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Species Variation and Some Properties of Renal Glutathione *S*-Transferase of Fish from Arabian Gulf

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The aquatic environment is a sink for a wide variety of inorganic and organic chemical contaminants or xenobiotics which often have detrimental impact on marine life. As in mammals, the major organ involved in the metabolism of lipophilic compounds to polar and easily excretable products in fish is liver, where the first step is often catalyzed by microsomal cytochrome p-450-dependent monooxygenases and the subsequent conjugation reactions mainly by microsomal UDP-glucuronyltransferases and cytosolic glutathione *S*-transferases (GST) (Short et al. 1988; Perdu-Durand and Cravedi 1989; Stegeman and Lech 1991). These enzymes have also been employed as biomarkers for the monitoring of environmental pollution and associated toxic manifestations in mammals and marine organisms including fish (Stegeman and Lech 1991; Martinez-Lara et al. 1996; Otto et al. 1996).

Available literature on the status of GSH-mediated detoxification of electrophilic xenobiotics in aquatic organisms mainly describes the role and characteristics of hepatic GST in a variety of fresh and salt water fish occurring in mostly temperate climates (Ramage and Nimmo 1984; Pesonen and Andersson 1987; Lauren et al. 1989; Perdu-Durand and Cravedi 1989; Gallagher and Di Giulio 1992). These studies have also reported the presence of about 80% of the whole homogenate GST activity in the kidney and gill cytosol, but its nature and function was not investigated. Since the gill and kidney are the organs to be exposed to xenobiotic stress and excrete waste products, these tissues are of importance in the detoxification and elimination of aquatic contaminants. There is a gap in our knowledge about the xenobiotic metabolism in marine fish native to tropical and subtropical Arabian Gulf region, where fish have been reported to exhibit many biological aspects different from those observed in fish occurring in temperate waters (Al-Ghais 1993).

Recently, it has been shown that there is substantial gill cytosolic GST activity in the fish from Arabian Gulf along the UAE coast, but its properties and kinetics differ from those of hepatic forms reported in other species (Al- Ghais and Ali 1995). Moreover, the enzyme activity investigated in three commercially important fish, *Scolopsis bimaculatus, Lethrinus mahsenoides* and *Lutjanus fulviflamma* exhibited marked species variations. The present study was designed to examine the properties of renal GST in the marine fish referred above along with *Lutjanus russellii.* The xenobiotic metabolizing capacity of kidney was also compared with that of liver in these species.

MATERIALS AND METHODS

1-chloro-2,4dinitrobenzene (CDNB), reduced glutathione (GSH), bovine serum albumin and tromethamine (Tris) were obtained from Sigma Chemicals Co., St. Louis, MO, USA. All other chemicals were of analytical grade.

Four commercially important fish species namely *S. bimaculatus* (Nemipteridae, Butterfly Bream), *L. mahsenoides* (Lethrinidae, Emperors), *L. fulviflamma* and *L. russellii* (Lutjanidae, Snappers) were captured with the help of a trap from southern Arabian Gulf along the UAE coast. Samples were immediately placed in ice boxes while they were still alive and transported to the laboratory for study.

Liver and kidney from each fish were dissected out, washed with ice-cold 1.15% KCl buffered with 0.01M Tris-HCl, pH 7.4 and processed separately. Tissues were homogenized in chilled buffered KCl in a Potter-Elvehjem homogenizer having teflon pestle and kidney cytosolic fraction was prepared as described previously (Khanna et al. 1992). The activity of GST in kidney cytosol and whole homogenate of kidney and liver was determined spectrophotometrically by following the formation of GSH-CDNB conjugate at 340 mn (Siddiqui et at. 1993). The reaction mixture (3 ml), unless otherwise stated, containing 0.1 M acetate buffer, pH 6.5, 2 mM GSH, 2 mM CDNB (in 0.05 ml methanol) and suitable amount of tissue preparation was incubated for 5 min at 28°C. Non-enzymatic increase in the absorbance at 340 mn was corrected by running the parallel controls in all the experiments. The rate of increase was linear with respect to time and protein under the experimental conditions. Protein was measured using bovine serum albumin as standard (Lowry et al. 1951). The enzyme activity was expressed as specific activity (mnole GSH-CDNB conjugate formed/min/mg renal cytosolic protein) or total activity (mnole conjugate formed/min/g wet weight of kidney or liver) calculated from the homogenate activity.

RESULTS AND DISCUSSION

Characterization of renal cytosolic GST activity in *S. bimaculatus*, a species possessing highest activity among the marine fish examined, was based on the determination of its pH and temperature optima, protein and time linearity and Michaelis-Menten constant (Km) and maximum velocity (Vmax). The present study also evaluated the comparative status of GST-mediated xenobiotic metabolism in the kidney and liver of *S. bimaculatus, L. mahsenoides, L. fulviflamma* and *L. russellii.*

Many characteristics of *S. bimaculatus* kidney GST have been found to be similar to those of gill enzyme reported earlier (Al- Ghais and Ah 1995), but different from those of liver, kidney and gill GST present in other species. The pH-activity profile of *S. bimaculatus* renal GST shows an increase in activity with rise in pH from 4.5 to 6.5 and decline thereafter (Fig. 1A).The pH optimum of 6.5 for both kidney and gill GST observed in the Arabian Gulf species does not concur with the values documented for other fish species. The enzyme activity towards CDNB in fish tissues including kidney exhibited an increasing trend with a rise in pH from 6.0 to 8.5 in rainbow trout

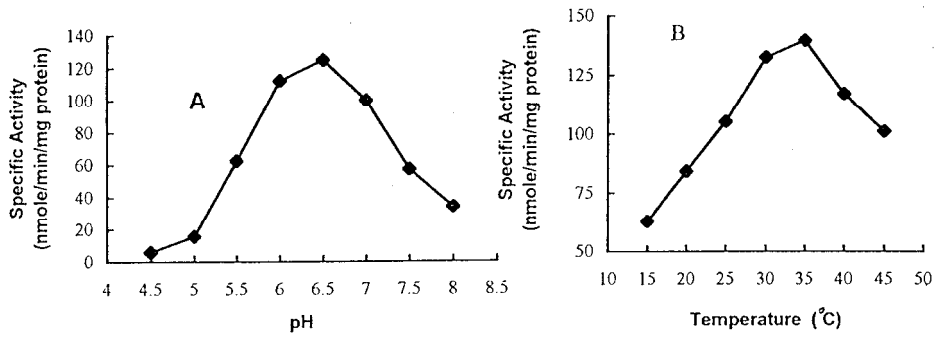

Figure 1. Glutathione *S*-transferase activity in *S. bimaculatus* kidney. Points represent the mean values from 3 fish investigated separately. (A) Effect of pH. Acetate and Tris-HCl buffers (0.1 M) were used for the pH range of 4.5 to 6.5 and 7.0 to 8.5, respectively. (B) Effect of temperature.

(Nimmo et al. 1979; Nimmo and Spalding 1985; Lauren et al. 1989), whereas an optimum between pH 6.5 and 7.5 in sturgeon (Perdu-Durand and Cravedi 1989) and 7.0 and 8.0 in channel catfish (Gallagher and Di Giulio 1992). The rate of GSH conjugation with chlorothalonil, a fungicide, in liver and gill cytosol from channel catfish reaches maximum at pH 8.0 and 8.5 - 9.0, respectively (Gallagher et al. 1991).

Temperature optima for *in vitro* metabolism of xenobiotics in fish have been found to vary considerably with the type of enzyme/isoenzyme, substrate, species and temperature of acclimatization. Several studies on the hepatic xenobiotic metabolism in fish have demonstrated that, in general, Phase I reactions catalyzed by cytochrome P-450-dependent monooxygenases achieve maximal rate at temperatures lower than 30 °C, whereas Phase II conjugation reactions represented by GST, UDP-glucuronyltransferase and acetyltransferase display peak activities at temperatures higher than 35 °C (Pohl et al. 1974; Pesonen and Andersson 1987; Short et al. 1988; Lauren et al. 1989). However, in many of these studies enzyme assays were performed at temperatures close to acclimatization temperature of fish. Temperature optima of 30 - 35 °C for GSH-mediated conjugation reaction recorded in this study (Fig. 1B) distinguishes *S. bimaculatus* renal enzyme from hepatic cytosolic GST which exhibited a linear increase in activity with temperature rise from 15 to 37 °C in rainbow trout (Nimmo et al. 1979; Lauren et al. 1989) and peak activity at 35-45 °C in sturgeon (Perdu-Durand and Cravedi 1989). The enzyme activity was linear up to 120 µg cytosolic protein (Fig. 2A). Marked thermal stability of renal GST observed up to 15 min at incubation temperatures of 25 and 35 °C (Fig. 2B) further differentiates it from other cytosolic forms of enzyme studied in liver and gill of channel catfish (Gallagher et al. 1991; Gallagher and Di Giulio 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. Comparatively greater stability of renal GST at higher temperatures

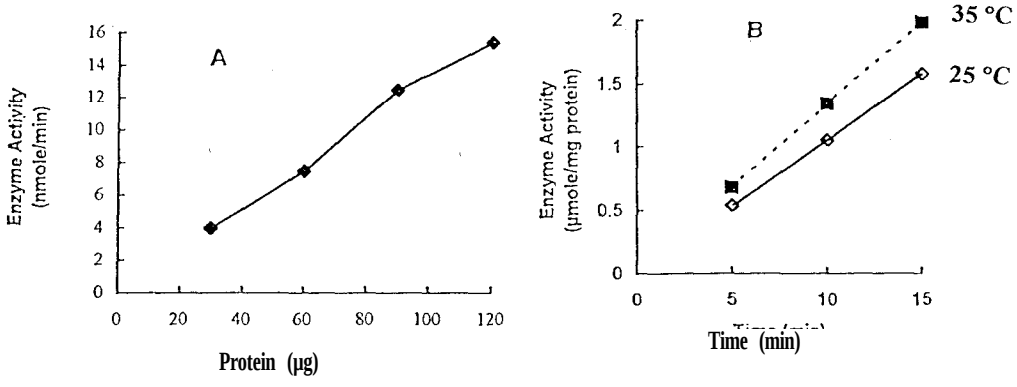

Figure 2. Renal cytosolic GST activity in *S. bimaculatus* as a function of (A) protein and (B) incubation time at 25 and 35 °C. Points represent the mean values obtained from 4 individual fish.

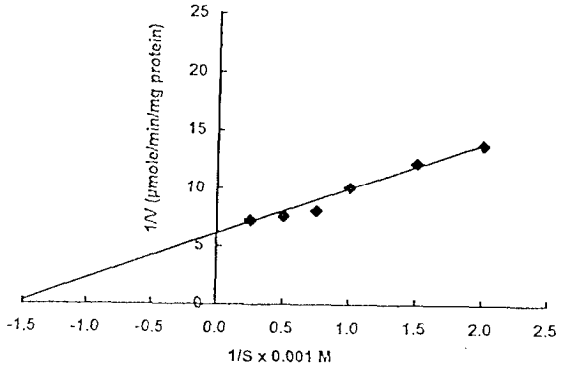

Figure 3. Lineweaver-Burk plot showing the effect of glutathione concentration on GST activity. Points represent the mean of values from 3 *S. bimaculatus* examined individually.

for prolonged period, as reported in the gill of *S. bimaculatus* (Al-Ghais and Ali 1995) may be the characteristic feature of these tissue enzymes in marine fish of tropical origin and related to the mechanism of adaptation.

Substrate saturation kinetics of renal cytosolic GST was investigated by Lineweaver-Burk plot (1934), according to which the binding affinities of the enzyme (apparent Km values) for GSH and CDNB were 0.66 and 1.1 mM, respectively (Fig. 3 and 4). Much less attention has been focused on the characterization of multiple forms and kinetic constants of fish GST as compared to cytochrome P-450-dependent

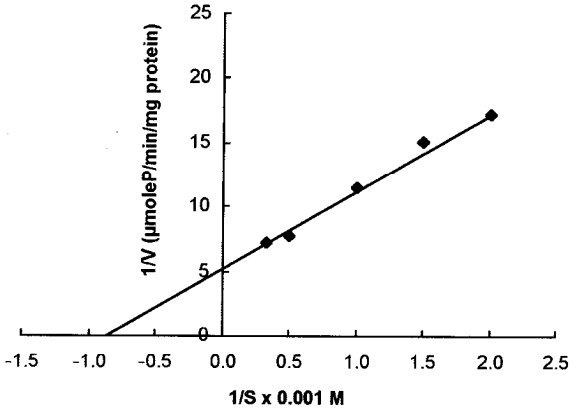

Figure 4. Lineweaver-Burk plot depicting the effect of CDNB concentration on GST activity. Points represent the mean of values from 3 *Scolopsis bimaculatus* examined individually.

monooxygenases. The GSH affinity chromatography resolved the rainbow trout kidney GST into two groups of activity having different kinetic constants (Nimmo and Spalding 1985). The major group of activity resembled the major cationic form from rainbow trout liver GST in terms of Km for GSH (0.4 and 0.2 mM, respectively) and subunit molecular weight of dimeric isozymic forms (22,900 and 22,400, respectively), but differed from the latter in terms of Km values for CDNB (4.5 and 0.4 mM, respectively) and selective substrate specificity towards CDNB, as there was no detectable activity with any of the other potential GST substrates examined namely 1,2-epoxy-3-(p-nitrophenoxy) propane, ethacrynic acid, p-nitrobenzylchloride or p-nitrophenyl acetate (Nimmo and Spalding 1985; Lauren et al. 1989). The Km values of minor group of kidney GST activity for GSH (3.0 mM) and CDNB (5.1 mM) were closer to the respective Km values of 1.9 and >1.0 mM recorded for rainbow trout gill enzyme (Nimmo and Spalding 1985). However, the Km values of rainbow trout liver GST determined with cytosolic fraction have been reported to be 0.4 and 1.0 mM for GSH and CDNB, respectively (Nimmo et al. 1979). Wallace (1989) has reported higher Km values of hepatic cytosolic GST for GSH and CDNB in fathead minnows (0.80 and 6.15 mM, respectively) than in rainbow trout (0.49 and 0.43 mM, respectively). The present study indicates a similarity between the Km values of kidney (0.66 and 1.1 mM, for GSH and CDNB, respectively) and gill (0.71 and 0.80 mM, respectively) enzymes of *S. bimaculatus.* The specific activity of renal cytosolic GST in *S. bimaculatus* was 134 mnole GSH-CDNB conjugate formed/min/mg protein which was 2.4-6.2 fold higher than that noted in *L. mahsenoides, L. fulviflamma* and *L. russellii,* indicating species variation in the renal metabolism of xenobiotics (Table 1). The GST activity in *S. bimaculatus* is comparable to 122 nmole/min/mg protein reported in rainbow trout kidney cytoplasm with CDNB (Lauren et al. 1989), but approximately 2, 3 and 5 fold lower than that found in kidney cytoplasm from brown bullheads (Otto and Moon 1996), sturgeon (Perdu-Durand and Cravedi 1989) and channel cattish (Gallagher and Di Giulio 1992), respectively, against CDNB.

Table 1. Glutathione *S*-transferase activity in tissues of marine fish from Arabian Gulf

Fish species	Specific activity nmole /min/mg protein	Total activity nmole /min/g wet tissue	
	Kidney	Kidney	Liver
Scolopsis bimaculatus,	134.7 ± 6.6	4997 ± 146	16562 ± 1354
Lethrinus mahsenoides	56.2 ± 3.3	2262 ± 130	5938 ± 647
Lutjanus fulviflamma	44.8 ± 2.3	1055 ± 85	3432 ± 344
Lutjanus russellii	21.7 ± 1.7	811 ± 52	1201 ± 100

Values are the mean ± SE of fish (n=5-7) per group investigated separately.

Comparative assessment of GST activity in kidney and liver homogenates (Table 1) showed 1.5-3.5 fold lower renal activity in the marine species investigated, which is consistent with the distribution pattern observed in rainbow trout (Lauren et al. 1989), sturgeon (Perdu-Durand and Cravedi 1989) and channel catfish (Gallagher and Di Giulio 1992). However, the enzyme activity in kidney was significantly higher than that reported in the gill of *S. bimaculatus, L. mahsenoides, L. .fulviflamma* (Al- Ghais and Ali 1995).

In conclusion, this study shows that in addition to liver and gill, kidney also plays an important role in GST-mediated metabolism of xenobiotics in *S. bimaculatus, L. mahsenoides, L. fulviflamma* and *L. russellii.* There was species dependent variation in the levels of GST in the tissues examined, however, the relative xenobiotic metabolizing efficiency of these species was in the same order when examined in different tissues. Comparatively greater resistance exhibited by renal GST to thermal inactivation observed in this study may be an expression of metabolic adaptation to warmer climate. Considerably lower catalytic activity of GST in *L. mahsenoides, L. fulvifamma* and *L. russellii* is indicative of the possibility that these species may be at disadvantage when exposed to deleterious environmental pollutants. This thesis gets support from a recent study where the deficiency of constitutive hepatic GST has been implicated in pollution-associated liver neoplasms in white suckers (*Catostomus commersoni*) (Stalker et al. 1994). Similar suggestions were made in another study where English sole, the species showing higher prevalence of contaminant-associated hepatic neoplasms, had l-2 fold higher hepatic activities of aromatic hydrocarbon hydroxylase, an enzyme responsible for bioactivation of carcinogens, and 0.8 and 1.8 fold lower activities of epoxide hydrolase and GST, respectively, than those of starry flounder, the species living in the same area but exhibiting considerably lower incidence of neoplastic and preneoplastic liver lesions (Collier et al. 1992).

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