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ScienceDirect
Ecotoxicology and Environmental Safety 69 (2008) 58–63
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Characterization of cytosolic glutathione *S*-transferases in striped bass (*Morone saxitilis*)

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Received 23 August 2006; received in revised form 4 February 2007; accepted 7 February 2007
Available online 28 March 2007

Abstract

Electrophilic compounds are ubiquitous in the environment and aquatic life is inevitably affected. Glutathione *S*-transferases (GSTs) are a class of enzymes that facilitate the detoxification of these electrophiles in phase II metabolism. In this study, cytosolic GSTs were isolated and characterized from striped bass liver (*Morone saxitilis*). Nanospray liquid chromatography–tandem mass spectrometry (LC–MS/MS) was used to elucidate peptide sequences, and the proteins were found to have homology to ρ and α by searching against the NCBI non-redundant database (nrDB). Catalytic activities of the cytosolic GSTs towards 1-chloro-2,4-dinitrobenzene (CDNB) were determined to be 141 ± 34 and 155 ± 65 nmol/min/mg for males and females, respectively (both $n = 3$). However, sex differences in classes expressed and activity toward CDNB were not statistically significant ($p > 0.05$).

Keywords: Glutathione *S*-transferase; Glutathione; Striped bass; *Morone saxitilis*; CDNB; Rho; Alpha; LC–MS/MS; NCBI non-redundant database (nrDB)

1. Introduction

Electrophilic pesticides such as diazinon and atrazine are commonly applied to fields in the Sacramento Valley. Many of these pesticides enter the Sacramento and San Joaquin Rivers (Sacramento River Watershed Program Annual Monitoring Report, 2005). In general, over one-half of all agricultural pesticides applied annually in California are electrophilic by nature (Sacramento River Watershed Program Annual Monitoring Report, 2005). Particularly reactive with nucleic acids and proteins, electrophilic pesticides represent a toxic hazard to potentially all resident species (Klaassen, 1996). The striped bass (*Morone saxitilis*) is one of several anadromous fish species that complete the spawning portion of their lifecycle in both the Sacramento and San Joaquin Rivers. Therefore, striped bass are invariably exposed to reactive electrophiles during one of the most sensitive periods of their development.

Glutathione *S*-transferases (GSTs) are a multigene family of enzymes that target electrophilic xenobiotics during phase II biotranformation. GSTs catalyze the nucleophilic conjugation of the endogenous tripeptide glutathione (GSH) with reactive electrophiles, facilitating their excretion (Hayes and Pulford, 1995). Due to the substrate specificity of the various GST classes, the more classes a species possesses, potentially the better it may be protected against a variety of electrophilic compounds (Hayes and Pulford, 1995).

GST activity varies greatly depending on species, environmental factors, age, diet, and sex, as well as a variety of other variables such as organ of interest and water salinity. For instance, in the African river prawn (*Macrobrachium vollenhovenii*), distribution of GSTs was found to vary greatly between tissues (Adewale and

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0147-6513/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2007.02.002

Afolayan, 2005). In addition, GST activity in juvenile white sturgeon (*Acipenser transmontanus*) was found to significantly increase when water salinity was elevated to 15‰; however, a similar change has not been observed in Chinook salmon (*Oncorhynchus tshawytsch*; Donham et al., 2006).

Limited information exists regarding GST activity and expression in piscine species, especially in anadromous fishes, thus striped bass investigation is vital. Obtaining a better understanding of the chemical susceptibility of these fishes by characterizing differences in GST activity and expression may facilitate protection of their impacted population.

2. Materials and methods

2.1. Chemicals

Reduced GSH, 1-chloro-2,4-dinitrobenzene (CDNB), ethylene diamine tetraacetic acid (EDTA), dithiothreitol (DTT), Tris–HCl, Tris–base, NaCl, sodium dodecyl sulfate (SDS), ammonia persulfate, TEMED, Millipore Ultrafree centrifugational filters (5 K NMWL membrane) and bovine serum albumin were purchased from VWR Scientific (San Francisco, CA). Tricaine methane sulfonate (MS-222) was obtained from Argent Chemical Laboratories (Redmond, WA). Bradford reagent, GSH–agarose (attached through the sulfur to epoxide-activated 4% cross-linked beaded agarose), and brilliant blue G-colloidal stain were purchased from Sigma (St. Louis, MO). Duracryl was from Genomic Solutions (Ann Arbor, MI) and HPLC solvents were from Burdick & Jackson (Muskegon, MI). BioRad Precision Plus protein standards were purchased from BioRad (Hercules, CA).

2.2. Animals

Adult striped bass (*Morone saxitilis*, 2 years in age, 28–36 cm FL) were purchased from Professional Aquaculture Services (Chico, CA), transported to the UCD Center for Aquatic Biology and Aquaculture, housed in flowing freshwater tanks (19 °C, 2‰) for 1 week, fed Purina Trout Chow, and euthanized with MS-222. All animals were treated ethically according to the UC Davis Animal Use and Care protocol.

2.3. GST isolation and purification

Striped bass were euthanized with MS-222 and dissected for sex determination. Livers from four females and four males were excised, weighed, and homogenized 1:3 (w/v) in ice-cold homogenization buffer (0.25 M sucrose, 10 mM Tris, 1 mM EDTA, and 0.2 mM DTT; pH 7.4) with a Tekmar (Cincinnati, OH) tissue homogenizer (speed setting 8; 1 min) as described previously (Donham et al., 2005a). All subsequent steps were carried out at 4 °C. Cytosol was prepared by centrifugation (10,000*g* for 10 min, then 100,000*g* for 1 h) using a Beckman L-70 refrigerated ultracentrifuge. The cytosolic supernatant was decanted and set aside on ice.

The cytosol from three fishes from each sex was diluted 1:10 with 50 mM Tris–buffer, 1 mM EDTA and 1 mM DTT (pH 6.5; Buffer A) and individually applied to a GSH–agarose affinity column (1.0 cm × 1.5 cm i.d.) equilibrated in Buffer A, and eluted with gravity. The column was washed using 15 mL of Buffer A with 0.5 M NaCl (Buffer B); non-retained effluent was collected. Retained GSTs were then eluted with 6 mL of 50 mM Tris–base, 0.5 M NaCl, 1 mM EDTA, 1 mM DTT, and 50 mM GSH (pH 9.6; Buffer C). Fractions were sampled for activity towards CDNB and assayed for protein using the Bradford Assay (Bradford, 1976). Retained and non-retained fractions were then frozen at −80 °C for future analysis.

2.4. Enzyme assays

Hepatic GST activity towards CDNB was determined by the methods of Habig et al. (1974), optimized for use with fish using a microplate reader (Gallagher et al., 2001). Briefly, 1 mM CDNB in ethanol and 1 mM GSH (in 0.1 M sodium phosphate buffer, pH 6.5) were prepared as substrates. An extinction coefficient of 9.6 mM^{-1} cm^{-1} was used for CDNB. Absorbance was collected for 1 min to result in a linear table. Changes in absorbance at 340 nm were monitored for product formation using a Tecan Sunrise microplate reader at 25 °C (Tecan, San Jose, CA). Activity was calculated using protein concentrations determined via the Bradford assay (Bradford, 1976), with bovine serum albumin used for standardization.

2.5. HPLC and fraction collection

GSH affinity column-retained samples were concentrated and buffer exchanged to low salt concentration with Millipore Ultrafree centrifugational filters (5000 NMWL membrane). Sample (10 μL) was manually injected onto a VYDAC protein/peptide column (model #218 TP 52; C-18, 5 μm 250 × 2.1 mm) and processed on a Waters Assoc. model 510 solvent delivery system with gradient control (0.4 mL/min). A Waters Assoc. variable wavelength UV/VIS 486 detector (214 nm) was used and data collection was via Waters Assoc. Millennium Software. Mobile phase A consisted of 99.95% water and 0.05% TFA, while mobile phase B consisted of 99.95% ACN with 0.05% TFA. The initial mobile phase consisted of 62% A and 38%B. GSTs were separated using a linear gradient from 38% B to 50% B over 90 min, and from 50% B to 60% B over 105 min and from 60%B to 38% B over 110 min. Eluents of detected peaks were hand collected and centrifugally evaporated to dryness.

2.6. Gel electrophoresis

Samples were reconstituted in 1 × Lamelle's running buffer and run on an 11% Duracryl gel, with a 4% stacking gel. Bands were stained for protein with brilliant blue G-colloidal stain. Band cutting, in-gel digestion, and liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis were conducted at the UCD Molecular Structure Facility. Approximate molecular masses were determined using BioRad Precision Plus protein standards.

2.7. In-gel digestion and analysis by LC–MS/MS

2.7.1. Protein in-gel digestion

Protein bands or spots of interest were washed thoroughly four times with Milli-Q (Millipore, MA) water, diced into approx. 1-mm squares, and dried in a SpeedVac (Savant, Holbrook, NY). Proteins were reduced and alkylated according to an established procedure (Shevchenko et al., 1996). Briefly, proteins were reduced with 10 mM DTT in 100 mM NH_4HCO_3 (pH 8, 55 °C) for 1 h, then alkylated with 55 mM iodoacetamide in 100 mM NH_4HCO_3 for 45 min in the dark at room temperature. Excess reagent was removed, and gel pieces were washed with 100 mM NH_4HCO_3, and partially dehydrated with acetonitrile; complete dehydration was via SpeedVac. Finally, proteins were digested in 50% NH_4HCO_3 containing sequence-grade, modified trypsin (Promega, Madison, WI) at a final concentration range of 10–25 ng/μL (37 °C) for 17 h. Peptides were extracted once each with 0.1% trifluoroacetic acid (TFA) and then 5% formic acid–acetonitrile (50:50). The extraction volume was carefully controlled to never exceed 50 μL via SpeedVac. The volume was then reduced to 15 μL for mass spectrometric analysis and nanospray LC–MS/MS.

2.7.2. Nanospray LC–MS/MS

The trypsin-digested samples were analyzed using a fully automated LC–MS/MS system to obtain additional peptide sequences. First, samples were placed into the autosampler of a thermo electron automated HPLC system (San Jose, CA). The injected peptides (1 mL) were first loaded onto a self-packed 100 μM reverse-phase peptide trap (Michrom Bioresources,