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The characterization of cytosolic glutathione transferase from four species of sea turtles: Loggerhead (*Caretta caretta*), green (*Chelonia mydas*), olive ridley (*Lepidochelys olivacea*), and hawksbill (*Eretmochelys imbricata*)

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ABSTRACT

Glutathione s-transferases (GST) play a critical role in the detoxification of exogenous and endogenous electrophiles, as well as the products of oxidative stress. As compared to mammals, GST activity has not been extensively characterized in reptiles. Throughout the globe, most sea turtle populations face the risk of extinction. Of the natural and anthropogenic threats to sea turtles, the effects of environmental chemicals and related biochemical mechanisms, such as GST catalyzed detoxification, are probably the least understood. In the present study, GST activity was characterized in four species of sea turtles with varied life histories and feeding strategies: loggerhead (*Caretta caretta*), green (*Chelonia mydas*), olive ridley (*Lepidochelys olivacea*), and hawksbill (*Eretmochelys imbricata*). Although similar GST kinetics was observed between species, rates of catalytic activities using class-specific substrates show inter- and intra-species variation. GST from the spongivorous hawksbill sea turtle shows 3–4.5 fold higher activity with the substrate 4-nitrobenzylchloride than the other 3 species. GST from the herbivorous green sea turtle shows 3 fold higher activity with the substrate ethacrynic acid than the carnivorous olive ridley sea turtle. The results of this study may provide insight into differences in biotransformation potential in the four species of sea turtles and the possible health impacts of contaminant biotransformation by sea turtles.

1. Introduction

Glutathione transferases (GSTs, EC 2.5.1.18) are a multigene family of enzymes that play a critical role in the detoxification of the exogenous and endogenous electrophiles, as well as the products of oxidative stress. GST proteins have been found in a wide range of eukaryotes, as well as certain prokaryotes, such as cyanobacteria, which use glutathione (GSH) as their major intracellular thiol (Vuilleumier and Pagni, 2002). GSTs have been assigned to various classes based on sequence homology. Generally, GSTs share 40% or greater homology within a class (and between classes, less than 25% sequence identity) (Hayes and Pulford, 1995). The major mammalian classes include alpha (GSTA), mu (GSTM), pi (GSTP), sigma (GSTS), theta (GSTT), zeta (GSTZ), and omega (GSTO) while additional classes of GSTs, including beta (GSTB), delta (GSTD), epsilon (GSTE), lambda (GSTL), phi (GSTF), rho (GSTR), and tau (GSTU) have been identified in nonmammalian organisms, such as fungi, plants, insects, and fish (Blanchette et al., 2007).

The primary reaction catalyzed by GSTs is the nucleophilic attack by reduced GSH on a diverse group of hydrophobic compounds which contain an electrophilic carbon, nitrogen, or sulfur atom. GSTs are present in most tissues at levels ranging from 5 mM to 100 μM (Hayes and Pulford, 1995). While nuclear, microsomal and mitochondrial GSTs have been identified, cytosolic GSTs are the most prominent. In fact, GSTs can account for up to 10% of cytosolic proteins (Will, 1999). In vertebrates, GSTs are distributed throughout most tissues – especially lungs, heart, intestines, and liver – and are expressed in a tissue-specific manner through constitutive and responsive mechanisms (Vos and Van Bladeren, 1990; Awasthi et al., 1994).

Environmental toxicants detoxified by GSTs include polyaromatic hydrocarbons, pesticides, and reactive intermediates produced by phase I biotransformation and other biochemical reactions, and thus GST expression is of importance when considering susceptibility to toxicity by environmental chemicals. For example, GST is a major contributor to the cellular defense mechanism against DNA damage caused by diol epoxides of PAHs (Xiao and Singh, 2007). Furthermore, exposure to environmental toxicants can affect the biochemical responses of exposed organisms. Particularly interesting is the determination of two mechanisms of GST induction by xenobiotics: these enzymes can be regulated by transcription factors which are directly activated by xenobiotics, or regulation can occur through signaling cascades which are activated by cellular stress (Xu et al.,

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2005). Thus, GST expression and activity contributes to an adaptive response to toxic stress within an organism (Hayes and Pulford, 1995).

GSTs also contribute to the detoxification of natural products. In humans, several GSTs contribute to the detoxification of carcinogenic heterocyclic amines produced by cooking protein-rich food (Coles et al., 2001). Furthermore, the ability of insects to tolerate dietary phytotoxins has been linked to constitutively high levels of GST activity (Li et al., 2007). GST expression and activity may help explain the evolution of dietary preferences, and the ability of certain animals to exploit chemically-defended prey.

Little is known about GST expression or activity in sea turtles. In fact, only one paper reports GST activity in the green sea turtle, *Chelonia mydas* (Valdivia et al., 2007). Most sea turtle populations throughout the world are considered threatened or endangered (IUCN, 2008), facing both anthropogenic and natural stressors throughout their life. Sea turtles are slow growing, late maturing, and long lived organisms, which make them particularly susceptible to population decline caused by toxicity associated with bioaccumulation (Rowe, 2008). In sea turtles, harmful effects from anthropogenic stressors include chronic stress, compromised physiology, impaired immune function, and increase in susceptibility to disease (Aguirre and Lutz, 2004). Because of their role in detoxification of endogenous and exogenous chemicals, GSTs may play a critical role in mitigating the potential toxic insult from anthropogenic stressors in sea turtles.

The aims of the present study were to characterize and compare cytosolic GST activity and kinetics in four species of sea turtles with varied life histories and feeding strategies found in the coastal regions of Mexico — green sea turtle (*C. mydas*), loggerhead sea turtle (*C. caretta*), olive ridley sea turtle (*Lepidochelys olivacea*), and hawksbill sea turtle (*Eretmochelys imbricata*) in order to obtain a better understanding of the potential role GSTs may play in the physiological response of sea turtles to xenobiotic exposure.

2. Methods

2.1. Chemicals

Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid (ECA), and 4-nitrobenzyl chloride (NBC) were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). Δ5-Androstene-3,17-dione (ADI) was purchased from Steraloids (Wilton, NH, USA). Various buffers, salts, and cofactors used at UCR were purchased from Fisher Scientific (Pittsburgh, PA, USA) or Sigma-Aldrich, while those used at Cinvestav were purchased from JT Baker (Phillipsburg, NJ, USA), Productos Químicos Monterrey (Mexico City, DF, MX), Invitrogen (Carlsbad, CA, USA), or Sigma Chemical.

2.2. Sample collection

Four species of sea turtles, loggerhead, olive ridley, green, and hawksbill, inhabitating the coastal regions of Mexico were examined in this study. The four species utilized in this study demonstrate different life histories. The dietary preference of adult sea turtles has been extensively studied through stomach content analysis (Bjorndal, 1997), and the various species generally exhibit preferred food sources as shown in Table 1.

Tissue samples were obtained as a result of natural mortality, incidental fisheries or unknown causes of death and were only collected from animals for which time of death could be approximated. Turtles were necropsied, the liver was removed, and placed on dry ice for subsequent subcellular fractionation. Liver samples from 4 loggerhead, 4 green, 4 olive ridley, and 3 hawksbill samples were used for these analyses, and information on each individual specimen is included in Table 1. Methods for the collection of liver tissue from wild loggerhead, green, and olive ridley turtles was described elsewhere (Richardson et al., in press). In July 2007, samples of liver tissue from wild hatchling hawksbill sea turtles were collected from nesting beaches near Celestun, Yucatan, Mexico from animals for which time of death could be approximated within 6 h.

2.3. Isolation of subcellular fractions

Liver subcellular fractions were isolated from liver samples using methods similar to those described elsewhere (Richardson et al., in press). Briefly, each liver sample (approximately 0.5 g) was homogenized then subjected to sequential centrifugation at 4 °C to isolate microsomal and cytosolic fractions. The cytosolic fraction was aliqouted, transported on dry ice, and stored at −80 °C. Protein concentrations of the fractions were determined using the microassay Bradford method (Brogdon and Dickinson, 1983), stained with Coomassie Plus and bovine serum albumin as a standard (both Pierce Biotechnology Inc, Rockford, IL, USA).

2.4. GST kinetics studies

GST enzyme kinetics for CDNB were examined spectrophotometrically by varying concentrations of GSH and CDNB using the assays of Habig and Jakoby (1981). All assay incubations were conducted at 25 °C. CDNB was dissolved in ethanol, with the final reaction concentration less than 0.01%, and GSH was dissolved in buffer. For all assays, the reaction mixture (1 mL final volume) contained 30 μg of cytosolic protein, along with substrate, GSH, and assay buffer — 0.1 M phosphate buffer for CDNB (pH 7.2). The approximate pH optima for freshwater turtle GSTs was reported as 7.2 (Willmore and Storey, 2005), so this pH was selected for the general substrate CDNB. The reaction was started, after 7-min preincubation of GSH and protein in buffer, by adding the appropriate substrate, and the linear portion of change of absorbance, as determined through preliminary studies, was monitored over time using a Shimadzu 1601 UV/Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The apparent enzyme substrate affinity (K_m) and maximum velocity (V_{max}) values for GSH were determined using a GSH range of 0.0625 to 1 mM and a fixed CDNB concentration of 1 mM. The apparent K_m and V_{max} for CDNB were determined using a CDNB range of 0.0625 to 1 mM and a fixed GSH concentration of 1 mM. The kinetic parameters were determined using non-linear regression in Graphpad Prism (Graphpad Software, San Diego, CA, USA).

Table 1
Dietary preferences of adult sea turtles (adapted from Bjorndal, 1997) and information on individual specimens used in this study.

Species	Common name	Preferred food	Specimen information		
			ID	Carapace length (cm)	Sex
Chelonia mydas	Green	Sea grass, algae	CM-1	52.5	Female
			CM-2	58.5	Unknown
			CM-3	50	Unknown
			CM-4	55	Male
Caretta caretta	Loggerhead	Benthic invertebrates	CC-1	55	Male
			CC-2	66.5	Unknown
			CC-3	62	Female
			CC-4	57	Unknown
Eretmochelys imbricata	Hawksbill	Sponges	EI-1	Immature	
			EI-2	Immature	
			EI-3	Immature	
Lepidochelys olivacea	Olive ridley	Fish, salps	LO-1	61	Unknown
			LO-2	57	Unknown
			LO-3	61.5	Unknown
			LO-4	53	Unknown