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CHARACTERIZATION OF PHASE I BIOTRANSFORMATION ENZYMES IN COHO SALMON (*ONCORHYNCHUS KISUTCH*)

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Abstract

Wild stocks of Pacific salmon in the Northwestern United States have declined in recent years, and the major factors contributing to these losses include water pollution and loss of habitat. In salmon, sublethal chemical exposures may impact critical behaviors (such as homing, feeding, predator-avoidance) that are important for species survival. Therefore, understanding the potential for these species to biotransform organic compounds within sensitive target tissues such as liver, gills and olfactory region can help estimate or predict their susceptibility to pollutants. In this study, we used real-time quantitative polymerase chain reaction (Q-PCR), Western blotting, and catalytic assays to characterize the expression of Phase I biotransformation enzymes in coho salmon (*Oncorhynchus kisutch*), a sensitive species in the Pacific Northwest. Gene expression analysis using Q-PCR assays developed for coho genes revealed the presence of the predominant cytochrome P450 mRNAs (CYP1A, CYP2K1, CYP2M1, CYP3A27) in the olfactory rosettes and provided quantitative mRNA expression levels in coho liver and gills. Q-PCR analysis revealed relatively high expression of the major CYP isoforms in the liver and olfactory rosettes, which was generally confirmed by Western blotting. Extrahepatic CYP expression was generally higher in the olfactory rosettes as compared to the gills. Catalytic studies demonstrated functional CYP1A-dependent ethoxyresorufin-*O*-deethylase, CYP2-dependent pentoxyresorufin-*O*-dealkylase, CYP2K1-dependent testosterone 16 β -hydroxylase, and CYP3A27-dependent testosterone 6 β -hydroxylase activities in liver, but not at detectable levels in gills. In contrast, flavin-containing monooxygenase (FMO)-dependent thiourea *S*-oxidase activity was readily observed in the gills and was substantially higher than that observed in liver. Collectively, the results of this study suggest that the olfactory rosettes are important sites of extrahepatic biotransformation in coho salmon, and that tissue specific-differences in Phase I metabolism may lead to contrasting tissue-specific biotransformation capabilities in coho salmon.

Keywords

Biotransformation; coho salmon; cytochrome P450; flavin-containing monooxygenase; olfactory rosettes

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1. Introduction

Biotransformation occurs through biochemical processes in which hydrophobic compounds of both endogenous and exogenous nature undergo steps towards becoming more hydrophilic, thus promoting/facilitating their metabolism. Such processes are often categorized into Phase I and Phase II reactions. While Phase I involves mostly hydrolysis, oxidation, and reduction reactions, by adding or exposing functional groups (e.g., hydroxyl, sulfhydryl, carboxyl, amino), Phase II comprises conjugation or synthetic reactions (e.g., methylation, glucuronidation, sulfation) to further increase solubility of the compound (Kleinow et al., 1987; Livingstone, 1998).

The cytochrome P450 and flavin-containing monooxygenase enzymes are the major oxidative enzymes in Phase I metabolism. Cytochrome P450s constitute a superfamily of heme-containing proteins best studied for their role in oxidative metabolism (Stegeman, 1989; Guengerich, 1992). Cytochrome P450s (CYPs) metabolize a multitude of both endogenous and exogenous compounds, ranging from hormones to organic pollutants. The typical reaction catalyzed by CYP is a monooxygenase reaction, in which an atom of oxygen is inserted into an organic substrate while the other oxygen atom is reduced to water. The name cytochrome P450 originated from the peak formed at a wavelength of 450 nm when the heme group is reduced and complexed to carbon monoxide. The flavin-containing monooxygenases (FMOs) catalyze numerous monooxygenase reactions using reducing equivalents provided by NADPH to a flavoprotein-containing enzyme system which undergoes nucleophilic attack by the substrate. Although the physiological function of FMO remains unknown, some studies have shown that these enzymes are capable of oxygenating numerous endogenous and dietary compounds (Cashman, 1997). From a toxicological standpoint, FMOs play an important role in the toxicity of various heteroatom-containing xenobiotics such as organic pesticides (Schlenk, 1998). Both CYPs and FMOs are NADPH-dependent and require oxygen for catalytic reactions. Phase I biotransformation is essential for the detoxification of xenobiotics, but in some cases, metabolic activation occurs, rendering the intermediate compounds even more toxic than the parent compounds (Guengerich, 1992).

In fish, biotransformation enzymes are mostly distributed in the liver, although extra-hepatic sites also include the gut, the kidneys, the gills and the olfactory system (Stegeman, 1989; Smolowitz et al., 1992; Monod et al., 1994; Buhler & Wang-Buhler, 1998). The gills and the olfactory tissues, in particular, constitute direct target sites for waterborne pollutants given their intimate contact with the external environment (Klaprat et al., 1992; Wood, 2001). The ability of fish to biotransform xenobiotics can help predict their susceptibility to contaminants in the environment (Nabb et al., 2006). Despite extensive investigation on the role of Phase I enzymes in liver and gills, little is known about the expression of these enzymes in the olfactory system of fish. Hara (1992) suggested that olfaction is the predominant chemical sense in fish, playing a remarkable role in behavioral aspects such as predator avoidance, prey selection, reproductive timing, and homing.

Pacific salmon populations have declined markedly in the Western United States, due to a multitude of factors such as water pollution, loss of habitat, over-fishing, dam construction/operation, predation, diseases, parasites, climatic and oceanic shifts (reviewed in Lackey, 2003). The widespread contamination of surface waters and sediments, in particular, appears to be a limiting factor for the recovery of some of these threatened wild salmon stocks. Water quality monitoring conducted by the United States Geological Survey (Gilliom et al., 2006) have indicated that many Pacific Northwest surface waters contain pesticide residues, oftentimes in river beds used by salmon for spawning and during the early life stages of the fry (Scholz et al., 2006). Pollutants in water may affect the physiology of fish olfaction, disrupting biologically-relevant signals essential in their behavior that ultimately affect species

survival (Klaprat et al., 1992; Moore & Waring, 1996; Scott et al., 2003). Accordingly, it is important to understand the expression and catalytic activities of the gene products of biotransformation enzymes in olfactory, branchial, and hepatic tissues to help understand the susceptibility of Pacific salmon to aquatic pollutants.

Recently, Trute et al. (2007) reported a complex glutathione *S*-transferase isoenzyme profile in coho salmon (*Oncorhynchus kisutch*), a sensitive and economically important salmonid species in the Pacific Northwest. The current study was initiated to characterize the expression of Phase I biotransformation enzymes in coho. Using real-time quantitative polymerase chain reaction (Q-PCR) and Western blotting, we characterized the expression pattern of CYP isoforms (CYP1A, CYP2K1, CYP2M1, and CYP3A27) and FMO. Particular attention was given to the olfactory region, given its fundamental importance in migratory salmonids. In addition, we measured the basal catalytic activities of CYP1A-dependent ethoxyresorufin-*O*-deethylase (EROD), CYP2-dependent pentoxyresorufin-*O*-dealkylase (PROD), CYP2K1-dependent testosterone 16 β -hydroxylase, CYP3A27-dependent testosterone 6 β -hydroxylase, and FMO-mediated thiourea *S*-oxidase activities in microsomal fractions isolated from liver and gills.

2. Materials and Methods

2.1. Experimental animals

All animal use and procedures were approved by the University of Washington Institutional Animal Care and Use Committee. Juvenile coho salmon (*Oncorhynchus kisutch*) (10–13 cm, approximately 6 months of age, total of 80 fish) were held in cylindrical tanks, with recirculating dechlorinated city water under filtration. Although flow rates were not measured, water flows were maintained at rates to minimize stress on the fish and ensuring minimal ammonia accumulation. Typical water conditions were ~120 mg/L as CaCO₃, pH 6.6, at 11–12°C, under normoxic conditions. Fish were fed commercial dry food pellets (BioOregon, Warrenton, OR, USA) once a day *ad libitum*. Fish were sacrificed by severing the spinal cord and tissues were immediately harvested in the following order: olfactory rosettes, livers, and gills. All tissues, with the exception of the olfactory rosettes, were rinsed in 100 mM phosphate buffer (pH 8.0), blotted dry, and snap-frozen on dry ice. A subset of *N*=6 samples from individual fish was stored separately for RNA extractions and subsequent real-time Q-PCR analyses, whereas the remainder of the samples were shipped to the University of California (Riverside, CA, USA) for further processing. Tissues were stored in a –80°C freezer until proceeding with microsomal isolation for protein work.

2.2. Real-time Quantitative Polymerase Chain Reaction

Total RNA was extracted from snap-frozen tissues (~50 mg; *N*=6) from each individual fish using a standard TRIzol procedure (Invitrogen, Carlsbad, CA, USA). Following determination of RNA concentrations by UV absorbance and insurance of optimal 260/280 ratios, the integrity of each RNA sample was verified using a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Two μ g of RNA was used to generate first strand cDNA (Superscript First Strand Synthesis kit, Invitrogen) which was stored at –20°C until proceeding with Q-PCR analyses.

Gene-specific primers and probes specific for coho salmon CYP1A, CYP2K1, CYP2M1, and CYP3A27 were designed against phylogenetically similar species such as rainbow trout (*Oncorhynchus mykiss*) using Primer Express (Applied Biosystems, Foster City, CA, USA). The resulting PCR products were electrophoretically separated, purified and sequenced. TaqMan real-time quantitative PCR was performed using 4 μ L of 1 μ g/ μ L cDNA, Taq antibody, TaqMan polymerase (Invitrogen), and gene-specific primers and probes (Table 1). The sequences were verified for specificity using BLAST software. Because of the extensive

homology between salmonid CYP1A1 and CYP1A3 cDNAs, and the difficulty to discriminate the two sequences, we refer to these genes as CYP1A throughout the text.

Standard curves of the housekeeping gene β -actin were run on each plate to account for inter-plate variability and quantification of each gene of interest was determined by interpolation from standard curves. Thermocycling was performed for 40 cycles and the increase in fluorescence during each replication cycle was plotted by the instrument against cycle number (Applied Biosystems Model 9700). Ct values for a series of standards (0.1 ng-1.0 pg) that were simultaneously obtained using coho β -actin cDNA as PCR template. The resulting standard curve values were generated by plotting Ct versus the log of the amount of cDNA added to the reaction. Triplicates were conducted for each gene and each sample, and products from Q-PCR reactions without reverse transcriptase were included as a control for undesired DNA amplification.

2.3. Microsomal isolation

Tissue samples were defrosted on ice and homogenized in 5 to 6 volumes of ice-cold buffer (100 mM potassium phosphate, pH 7.8, 100 mM KCl, 1 mM EDTA), using a Potter-Elvehjem tissue homogenizer (Wheaton, Millville, NJ, USA) at a 1,600 rpm speed, 8 to 10 passes per sample. For gills, filaments were clipped with scissors to avoid cartilage pieces prior to homogenization. For olfactory rosettes, samples were homogenized using a microcentrifuge tube-adapted pestle (Disposable Pestle System, Fisher Scientific, Fair Lawn, NJ, USA) due to the small tissue amount and buffer volume (6 to 10 individual samples were pooled for rosette microsomes; $N=4$). Tissue homogenates were centrifuged at 13,000 g for 20 min at 4°C. Supernatants were then transferred to clean tubes and centrifuged at 100,000 g for 90 min. The resulting microsomal pellets were washed in ice-cold buffer and resuspended in approximately 1 mL of buffer (100 mM phosphate buffer, pH 7.8, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol) using a manual homogenizer (for rosette microsomes, buffer volume for resuspension was about 0.1 mL per pooled sample). Microsomes were then aliquoted in centrifuge tubes and stored in a -80°C freezer for further use. Protein concentration was determined in microsomal fractions using the Bradford method (Coomassie Plus, Pierce, Rockford, IL, USA).

2.4. Immunoblotting of proteins

Microsomal proteins (40 μ g per lane), along with stained molecular weight marker (SeeBlue Plus2, Invitrogen) were resolved in polyacrylamide gels (SDS-PAGE, 10% gradient). Positive controls for CYP isoforms and FMO1 consisted of microsomes of the following: for CYP1A, β -naphthoflavone-treated rainbow trout liver; for CYP2K1, CYP2M1, and CYP3A27, rainbow trout liver; and for FMO, microsomes from rat kidney. Resolved proteins were transferred to 0.45 μ m nitrocellulose membrane using semi-dry transfer. Membranes were stained with Ponceau solution (Pierce) to confirm protein transfer, and then placed in blocking solution for a minimum of 1 h. Primary antibodies for CYPs or FMO1 consisted of: mouse anti-fish monoclonal CYP1A antibody (Biosense, Bergen, Norway), rabbit anti-rainbow trout polyclonal CYP2K1, CYP2M1, and CYP3A27 antibodies (provided by Dr. D.R. Buhler, Oregon State University), and rabbit anti-guinea pig polyclonal FMO1 antibody (provided by Dr. A. Rettie, University of Washington, Seattle). Goat anti-rabbit IgG alkaline phosphatase was used as the secondary antibody (BioRad, Hercules, CA, USA). Immunoreactive bands were visualized using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium from a commercial alkaline substrate conjugation kit (BioRad). Immunoblots were then scanned and densitometrically analyzed using Quantity One software (BioRad). Semi-quantitative measurements of protein expression as reflected by optical density were plotted in a bar graph for tissue-specific comparisons.

2.5. Enzyme assays

Phase I biotransformation enzyme catalytic activities were analyzed in coho gill and liver microsomes. However, the extremely small mass of the olfactory rosettes (<20 mg) precluded a detailed analysis of Phase I catalytic activities in these tissues.

2.5.1. EROD and PROD activities—EROD and PROD activities were measured kinetically using a fluorimetric microplate method modified from Kennedy et al. (1993). Excitation and emission wavelengths for measuring resorufin formation were, respectively, 560 and 590 nm (Omega Filters, Brattleboro, VT, USA). Resorufin formation was measured over 10 min and the rate of product formation in samples was obtained from the linear portion of the delta-fluorescence measurements over time. Based on the slope obtained by the linear regression of standards, EROD and PROD activities were normalized to the protein concentration under initial rate conditions and expressed as pmol of resorufin/mg protein/min.

2.5.2. Testosterone hydroxylation activities—CYP-mediated testosterone hydroxylation activities were measured using high performance liquid chromatography (HPLC) by incubating microsomes with ^{14}C -testosterone, as described in Martin-Skilton et al. (2006). Testosterone (Sigma Aldrich, St. Louis, MO, USA), testosterone 6 β - and 16 β -hydroxylase (Steraloids, Newport, RI, USA) were detected at 254 nm on spiked samples, and retention times were compared to peaks obtained in liver and gill microsomal incubations with ^{14}C -testosterone. Catalytic activities were measured under initial rate conditions and expressed as pmol/mg protein/min.

2.5.3. Thiourea S-oxidase activity—The thiocholine-dependent measurement of thiourea oxidation has been shown to be a sensitive measure of microsomal FMO activity in trout (Schlenk, 1995). FMO activities in coho tissues were measured spectrophotometrically according to Guo & Ziegler (1991) as modified by Schlenk et al. (1996). Calculations for thiourea S-oxidase activity were based on a millimolar absorptivity of 13.6 cm^{-1} for 5,5'-dithiobis(-2-nitrobenzoic acid) (DTNB). Results were normalized to protein concentration in microsomes and incubation time.

2.6. Statistical Analyses

All Q-PCR and semi-quantitative Western blotting data is reported as mean $\pm$ SEM for multiple individuals as designated in the legends. Tissue-specific differences in gene and protein expression for the various CYP isoform were analyzed by ANOVA. When differences proved to be significant at $P < 0.05$, a Dunnett's multiple comparison test was applied to identify the source of significance. Differences in basal catalytic levels for CYPs and FMO among coho liver and gills were compared using Student's *t*-tests, with differences being considered significant at $P < 0.05$.

3. Results

3.1. Real-time Q-PCR analysis of CYP isoforms

The results of the Q-PCR analysis of CYP isoform expression in coho tissues are presented in Fig. 1. As observed, CYP1A, CYP2M1, and CYP3A27 isoforms were present in all tissues analyzed, whereas CYP2K1 was observed in liver and olfactory rosettes, but was not detected in gills. We also observed significant tissue-specific differences with regard to the expression of CYP genes. For example, in liver, the relative expression of the various isoforms was $\text{CYP3A27} \gg \text{2M1} > \text{2K1} > \text{1A}$. In contrast, the relative level of CYP expression in the gills was $\text{CYP3A27} > \text{2M1} > \text{1A}$, and in the olfactory rosettes, CYP expression was $\text{CYP3A27} > \text{2K1} > \text{2M1} > \text{1A}$ (Fig. 1). Of note was the relatively high expression of all isoforms in the olfactory rosettes of coho. Among the various CYP isoforms, the expression of the PAH-

inducible CYP1A was consistently low, and there were no significant differences among liver, gill, and olfactory rosette CYP1A expression.

3.2. Immunoblotting and CYP catalytic activities

Western blots of coho salmon microsomes confirmed the presence of CYP2K1-, CYP2M1-, and CYP3A27-like proteins in both olfactory rosettes and liver (Fig. 2a). The molecular weights of these isoforms were estimated at 49, 52, and 54 kDa, respectively. In contrast, we could not detect any CYP isoform expression in gills, even at microsomal protein loads above 40 µg/lane. This may have been a result of CYP protein expression being below the detection limit of the immunoblotting method, as CYP1A-dependent EROD activity was detected in both coho gill and liver microsomes (Table 2) despite the lack of CYP1A-immunoreactivity in gills and in other tissues. PROD activity, a marker for CYP2 activity in mammals (Burke et al., 1994), was observed at very low levels in coho salmon liver microsomes, and was not detected in gills (limit of detection: <0.5 pmol/mg protein/min). As observed, the semi-quantitative analysis of constitutive CYP proteins (Fig. 2b) revealed similar expression patterns that were detected by the more quantitated Q-PCR methodology. Consistent with the results of our western blotting studies, CYP2K1-dependent activity of 16β-hydroxytestosterone and CYP3A27-dependent activity of 6β-hydroxytestosterone was readily detected in liver, but not in gills (Table 2), given their low limit of detection (<0.3 pmol/mg protein/min).

In addition to the CYP substrates analyzed, FMO-mediated thiourea *S*-oxidase activities were readily apparent in coho gills, and initial rate of branchial FMO activity was significantly greater than that observed in liver (Table 2). However, we were unable to detect the presence of an FMO-like isoform in either liver or gills by Western blots (data not shown), likely due to poor antibody recognition of the coho FMO protein.

4. Discussion

This is the first study to show the presence of constitutive CYP isoforms in olfactory rosettes of fish. CYP2K1 (LMC2), CYP2M1 (LMC1), and CYP3A27 (LMC5) represent constitutive CYP isoforms ubiquitous in rainbow trout liver, with relative molecular weights of 54, 50, and 59 kDa, respectively (Miranda et al., 1989). The role of CYP2K1 in the biotransformation of endogenous compounds has been linked to hormones (catalysis of the 2-hydroxylation of 17β-estradiol, the 16β-hydroxylation of testosterone, the 16α-hydroxylation of progesterone, and hydroxylation at the (ω-1)-position of lauric acid, a long chain fatty acid (Miranda et al., 1989). As for xenobiotic biotransformation, CYP2K1 has been shown to activate aflatoxin B1 to its carcinogenic epoxide form (Williams & Buhler, 1983). CYP2M1 is also involved in the hydroxylation of lauric acid, however, more specifically at the (ω-6)-position (Buhler et al., 1997). CYP3A27 is involved in the metabolism of both testosterone and progesterone by hydroxylation in the 6β- position (Miranda et al., 1989). In addition, CYP3A27 has also been shown to catalyze *N*-demethylation of benzphetamine in rainbow trout (Miranda et al., 1989). Buhler & Wang-Buhler (1998) suggested that fish CYP3A27 and the mammalian CYP3A4 (the major CYP isoform studied in drug metabolism) are very similar in structure and catalytic function. Our findings on the constitutive CYP isoforms emphasize the role of the olfactory system, not only for normal development and behavior, but also its relevance from an environmental toxicology perspective. For example, estrogen-like endocrine disruptors are known to inhibit expression of CYP2K1, CYP2M1, and CYP3A27 transcripts in juvenile rainbow trout, and are therefore likely to affect numerous physiological pathways that depend on the normal expression of specific P450 isozymes (Buhler et al., 2000; Katchamart et al., 2002). The expression pattern of the various CYP isoforms in coho salmon, specifically in the olfactory tissues, suggest that these target sites may play an important role in chemical toxicity when fish face waterborne xenobiotics. We also speculate that detection of key odorant cues

during migration may be disrupted when coho salmon come across pollutant exposures, for example, during their return to natal streams. We are pursuing detailed studies of the impacts of chemical exposures on salmon olfaction in our laboratories.

CYP1A expression and its associated catalytic activities were relatively low in coho salmon microsomes, but were generally consistent with numerous other studies of basal CYP1A enzyme levels in aquatic species. It must be noted that in the current study, we did not discriminate among coho CYP1A isoforms. In rainbow trout, CYP1A1 and CYP1A3 genes share 96% amino acid identity and have similar enzymatic activity, and both genes are inducible on exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (Cao et al., 2000). In trout liver, the CYP1A1 isoform predominates (Cao et al., 2000). CYP1A1 mRNA is also constitutively expressed in Atlantic salmon and is inducible on exposure to polycyclic aromatic hydrocarbons (Buhler and Wang-Buhler, 1998; Rees and Li, 2004). Although we could not discriminate between the two isoforms in the present study, it is reasonable to assume CYP1A1 mRNA was detected in our assays due to the predominant presence of this isoform in the closely related rainbow trout. Induction of CYP1A by aryl hydrocarbon receptor agonists has been shown in olfactory tissues of *Poeciliopsis* spp. exposed to benzo[a]pyrene (Smolowitz et al., 1992) and rainbow trout exposed to β -naphthoflavone (Monod et al., 1994; Saucier et al., 1999). To this end, we are pursuing the relevance of modulation of CYP gene expression in Pacific salmon inhabiting polluted waterways in other field studies.

Levels of thiourea *S*-oxidase in coho salmon were about twice as high compared to data previously reported for rainbow trout (Larsen & Schlenk, 2001). That FMO catalytic activities were significantly higher in gills relative to livers suggests that the branchial pathway is a primary route for FMO-mediated biotransformation in coho salmon. Because the gills of fish are directly in contact with the external environment, any changes in water chemistry may affect the normal physiology and biochemistry at these target sites (Wood, 2001). Therefore, the gills can increase the susceptibility of coho salmon to pollutants, especially when fish encounter waterborne chemicals and polluted waterways during their life cycle. Many thioether-containing pesticides present in salmon waterways (e.g., Aldicarb, fenthion, phorate) are substrates for FMO. The *S*-oxidation of these thioether pesticides by FMO results in bioactivation and formation of more toxic metabolites (Schlenk & Buhler, 1991; Cashman, 1997; Wang et al., 2001). In addition, Wang et al. (2001) reported that toxicity of Aldicarb in rainbow trout increases at higher salinities. Because coho salmon, among other anadromous species, face significant salinity changes during migration, its concomitant exposure to increased salinity in polluted waterways (such as Superfund sites) can drastically affect species susceptibility to contaminants. Given that FMO activity is associated with increased oxidation of these toxic substrates and higher toxicity to trout, it is possible that the expression of FMO may modulate susceptibility to pesticide injury in coho salmon.

5. Conclusion

The results of the study substantiate the presence of constitutive CYP isoforms in coho salmon olfactory tissues, suggesting substantial biotransformation capabilities at this site that may contribute to detoxification/bioactivation of waterborne chemicals and potentially control chemical interactions with sensitive neuronal targets. In addition, our study supports the hypothesis that the gills are a major biotransformation route for FMO-mediated oxidation, whereas the predominant Phase I enzymes in the liver are CYP isoforms. As an ongoing effort to understand coho salmon susceptibility to pollutants, we are currently investigating the toxicological ramifications of tissue-specific expression of Phase I and Phase II biotransformation pathways on chemical injury in coho salmon.

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Fig. 1. Quantitative PCR data in CYP isoforms of juvenile coho salmon (mean ± SEM; $N=6$) normalized to the expression of housekeeping gene β -actin. Differential tissue expression of CYP isoforms was seen for the constitutive CYP2K1 (b), CYP2M1 (c), and CYP3A27 (d), but not for the inducible CYP1A (a). Significant differences ($P<0.05$) are represented by **a** = relative to liver, **b** = relative to gills, and **c** = relative to olfactory rosettes. ND = not determined.

Fig. 2b

Fig. 2.

a) Western blots of CYP2K1-, CYP2M1, and CYP3A27-like proteins in microsomes from juvenile coho salmon tissues. CYP1A and FMO1 were not detected by immunoblotting (not shown). Protein load: 40 μ g/lane. OR = olfactory rosette; L = liver. Molecular weights of bands are shown by arrows; b) Relative optical density of CYP-like isoforms detected by Western blotting illustrating tissue-specific differences in isoform expression.

Table 1

Primer pairs and fluorescently labeled probes used in CYP TaqMan analyses of juvenile coho salmon tissues.

Gene	Type of oligo	Sequence (5'-3')	(Species) GenBank Accession Number Position
β-actin			(<i>O. mykiss</i>) AF157514
	primer (forward)	gacccacacagtgccatct	528-547
	primer (reverse)	gtgccatctcctgtctaaa	767-718
	probe	acggagcgaggctacagctcacca	631-655
CYP1A			(<i>O. mykiss</i>) AF059711
	primer (forward)	agtgtgatggcacagaactcaa	1441-1463
	primer (reverse)	agctgacagcgctgtgtctt	1658-1639
	probe	cctcttctggctatcctgtccaaaggc	1548-1576
CYP2K1			(<i>O. mykiss</i>) AF0455053
	primer (forward)	ctcacaccaccagccgagat	1231-1250
	primer (reverse)	cttgacaaatcctccctgtcat	1394-1372
	probe	tcgtatcatcctgtaggaccgacgtcag	1330-1302
CYP2M1			(<i>O. mykiss</i>) OMU16657
	primer (forward)	gctgtatatcacactcacctgcttg	1811-1836
	primer (reverse)	cccctaagtgttgcattgatagat	2005-1980
	probe	acacctgaaacttttgcctt	1918-1897
CYP3A27			(<i>O. kisutch</i>)
	primer (forward)	tctgtgatgcccacga	
	primer (reverse)	cggtgtggactctcagagtgta	
	probe	tttctacgggcctccagcctcagttt	

Table 2

Catalytic activities of Phase I biotransformation enzymes associated with CYPs and FMO in juvenile coho salmon (mean $\pm$ SEM; $N=5-7$).

Enzyme	Associated isoform	Gills (pmol/mg protein/min)	Liver (pmol/mg protein/min)
EROD	CYP1A	1.43 $\pm$ 0.35	53.8 $\pm$ 8.1 (*)
PROD	CYP2	< 0.5	0.87 $\pm$ 0.1 (*)
6 β -hydroxytestosterone	CYP3A27	< 0.3	13.2 $\pm$ 2.7 (*)
16 β -hydroxytestosterone	CYP2K1	< 0.3	4.3 $\pm$ 1.1 (*)
thiourea S-oxidase	FMO	458 $\pm$ 50	163 $\pm$ 20 (*)

* Asterisks (*) indicate significant differences in microsomal activity levels of liver relative to gills ($P<0.05$).