and Western immunoblot analysis

SDS-PAGE was performed according to the method of Laemmli (1970) using a BioRad (UK) Mini protean II electrophoresis system. Samples were heat denatured at 95 °C in 4× loading buffer (250 mM Tris-HCl, pH 6.8, 4% (w/v) SDS, 30% (v/v) glycerol, 2% (v/v) β-mercaptoethanol, 2% (v/v) bromophenol blue) and a 10 µl aliquot of each sample applied to a 3% stacking, 12% resolving polyacrylamide gel. Protein bands were visualised by staining with Coomassie Brilliant Blue as described by Blakesley and Boezi (1979).

For the Western blots, 10 µg GST was loaded per lane and electrophoretic transfer performed using standard protocols (Harlow and Lane, 1988). Blots were then incubated with pooled serum collected from five sheep that had received two *P. ovis* infestations. Following the second infestation, the sera were tested for IgE and IgG antibody responses by ELISA and samples collected from the peak of the antibody response (Van den Broek et al., 2000). Sera taken from the same five animals prior to infection (scab naïve) served as controls. Blots were then sequentially incubated in each of the following: either mouse anti-sheep IgE (see Kooyman et al., 1997) diluted 1/10 in PBS containing 0.5% (w/v) Tween-80 and 0.5 M NaCl (PBS/T80) or mouse anti-sheep IgG, for 1 h at room temperature; horse anti-mouse IgE or IgG-biotin conjugates (diluted 1/1000 in PBS-T80) and streptavidin conjugated horse-radish peroxidase (Boehringer Mannheim, UK). The blots were washed three times with PBS/T80 after each incubation. Specific antibody binding was detected using the enhanced chemiluminescence plus system (ECL Plus, Amersham International PLC, UK).

2.6. Prausnitz–Küstner (PK) test and histopathology

Five, 1-year-old sheep (three Texel cross and two Suffolk cross) were used in this study. The left flank was clipped and marked with a 5 × 2 grid. 50 µl of each of the following was injected intradermally in two randomised sites: pooled naïve serum (NS) collected from

sheep before infestation, pooled positive serum (PS) from sheep infested with *P. ovis* and heat-treated PS (56 °C for 120 min). After 24 h, 0.5 µg rGST in 50 µl PBS was injected intradermally into each of these sites. Two further sites were injected with 0.5 µg rGST in 50 µl PBS and two sites injected with PBS alone. Weal diameters were measured 0.25, 0.5, 1, 2, 3, 6 and 24 h post-injection.

6 h after intradermal challenge, a local anaesthetic (Lignol, Arnolds Veterinary Products, Harlescott, Shrewsbury, UK) was injected subcutaneously and a 6 mm punch biopsy was collected from one of each of the two test sites and from the remaining test site after 24 h. Biopsies were fixed in 4% (w/v) paraformaldehyde in PBS pH 7.4 for 6 h (Newlands et al., 1984), then stored in 70% (w/v) ethanol at 4 °C (Miller et al., 1983), processed and embedded in paraffin wax. Serial 5 µm tissue sections were cut. Neutrophil counts were determined in sections stained with haematoxylin and eosin. Mast cells were stained with toluidine blue 0.5% in 0.5 N HCl, pH 0.5 (Enerback, 1966) for 18 h. Eosinophils were stained with Lendrums carbol chromotrope solution (Sigma, UK) for 1 h (Lendrum, 1944) and counterstained for 10 s with haematoxylin. Dermal eosinophils, neutrophils and mast cells were counted, in a minimum area of 1.25 mm², using an Olympus BX 50 microscope at 400× magnification. Intravascular cells were excluded from the count. Basophils, identified as small, round cells with large granules were also detected but poor morphology frequently made differentiation from mast cells impossible and, therefore, they were included with mast cells as toluidine blue staining cells. The results are expressed graphically as mean ± SEM. Differences between the groups were determined by analysis of variance using a GenStat Release 4.2 programme (5th edition) (Lawes Agricultural Trust; Numerical Algorithms Group, Oxford). Values of $P < 0.05$ were considered significant.

2.7. GST activity determination

GST conjugation activity was determined according to the spectrophotometric method of Habig and Jakoby (1981) using the substrates 1-chloro-2,4-dinitrobenzene (CDNB; Sigma, UK) and 1,2-dichloro-4-nitrobenzene (DCNB). The formation of the glutathione-CDNB and -DCNB conjugates was monitored at 340 and 345 nm, respectively (Spectra Max 340pc, Molecular devices, CA). The reaction was started by the addition of substrate and the increase in absorbance was measured for 10 min. All reactions were corrected for non-enzymatic conjugation, with reaction mixtures without enzyme serving as controls. $V_{\max}$ and K_m values, and standard errors were determined using a the Pfit computer software (Biosoft, UK). The specific activity of the enzyme was expressed as nmol min⁻¹ µg⁻¹ protein.

3. Results

3.1. Phylogenetic analysis

Phylogenetic analysis (Fig. 2) of representative sequences from all GST classes, including the *P. ovis* sequence, shows that the *P. ovis* sequence clusters with the mu-class GSTs and is closely related to the two acarine GST sequences that have been published to date, the house dust mite (*D. pteronnyssinus*) and cattle tick (*B. microplus*).

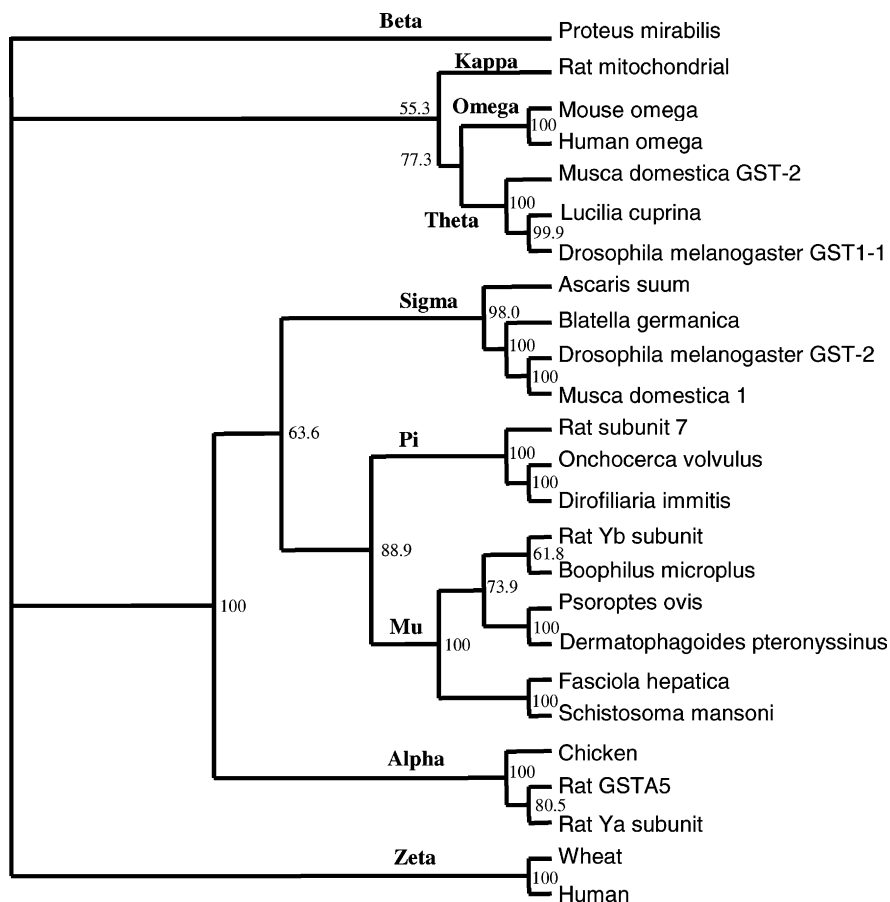


Fig. 2. Phylogenetic analysis of GST protein sequences. The *P. ovis* GST protein sequence was aligned with representative sequences from the nine GST classes using ClustalX with the default parameters (Thomson et al., 1997). The resulting neighbour-joining tree was bootstrapped 1000 times in ClustalX and the tree plotted using the TREEVIEW programme (Page, 1996). Bifurcations with a bootstrap value of less than 50% have been collapsed in the figure. The sequences with their GenBank accession numbers are *Proteus mirabilis*, P15214; Mouse omega, U80819; Human omega, AF212303; *Musca domestica* GST-2, P46431; *L. cuprina*, P42680, *Drosophila melanogaster* GST1-1, P20432; Rat mitochondrial, P24473; *Ascaris suum*, CAA53218; *B. germanica*, O18598; *Drosophila melanogaster* GST-2, P41043; *Musca domestica* 1, AAA03434; Rat subunit 7, P04906; *Onchocerca volvulus*, AAA53575; *Dirofiliaria immitis*, P46426; Rat Yb subunit, P04905; *B. microplus*, AAD15991; *P. ovis*, AAF19264; *D. pteronyssinus*, P46419; *F. hepatica*, P56598; *Schistosoma mansoni*, P35661; Chicken, Q08393; Rat GSTA5, A54858; Rat Ya subunit, P00502; Human, AAB96392; Wheat, AAD09190.

3.2. Expression and purification of recombinant protein

Growth of Top10 *E. coli* cultures containing the pBAD/GST construct at 30 °C resulted in the protein being expressed in both the soluble fraction and insoluble inclusion bodies. Both SDS-PAGE analysis and a CDNB assay indicated that the concentration of rGST in

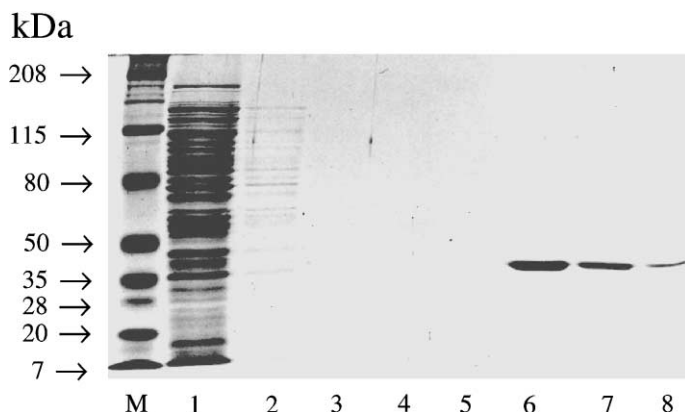


Fig. 3. Purification of rGST. Proteins denatured in the presence of β -mercaptoethanol were separated on a 12% SDS-PAGE gel and visualised by staining with Coomassie blue R-250. Molecular sizes of protein standards (M) in kilodaltons are indicated on the left. Lane 1, Sonicated lysate of *E. coli* Top 10 clone expressing *P. ovis* GST (10 μ l); lane 2, material not bound to the affinity column (10 μ l); lanes 3–5, 10 μ l PBS column washes; lanes 6–8, purified rGST, 10 μ l of elutions 1, 2 and 3 with 0.3 ml GST elution buffer.

the soluble fraction was of sufficient concentration to employ glutathione-sepharose affinity chromatography to purify the soluble protein. Using this method, we were able to produce 1 mg of recombinant protein from a 250 ml culture of *E. coli* induced with 0.2% arabinose. SDS-PAGE analysis indicated that the purified protein was homogeneous (Fig. 3) and had a molecular mass of 30 kDa, which is consistent with a molecular mass of 29.8 kDa deduced from the amino acid sequence.

3.3. GST conjugation activity

To establish whether the *P. ovis* GST cDNA encodes a catalytically active GST, the CDNB and DCNB conjugating activity of the purified recombinant protein was measured in the presence of 1 mM reduced glutathione. Whereas conjugation of CDNB with glutathione was easily detected spectrophotometrically, no GST activity was demonstrated using DCNB as the substrate (Fig. 4). The K_m and k_{cat} values for CDNB conjugation were analysed by varying the concentration of CDNB whilst keeping the glutathione concentration constant at 1 mM and by observing the effect of varying the glutathione concentration whilst the CDNB concentration was held at 1 mM (Fig. 5A and B; Table 1). Under these experimental conditions the K_m values for CDNB and glutathione were 0.431 and 0.658 mM, respectively.

3.4. Western blot analysis

The presence of IgE and IgG antibody to rGST in the blood of parasitised sheep was examined by immuno-electrophoretic analysis using sera taken from sheep prior to and after the *P. ovis* infestation. The binding of IgE or IgG antibody to rGST was not detected in either the NS or the post-infection serum (data not shown).

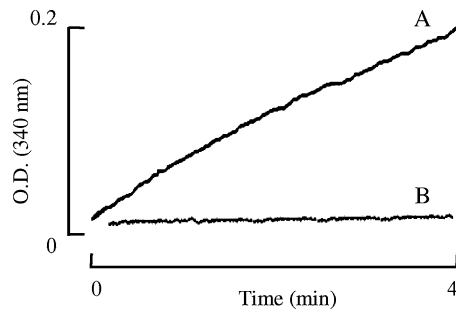


Fig. 4. Time course for the conjugation of CDNB (A) and DCNB (B) by purified rGST. Enzymatic activity was measured by monitoring the increase in absorbance at 340 nm in the presence 0.3 μ g of rGST, 1 mM GSH and 1 mM of either CDNB or DCNB. Figure is representative of three independent experiments.

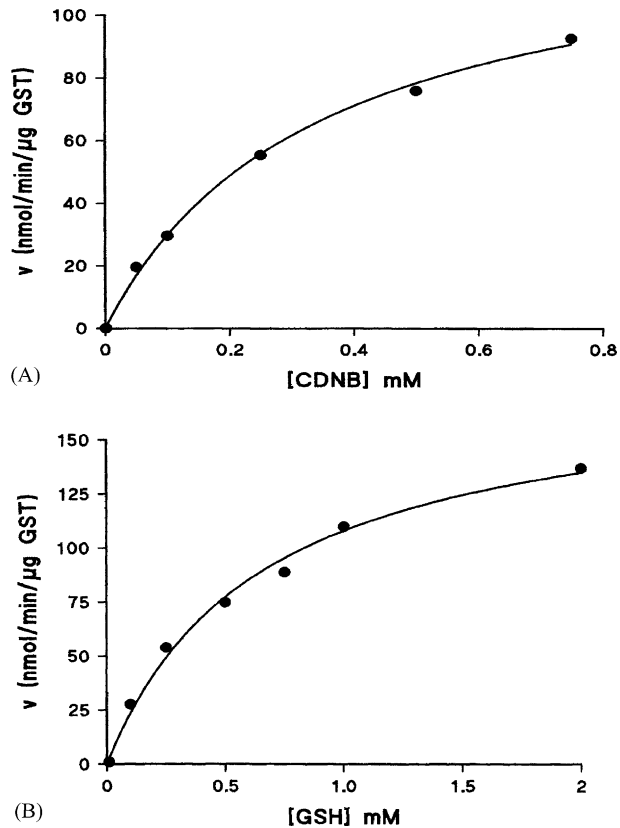


Fig. 5. (A) Plot of reaction velocities v as a function of substrate concentration $[S]$ for the substrate CDNB in the presence of 1 mM glutathione. (B) Plot of reaction velocities v , as a function of substrate concentration $[S]$ for the substrate glutathione in the presence of 1 mM CDNB. Each point is the mean of duplicate determinations from a single experiment and is representative of at least three independent experiments.

Table 1

Kinetic values for the conjugation of CDNB^a

Substrate	K_m (mM)	V_{max} (nmol min ⁻¹ μ g ⁻¹ protein)	k_{cat} (s ⁻¹)	k_{cat}/K_m (s ⁻¹ mM ⁻¹)
CDNB	0.431 $\pm$ 0.074	149 $\pm$ 11	65	150
GSH	0.658 $\pm$ 0.09	179 $\pm$ 10	77	117

^a Enzyme assays were performed as described in Section 2 and in Fig. 5. The K_m and V_{max} values $\pm$ standard errors were determined using the Pfit computer software (Biosoft, UK).

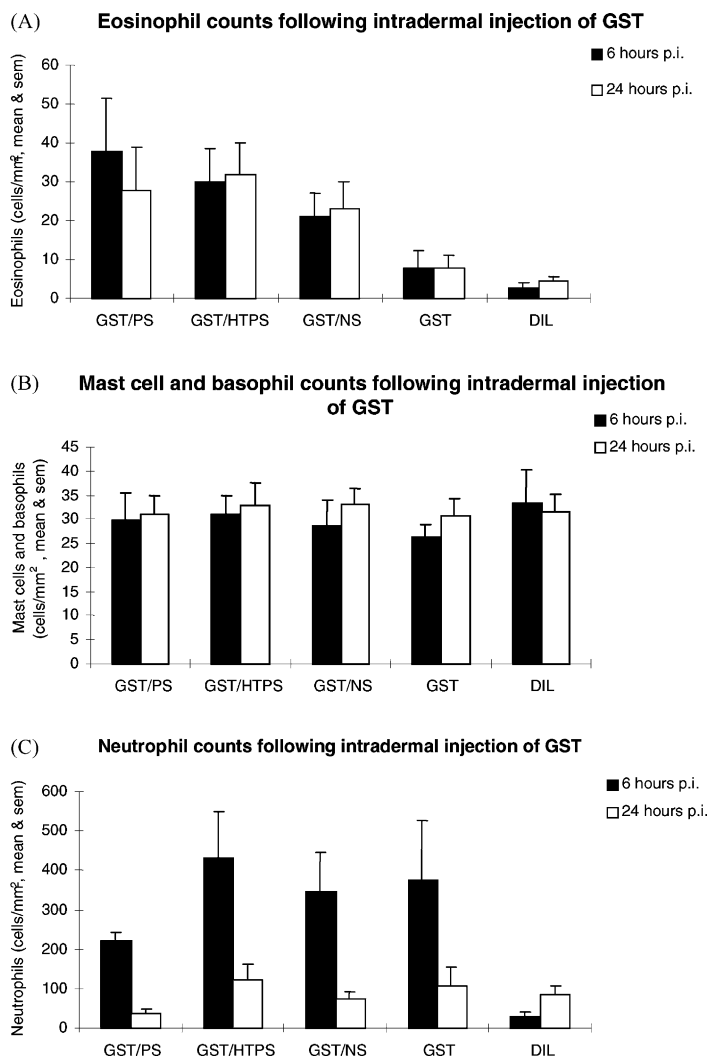


Fig. 6. (A) Eosinophil counts, (B) mast cell and basophil counts, and (C) neutrophil counts, after administration of PS, heat-treated positive serum (HTPS) and NS followed by intradermal challenge with rGST. Cell counts following administration of rGST and the diluent (DIL) alone are also shown.

3.5. PK test

No measurable weal reactions were obtained at any of the sites challenged with rGST.

3.6. Histopathology

Challenge of the naïve, positive and heat-treated serum sites with rGST elicited a pronounced influx of eosinophils that was evident at 6 h and persisted until 24 h after challenge (Fig. 6A). Eosinophil counts at these sites were not significantly different, but at both 6 and 24 h they were significantly higher ($p < 0.02$) than at the rGST and diluent control sites. In contrast, at 6 and 24 h mast cell and basophil counts at sites challenged by intradermal injection of rGST were not significantly different from the diluent control (Fig. 6B). At 6 h, there was no significant difference in neutrophil counts at sites challenged by rGST but at all these sites the influx of neutrophils was significantly greater ($p < 0.001$) than at the diluent control site. By 24 h p.i., the neutrophil count had fallen and was not significantly different from the diluent control at any site (Fig. 6C).

4. Discussion

In this paper, we report the successful expression and purification of a recombinant *P. ovis* GST. Furthermore, the recombinant enzyme is catalytically active, as demonstrated by its ability to conjugate reduced glutathione to the standard GST substrate CDNB. It should be noted that the strategy used to subclone the cDNA into the pBAD expression vector resulted in Ser² being altered to a glycine residue. Although this mutation may have an effect on activity, alignment of the *P. ovis* GST amino acid sequence with other cytoplasmic GST sequences (Lee et al., 1999) shows that this residue is not conserved suggesting that it is unlikely to have a critical role. It would be impractical to compare the activity of the recombinant with the native enzyme owing to the difficulty in obtaining the quantity of mites that is required to prepare sufficient protein for analysis. Furthermore, as with most eukaryotes (Mannervik, 1985; Clark, 1989), it is likely that *P. ovis* possesses multiple GST isoforms. These would need to be differentiated and identified before any comparison could be made.

A phylogenetic analysis of the *P. ovis* sequence shows that the protein is closely related to the mu-class GSTs of the cattle tick, *Boophilus microplus*; and the house dust mite *D. pteronyssinus* (Fig. 2). Like the *B. microplus* enzyme, the *P. ovis* GST displayed activity towards CDNB, but very low activity towards the substrate DCNB (He et al., 1999). However, it is not possible to compare directly the two recombinant proteins since the kinetic constants for the tick GST were not reported. To date, we are unaware of any reports providing data on the enzyme activity of the *D. pteronyssinus* GST.

Little is known about the nature of the *P. ovis* antigenic or allergenic proteins that elicit an immune response in sheep. Some investigations have been carried out using gel electrophoresis to resolve proteins in *P. ovis* extracts followed by immunoblotting. Boyce and Brown (1991) resolved crude soluble proteins from *P. ovis* and *P. cuniculi*, and observed that sera from *Psoroptes* spp. infested sheep recognised over 30 antigens, with predominant bands in the regions of 8–12 kDa, 26–34 kDa and >97 kDa. In a more recent

study, *P. ovis*-specific sheep antibody IgG recognised six antigenic bands in extract of *P. ovis*, which had molecular weights ranging from 12 to 183 kDa (Jayawardena et al., 1998). However, the identity of these proteins was not established. Currently, one *P. ovis* allergen has been identified (Pruett, 1999). The first 30 amino acids sequence of this 16 kDa protein have been reported and display significant sequence homology to the mite allergens *Lep d* II and *Der f* II, isolated respectively, from the mites *Lepidoglyphus destructor* (Schmidt et al., 1995) and *D. pteronyssinus* (Yuuki et al., 1991). These belong to a group of homologous mite allergens which have been classed as the Group II allergens. The biological function of these proteins remains to be determined, however, they exhibit homology to a human epididymal gene product (HE1), suggesting that they might play a role in mite reproduction (Thomas and Chua, 1995).

Experimental studies in sheep indicate that mite products stimulate the production of *P. ovis* antigen-specific serum IgE antibody, a marked cutaneous eosinophil infiltration and mast cell hyperplasia, which is consistent with an IgE-mediated Type-1 allergic reaction (Van den Broek et al., 2000). GSTs from the cockroach, *B. germanica*, and house dust mite, *D. pteronyssinus* are potent allergens (O'Neill et al., 1994; Arruda et al., 1997), which raises the possibility that these enzymes have a role in allergic disease. However, this is unlikely in the case of the *P. ovis* GST cloned and expressed in this study since IgE antibody binding was not detected in post-infestation sera. Moreover, intradermal injection of rGST did not induce a weal reaction and did not provoke the histopathological changes that are characteristic of a cutaneous inflammatory response. While eosinophil counts following administration of the various sera and rGST were significantly higher than at the two control sites, it is clear that this is not due to an IgE response.

Several vaccine studies have indicated that GSTs are effective protective antigens. In trials using purified or rGSTs from schistosomes (*S. japonicum*, *S. bovis* and *S. mansoni*) and liver flukes (*Fasciola hepatica* and *F. gigantica*) significant protection against worm infections was achieved (Spithill et al., 1997; Riveau et al., 1998; Shuxian et al., 1998). In contrast, antisera raised to GST purified from the blowfly *Lucilia cuprina* had no effect on the survival of the parasite (Sharp et al., 1991). By immunoblotting, we did not detect a reaction between *P. ovis*-specific IgG with rGST. However, this does not preclude the rGST from being a candidate vaccine antigen. It is possible that the native protein is located in the internal organs of the parasite and is not exposed to the host and, therefore, could be regarded as a potential concealed antigen. Vaccination with concealed antigens, where subsequent ingestion of immunoglobulin interferes with the function of the antigen resulting in damage to the parasite, has been successfully exploited to control the cattle tick *B. microplus* (Kemp et al., 1986; Willasden and Kemp, 1988; Riding et al., 1994).

Finally, it must also be noted that it is likely that *P. ovis* contains a GST gene family and we do not rule out the possibility that another member of the GST family contributes to the ovine immune response.

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