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The purification and properties of glutathione S-transferase from *Ditylenchus myceliophagus*

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Summary – The cytoplasmic glutathione S-transferase (GST) activity of *Ditylenchus myceliophagus* was resolved into four isoforms, DmI, DmII, DmIII and DmIV, by a combination of chromatofocusing and hexylglutathione affinity chromatography. An endogenous cytoplasmic factor interfered with the binding of the cytosolic transferases to glutathione and hexylglutathione affinity matrices and binding only occurred after initial purification. The four isoforms were homodimers with subunit molecular weights 25.3, 24, 26 and 25.7 kDa for DmI, II, III and IV, respectively. The pIs for DmI, II and III were 7.28, 5.04 and 4.88. The N-terminal sequence of the major form (DmIII) had a strong sequence similarity to the mammalian alpha class GSTs. However, substrate specificities and inhibitor profiles of Dm II, III and IV showed an overall resemblance to the mammalian mu class, whilst DmI showed more alpha class characteristics.

Résumé – ***Purification et propriétés de la glutathion S-transférase isolée de* Ditylenchus myceliophagus** – L'activité de la glutathion S-transférase cytoplasmique chez *Ditylenchus myceliophagus* a été attribuée à quatre isoenzymes, DmI, DmII, DmIII et DmIV, par combinaison de la chromatographie focalisante et de la chromatographie d'affinité au moyen de l'hexyl-glutathion. Un facteur endogène cytoplasmique a interféré avec les matrices d'affinité de la liaison entre les transférases cytosoliques et le glutathion, et la liaison ne s'est produite qu'après une purification préliminaire. Les quatres isoenzymes sont apparues être des homodimères avec des subunités de poids moléculaires respectifs de 25,3, 24, 26 et 25,7 kDa pour DmI, DmII, DmIII et DmIV. Les points isoélectriques pour DmI, DmII et DmIII sont de 7,28, 5,04 et 4,88. La séquence N-terminale de l'enzyme principale (DmIII) présente une très forte similarité de séquence avec la classe alpha GSTs des mammifères. En tout état de cause, les spécificités de substrats et les profils inhibiteurs de DmII, DmIII et DmIV montrent une ressemblance générale avec la classe mu des mammifères alors que DmI montre des caractéristiques plus proches de la classe alpha.

Key-words : detoxification *Ditylenchus myceliophagus*, glutathione S-transferase, nematode.

The glutathione S-transferases (GSTs) play a key role in detoxification processes by catalysing the conjugation of glutathione with a wide range of exogenously and endogenously derived toxic compounds, the result of which is to increase water solubility and reduce toxicity (Mannervik, 1985; Wilce & Parker, 1994). The diets of fungivorous and phytophagous nematodes expose them to a range of secondary metabolites, some of which may have specific defensive functions. The ability of nematodes to detoxify these host metabolites could be a factor in determining host range and cultivar resistance. Detoxification enzymes are also potential targets for selective inhibition by novel nematicides. However, previous studies on nematode GSTs have concentrated on animal parasitic and free-living species. *Ditylenchus myceliophagus* is an economically important parasite of cultivated mushrooms (Cayrol, 1970), and may prove to be a useful biochemical model for other economically important species of *Ditylenchus* such as *D. dipsaci* and *D. angustus*. In this paper we report the purification and characterization of one major and three minor isoforms of GST from *D. myceliophagus*.

Materials and methods

NEMATODES

Peak-heated mushroom-phase unspored compost, in 350 ml plastic containers, was sown with rye grain mushroom spawn of *Agaricus bisporus* and the mycelia allowed to develop for 2-3 weeks at 23 °C before 50-100 active nematodes (mixed stages) were added to each culture. The *D. myceliophagus* were harvested when "curds" of nematodes were visible on the lid and sides of the culture vessel. These worms were removed by gentle washing with distilled water and allowed to sediment. Washing/sedimentation was repeated until the supernatant was clear. The worms were finally cleaned by migration through two layers of moist filter paper resting on a miniature sieve (1 mm mesh). The nematodes were initially retained by the filter paper, which was then transferred to a

collecting dish containing water up to the level of the filter paper. Worms were concentrated by centrifugation at 250 *g* for 5 min.

Ditylenchus myceliophagus were also recovered from the compost using a mistifier (Webster, 1962). Worms recovered in this way were often contaminated with fine particles of inert material which could only be removed by passing through 250 µm and 65 µm sieves, and washing onto a 35 µm sieve to retain the worms. Nematodes which were not used immediately were stored in liquid nitrogen.

Subcellular fractionation

Nematode material was homogenized in two volumes of extraction buffer (10 mM potassium phosphate buffer, pH 7.4, 1 mM ethylene diamine tetra-acetic acid (EDTA), 0.2 mM dithiothreitol (DTT)) using an MSE sonicator, medium power in 20 s bursts, with the sample cooled in ice. The crude homogenate was centrifuged at 1000 *g* for 10 min at 4 °C to remove nuclei and large cell debris. The supernatant was further centrifuged at 14 000 *g* for 20 min at 4 °C to pellet the mitochondria. The supernatant was then centrifuged at 100 000 *g* for 90 min at 4 °C, which resulted in a microsomal pellet and a cytosolic fraction. The cytosolic fraction contained a layer of lipid which was removed by filtration through glass wool.

Enzyme assays

All assays were performed at 30 °C using a Gilford Response Spectrophotometer and the results are means of five replicates. Routine assay of GST (EC 2.5.1.18) with 1-chloro-2,4 dinitrobenzene (CDNB) as the second substrate was carried out as described by Habig *et al.* (1974). The reaction mixture (1 ml) contained (final concentrations): 100 mM potassium phosphate buffer, 1mM glutathione (reduced, neutralised), protein (10-50 µg). After 2-3 min pre-incubation the reaction was started by the addition of 1mM CDNB. The formation of the glutathione conjugate was followed by the increase in optical density (OD) at 340 nm ($\varepsilon = 9.6\ mM^{-1}\ cm^{-1}$) due to the formation of the thiol ester. Glutathione conjugating activity with the model substrates 1, 2 dichloro-4-nitrobenzene, *trans*-4-phenyl-3-buten-2-one, ethacrynic acid, bromosulphophthalein and 1, 2 epoxy-3-(*p*-nitrophenoxy) propane was measured in the same assay. The non-enzymatic rate between glutathione and the second substrate was subtracted from the enzymatic rate. GST activity towards products of lipid peroxidation (members of the *trans,trans* alka 2,4-dienal and *trans* alk-2-enal series and 4-hydroxy-alk-2-enals were measured by the methods of Alin *et al.* [1985], Brophy *et al.* [1989] and Brophy and Barrett [1990]). 4-Hydroxynon-2-enal was a generous gift from Professor H. Esterbauer (University of Graz, Austria). Where it was necessary to dissolve the second substrate in ethanol, the concentration of solvent in the final reaction mixture was less than 5 % and an equal volume of solvent was added to the control.

Selenium - independent glutathione peroxidase activity was determined as described by Jaffe and Lambert (1986). The reaction mixture (1 ml) contained (final concentrations): 50 mM potassium phosphate buffer, pH 7.0, 0.5 mM EDTA, 0.24 units glutathione reductase (Sigma), 0.15 mM NADPH, protein 10-50 µg. The peroxide independent oxidation of NADPH was followed at 340 nm for 3 min, the reaction was then started by the addition of 1 mM hydroperoxide. A mixture of linoleic hydroperoxides was prepared by the auto-oxidation of linoleic acid at pH 8.5 (Preston & Barrett, 1987). The competency of the second substrate was confirmed using rat liver cytosolic extract as a positive enzyme control. Protein concentration was determined by the Coomassie Blue G-250 binding assay (Sedmak & Grossberg, 1977) using bovine serum albumin as the protein standard.

Inhibition studies

Inhibition was assumed to be reversible and the I_{50} value estimated by probit analysis; the I_{50} is the concentration of inhibitor giving 50% inhibition with CDNB as the second substrate. Inhibitors were dissolved in either ethanol or dimethylsulphoxide. Haematin was initially dissolved in 200 mM sodium hydroxide and stock solutions prepared by diluting with 200 mM potassium phosphate buffer, pH 6.5. In all cases the final concentration of solvent in the assay mixture was less than 5 % and constant for each inhibitor. An equal volume of solvent was added to the control. The enzyme and inhibitor were pre-incubated for 5 min at 30 °C prior to the start of the assay and each value is the mean of three replicates.

Principal component analysis of activity with model substrates (1-chloro-2, 4 dinitrobenzene, 1, 2 dichloro-4-nitrobenzene, *trans*-4-phenyl-3-buten-2-one, ethacrynic acid, bromosulphophthalein, cumene hydroperoxide) and model inhibitors (Cibacron blue, haematin, triphenyl tin) was performed using MINITAB.

Column chromatography

All chromatographic procedures were carried out at 4 °C except for gel filtration and fast protein liquid chromatography (FPLC) which were performed at room temperature. The columns and media were obtained from Pharmacia, except the affinity gels which were from Sigma: the procedures were performed according to the manufacturers' instructions. Chromatofocusing in the range pH 7-4 was carried out using a polybuffer exchange column 9-4 (0.9 × 30 cm matrix) with polybuffer 7-4, pH 4 as the run-

ning buffer (10 ml h^{-1}). Eluted fractions were collected into 0.2 M potassium phosphate buffer, pH 7.4 to neutralize the polybuffer and increase enzyme stability. Affinity chromatography was performed using glutathione and S-hexylglutathione affinity gels (0.9 × 15 cm matrix). Bound proteins were eluted from the glutathione column by adding 10 mM glutathione to the elution buffer (50 mM Tris-HCl, pH 9.6, 1 mM EDTA, 0.2 mM DTT, 10 % v/v glycerol), flow rate 20 ml h^{-1}. Proteins bound to the hexylglutathione column were eluted by adding 5 mM S-hexylglutathione to the elution buffer (10 mM Tris-HCl, pH 7.8, 200 mM NaCl, 1 mM EDTA, 0.2 mM DTT, 10 % v/v glycerol), flow rate 20 ml h^{-1}. Active fractions from the hexylglutathione column were dialysed at 4 °C for 14 h against 500 ml of 200 mM potassium phosphate buffer, pH 7.0 containing 1 mM EDTA, 0.2 mM DTT, 5 mM reduced glutathione, 20 % v/v glycerol, to remove the hexylglutathione which interferes with the enzyme assay.

Native molecular mass was determined by Sephadex G-75 superfine chromatography (2.5 × 50 cm matrix) using 20 mM potassium phosphate buffer, pH 7.4 containing 0.1 M KCl as the running buffer (16 ml h^{-1}). The column was calibrated using standard proteins (bovine serum albumin, ovalbumin, carbonic anhydrase, cytochrome c).

FPLC ion-exchange chromatography was carried out using a Sepharose Q Fast Flow matrix (1.6 × 20 cm) with 50 mM Tris-HCl, pH 8.0 containing 1 mM EDTA, 0.2 mM DTT as the eluting buffer with a 0 to 1 M linear NaCl gradient (2 ml min^{-1}).

GEL ELECTROPHORESIS

SDS polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing (IEF) were carried out using a Pharmacia Phastsystem. SDS-PAGE was performed on 20 % polyacrylamide homogeneous gels. The sample buffer, which was used for standards as well as samples, contained 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Samples were concentrated prior to electrophoresis using 3K Microconcentrators, Amicon (350 *g* for 10 min). Immediately before application of the sample protein to the gel, SDS (2.5 % w/v) and 2-mercaptoethanol (5 % v/v) were added and the samples heated to 100 °C for 5 min to dissociate subunits. Bromophenol blue (0.01 % w/v) was added and the samples briefly centrifuged before being applied to the gel. The gels were calibrated with Bio Rad low molecular weight standards. The protein bands were visualized either with Brilliant Blue G-Colloidal Concentrate (Sigma) or by silver staining (Pharmacia) according to the manufacturers' instructions.

Isoelectric focusing was performed on Phastgel 3-9 gels and calibrated with a Pharmacia broad range calibration kit pH 3-10. Proteins were visualized by silver staining.

N-TERMINAL SEQUENCING

Purified GST was exchanged into 10 mM ammonium formate buffer pH 7.0 and lyophilized. The N-terminal amino acids were determined by automated Edman degradation using an Applied Biosystems Model 477A automatic sequencer (Eurosequence, Groningen, The Netherlands).

Results

INTRACELLULAR DISTRIBUTION

More than 90 % of the total GST activity was found in the cytosolic fraction, with less than 2.5 % in the microsomes (Table 1). Triton X-100 can be used to release enzymes trapped in vesicles formed during the sonication process but only the cell debris fraction showed an increase in activity after treatment with detergent.

Table 1. *Subcellular distribution of glutathione S-transferase in* Ditylenchus myceliophagus.

Cell fraction	% of total recovered activity with CDNB	% increase in activity with 0.1 % Triton X-100
Cell debris and nuclei	0.59	590
Mitochondrial	0.95	0
Microsomal	2.46	0
Cytosolic	96	0

PURIFICATION

When *D. myceliophagus* cytosolic extract was applied to the reduced glutathione affinity column, 87 % of the GST activity failed to bind and was recovered in the flow through fraction. The enzyme also failed to bind to the hexylglutathione column. Preliminary purification prior to affinity chromatography was tried in an attempt to remove the blocking factor. Using ion-exchange FPLC chromatography, one major and two minor peaks of activity were resolved, but more than 50 % of the original GST activity applied to the column was lost. The major peak of activity from ion-exchange chromatography still failed to bind to the GSH affinity matrix. However, there was improved binding to the hexylglutathione affinity matrix (56 % bound) and binding could be further improved by changing the pH of the eluting buffer from pH 8.0 to pH 10. Despite the improved binding to the hexylglu-

tathione column, the loss of activity during FPLC made this approach impractical.

Chromatofocusing of *D. myceliophagus* cytosol resolved four main peaks of activity (Fig. 1), all acidic or near neutral and no unresolved basic activity was detectable. The isoforms were labelled, in order of elution, DmI, DmII, DmIII and DmIV. Form III was the most prominent peak, accounting for approximately 48 % of the total activity recovered. Forms I and II represented 9 and 12 % of the activity, respectively. The levels of form IV varied, from preparation to preparation, between 2 and 20 % of the total activity recovered. Storing the worms in liquid nitrogen did not appear to affect the chromatofocusing profile. Following chromatofocusing, the isoforms were purified by affinity chromatography on a hexylglutathione matrix, with the elution buffer adjusted to pH 10. The small peak of activity which eluted between Dm II and DM III did not bind to the affinity column. Recovery of the different isoforms from the affinity column varied, but was approximately 50 % (Table 2). The purified GSTs all showed a single band on SDS-gels (Fig. 2).

Substrate and inhibitor profiles

Based on substrate specificities and inhibitor sensitivities, mammalian GSTs can be grouped into four supergene families, alpha, mu, pi and theta. The activities of the different isoforms of *D. myceliophagus* GST with the model substrates used to distinguish the mammalian transferase groups is shown in Table 3. All of the isoforms showed highest activity with CDNB, but only low activity with *trans*-4-phenyl-3-buten-2-one. DmI, DmII and DmIII showed activity with ethacrynic acid, but with considerable variation between the isoforms. No conjugating activity was detected with bromosulphophthalein or 1,2 dichloro-4-nitrobenzene in any of the isoforms. Comparatively high transferase activity was detected with DmIII using 1,2,epoxy-3-(p-nitrophenoxy)propane, a characteristic of theta type transferases (Meyer *et al.*, 1991).

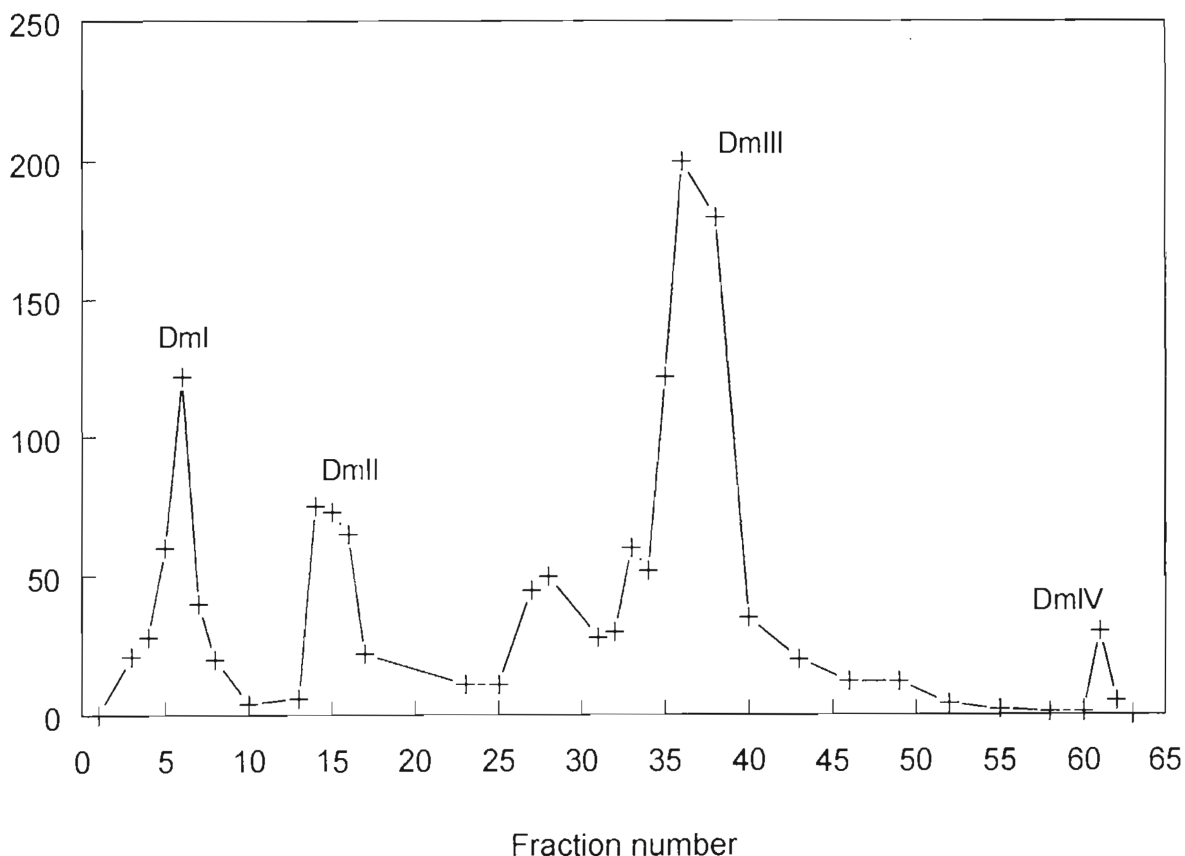

Fig. 1. *Chromatofocusing pH 7-4 profile of cytosolic glutathione S-transferase activity from* Ditylenchus myceliophagus *(For details of the column procedure, see methods).*

Table 2. *Typical chromatofocusing profile of* Ditylenchus myceliophagus *cytosolic glutathione S-transferases.*

Apparent isoform	Total activity* (μ Mol.min^{-1})	Total protein (mg)	Specific activity (μ mol.min^{-1} mg^{-1} protein)	% Total activity recovered
DmI	0.83	36.7	0.023	16.6
DmII	0.368	23.46	0.015	7.2
DmIII	2.87	21.73	0.132	57.3
DmIV	0.29	14.98	0.019	6.4
Unresolved activity	0.697	–	–	13

–: not determined;
*: with CDNB as substrate.

The ability to conjugate lipid hydroperoxides was limited in *D. myceliophagus* transferases; only DmIII showed measurable activity with cumene hydroperoxide (Table 3). DmIII showed only limited activity with alkenals, but activity was two- to ten-fold higher with the minor isoform DmIV. Activity with alkdienals was generally less than with alkenals for all isoforms tested (I, III and IV) and there was little correlation between activity and chain length (Table 3).

The inhibition profiles for the transferases of *D. myceliophagus* are shown in Table 4. The different isoforms differed in their sensitivities to different inhibitors. Compared to mammalian isozymes, the transferases were relatively insensitive to inhibition by bromosulphophthalein whilst the plant phenols tested (quercetin, ellagic acid, purpurogallin and alizarin) were relatively poor inhibitors compared with Cibacron blue.

PHYSICAL PROPERTIES

The native molecular mass of *D. myceliophagus* cytoplasmic GST activity determined by gel filtration was 51 kDa. The apparent sub-unit weights of the four isoforms determined by SDS- PAGE were 25.3, 24, 26 and 25.7 kDa for DmI, DmII, DmIII and DmIV, respectively. Since on SDS-PAGE there was a single band for each isoform, the *D. myceliophagus* isoforms are homodimers. The isoelectric points of isoforms DmI, DmII and DmIII were estimated as 7.28, 5.04 and 4.88, respectively, using IEF-PAGE. The pH optimum for DmIII with CDNB as the second substrate was approximately pH 8.0, with a broad peak of activity between pH 6 and 9 (Fig. 3).

The N-terminal sequence of DmIII is shown in Fig.4. DmIII shows a shared sequence similarity of 65 % with mammalian alpha GST and similarities of 55 % with mammalian mu and pi class GSTs, but only a limited sequence identity with the theta class (30 %).

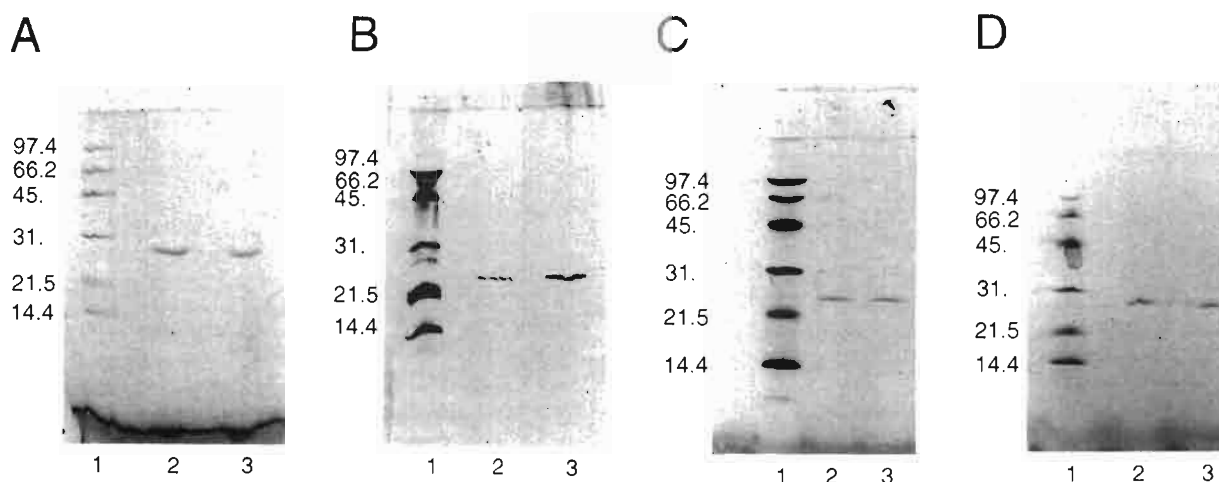

Fig. 2. *SDS PAGE gels of* Ditylenchus myceliophagus *glutathione S-transferase isoforms. Lane 1: Low molecular weight markers (kDa); Lanes 2 and 3: Purified protein (A: DmI; B: DmII; C: DmIII; D: DmIV).*

Table 3. *Substrate specificity of purified* Ditylenchus myceliophagus *glutathione S-transferases.*

Substrates	Activity μmol.min^{-1} mg^{-1} protein*			
	Dm I	Dm II	Dm III	Dm IV
Model Substrates				
1-Chloro,2,4-dinitrobenzene	0.66 ± 0.14	0.40 ± 0.06	6.15 ± 1.11	1.19 ± 0.36
1,2 Dichloro-4-nitrobenzene	N/D	N/D	N/D	N/D
trans-4-Phenyl-3-buten-2-one	0.09 ± 0.03	N/D	0.26 ± 0.06	0.03 ± 0.01
Ethacrynic acid	0.18 ± 0.04	N/D	7.08 ± 1.77	4.35 ± 0.91
Bromosulphophthalein	N/D	N/D	N/D	N/D
1,2 Epoxy-3-(p-nitrophenoxy) propane	N/D	-	3.11 ± 0.93	0.33 ± 0.01
Lipid hydroperoxides				
Cumene hydroperoxide	N/D	0.0028 ± 0.001	0.54 ± 0.19	N/D
Butyl hydroperoxide	N/D	-	N/D	N/D
Linoleate hydroperoxide	N/D	-	N/D	N/D
Reactive carbonyls		-		
trans-Hex-2-enal	0.0006 ± 0.00019	-	0.06 ± 0.005	0.52 ± 0.16
trans-Oct-2-enal	0.0008 ± 0.00024	-	0.047 ± 0.011	0.12 ± 0.04
trans-Non-2-enal	0.00095 ± 0.00024	-	0.0554 ± 0.01	0.23 ± 0.095
Hexa-2,4-dienal	0.00093 ± 0.00016	-	0.021 ± 0.003	0.07 ± 0.021
trans,trans Hepta-2,4-dienal	0.01 ± 0.0023	-	0.036 ± 0.003	0.06 ± 0.018
trans,trans Nona-2,4-dienal	0.0092 ± 0.0027	-	0.046 ± 0.011	0.075 ± 0.012
trans,trans Deca-2,4-dienal	0.01 ± 0.00292	-	0.0047 ± 0.0017	0.089 ± 0.02
Hydroxynon-2-enal	N/D	-	0.022 ± 0.005	-

*: mean of five replicates ± S.D;
N/D: not detected;
- : not determined.

Discussion

Glutathione S-transferase activity in *D. myceliophagus*, as in other nematodes, is primarily cytoplasmic, with little activity associated with the microsomes (Barrett, 1995). The cytoplasmic glutathione transferases of *D. myceliophagus* can be resolved into four acidic isoforms DmI, DmII, DmIII and DmIV. This is different from the free-living nematode *Panagrellus redivivus* where the major isoforms are basic (Papadopoulos *et al.*, 1989). An endogenous cytoplasmic factor interfered with the binding of *D. myceliophagus* isoforms to glutathione and hexylglutathione affinity columns and binding only occurred after initial purification. Similar "blocking factors'" have been described during the purification of GST from a number of invertebrates (Papadopoulos *et al.*, 1989; Brophy & Barrett, 1989; Brophy *et al.*, 1989). Recently, Brophy *et al.* (1994) purified a series of 70-80 kDa "blocking proteins" from the animal parasitic nematode *Heligmosomoides polygyrus*. These blocking factors do not show glutathione transferase activity and may possibly be binding proteins (ligandins) for glutathione conjugates, since the latter can be potent enzyme inhibitors.

The GST isoforms from *D. myceliophagus* are homodimers and their subunit and native molecular weights are similar to those of other GSTs (Wilce & Parker, 1994). Like other nematode GSTs, the *D. myceliophagus* isoforms cannot be classified easily as alpha, mu, pi or theta type, but exhibit a mosaic of substrate specificities and inhibitor sensitivities. DmI, II, III and IV show no, or low, activity with the mu class marker substrates bromosulphophthalein, *trans*-4-phenyl-3-buten-2-one and 1,2-dichloro-4-nitrobenzene; similarly, there was little or no activity with cumene hydroperoxide, an alpha class marker substrate. DmIII and IV showed relatively high activity with the pi class marker ethacrynic acid, whilst DmIII also showed activity with the theta class substrate 1,2 epoxy-3-(p-nitrophenoxy)propane. DmIII and IV were sensitive to inhibition by Cibacron blue and

Table 4. Inhibitor sensitivities of purified Ditylenchus myceliophagus *glutathione S-transferases.*

Inhibitor	I_{50} Value (μM)*			
	Dm I	Dm II	Dm III	Dm IV
Cibacron Blue	4.88	0.14	0.27	0.15
Bromosulpho-phthalein	66.56	12.9	8.2	9.45
Haematin	4.24	0.03	0.22	0.71
Triphenyltin chloride	57.00	0.004	0.029	0.012
Chalcone	131	–	52.67	47.30
Ellagic acid	N/I	–	16.06	117
Quercetin	123	–	16.38	30.89
Purpurogallin	167	–	129	-
Alizarin	N/I	–	N/I	N/I

*: mean of three replicates
N/I: no inhibition up to 0.1 mM
–: not determined

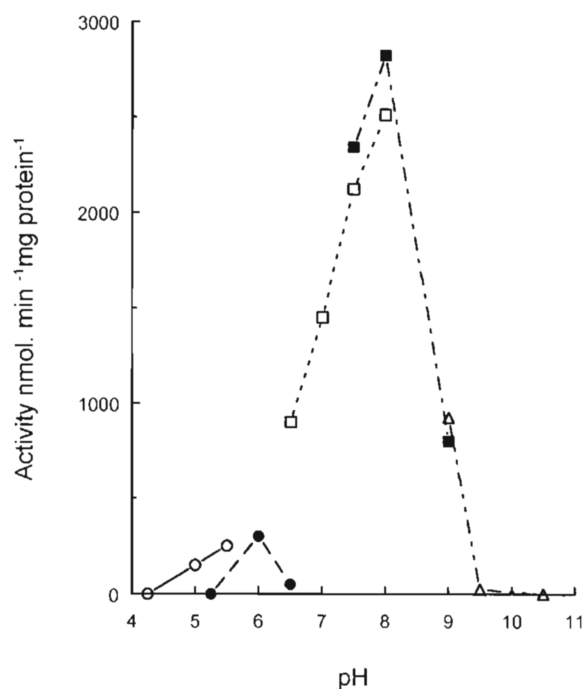

Fig. 3. The effect of pH on glutathione S-transferase activity (Dm III) from Ditylenchus myceliophagus. *Each point is the mean of five replicates, st. dev. less than 10 % of the mean.* (-○-) *acetate buffer,* (-●-) *citrate/phosphate buffer,* (-□-) *sodium phosphate buffer,* (-■-) *tris-HCL buffer,* (-Δ-) *carbonate/bicarbonate buffer (all 100 mM).*

triphenyltin, typical mu class markers, and to inhibition by haematin, an alpha class marker.

Although class discriminating substrates and inhibitors are useful, no single compound can give an unambiguous distinction (Mannervik *et al.*, 1985). A more comprehensive analysis of the enzymatic properties of the four *D. myceliophagus* isoforms with those of the three major mammalian GST classes can be made by means of multi-variate analysis. This allows the variables to be represented, as a reasonable approximation, by two derived variables (Wold *et al.*, 1984). The first and second principal components were calculated for DmI, II, III and IV on the basis of their specific activities with model substrates and sensitivities to the model inhibitors and plotted, together with the first and second principal components of the mammalian alpha, mu and pi GST classes (Fig. 5). The three mammalian GST classes are localized in three distinct domains in the plane defined by the two

Dm III - V K Y E L H Y F D T R G L G E A I R L I
Alpha (rat 2-2) P G * P V * * * * * G * * R M * P * * W L
Mu (rat 3-3) - - P M I * G * W N V * * * T H P * * * L
Pi (rat 7-7) - P P * T I V * * P V * * R C * * T * M L
Theta (rat 13-13) - - - G L E L * L * L L S Q P S R A V Y *

Fig. 4. Comparison of the N-terminal sequence of Ditylenchus myceliophagus *glutathione S-transferase (Dm III) with mammalian alpha, mu, pi and theta class glutathione S-transferase sequences. Asterisk (*) indicates an exact match, letters in bold indicate conserved substitutions. The boxes indicate regions of identity involved in glutathione binding. The amino acids are represented by their one letter symbols.*

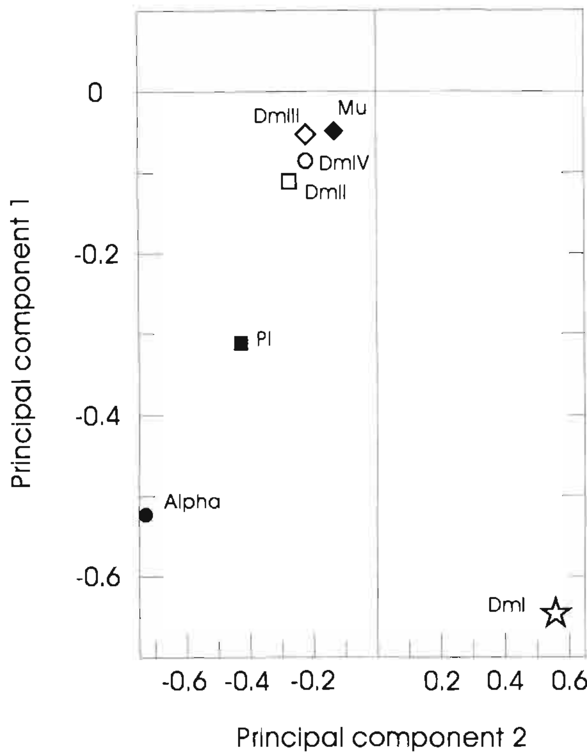

Fig 5. *Multivariate analysis of the biochemical characteristics of the purified glutathione S-transferases from* Ditylenchus myceliophagus *(Dm I, II, III, IV) and the mammalian classes alpha, mu and pi. Mammalian class theta would appear as an outlier on the diagram (For details see text).*

principal components. The location of DmII, III and IV suggest an overall catalytic homology with mammalian mu class GSTs, whilst DmI appears spatially separate from the mammalian classes and enzymatically different from transferases II, III and IV.

Despite its catalytic affinity to the mammalian mu class glutathione transferases, DmIII showed the highest shared N-terminal sequence similarity with the mammalian alpha class. When compared with other nematode GSTs (Fig. 6), the N-terminal sequence of DmIII showed 70 % similarity with *Ascaris suum* GST (Liebau *et al.*, 1994 *a*) and 60 % similarity with GST1 and GST2 from *H. polygyrus* (Brophy *et al.*, 1994). The predicted amino acid sequences from cDNA encoding GST1 and GST2 from *Onchocerca volvulus* showed similarities of 60 and 65 % respectively with DmIII (Liebau *et al.*, 1994 *b*). There is a lower sequence identity of 50 % with GSTs from *Caenorhabditis elegans* (Weston *et al.*, 1989). Other helminth GSTs show only low sequence similarity: *Fasciola hepatica* 30 % (Wijffels *et al.*, 1992), *Echinococcus granulosus* 33 % (Fernandez & Hormaeche, 1994), *Schistocephalus solidus* 26 % (Brophy *et al.*, 1989), and *Schistosoma mansoni* 20 % (Henkle *et al.*, 1990).

An integral part of plant and fungal defence mechanisms consists of chemicals that impose oxidative stress on invading pathogens. DmIII showed peroxidase activity with cumene hydroperoxide, whilst DmIII and IV showed relatively high conjugating activity with members of the *trans* alk 2-enal, *trans,trans* alka 2,4-dienals and 4-hydroxyalk 2-enal series of reactive carbonyls. Although the ability of the *D. myceliophagus* isoforms to detoxify lipid hydroper-

Dm III	V	K	Y	E	L	H	Y	F	D	T	R	G	L	G	E	A	I	R	L	I
1	-	V	*	K	*	T	*	*	*	I	*	*	*	*	*	**G**	A	*	*	*
2	-	*	*	T	*	T	*	*	**N**	G	*	*	R	A	*	V	*	*	*	**L**
3	M	S	*	K	*	T	*	*	S	I	*	*	*	A	*	P	*	*	*	**F**
4	-	-	L	K	*	T	*	*	*	I	H	*	*	A	*	P	*	*	*	**L**
5	*	H	*	K	*	T	*	*	**N**	G	*	*	-	-	-	-	-	-	-	-

Fig 6. *Comparison of the N-terminal sequence of Ditylenchus myceliophagus glutathione S-transferase (Dm III) with other nematode glutathione S-transferase sequences. 1. Ascaris suum (Liebau et al., 1994 a); 2. Onchocerca volvulus (Salinas et al., 1994); 3. Onchocerca volvulus (Liebau et al., 1994 b); 4. Caenorhabditis elegans (Weston et al., 1989); 5. Heligmosomoides polygyrus (Brophy et al., 1994). Asterisk (*) indicates an exact match, letters in bold indicate conserved substitutions. The boxes indicate regions of identity involved in glutathione binding. The amino acids are represented by their one letter symbols.*

oxides is limited, their ability to detoxify reactive carbonyls (themselves products of lipid hydroperoxide breakdown) suggests that they could play a role in protection against oxidative tissue damage.

Several isoforms of GST with different substrate specificities occur in *D. myceliophagus*. A range of isoforms are presumably required to deal with the high number of potential toxicants in the nematodes' environment. Fungi produce a tremendous range of secondary metabolites including phenolic compounds, quinones and organic acids. It is not known if the different isoforms occur in different tissues of the nematode, nor if their expression is environmentally or developmentally controlled, although the variable levels of DmIV do suggest some form of regulation.

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