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Xenobiotic Metabolism by Glutathione S-transferase in Gill of Fish from Arabian Gulf

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Fish and other marine organisms inhabiting polluted aquatic environment may be exposed to a variety of foreign chemicals which undergo reduced glutathione (GSH)-mediated detoxication catalyzed by glutathione *S*-transferase (GST, E.C. 2.5.1.18) (Wallace 1989, Lauren et al. 1989). These cytotoxic electrophilic compounds include chlorinated and nitroaromatics, arene and alkene oxides present as such in the environment or generated as intermediates during oxidative metabolism of xenobiotics by cytochrome P-450-dependent mixed function oxidases (MFO) in the body (Gelboin 1980, Lauren et al. 1989). The xenobiotic metabolizing enzymes have been used as biomarkers for chemical pollution and chemical-induced cytotoxicity including carcinogenesis in mammals and aquatic communities (Hendrich and Pitot 1987, Stegeman and Lech 1991).

Several studies have been undertaken in the recent past to investigate the nature and function of hepatic GST in rainbow trout, *Salmo gairdneri/ Oncorhynchus mykiss* (Ramage and Nimmo 1984, Lauren et al. 1989); sturgeon, *Acipenser baeri* (Perdu-Durand and Cravedi 1989); channel catfish, *Ictalurus punctatus* (Gallagher and Di Giulio 1992) and other fish found in mostly temperate waters. These studies have demonstrated the localization of 80-90% GST activity in the cytosolic fraction of gills, however, no attempt has been made to study the properties of the enzyme in these species. Moreover, little information is available regarding the status of xenobiotic metabolism in the species inhabiting tropical and subtropical marine environment and in particular the Arabian Gulf

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region, where fishes have been reported to exhibit many biological aspects different from those observed for temperate counterpart (Al-Ghais 1993).

The purpose of the present investigation was to evaluate the xenobiotic metabolizing ability of gill GST in marine fishes namely *Scolopsis bimaculatus, Lethrinus mahsenoides* and *Lutjanus fulviflamma* inhabiting the Arabian Gulf along UAE coast. Attempts have been made to characterize Gill GST from *Scolopsis bimaculatus* in detail by determining the enzyme kinetics using 1-chloro-2,4-dinitrobenzene as substrate and the factors influencing glutathione conjugation reaction.

MATERIALS AND METHODS

Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB) and bovine serum albumin were procured from Sigma Chemical Company, St. Louis, USA. All other chemicals were of analytical grade and obtained from commercial sources.

Three commercially important fish species namely *Scolopsis bimaculatus* (Nemipteridae, Butterfly bream), *Lethrinus mahsenoides* (Lethrinidae, Emperors) and *Lutjanus fulviflamma* (Lutjanidae, Snappers) were captured with the help of a trap from Arabian Gulf along UAE coast during the period November 1993 to January 1994. Samples were placed in ice boxes while they were still alive and transferred to laboratory for study.

Gills were excised and washed thoroughly with ice-cold 1.15% KCl buffered with 0.01 M Tris-HCl, pH 7.4. Gill filaments were trimmed from the gill arches of each fish separately and pooled, and the arches were discarded. Filaments were cut into small pieces with a scissor and homogenized in chilled buffered KCl referred above in a Potter-Elvehjem homogenizer having teeth bearing teflon pestle. Cytosolic fraction was prepared as described previously (Khanna et al. 1992). The activity of GST in gill cytosol or whole homogenate was determined spectrophotometrically by following the formation of GSH conjugate with CDNB at 340 nm using an extinction coefficient of 9.6 /mM /cm (Siddiqui et al. 1993). The reaction mixture (3ml) containing 0.1 M acetate buffer, pH 6.5, 2 mM GSH, 2 mM CDNB (in 0.05 ml ethanol) and suitable aliquot of gill homogenate or cytosol was incubated for 5 min at 28 °C. Under the assay conditions the rate of reaction was linear with respect to incubation time and amount of protein which was

determined using bovine serum albumin as standard (Lowry 1951). The enzyme activity was expressed as specific activity (nmole GSH-CDNB conjugate formed/min/mg cytosolic protein) or total activity (nmole conjugate formed/min/g tissue) calculated from homogenate activity.

RESULTS AND DISCUSSION

In order to characterize GST activity in gill cytosol from *Scolopsis bimaculatus,* a fish species exhibiting highest activity among the species examined in this study, pH and temperature dependence, incubation time and protein linearity, Michaelis-Menten constant (Km) and maximum velocity (Vmax) of glutathione conjugation with CDNB, a widely used nonspecific reference substrate for all forms of fish liver GST (Ramage and Nimmo 1984), was investigated.

There was an increase in GST activity with rise in pH from 5.5 to 6.5 and a fall thereafter (Fig. 1A). Hence, the enzyme was routinely assayed at optimum pH 6.5. The pH-activity profile of gill GST observed in *Scolopsis bimaculatus* differs from that reported in other species. An increase in the rate of glutathione conjugation with CDNB was reported between pH 6.0 and 8.5 in rainbow trout liver cytosol, however, the enzyme activity was routinely determined at pH 6.5 to exclude appreciable nonenzymatic activity observed above pH 7.0 (Nimmo et al. 1979, Ramage and Nimmo 1984). Similarly, the pH optima for gill cytosolic GST activity in channel catfish was found to range between 7.5 and 8.5 (Gallagher et al 1991, Gallagher and DiGiulio 1992). The hepatic GST activity in liver cytosol towards CDNB in sturgeon was independent of pH change from 6.5 to 7.5 (Perdu-Durand and Cravedi 1989). Temperature-dependence studies demonstrated an increase in gill GST activity up to 30 °C reaching a plateau between 30 and 40°C and a fall thereafter (Fig. 1B). As in all experiments corrections were made for non-enzymatic increase in absorbance noted with temperature rise. In accordance with our results, the temperature optimum for hepatic GST activity against CDNB in sturgeon was found to be 35-45 °C after which the enzyme activity declined Perdu-Durand and Cravedi 1989). However, the rate of glutathione conjugation was linear with temperature from 15 to 37 °C in rainbow trout liver cytosol both with CDNB (Nimmo et al. 1979) and *cis* -stilbene oxide (Lauren et al. 1989) as substrates which was comparable to the pattern observed in rat liver cytosol (Nimmo et

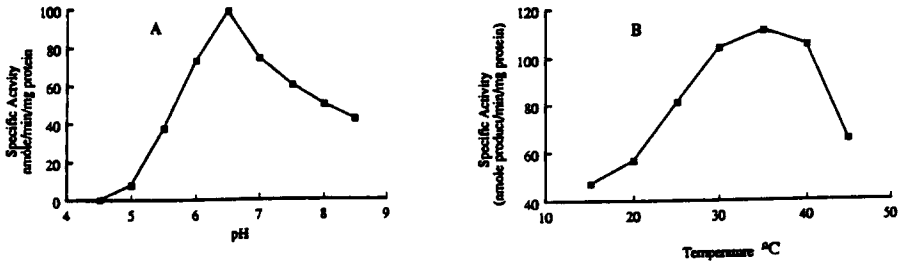

Figure 1. Glutathione *S*-transferase activity in gill cytosol from Scolopsis *bimaculatus* . Values are the mean of 3-4 fish investigated separately.
(A) Effect of pH. Acetate and Tris-HCl buffers (0.1M) were used for the pH range of 4.5 to 6.5 and 7 to 8.5 respectively.
(B) Effect of temperature.

al. 1979). As is evident from Figure 2A and B the rate of glutathione conjugation with CDNB in gills of *Scolopsis bimaculatus* was linear up to 100 μg cytosolic protein and 20 min at both 28 and 40 °C. Earlier studies have also found that xenobiotic metabolizing fish transferases are more resistant to higher temperatures than MFO (Bend and James 1978, Perdu-Durand and Cravedi 1989). Remarkable thermal stability of gill cytosolic GST recorded up to 20 min in *Scolopsis bimaculatus* discrimates it from other forms of enzyme studied in the cytosolic fraction of liver and gill of channel catfish (Gallagher et al 1991, Gallagher and DiGiulio 1992), liver of rainbow trout (Nimmo et al. 1979, Ramage and Nimmo 1984) and liver of sturgeon and rat (Perdu-Durand and Cravedi 1989) where the enzyme activity was linear up to 2-5 min at 25-30 °C. It is likely that comparatively greater stability of gill GST at high temperatures for prolong period is an expression of adaptation potential of marine fishes of tropical origin.

Kinetic constants of the gill GST for GSH and CDNB were determined by Lineweaver-Burk plot (1934). Apparent Michaelis constant (Km) derived by varying the concentration of one substrate at a fixed concentration of the other were found to be 0.71 and 0.80 mM for GSH and CDNB respectively (Fig. 3 and 4). The Km values of rainbow trout liver GST determined with cytosolic fraction as the enzyme source, for GSH and CDNB have been reported to be 0.4 and 1.0 mM respectively (Nimmo et al. 1979). Further studies undertaken to partially purify and characterize the cytoplasmic GST in trout liver have demonstrated the presence of 6-7 dimeric forms (monomer M.W. 21,700-24,400) exhibiting Km

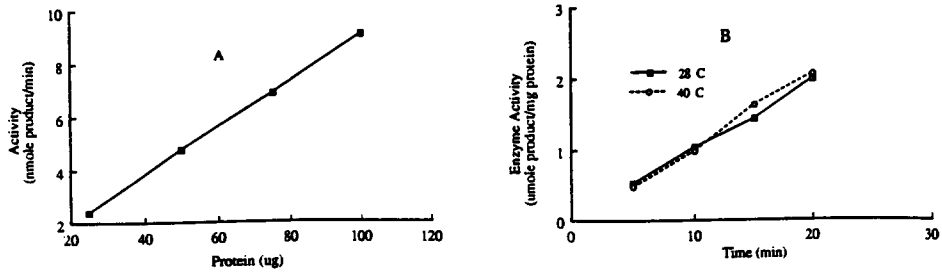

Figure 2. Gill GST activity in *Scolopsis bimaculatus* as a function of (A) enzyme protein and (B) incubation time at 28 and 40°C Values are the mean of 3-4 fish investigated separately.

Table1.Glutathione *S*-transferase activity in the gill of marine fishes from Arabian Gulf

Fish species	Specific activity nmole/min/mg protei	Total activity nmole/min/g tissue
Scolopsis bimaculatus	91.9 ± 5.2	984 ± 38
Lethrinus mahsenoides	30.3 ± 0.9	238 ± 11
Lutjanus fulviflamma	26.9 ± 1.2	93 ± 8

values are mean of 5-6 fish investigated separately (n=5-6) per group.

values for GSH and CDNB in the range of 0.2-0.3 and 0.4-0.5 mM respectively (Ramage and Nimmo 1984). This suggests that the gill enzyme has relatively less affinity towards the substrates used in the current study. Substrate specificity studies in trout have shown that gill GST, unlike liver cytosolic GST, is active against only CDNB but not *cis*-stilbene oxide indicating its inability to metabolize arene oxides (Lauren et al. 1989). Under the optimal assay conditions the specific activity and total activity of GST/g wet weight of gills were determined in *Scolopsis bimaculatus* , *Lethrinus mahsenoides* and *Lutjanus fulviflamma* (Table 1). There was marked species variation in GSH-mediated xenobiotic metabolism in the marine species studied.

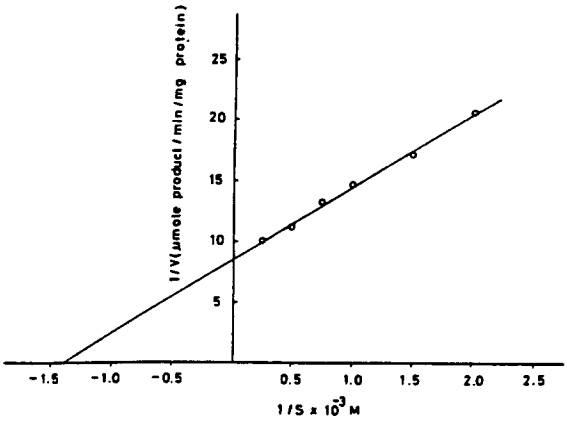

Figure 3.Lineweaver-Burk plot showing the effect of glutathione concentration on GST activity. Each point represents the mean of 3 *Scolopsis bimaculatus* .

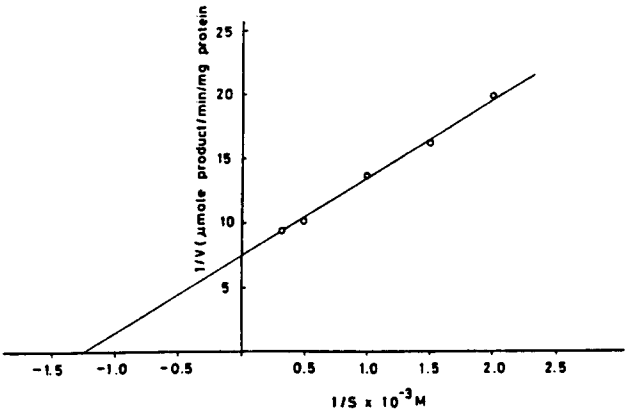

Figure 4. Lineweaver-Burk plot showing the effect of CDNB concentration on GST activity. Each point represents the mean of 3 fish.

The specific activity of GST against CDNB (9.19 nmole/min/mg cytosolic protein) registered in *Scolopsis bimaculatus* was comparable to 70-160 nmole and 84 nmole/min/mg protein reported in the gill cytoplasm of rainbow trout (Nimmo et al. 1979, Lauren et al. 1989) and sturgeon (Perdu-Durand and Cravedi 1989) respectively, but 7-8 fold less than that noted in channel catfish (Gallagher et al. 1991, Gallagher and DiGiulio 1992). Interestingly, *Lethrinus mahsenoides* and *Lutjanus fulviflamma* gills had low levels of GST activity. In case of *Lutjanus fulviflamma* this difference was more pronounced when the total activity/g tissue was compared. No apparent explanation can be offered for this finding, it may be due to some differences in the structural configuration and composition of gill filaments in *Lutjanus fulviflamma* from that present in other two species and consequently the extent of cell disintegration during homogenization of tissue.

In summary this study suggests that the gills of marine fishes inhabiting tropical water of Arabian Gulf play an important role in GSH-mediated detoxication of waterborn chemical pollutants. Comparatively higher thermal stability of gill GST observed in these species may be an expression of metabolic adaptation to warmer climate. Considerable difference in the catalytic activity of GST towards CDNB in the gill of three species investigated suggests marked species variation in the branchial GSH-mediated detoxication of xenobiotics.

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