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Cloning, expression and analysis of the olfactory glutathione *S*-transferases in coho salmon

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ABSTRACT

The glutathione *S*-transferases (GSTs) provide cellular protection by detoxifying xenobiotics, maintaining redox status, and modulating secondary messengers, all of which are critical to maintaining olfaction in salmonids. Here, we characterized the major coho salmon olfactory GSTs (OlfGSTs), namely omega, pi, and rho subclasses. *OlfGST omega* contained an open reading frame of 720 bp and encoded a protein of 239 amino acids. *OlfGST pi* and *OlfGST rho* contained open reading frames of 627 and 681 nt, respectively, and encoded proteins of 208 and 226 amino acids. Whole-protein mass spectrometry yielded molecular weights of 29,950, 23,354, and 26,655 Da, respectively, for the GST omega, pi, and rho subunits. Homology modeling using four protein-structure prediction algorithms suggest that the active sites in all three OlfGST isoforms resembled counterparts in other species. The olfactory GSTs conjugated prototypical GST substrates, but only OlfGST rho catalyzed the demethylation of the pesticide methyl parathion. OlfGST pi and rho exhibited thiol oxidoreductase activity toward 2-hydroxyethyl disulfide (2-HEDS) and conjugated 4-hydroxynonenal (HNE), a toxic aldehyde with neurodegenerative properties. The kinetic parameters for OlfGST pi conjugation of HNE were $K_M = 0.16 \pm 0.06$ mM and $V_{\max} = 0.5 \pm 0.1$ μmol min^{-1} mg^{-1}, whereas OlfGST rho was more efficient at catalyzing HNE conjugation ($K_M = 0.022 \pm 0.008$ mM and $V_{\max} = 0.47 \pm 0.05$ μmol min^{-1} mg^{-1}). Our findings indicate that the peripheral olfactory system of coho expresses GST isoforms that detoxify certain electrophiles and pesticides and that help maintain redox status and signal transduction.

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1. Introduction

The glutathione *S*-transferases (GSTs) are a multifunctional family of phase II detoxification enzymes that protect cells against harmful endogenous toxic metabolites, superoxide radicals, and exogenous toxic chemicals [1–3]. To date, at least 14 classes of mammalian GSTs have been identified based on primary amino acid sequences with GSTs sharing more than 40% identity being assigned to the same class, and those sharing less than 30% being assigned to different classes [4]. Additionally, the structurally distinct membrane-bound microsomal GSTs are a separate GST subfamily belonging to the Membrane Associated Proteins in Eicosanoid and Glutathione (MAPEG) metabolism pathway [5]. Although the predominant catalytic activity of GSTs involves the conjugation of exogenous and endogenous compounds by facilitating nucleophilic attack by reduced glutathione (GSH), several GSTs combat oxidative stress by GSH-dependent peroxidase activity or via conjugation of reactive α-β-unsaturated aldehydes produced during lipid peroxidation. In addition, certain environmental chemicals or their metabolites are also GST substrates, and species' differences in GST expression have been linked to susceptibility to chemical-induced injury [6].

Although fish GSTs have not been well characterized relative to mammals, all fish have GST activity and express multiple GST isoforms. The predominant GST in carp (cyprinids), salmon (salmonids) and cod (gadoids) is a pi-class GST, whereas the major isoform in flatfish (pleuronectids), mullet (mugilids), sea bream (sparids) and bass (centrarchids) is a rho-class GST [7]. Coho salmon exhibit a complex GST tissue profile encompassing 9 subfamilies, including alpha, mu, pi, theta, omega, kappa, rho, zeta and microsomal GSTs [8,9] and have notable tissue differences in constitutive expression patterns [8]. For example, omega-, pi-, and rho-class GST mRNA transcripts predominate in the olfactory system of coho salmon, and of the three forms, rho GSTs are an evolutionarily distinct subclass of GSTs restricted to aquatic organisms. Rho GSTs were initially characterized in pleuronectid flatfish and largemouth bass [10] and originally misidentified as theta-class-like GSTs [11]. As a group, rho GSTs share >80% homology among aquatic animals [12], and can dominate total tissue GST composition [13]. Although the endogenous function of

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rho GSTs has not been determined, rho GSTs from several aquatic species conjugate 4-hydroxynonenal (HNE) [7], a reactive aldehyde produced during lipid peroxidation. Fish can be particularly vulnerable to membrane damage due to high levels of polyunsaturated fatty acids that are susceptible to peroxidation. Accordingly, GST rho may have functionality in protecting fish tissues against oxidative damage associated with changes in membrane fluidity. Furthermore, the fact that HNE also exhibits neurodegenerative properties suggests that the expression of HNE-conjugating GSTs, such as GST rho, in the peripheral or central nervous system tissues of fish is also of physiological importance. However, not all rho GSTs exhibit HNE-conjugating activity, suggesting that minor differences in protein structure can markedly affect HNE catalytic specificity. Notwithstanding, the fact that HNE plays a major role in oxidative stress-induced signaling indicates that certain GST isoforms that are active in HNE metabolism may also regulate cellular signal transduction.

Although fish GSTs have been studied most in liver tissues, the peripheral olfactory system is in direct contact with the environment and is vulnerable to waterborne chemical exposures. For this reason, maintenance of intact olfaction in the presence of chemical exposures is critical for uninterrupted olfactory signaling, which in turn triggers sensory motor-dependent functions (i.e. predator avoidance, prey selection, and homing) critical to survival [14–17]. The present study describes the identification, expression and catalytic function of the three major olfactory GST isoforms in coho salmon. We were particularly interested in understanding the role of GSTs in mediating the effects of oxidative stress and the metabolism of HNE due to its critical role in cellular oxidative damage as well as mediating signal transduction processes that may be important in salmon olfaction.

2. Materials and methods

2.1. Chemicals

MS-222 (Tricaine methanesulfonate) was obtained from Argent Chemical Laboratories (Redmond, WA). TRIzol® reagent, the SuperScript® First-Strand Synthesis System, and the Zero Blunt® TOPO® PCR Cloning Kit for Sequencing were purchased from Invitrogen (Carlsbad, CA). Finnzymes Phusion® Hot Start was purchased from Thermo Fisher Scientific, (Waltham, MA). T4 DNA Ligase and all cloning restriction endonucleases were purchased from New England Biolabs, Inc. (Ipswich, MA). The SMART™ RACE cDNA Amplification Kit was obtained from Clontech (Mountain View, CA). Primers were obtained from Eurofins MWG Operon (Huntsville, AL). The pET-28a (+) vector, the *Escherichia coli* bacterial strain BL21 DE3, and restriction grade Thrombin Protease were obtained from Novagen (Madison, WI). Ni Sepharose™ High Performance Beads were purchased from GE Healthcare (Pittsburg, PA). Ampicillin, kanamycin, isopropyl β-D-1-thiogalactopyranoside(IPTG), imidazole, 1-chloro-2,4-dinitrobenzene (CDNB), 2,4-dichloro-nitrobenzene (DCNB), ethacrynic acid (ECA), cumene hydroperoxide (CuOOH), 2-hydroxyethyl disulfide (2-HEDS), methyl parathion, and reduced GSH were purchased from Sigma (St. Louis, MO). HNE was purchased from Cayman Chemicals (Ann Arbor, MI). Glutathionyl 4-hydroxynonanal (GSHNE) was biosynthesized as described previously [18]. All other chemicals and solvents were of analytical grade and purchased from standard sources (Thermo Fisher Scientific, Waltham, MA; Sigma, St. Louis, MO).

2.2. Animals and tissue processing

Juvenile coho salmon (~1 year of age) were provided by the National Oceanic and Atmospheric Administration (NOAA), Seattle, WA. Fish were raised in cylindrical tanks with recirculated freshwater under natural photoperiod in dechlorinated municipal water and fed commercial dry food pellets (BioOregon, Warrenton, OR, USA) daily. Animal welfare and experimental procedures were carried out in strict accordance with the University of Washington Institutional Animal Care and Use Committee (IACUC) guidelines. For RNA preparations, olfactory rosettes were collected from individuals euthanized with MS-222 (Argent Chemical Laboratories, Redmond, WA), rinsed in 1× PBS (pH 7.0), placed in 1 mL Trizol, and snap-frozen in liquid nitrogen. For subcellular preparations, olfactory rosettes were pooled from 20 juvenile coho, rinsed in 1× PBS (pH 7.0), and snap-frozen in liquid nitrogen. For catalytic activity analysis, liver tissue was collected from juvenile coho salmon, snap-frozen in liquid nitrogen, and stored at −80 °C until processing.

2.3. Cloning of olfactory GST isoforms by RACE

An olfactory cDNA library was synthesized from 1 μg of total RNA (DNAse treated) using oligo (dT) primers and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). Rapid amplification of cDNA ends (RACE-PCR) using the SMART™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA) together with MMLV Reverse Transcriptase (Clontech, Mountain View, CA) was used to amplify the *GST omega*, *pi*, and *rho* partial cDNAs according to the supplier's protocol. Gene-specific RACE primers for *GST pi* were designed against Salmo salar *GST pi* full-length sequence (Table 1). Primers for *GST rho* were designed against both Oncorhynchus mykiss and Salmo salar *GST A* full length sequences (Table 1). Similarly, gene-specific RACE primers for *GST omega* were designed against Oncorhynchus mykiss and Salmo salar *GST omega 1* full-length sequences (Table 1).

All amplified PCR products were cloned into the pCR4Blunt-TOPO cloning vector (Invitrogen, Carlsbad, CA) and sequenced at

Table 1
Sequences of PCR primers used in this study.

Function	Primer (5′–3′)	Source accession no.
GST pi 5′ Race	Reverse: GGACGGGCTGACATCTTCTCGAC	BT059949.1
GST pi 3′ Race	Forward: GTCAGCCCGTCCCAAAATCAAGG	BT059949.1
GST pi full-length	Forward: CGGTAGCTAGCATGGGGCCATACACAATCACC	JX416477
	Reverse: GGCCCAAGCTTTCACTGTTTGCCATTGCC	
GST rho 5′ Race	Reverse: TTTCCTGCCAGGTAAGAACCCAC	BT073173.1; BT057296.1
GST rho 3′ Race	Forward: GTGGGTTCTTACCTGGCAGGAAA	BT073173.1; BT057296.1
GST rho full-length	Forward: CGGTAGCTAGCATGGCCAAGGACATGACACTTCTC	JX416475
	Reverse: GGCCCAAGCTTTCAGAACTCCTTGAGAGTGTCCCC	
GST omega 5′ Race	Reverse: TCCACTTCTTCAGCTCAGGGGT	BT073404.1; BT049799.1
GST omega 3′ Race	Forward: ACCCCTGAGCTGAAGAAGTTGAC	BT073404.1; BT049799.1
GST omega full-length	Forward: CGGTAGCTAGCATGGCCTCTGAAAAATGTTTTGCG	JX416476
	Reverse: GGCCCAAGCTTCTACAGGCCATAGTCATAATTGGG	

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