

The Kinetics and Inhibition Properties of Partially Purified Glutathione Transferase from the Head of Palm Weevil (*Rynchophorus Phoenicis*) Larva

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Abstract: An insect cytosolic glutathione transferase (GST) was partially purified from the head of palm weevil (*Rynchophorus phoenicis*) larvae using DEAE- cellulose ion exchange chromatography. The purified GST showed conjugating activity readily with 1-chloro-2, 4 – dinitrobenzene (CDNB) and the activity was inhibited by an insecticide 2, 2-dichloroethyl dimethyl phosphate (DDVP). Palm weevil GST displayed a Michealian behavior at low (0.1-1.0 mM) substrate concentration. $K_m^{(GST)}$, V_{max} was calculated to be 0.70 mM and 40.98 $\mu\text{mol}/\text{min}/\text{mg}$ whereas K_m and $V_{max}^{(CDNB)}$ was 33.2 $\mu\text{mol}/\text{min}/\text{mg}$ and 1.55 mM respectively. GST from the head of palm weevil larva probably possesses ability to catalyse external toxic substances and may be implicated in GST-based resistance.

Keywords: Kinetics, Inhibition, glutathione transferase, *Rynchophorus phoenicis*.

1. Introduction

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) are a large family of multifunctional enzymes found ubiquitously in aerobic organisms (Clark, 1990). The majority of studies on insect GSTs have focused on their role in detoxifying xenobiotic compounds, in particular insecticides and plant allelochemicals and, more recently, their role in mediating oxidative stress responses (Fournier *et al.*, 1992; Ranson *et al.*, 2001; Vontas *et al.*, 2001). Increased GST activity has been detected in strains of insects resistant to organophosphates and organochlorines (Fournier *et al.*, 1992). and this enzyme family has recently been implicated in resistance to pyrethroid insecticides (Kostaropoulos *et al.*, 2001; Vontas *et al.*, 2001). What's more, GST-based resistance to insecticides was described to be facilitated by the increase in the level of expression of one or more GSTs.

The African black palm weevil (*Rynchophorus phoenicis*) is a species of snout beetle. At its developmental stage palm weevil larvae can excavate holes in the trunk of a palm tree up to a meter long thereby weakening and eventually killing the host plant, as a result, the weevil is considered as a major pest in palm plantations including coconut palm, date palm and oil palm. The adaptation of this insect pest to its host environment might be due to presence of GST in the insect particularly at the larva stage (Bozbuga and Hazir, 2008). This study therefore seeks to determine the kinetics and inhibition of partially purified Glutathione transferase from the head of palm weevil (*Rynchophorus phoenicis*) larva

2. Materials and Methods

Experimental insects

The Palm weevil larva used for this work was obtained from infected palm tree in Iye area of Ekiti State, Nigeria. The larvae were randomly selected for measuring the enzyme activity.

Chemicals

Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), 2,2-dichloroethyl dimethyl phosphate (DDVP), ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA) and DEAE-Sephadex were purchased from Sigma. Reduced glutathione (GSH) was used as a distilled water solution. 1-chloro-2, 4-dinitrobenzene (CDNB) solution was prepared in ethanol. Other chemicals were of the highest purity commercially available.

Dissection of larvae

The larvae were kept in the freezer to demobilize the organism in order for the dissection to be possible and easy. After freezing, the larva was removed and placed on a board laid with aluminium foil and dissected using dissecting kits – forceps, knife, e.t.c. The dissected larva was then separated into three fractions; Head, Gut and the Body Tissue, based on their respective grouping. The head was well labelled and were kept in the research refrigerator until required

Preparation of crude extract

Head of palm weevil larva was homogenised at 4°C in a ratio of 1:4 (w/v) with 100 mM potassium phosphate buffer, pH 7.2. The homogenate was centrifuged at 10,000 g for 10 min and the pellet was discarded. The supernatant was filtered through glass wool and the cytosolic glutathione S-transferase was obtained. The enzyme solution was stored in the cold at -20°C until needed. (Cohen, 1987).

Purification of GST by glutathione ion exchange chromatography

The crude extract was applied to a DEAE-Sephadex A₅₀ ion exchange column (20 x 15 cm) previously equilibrated with 20 mM phosphate buffer, pH 7.2 and the protein fraction was eluted with stepwise increase molarity of phosphate buffer pH 7.2 (0.02-0.2M). The active were pooled and stored at -20°C (Cohen, 1987).

Enzyme Assay

GST catalytic activity was determined spectrophotometrically with the aromatic substrate 1-chloro-2, 4 dinitrobenzene (CDNB) by monitoring the change

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in absorbance, due to thioether formation at 340nm and 25°C as described by (Habig et al; 1974). The assay mixture contained in a total volume of 1ml. 0.1M potassium phosphate buffer at pH 7.4, 1 mM CDNB in ethanol, 1 mM GSH and the enzyme solution. The increase of absorbance at 340nm of the complete assay reaction was monitored against a control containing buffer instead of the enzyme and treated similarly. The product extinction coefficient was taken to be 9.6mM^{-1} defined as the amount of enzyme which catalyses the formation of $1\mu\text{mol}/\text{min}/\text{mg}$ protein. Protein concentration was determined by the method of Using bovine serum albumin (BSA) as a standard. (Lowry, 1971)

Kinetic analysis

The kinetic parameters K_m and V_{max} were determined for purified palm weevil GST using reduced glutathione and CDNB as substrates. When CDNB constants were measured, the GSH concentration was held at 1mM while the CDNB concentration was varied from 0.1 to 1.0mM. When GSH constants were measured, the CDNB concentration was held at 1 mM while the GSH concentration was varied from 0.1 to 1 mM. Maximal velocity (V_{max}) and Michaelis constant (K_m) values for each substrate were calculated from Lineweaver Burk double reciprocal plot

Protein content determination

Protein contents of the enzyme homogenate were determined according to the method of using bovine serum albumin as the standard. The measurement was performed with the wavelength of 340 nm (Lowry, 1971).

3. Result

Purification of GST

Two major protein peaks were obtained in the flow through fractions while two minor peaks were observed to bind to the column after washing the column with NaCl solution. These were done by reading each fraction at the absorbance of 280 nm. Estimation of GST in all the fraction showed two major activity peaks (Fig 4).

Kinetic of palm weevil GST

The kinetic properties of partially purified GST from head of a palm weevil larva were studied. Line weaver burk plot of enzyme catalysed reactions with varying concentrations of glutathione (0.1-1 mM) at a fixed concentration of CDNB (1 mM) or glutathione (1mM) respectively as shown in fig 5a and 5b and the K_m and V_{max} were calculated as shown in table 1. $K_m^{(GSH)}$, V_{Max} was calculated to be (0.70 mM and $40.9\mu\text{mol}/\text{min}/\text{mg}$) whereas K_m and $V_{max}^{(CDNB)}$ was 1.55 mM and $33.2\mu\text{mol}/\text{min}/\text{mg}$

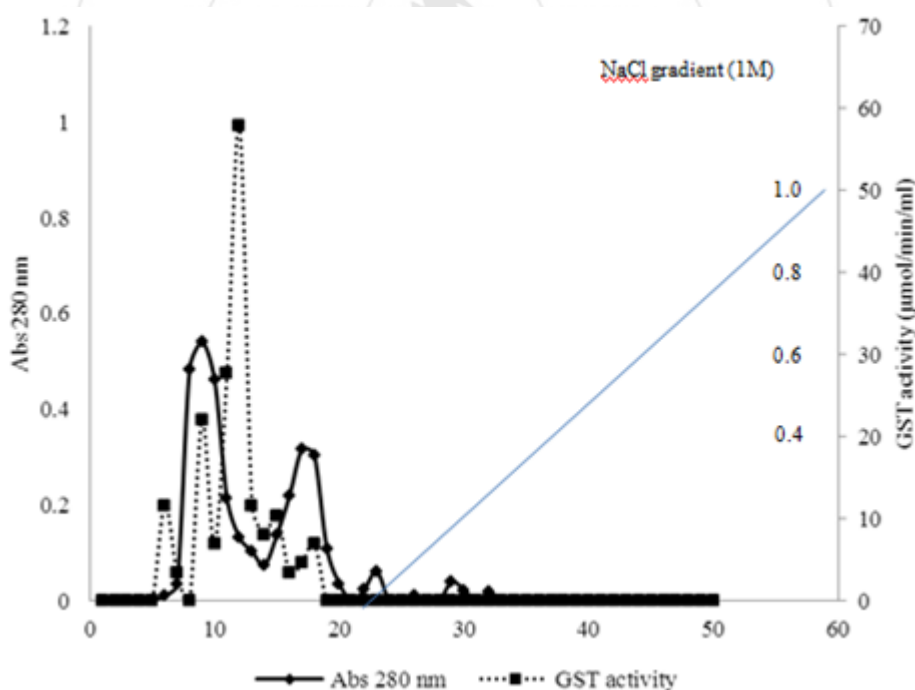


Figure 4: Elution profile of glutathione transferase from the head of palm weevil (*Ryhncophorus phoenicis*) larva using ion exchange chromatography. Crude enzyme was applied on CMC-cellulose column (1.5 x 20 cm) previously equilibrated with 20 mM phosphate buffer, pH 7.2 containing 1 mM EDTA and 1 mM mercaptoethanol. 5 ml fractions were collected at a flow rate of 10 ml/h

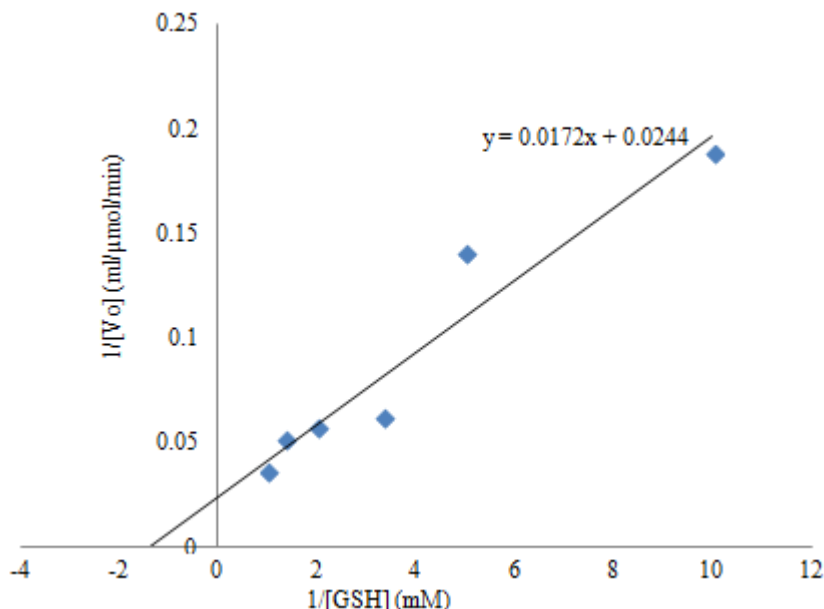


Figure 5A: Line-weaver Burk plot of partially purified glutathione transferase from the head of palm weevil.

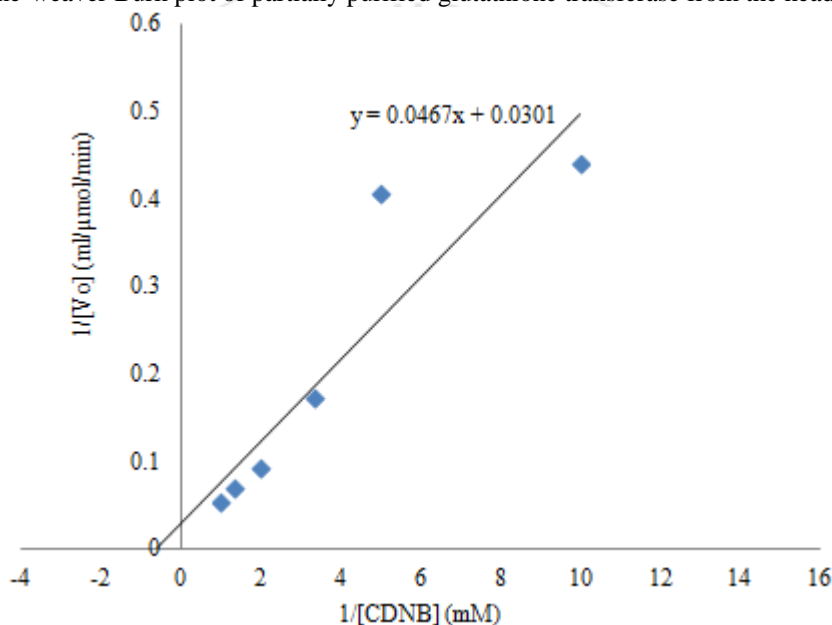


Figure 5B: Line-weaver Burk plot of partially purified glutathione transferase from the head of palm weevil.

Table 1: Kinetic parameters of partially purified GST from head of palm weevil

Substrate	K_M (mM)	V_{MAX} (μ mol/min/mg)
CDNB	1.55	33.22
GSH	0.70	40.98

Table 2: In vitro inhibition of partially purified palm weevil larva GST

Set-up (inhibition assay)	Activity (μ mol/min/ml)	Residual activity (%)
Enz + GSH+CDNB	25.23 \pm 0.14	100
Enz +GSH+DDVP	9.36 \pm 0.45	37.10
Enz +GSH+DDVP+CDNB	15.23 \pm 0.29	61.45

Inhibition Studies

The result of GST inhibition with a known insecticides 2, 2-dichloroethenyl dimethyl phosphate (DDVP) is shown in Table 2. The strongest of inhibition 37% was observed with DDVP in absence of CDNB and the weakest of 61.45% with presence of insecticides 2,2-dichloroethenyl dimethyl phosphate (DDVP) CDNB, Enzyme and GSH, The performed inhibition assay shows that the activity of palm weevil larva GST was inhibited to various extent by 2,2-dichloroethenyl dimethyl phosphate (DDVP)

4. Discussion

Analysis of Lineweaver-burk plot (Table1 and figure 5), provide the information regarding the mode of action of GST in palm weevil larva which was enzymatically characterised with CDNB and GSH as substrates. K_m and V_{max} GST from the head of palm weevil larva were comparable to values reported for other insect GST_s (Yu, 1996). the K_m and V_{max} from palm weevil larva were higher than other lepidoptoreous insect (Ranson et al., 2001). and most similar to corresponding constant reported in GST

from *Bulinus truncatus* (Yu, 1996). The apparent K_m values for glutathione and CDNB appeared to be similar, while the apparent V_{max} for CDNB was lower than that of glutathione. This suggests that GST of palm weevil larva had a higher affinity for glutathione than for CDNB.

The role of insect detoxification enzymes in the metabolism of insecticides, allelochemicals and other xenobiotics is well established (Ranson *et al.*, 2001). The table 2 shows that GST are present in palm weevil larva of concentrations comparable with those in other insects *in vitro*, and its sensitive to the inhibitory effect of DDVP. The strongest inhibition of 37.10% was observed with DDVP in the absence of substrate and 67.10% in the presence of substrate (CDNB), and this may be due to reaction of DDVP with essential residues related to GSH binding. Most GST have a cysteine residue near the active site, and modification of this residue alters the binding of GSH as been shown for some vertebrates (Stella *et al.*; 1998). It is possible that DDVP can react with GSH as it does with other thiols (Yu, 1996). to form a conjugate and the result indicated that DDVP can act as a substrate.

5. Conclusion

The GSTs in insects are primarily of interest because of their role in insecticide resistance. They are involved in the O-dealkylation or O-dearylation of organophosphorus insecticide. as a secondary mechanism in the detoxification of organophosphate metabolites and in the dehydrochlorination of organochlorines. This study presented some basic biochemical information of GSTs from palm weevil larva and surely will help in understanding the interaction of GSH with a known insecticide in the presence and absence of enzyme substrate and inhibition of partially purified GST from the head of palm weevil larva.

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