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Glutathione transferase in the free-living nematode *Panagrellus redivivus*

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The free-living nematode *Panagrellus redivivus* was found to have relatively high cytosolic glutathione transferase activity. Chromatofocusing indicated that at least four GSH transferase forms were present in the nematode cytosol. An endogenous molecular factor interfered with the binding of the cytosolic GSH transferases to a glutathione affinity matrix and binding only occurred after a partial purification step. The major resolved GSH transferase form B was a basic enzyme that showed no biochemical homology to the three mammalian multigene GSH transferase families and appeared to interact with plant phenols, possible natural substrates. A minor GSH transferase form AT showed a biochemical relationship to the mammalian α family including catalytic activity with a model lipid hydroperoxide substrate.

Glutathione transferase; Lipid peroxidation; (*Panagrellus redivivus*)

1. INTRODUCTION

Glutathione (GSH) transferases are multifunctional proteins detected in both the animal and plant kingdoms [1]. The enzymes are part of the phase II detoxification system that catalyses the glutathione conjugation of a multitude of exogenous and endogenous toxic compounds [2]. GSH transferases may also detoxify a number of toxic ligands by acting as non-catalytic intracellular binding proteins [3].

Mammalian cytosolic GSH transferases can be divided into three multigene families α, μ and π [4] and it is not confirmed if this general classification can be extended to non-vertebrates [5,6]. GSH transferase activity can be detected in a range of helminths [6] and it may be the major detoxification enzyme [7]. There is little literature information on nematode GSH transferases.

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2. MATERIALS AND METHODS

Panagrellus redivivus was maintained in culture on autoclaved porridge oats as described previously [8]. 1-Chloro-2,4-dinitrobenzene (CDNB) was used as the standard substrate at 30°C [9]. Assays for the conjugation of other second substrates were carried out as described in the literature [6,9,10]. Protein was determined using a Coomassie blue binding assay [11]. Microsomal and cytosolic fractions were prepared as previously described [12]. GSH transferases were purified by chromatofocusing (Pharmacia LKB, Uppsala, Sweden) and glutathione-agarose affinity chromatography [13].

3. RESULTS

3.1. *Purification*

Over 99% of the GSH transferase activity in *P. redivivus* was detected in the cytosol compared to the microsomes. The cytosolic GSH transferase level in *P. redivivus* was equivalent to the activity detected in rat liver cytosol when assayed under identical conditions. Between 30 and 75% of the *P. redivivus* cytosolic GSH transferase activity did not bind directly to a glutathione-agarose matrix even after a dialysis step (fig.1). The cytosolic GSH transferase activity also failed to bind directly to a *S*-hexylglutathione-affinity matrix [14].

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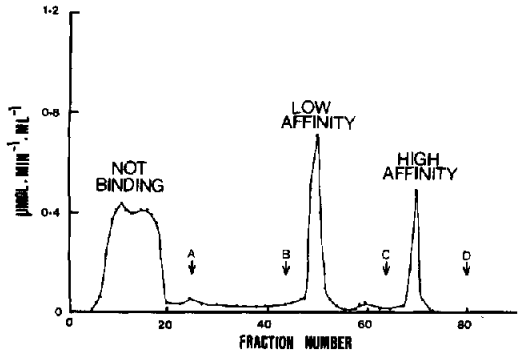

Fig.1. Glutathione-agarose chromatography of *P. redivivus* cytosolic GSH transferase activity. The technique was carried out as described previously [12]. (A) 20 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA, 0.2 mM DTT and 10% (v/v) glycerol. (B) 50 mM Tris-HCl buffer, pH 9.6, containing 1 mM EDTA, 0.2 mM DTT and 10% (v/v) glycerol. (C) Buffer B with 10 mM glutathione. (D) 3 M NaCl.

pH 7–4 chromatofocusing of the cytosolic fraction resolved three acidic forms of GSH transferase and indicated the presence of unresolved basic GSH transferase activity (fig.2). The active fractions of two major acidic forms AI and AIII from chromatofocusing were individually pooled and applied separately to a glutathione-agarose matrix. The AI form now interacted with the affinity matrix with no GSH transferase activity detectable in the flow through fractions. On refocusing the AI GSH transferase between pH 7 and 4 one peak was detected. The AIII form from pH 7–4 chromatofocusing still failed to interact with a glutathione-agarose matrix after this partial purification.

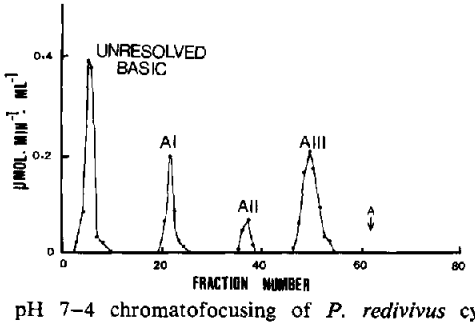

Fig.2. pH 7–4 chromatofocusing of *P. redivivus* cytosol showing GSH transferase activity. A 0.9 × 15 cm polybuffer exchanger 9–4 matrix was used at 4°C using a flow rate of 10 ml/h and a 1:8 dilution of polybuffer, pH 4.0. The activity was eluted into fractions (1.5 ml) containing 30% (v/v) glycerol and 200 mM potassium phosphate buffer, pH 7.8. (A) 1 M NaCl.

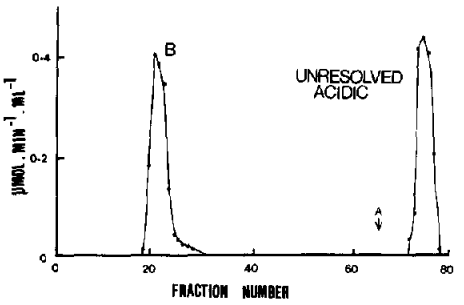

Fig.3. pH 9–6 chromatofocusing of *P. redivivus* cytosol showing GSH transferase activity. The method was carried out as described in fig.2 using a 1:8 dilution of polybuffer, pH 6.0. (A) 1 M NaCl.

On pH 9–6 chromatofocusing, a single basic cytosolic GSH transferase form was resolved (fig.3) which accounted for approximately 45% of the total cytosolic GSH transferase activity with CDNB. Following the initial chromatofocusing step the B form interacted with a glutathione-agarose matrix with 100% binding and showed a single band at 25 kDa on SDS-PAGE (results not shown).

The 'high affinity' activity described in fig.1 was found to consist only of the B GSH transferase form when analyzed by chromatofocusing. The 'low affinity' and 'not binding' GSH transferase activities in fig.1 consisted of a mixture of the GSH transferase forms. The AIII form was only present in the 'not binding' fractions.

3.2. *Biochemical characteristics*

The B and AI GSH transferase forms were

Table 1

Substrate specificity of B and AI GSH transferases of *P. redivivus* cytosol

Substrate	Activity ($nmol \cdot min^{-1} \cdot mg^{-1}$)	
	B	AI
Cumene hydroperoxide	N.D.	1120 ± 18.8 (5)
trans-2-Nonenal	N.D.	532 ± 201 (3)
trans-4-Phenyl-3-buten-2-one	N.D.	N.D.
Ethacrynic acid	400 ± 72 (5)	90 ± 22.5 (5)
1,2-Dichloronitrobenzene	N.D.	N.D.
Bromosulphophthalein	N.D.	N.D.

N.D., not detected (less than 10 $nmol \cdot min^{-1} \cdot mg^{-1}$). Activity was expressed as ± SD, with number of replicates in parentheses

Table 2

Inhibitor sensitivity of B and AI GSH transferases of *P. redivivus* cytosol

Inhibitor	$I_{50\mu M}$	
	B	AI
Cibacron blue	0.3	1.8
Haematin	0.05	1.5
Bromosulphophthalein	1.5	6.0
Triphenyltin chloride	0.05	0.6
Quercetin	18.0	45.0
Ellagic acid	8.0	no inhibition by 50 μM
Alizarin	18.0	no inhibition by 50 μM

The I_{50} values were determined graphically after using at least four concentrations of inhibitor and three independent experiments

preliminarily compared to the mammalian classification system [4] using model substrates (table 1) and model inhibitors (table 2). The AI form had significant glutathione peroxidase activity with cumene hydroperoxide indicating a relationship to the α family of mammalian GSH transferases. The B form showed no clear relationship to any one mammalian family.

Plant phenols may be natural catalytic or binding substrates of GSH transferases [15] and the standard assay of the B form was inhibited by plant phenols including alizarin, quercetin and ellagic acid (table 2).

4. DISCUSSION

The cytosol of *P. redivivus* appears to contain four forms of GSH transferase. The major form (B) was a basic enzyme with apparently limited catalytic capabilities. However, the natural catalytic substrate(s) of the B form may as yet be undetected and the interaction of the enzyme with plant phenols would appear to require further investigation. Haematin was a potent inhibitor of the B form suggesting that the nematode enzyme may have a role in the detoxification of haematin related compounds as has been suggested for mammalian GSH transferases [2].

The α family type acidic GSH transferase AI may help provide a defence against the cytotoxic products of lipid peroxidation, including lipid hydroperoxides, via their glutathione peroxidase activity, and by conjugating reactive carbonyls such as *trans*-2-nonenal. An α-like minor GSH transferase has been found in the plerocercoid stage of the cestode *Schistocephalus solidus* [12] and a GSH transferase isolated from the digenean *Schistosoma mansoni* had high lipid hydroperoxidase activity [16]. It appears that invertebrates, as has been suggested for mammals [2], may also require protection against the products of lipid peroxidation.

The endogenous interfering factor may occur in a number of other invertebrates [5] and could be a natural substrate or a regulatory protein/peptide.

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