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MALDI mass sequencing and characterization of filarial glutathione-*S*-transferase

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Abstract

Glutathione-*S*-transferase has been detected in the somatic extract and excretory–secretory products of different life stages of *Setaria cervi*, a bovine filarial parasite. The enzyme was subjected to MALDI-TOF followed by mass spectrometry and the nearest match found was *Pleuronectes platessa* GST. Molecular mass of the purified enzyme was ≈26 kDa as determined by SDS–PAGE and MALDI-TOF. *Setaria cervi* GST exhibited high activity towards 1-chloro-2,4-dinitrobenzene and ethacrynic acid. Kinetic analysis with respect to 1-chloro-2,4-dinitrobenzene and glutathione as substrate revealed a K_m of 2.22 mM and 0.61 mM, respectively. The activity was inhibited significantly by Cibacron blue and α-tocopherol.

Keywords: MALDI mass; Glutathione-*S*-transferase; Filariasis; *Setaria cervi*

Human lymphatic filariasis also known as elephantiasis is a mosquito borne parasitic disease caused by three nematode worms of the family filariadae *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. Filarial parasites are long lived and are capable of surviving in host's hostile environment by employing a variety of immune evasion strategies and defense mechanisms including the detoxification and repair mechanisms of glutathione-*S*-transferase.

Helminths have limited detoxification enzymes and appear to lack the important cytochrome P-450 dependent detoxification reactions therefore; the GSTs seem to be the major detoxification enzymes of this parasitic group [1]. Glutathione-*S*-transferases are multifunctional proteins that can function as enzymes catalyzing the conjugation of glutathione thiolate anion with a multitude of second substrates or as non-covalent binding proteins for a range of hydrophobic ligands [2].

Glutathione-*S*-transferase has been detected in adult female *Setaria cervi*, a bovine filarial parasite. In a previous publication the role of *S. cervi* GST antigen in inducing immunity in the host against *B. malayi* microfilariae and infective larvae was studied by in vitro antibody dependent cell mediated reaction as well as in situ inoculation of filarial parasites with in a micropore chamber implanted in Mastomys, suggestive of the fact that native *S. cervi* GST is effective in inducing protection against heterologus *B. malayi* filarial parasite and thus has immunoprophylactic potential [3].

In our earlier studies, *S. cervi* GST was also found to be inducible by xenobiotics and antifilarial drugs. In vitro maintenance of adult female *S. cervi* in medium containing butylated hydroxyanisole, phenobarbitone, and diethylcarbamazine, resulted in a significant increase in GST specific activity. This induction of GST was dose and time dependent. We had also studied the potential of GST as a drug target by studying the in vitro effects of some known GST inhibitors on the motility and viability of *S. cervi*

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adult females and microfilariae. Ethacrynic acid, ellagic acid, BHA, and Cibacron blue significantly reduced the viability and motility of the parasite suggesting that GST plays an important role in parasite survival [4].

Since *S. cervi* GST could be induced by exposure of the parasite to drugs and other xenobiotics or to reactive oxygen species indicates that intervention of this enzyme could surely lead to an innovative approach for parasite control. Therefore characterization of *S. cervi* GSTs is a pre-requisite for an elaborative study to further explore its potential as vaccine and drug target. This paper reports the identification of GST activity in different life stages of *S. cervi*, along with its purification, sequence homology with other filarial parasites' GST as well as its biochemical and immunological characterization in adult female worms. Since human filarial parasite is not available in substantial amount *S. cervi* GST could be used as model for drug target and in immunoprophylactic studies.

Materials and methods

Collection of parasitic material and preparation of homogenate. *Setaria cervi* male female and mf extract was prepared as described previously [5].

Protein estimation. Protein estimation was done by Bradford method [6]. Bovine serum albumin was used as protein standard.

Enzyme assay and substrate specificity. GST activity was routinely assayed by measuring enzymic conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB). Substrate specificity studies were done spectrophotometrically by using a range of substrates like 1,2-dichloro-4-nitrobenzene (DCNB), ethacrynic acid, and bromosulfophthalein (Sigma, USA) according to the method of Habig and Jacoby [7]. Glutathione peroxidase activity in purified enzyme was determined as described elsewhere using cumene hydroperoxide, *tert*-butyl hydroperoxide, and hydrogen peroxide as substrates [8].

Protein identification and sequencing. Adult female *S. cervi* homogenate was purified with GSH–agarose affinity column [3]. The purified *S. cervi* glutathione binding proteins were run on 12.5% SDS–PAGE and 26 kDa band was excised from the gel. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was done at The Centre for Genomic Application, New Delhi. Three major peptides of parent mass 899.525 and 1094.600, 1894.983 were then sequenced by mass spectrometry.

Purification. *Setaria cervi* GST-26 was purified using sequential column chromatography involving anion exchange and affinity chromatography. Adult female homogenate was applied on a DEAE–Sephadex column; bound proteins were eluted using stepwise increasing NaCl gradients in 50 mM Tris–HCl pH 7.5 buffer. Peak fractions containing GST activity were applied to a GSH–agarose (Sigma) affinity column and enzyme active fractions (GST-26) were collected.

Electrophoretic techniques. Native polyacrylamide gel electrophoresis and SDS–PAGE was done following Laemmli [9].

Determination of kinetic parameters. The effect of pH on purified GST-26 enzyme was measured over a range of pH using 100 mM Potassium Phosphate buffer (5.0–7.0) and 100 mM Tris–HCl (7.5–9.5). Aliquot's from pure enzyme preparation were withdrawn and exposed to various temperatures along with water and assay buffer for 3 min for determination of optimum temperature. GST activity was assayed taking different conc. of CDNB (0.05–5 mM) and GSH (0.05–2 mM). The K_m and V_{max} were calculated from double reciprocal plot of $1/V$ vs. 1/[S].

Inhibition studies. The enzyme activity was measured using CDNB substrate in the presence of varying concentrations of inhibitors and the I_{50} value was estimated graphically.

Results and discussion

Filarial nematodes have adopted a number of strategies to evade, modify or neutralize the host's defense mechanisms for ensuring its survival. GST activity in relation to these strategies has been identified in filarial nematodes and other helminths [10]. Similarly GST homologs have been cloned and expressed in filarial parasites [11–14] but the potential of GST's as molecular target for chemotherapy and their use to induce protective immunity is undetermined till date. A significant level of GST activity has been detected in various *S. cervi* life stages suggesting that the enzyme is ubiquitous in all stages of parasite's life cycle. The specific activity of male worms was twice to that of females (Table 1) indicating heterogeneity in sexes. However female ES had highest enzymatic activity followed by microfilariae then males showing differential expression of GST activity in different life stages. In contrast, enzyme activity was not detected in the mf and ES products of *S. digitata* [15].

Setaria cervi adult female GST (glutathione binding proteins) have already been studied as immunoprophylactic agents and chemotherapeutic targets hence sequence analysis using MALDI-TOF mass spectrometry was done to investigate its sequence homology with other filarial parasites (Fig. 1, lane 1). The digested sample was analysed with MALDI-TOF mass spectrometer, the subunit mass of the digested protein was 25.8 kDa. Fifty-nine peptide peaks were obtained and peptide mass fingerprint was used for database search using MASCOT. The most significant match found was *Pleuronectes platessa* GST with seven matching peptides (Fig. 2). Further three major peptide peaks of parent mass 899.525, 1894.983, and 1094.600 were sequenced by MS/MS and most significant match found was with *Onchocerca volvulus* 26.7 kDa, 54.80 kDa and *Ascaris suum* 38.50 kDa glutathione transferases, respectively (Fig. 3). *Onchocerca volvulus* and *W. bancrofti* belong to the same family that is onchocercidae, besides this *Onchocerca volvulus* GST resembles closely to *W. bancrofti* GST. Hence it can be speculated that drugs designed against Sc-GST-26 will also be effective against human parasites, besides this *S. cervi* is easily available in substantial amounts also.

Table 1
Glutathione-*S*-transferase activity in different life stages and excretory–secretory products (ESP) of *S. cervi*

Sample	Specific activity (U/mg) (mean ± SD)	
	Extracts	ESP
Microfilariae	8.13 ± 1.5	20.39 ± 0.98
Male	11.89 ± 1.14	18.54 ± 1.96
Female	5.29 ± 1.17	34.72 ± 2.31

The enzyme activity was measured by the method of Habig et al., using CDNB as substrate. Results are means ± SD of 10 different determinations.
One unit of enzyme activity is defined as the amount of enzyme catalyzing the oxidation of 1 μM of CDNB per milliliter per minute at 25 °C.