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SUBSTRATE SPECIFICITY AND INHIBITION STUDIES ON AFRICAN CATFISH (*Clarias gariepinus*) LIVER GLUTATHIONE S-TRANSFERASE

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ABSTRACT

The effect of some inhibitors of glutathione transferases was examined on a purified glutathione transferase from the liver of the African catfish (*Clarias gariepinus*). The ability of the enzyme to catalyze the conjugation of glutathione (GSH) with a variety of other compounds was also examined. The glutathione transferase (GST) from the African catfish was inhibited by all the compounds examined. Cibracron blue and triphenyltin chloride were the most potent inhibitors with I_{50} (concentration of inhibitors causing 50% inhibition) of 0.0875μM and 0.0525μM respectively. The enzyme displayed broad substrate specificity. The enzyme was able to catalyze the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid , 4-nitrobenzylchloride, p-nitrophenylacetate and hydrogen peroxide. The results obtained from this work suggest that the glutathione transferase from the liver of African catfish might possibly belong to the Pi and Mu class of isoenzymes and that the enzyme is homodimeric.

Keywords: Glutathione transferase; African catfish; *Clarias gariepinus*; substrate specificity; isoenzyme(s)

INTRODUCTION

Previous studies have indicated that fish liver contains glutathione S-transferase (GST) isoenzymes (George and Buchanan, 1990). Glutathione S-transferases are a family of enzymes involved in the initial stages of the cellular detoxification process of various endogenous and exogenous chemicals (Mannervik and Danielson, 1988). They conjugate electrophilic substrates to glutathione making the target molecule more water soluble for eventual excretion from the organism. Cytosolic GST exists as either homo- or heterodimer with a subunit molecular weight of about 25kDa (Armstrong, 1994). Based on substrate specificity, antibody cross reactivity and amino acid sequence data, cytosolic GSTs have been subdivided into seven main classes: alpha, mu, pi, sigma, theta, zeta and kappa (Cnubben *et al*, 2001). Each member of the classes has clearly different substrate specificities, indicating distinct roles of the various enzymes in detoxification reactions (Tahir *et al*, 1985). Furthermore, enzyme inhibition can be a source of insight for characterization of different isoenzymes (Tahir and Mannervik, 1986).

In view of the non-uniform tissue distribution of isoenzymes, it is important to be able to distinguish the type of transferase in previously uncharacterized sources. Isoforms of GST correlating with mammalian alpha, mu, and pi (by means of substrate affinity) are the primary isoforms expressed in fish species such as rainbow trout, brown bullhead and largemouth bass (George, 1994). Studies to examine the probable class and the dimeric structure of *C. gariepinus* hepatic GST isoform are therefore important.

MATERIALS AND METHODS

Materials

Glutathione (reduced form), 1-chloro-2,4-dinitrobenzene (CDNB), hematin, ethacrynic acid, 4-nitrobenzylchloride, p-nitrophenylacetate, Cibracron blue, were from Sigma chemicals company, St. Louis, USA. Tributyltin acetate and Triphenyltin chloride are products of Alfa, Karlerushe, Germany.All other reagents used were of the highest grade commercially available.

Enzyme Source

African catfish (*Clarias gariepinus*) hepatic GST purified to apparent homogeneity using standard method of gel permeation (Sephadex G-100) and affinity chromatography (Glutathione-agarose) was used for substrate affinity and inhibition studies. It was homogeneous on non-denaturing 10% gel in polyacrylamide gel electrophoresis (PAGE).

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Table 1: Substrate Specificity of *Clarias gariepinus* hepatic glutathione transferase

Substrate	Specific Activity (mM/min/mg)
1-chloro-2, 4-dinitrobenzene	0.49±0.02
Ethacrynic acid	0.20±0.03
Hydrogen peroxide	0.14±0.03
p-nitrophenylacetate	0.07±0.01
4-nitrobenzylchloride	0.34±0.07

Values are mean ± S. D generally based on n≥4.

Table 2: Inhibition effects of some inhibitors on GSH-CDNB conjugation of the *C.gariepinus* glutathione transferase.

Inhibitors	I_{50} (μM)
Ethacrynic acid	0.65
Cibracron blue	0.0875
Hematin	0.575
Tributyltin acetate	0.58
Triphenyltin chloride	0.0525

Substrate Specificities

The specific activities were determined by measuring the initial rates of enzyme catalyzed conjugation of GSH with CDNB, ethacrynic acid, hydrogen peroxide, p-nitrophenylacetate and 4-nitrobenzylchloride spectrohotometrically essentially as described by Habig and Jakoby (1981), Mannervik & Widerstern (1995) and Ajele & Afolayan (1992) using Biochrome 4060 spectrophotometer at 28°C. The specific activity of GST was defined as 1mM product formed per minute per milligram protein.

Inhibition Studies

Stock solutions of cibracron blue, hematin, triphenyltin chloride, ethacrynic acid and tributyltin acetate were prepared as described by Tahir *et al* (1985). Their inhibitory effects on the activity of the enzyme (*C. gariepinus* hepatic GST) was measured by preincubating inhibitors for 2 mins with 1mM GSH and 1mM CDNB in 0.1M potassium phosphate buffer of pH6.5 (their final concentration in 3ml cuvette) and initiating the reaction by addition of enzyme at 28°C. The concentration of inhibitor giving 50% inhibition (I_{50}) was determined from the plot of residual activity against the inhibitor concentration. The concentration of inhibitors used was in the range of 0.001μM to 10μM.

RESULTS

Substrate Specificities

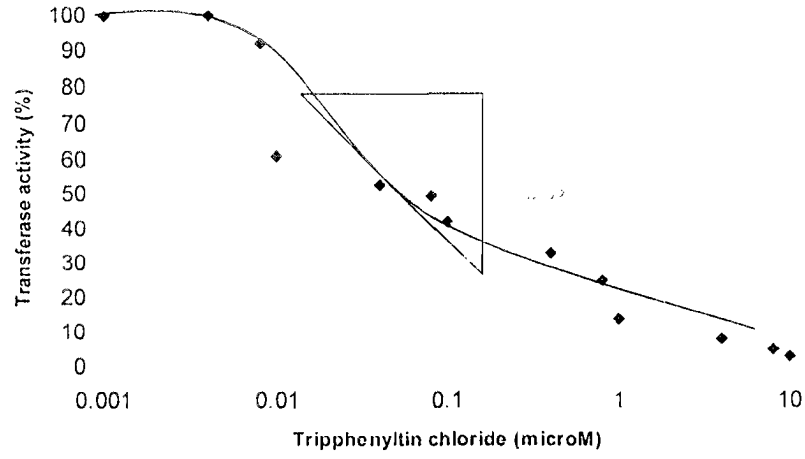
The specific activities for the various substrates used are presented in Table 1. The enzyme is quite efficient in catalyzing the conjugation of GSH to CDNB (a general substrate for GST). The enzyme was less efficient in GSH-conjugating activity towards 4-nitrobenzylchloride and ethacrynic acid. The enzyme was fairly active towards peroxides; exhibiting a peroxidase activity of 0.140 ± 0.03 mM/min/mg. The enzyme has a relatively low thiolytic activity with p-nitrophenylacetate with specific activity of 0.07 ± 0.01 mM/min/mg

Inhibition Effect

The I_{50} values of various inhibitors - ethacrynic acid, cibracron blue, hematin, triphenyltin chloride and tributyltin acetate -for the GSH-CDNB conjugating activities are presented in Table 2. The most potent inhibitors were cibracron blue and triphenyltin chloride with I_{50} values of 0.0875μM and 0.0525μM respectively. A plot of the fractional velocity against the concentration of inhibitors (triphenyltin chloride) is shown in Fig 1. The slope of the curve at 50% inhibition was -0.59

DISCUSSION

1-chloro-2,4-dinitrobenzene (CDNB) has previously been shown to react with a broad spectrum of GST isoforms with the exception of the theta class (George, 1994). *C.gariepinus* hepatic GST is significantly active with CDNB, the substrate most often used in the assay of GST. The activity of the enzyme for CDNB was significantly higher than those of Plaice (George and Buchanan, 1990) and rainbow trout (Nimmo *et al*, 1979) but close to human Mu class CDNB activity (Mannervik and Widerstern, 1995). Among the substrates used, the relatively high activity displayed with CDNB and ethacrynic acid is in agreement with the result obtained for catfish intestinal mucosa (James *et al*, 1998) and brown trout (*Salmo trutta)* GSTs (Egaas *et al*, 1998) and channel catfish intestine GST (Gadagbui and James, 2000). Ethacrynic acid reacts primarily with the Pi class (George, 1994). This indicates that catfish contain the Pi isoform. The fact that *C.gariepinus* liver GST displayed selenium-



Semi-log graph of the inhibition studies on Triphenyltin chloride

Fig 1. Inhibition of *Clarias gariepinus* glutathione transferases with triphenyltinchloride. Fractional velocity was determined by comparison with enzyme in the absence of inhibitor. Assays were made at pH6.5 with 1mM 1-chloro-2,4-dinitrobenzene and 1mM glutathione as substrates.

independent activity toward hydrogen peroxide, ethacrynic acid, 4-nitrobenzylchloride suggests that this enzyme is quite distinct from Plaice GST which was earlier reported to show no detectable peroxidase activity and very low activity towards etnacrynic acid and 4-nitrobenzylchloride (George & Buchanan, 1990). The high peroxidase activity is an indication that it is related to rat liver mu class GST (Mannervik, 1985).

Inhibition of the various isoenzymes by inhibitors has been employed in distinguishing various isoenzymes from humans, mouse and rat (Tahir and Mannervik, 1986). For instance, Tahir *et al* (1985) found that cibracron blue was most effective on the neutral isoenzyme from human liver whereas tributytin acetate was the most potent inhibitor of the basic human transferase. The I_{50} values obtained in this study suggest that glutathione transferase from *C.gariepinus* might be a near neutral isoenzyme. Hematin is a less effective inhibitor of *C. gariepinus*. The value of triphenyltin chloride I_{50} is also instructive, since organotin compounds are used as antifoulants on ships this sensitivity would pose toxicity problem for *C. gariepinus*. A value of -0.59 as the slope at 50% inhibition for the plot of fractional velocity versus inhibitor concentration suggests that the enzyme might be homodimeric. This is in line with the result of Tahir and Mannervik (1986).

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