Aquatic Toxicology 89 (2008) 94–102

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Identification and characterization of two novel cytosolic sulfotransferases, SULT1 ST7 and SULT1 ST8, from zebrafish

Tzu-An Liu[a,b], Shakhawat Bhuiyan[c], Rhodora Snow[d], Shin Yasuda[a], Tomoko Yasuda[a], Yuh-Shyong Yang[b], Frederick E. Williams[a], Ming-Yih Liu[a], Masahito Suiko[a], Glendora Carter[d], Ming-Cheh Liu[a,*]

[a] Department of Pharmacology, College of Pharmacy, The University of Toledo, Toledo, OH 43606, USA
[b] Department of Biological Science and Technology, College of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan, ROC
[c] Division of Arts and Sciences, Jarvis Christian College, Hawkins, TX 75765, USA
[d] School of Mathematics and Science, J. Sargeant Reynolds Community College, Richmond, VA 23285, USA

ARTICLE INFO

Article history:
Received 25 February 2008
Received in revised form 27 May 2008
Accepted 4 June 2008

Keywords:
Sulfotransferase
SULT
Sulfation
Environmental estrogen
Zebrafish

ABSTRACT

Cytosolic sulfotransferases (SULTs) constitute a family of Phase II detoxification enzymes that are involved in the protection against potentially harmful xenobiotics as well as the regulation and homeostasis of endogenous compounds. Compared with humans and rodents, the zebrafish serves as an excellent model for studying the role of SULTs in the detoxification of environmental pollutants including environmental estrogens. By searching the expressed sequence tag database, two zebrafish cDNAs encoding putative SULTs were identified. Sequence analysis indicated that these two putative zebrafish SULTs belong to the SULT1 gene family. The recombinant form of these two novel zebrafish SULTs, designated SULT1 ST7 and SULT1 ST8, were expressed using the pGEX-2TK glutathione *S*-transferase (GST) gene fusion system and purified from transformed BL21 (DE3) cells. Purified GST-fusion protein form of SULT1 ST7 and SULT1 ST8 exhibited strong sulfating activities toward environmental estrogens, particularly hydroxylated polychlorinated biphenyls (PCBs), among various endogenous and xenobiotic compounds tested as substrates. pH-dependence experiments showed that SULT1 ST7 and SULT1 ST8 displayed pH optima at 6.5 and 8.0, respectively. Kinetic parameters of the two enzymes in catalyzing the sulfation of catechin and chlorogenic acid as well as 3-chloro-4-biphenylol were determined. Developmental expression experiments revealed distinct patterns of expression of SULT1 ST7 and SULT1 ST8 during embryonic development and throughout the larval stage onto maturity.

1. Introduction

Cytosolic sulfotransferase (SULTs) constitute a group of enzymes that catalyze the transfer of a sulfonate group from the universal sulfate donor, 3′-phosphoadenosine-5′-phosphosulfate (PAPS), to acceptor substrate compounds containing hydroxyl or amino groups (Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994; Coughtrie, 2002). Such sulfation reactions, taking place in mammals and other vertebrate species, are generally thought to serve for the regulation of endogenous compounds such as steroid/thyroid hormones, catecholamine neurotransmitters, as well as the detoxification of dietary, therapeutic, and environmental xenobiotics (Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994; Coughtrie, 2002). In the latter case, sulfated products may become more water-soluble and can be more easily excreted from the body. From this perspective, the SULTs can be considered as a part of the chemical defense mechanism (Allali-Hassani et al., 2007; Gamage et al., 2006).

In recent years, environmental estrogens have been increasingly recognized as a potential hazardous factor for wildlife as well as humans (Roy et al., 1997). In general, they are able to bind to estrogen receptors and thereby mimicking estrogenic actions (Ridgway and Wiseman, 1998) or interfere with the action of enzymes that help regulate the level of endogenous estrogens (Kester et al., 2000). Some examples of this diverse group of compounds are diethylstilbestrol, bisphenol A, alkyl phenolic compounds, polychlorinated

Abbreviations: SULT, cytosolic sulfotransferase; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RT-PCR, reverse transcription-polymerase chain reaction; polychlorinated biphenyls, PCBs.

* Corresponding author at: Department of Pharmacology, College of Pharmacy, The University of Toledo, 2801 W. Bancroft Street, Toledo, OH 43606, USA.
Tel.: +1 419 530 1918; fax: +1 419 530 1909.
E-mail address: ming.liu@utoledo.edu (M.-C. Liu).

0166-445X/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2008.06.005

biphenyls (PCBs), herbicides, polystyrenes and plasticizers (Danzo, 1998). These environmental estrogens have been implicated in the malfunctioning of the reproductive system and abnormal embryonic development of wildlife, and in a worldwide debate on a decline in sperm quality in men (Carlsen et al., 1992; Auger et al., 1995) and an increased incidence of human breast cancer (Falck et al., 1992; Rogan, 2007).

Zebrafish has in recent years emerged as a popular animal model for a wide range of studies (Kari et al., 2007; Lieschke and Currie, 2007). Its advantages, compared with mouse, rat, or other vertebrate animal models, include the small size, availability of a large number of eggs, rapid development externally of virtually transparent embryos, and short generation time. These characteristics make the zebrafish an excellent model for a systematic investigation on the physiological involvement of the SULTs, including the sulfation of environmental estrogens. A prerequisite for using the zebrafish in these studies, however, is the identification of the various SULTs and their functional characterization. We have recently embarked on the molecular cloning of zebrafish SULTs. Sequence analysis via BLAST search revealed that the zebrafish SULTs we have cloned display sequence homology to mammalian SULTs. Of the ten zebrafish SULTs that have been cloned, six fall within the SULT1 gene gamily (Sugahara et al., 2003a,b; Liu et al., 2005; Yasuda et al., 2005a,b); three belong to the SULT2 gene family (Sugahara et al., 2003c; Yasuda et al., 2006); and one (designated SULT X) appears to be independent from all known SULT gene families (Sugahara et al., 2003d). The zebrafish SULT1 enzymes previously cloned and expressed displayed differential sulfating activities toward endogenous compounds including L-Dopa, dopamine, 17β-estradiol, estrone, and thyroid hormones, as well as a variety of xenobiotic phenolic compounds.

We report here the identification of two new zebrafish SULT1 enzymes, designated SULT1 ST7 and SULT1 ST8. Their enzymatic activities toward a variety of endogenous compounds and xenobiotics were examined. Kinetic parameters of the two enzymes in catalyzing the sulfation of representative substrates were determined. Moreover, their developmental expression during embryogenesis onto maturity was investigated.

2. Materials and methods

2.1. Materials

3,3′,5-Triiodo-L-thyronine (L-T_3), L-thyroxine (L-T_4), 17β-estradiol, estrone, cholesterol, dehydroepiandrosterone (DHEA), D-Dopa, L-3,4-dihydroxyphenylalanine (L-Dopa), dopamine, allopregnanolone, chlorogenic acid, kaempferol, genistein, β-naphthol, catechin, caffeic acid, daidzein, gallic acid, butylated hydroxylanisole, butylated hydroxytoluene, quercetin, myricetin, *n*-propyl gallate, *p*-nitrophenol, β-naphthylamine, acetaminophen, epicatechin, epigallocatechin gallate, mestranol, minoxidil, bisphenol A, *n*-octylphenol, *n*-nonylphenol, diethylstilbestrol (DTT), 17α-ethynylestradiol, 17β-estradiol, aprotinin, adenosine 5′-triphosphate (ATP), sodium dodecyl sulfate (SDS), sodium acetate, 2-morpholinoethanesulfonic acid (MES), 3-(*N*-morpholino)propanesulfonic acid (MOPS), *N*-2-hydroxylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 3-[*N*-tris-(hydroxymethyl)methylamino]-propanesulfonic acid (TAPS), 2-(cyclohexylamino)ethanesulfonic acid (CHES), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), reduced glutathione, and isopropyl β-D-thiogalactopyranoside (IPTG) were products of Sigma Chemical Company (St. Louis, MO). 3-Chloro-4-biphenylol and 3,3′,5,5′-tetrachloro-4,4′-biphenyldiol, both with a minimum purity of 95%, were obtained from Ultra Scientific (N. Kingstown, RI). TRI Reagent was from Molecular Research Center, Inc. (Cincinnati, OH). Unfertilized zebrafish eggs, embryos and larvae at different developmental stages were prepared by Scientific Hatcheries (Huntington Beach, CA). Total RNAs from zebrafish embryos and larvae at different developmental stages, as well as 3-month-old adult male or female fish, were isolated using the TRI Reagent, based on manufacturer's instructions. *Taq* DNA polymerase was a product of Promega Corporation (Madison, WI), and Takara *Ex Taq* DNA polymerase was from Fisher Scientific (Pittsburgh, PA). T_4 DNA ligase and Bam HI restriction endonuclease were from New England Biolab (Ipswich, MA). Oligonucleotide primers were synthesized by MWG Biotech (Huntsville, AL). pSTBlues-1 AccepTor Vector Kit and BL21 (DE3) competent cells were purchased from Novagen (Gibbstown, NJ). Prestained protein molecular mass standard was from Life Technologies (Gaithersburg, MD). First-strand cDNA Synthesis Kit, pGEX-2TK glutathione *S*-Transferase (GST) gene fusion vector, GEX-5′-and GEX-3′ sequencing primers, and glutathione-Sepharose 4B were products of Amersham Biosciences (Piscataway, NJ). Recombinant human bifunctional ATP sulfurylase/adenosine-5′-phosphosulfate kinase was prepared as described previously (Yanagisawa et al., 1998). Cellulose thin-layer chromatography (TLC) plates were products of EMD Chemicals (Gibbstown, NJ). Carrier-free sodium [^{35}S]sulfate, Ecolume scintillation cocktail, 17α-hydroxypregnenolone, 17α-hydroxyprogesterone, pregnenolone, progesterone, hydrocortisone, 4-androstene-3,17-dione, and corticosterone were from MP Biomedicals (Solon, OH). All other reagents were of the highest grades commercially available.

Table 1
Oligonucleotide primers used in the cloning and the RT-PCR analysis of zebrafish SULT1 ST7 and SULT1 ST8

Target sequence	Sense and antisense oligonucleotide primers
SULT1 ST7	
Sense	5′-CGCGGATCC**ATG**GATCTCCCAGACATATCCTCTATTAAA-3′
Antisense	5′-CGCGGATCC**TTA**AATCTTAGTGCGGAAATTGAGAGTGGT-3′
SULT1 ST8	
Sense	5′-CGCGGATCC**ATG**GCAAACCAAGACAAATCCTCTATTGAATTA-3′
Antisense	5′-CGCGGATCC**TTA**AATAATCACACAGAAGTATTAAATCTCAGT-3′
β-Actin	
Sense	5′-ATGGATGAGGAAATCGCTGCCCTGGTC-3′
Antisense	5′-TTAGAAGCACTTCCTGTGAACGATGGA-3′

Recognition sites of Bam HI restriction endonuclease in the oligonucleotides are underlined. Initiation and termination codons for translation are in bold type. The sense and antisense oligonucleotide primer sets listed were verified by BLAST Search to be specific for the zebrafish SULT1 STs or β-actin nucleotide sequence.

2.2. Cloning, bacterial expression, and purification of recombinant zebrafish SULT1 ST7 and SULT1 ST8

By searching the GenBank database, two zebrafish sequences (GenBank Accession # XM_688954 (SULT1 ST7) and AI384974 (SULT1 ST8)) encoding putative SULTs were identified. The former is a full-length coding sequence derived from an annotated genomic sequence (GenBank Accession # NW_635013), and the latter is a partial sequence covering the 5′-region of the coding sequence. A full-length cDNA clone containing the latter sequence was purchased from RZPD Deutsches Ressourcenzentrum fur Genomforchung GmbH (Berlin, Germany), and amplified, purified, and subjected to nucleotide sequencing to obtain its complete coding sequence. To subclone these two cDNAs into the pGEX-2TK prokaryotic expression vector, sense and antisense oligonucleotide primers designed based on 5′- and 3′- regions of the respective coding sequences were synthesized with Bam HI restriction site incorporated at the end (see Table 1). Using these primer sets, PCRs

were carried out under the action of *EX Taq* DNA polymerase, with the first-strand cDNA reverse-transcribed from either the total RNA of a 2-week-old zebrafish larvae (for SULT1 ST7) or the commercially obtained cDNA (for SULT1 ST8) as template. Amplification conditions were 2 min at 94 °C and 20 cycles of 94 °C for 35 s, 60 °C for 40 s, and 72 °C for 1 min. The final reaction mixtures were applied onto a 0.9% agarose gel, separated by electrophoresis, and visualized by ethidium bromide staining. The PCR product bands detected were excised from the gel, and the DNAs therein were isolated by spin filtration. Purified PCR products were subjected to Bam HI restriction and subcloned into Bam HI-restricted pGEX-2TK vector, and verified for authenticity by nucleotide sequencing (Sanger et al., 1977). To express the recombinant zebrafish SULT1 ST7 and SULT1 ST8, competent BL21 (DE3) cells transformed with pGEX-2TK harboring the cDNA encoding SULT1 ST7 or ST8 were grown in 1 L LB medium supplemented with 60 μg/ml ampicillin. After the cell density reached 0.6 $OD_{600\,nm}$, IPTG (0.1 mM final concentration) was added to induce the production of recombinant zebrafish SULT1 ST. After an overnight induction at room temperature, the cells were collected by centrifugation and homogenized in 25 ml ice-cold lysis buffer (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 1 mM EDTA) using an Aminco French Press. Twenty microlitres of 10 mg/ml aprotinin (a protease inhibitor) was added to the crude homogenate. The crude homogenate was subjected to centrifugation at 10,000 × *g* for 15 min at 4 °C. The supernatant collected was fractionated using 2.5 ml of glutathione-Sepharose, and the bound GST-SULT1 ST fusion protein was eluted with 3 ml of an elution solution containing 50 mM Tris–HCl, pH 8.0, and 10 mM reduced glutathione at 4 °C. For the preparation of GST-free SULT1 ST, the GST-SULT1 ST fusion protein bound on glutathione Sepharose was treated with 3 ml of a thrombin digestion buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 2.5 mM $CaCl_2$) containing 5 U/ml bovine thrombin at room temperature. Following a 10–15-min incubation with constant agitation, the preparation was subjected to centrifugation. The recombinant zebrafish SULT1 ST released into the supernatant and the GST-SULT1 ST fusion protein prepared as described above were analyzed by SDS-polyacrylamide gel electrophoresis (SDS–PAGE) and subjected to enzymatic characterization.

2.3. Enzymatic assay

The sulfating activity of recombinant zebrafish SULT1 ST7 or ST8 was assayed using radioactive PAP[^{35}S] as the sulfate donor. The standard assay mixture, with a final volume of 25 μl, contained 50 mM MOPS buffer at pH 7.0, 14 μM PAP[^{35}S] (15Ci/mmol), 1 mM DTT, and 50 μM substrate. Controls with DMSO or water, in place of substrate, were also prepared. The reaction was started by the addition of the enzyme, allowed to proceed for 5 min at 28 °C, and terminated by heating at 100 °C for 2 min. The precipitates formed were cleared by centrifugation, and the supernatant was subjected to the analysis of [^{35}S]sulfated product using the previously developed TLC procedure (Liu and Lipmann, 1984), with *n*-butanol/isopropanol/88% formic acid/water (3:1:1:1; by volume) as the solvent system. To examine the pH-dependence of the sulfation of chlorogenic acid by SULT1 ST7 or ST8, different buffers (50 mM sodium acetate at 4.5, 5.0, or 5.5; MES at 5.5, 6.0, or 6.5; MOPS at 6.5, 7.0, or 7.5; HEPES at 7.0, 7.5, 8.0; TAPS at 8.0, 8.5, 9.0; CHES at 9.0, 9.5. or 10.0; and CAPS at 10.0, 10.5, 11.0, or 11.5), instead of 50 mM MOPS (pH 7.0), were used in the reactions. For the kinetic studies on the sulfation of catechin (by SULT1 ST7), chlorogenic acid (by SULT1 ST8), and 3-chloro-4-biphenylol (by both SULT1 ST7 and ST8), varying concentrations (2, 2.22, 2.50, 2.86, 3.33, 4, 5 and 6.67 μM of catechin and 0.22, 0.25, 0.29, 0.33, 0.40, 0.50, 0.66 and 1 μM of 3-chloro-4-biphenylol for SULT1 ST7 and 12.5, 14.3, 16.7, 20, 25, 33.3, 50 and 100 μM of chlorogenic acid and 3.13, 3.57, 4.16, 5, 6.25, 8.33, 12.5 and 25 μM of 3-chloro-4-biphenylol for SULT1 ST8) of these substrate compounds and 50 mM MOPS buffer at pH 7.0 were used. The reactions were also carried out for 5 min at 28 °C, and terminated by heating at 100 °C for 2 min. The protein concentrations of SULT1 ST7 and ST8 (in GST fusion protein form) used in the final reaction mixtures in the kinetic studies were 0.03 and 0.08 mg/ml, respectively.

2.4. Analysis of the developmental expression of the zebrafish SULT1 ST7 and SULT1 ST8

RT-PCR was employed to investigate the developmental stage-dependent expression of the zebrafish SULT1 ST7 and SULT1 ST8. Aliquots containing 5 μg each of the total RNAs isolated from zebrafish embryos and larvae at different developmental stages as well as 3-month-old adult male or female fish were used for the synthesis of the corresponding first-strand cDNAs using the First-Strand cDNA Synthesis Kit (Amersham Bioscience). One microlitre aliquots of the 33 μl first-strand cDNA solutions prepared were used as templates for the subsequent PCR amplification. PCR reactions were carried out in 25 μl reaction mixtures using *EX Taq* DNA polymerase, in conjunction with gene-specific sense and antisense oligonucleotide primers (see Table 1). Amplification conditions were 2 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 35 s at 56 °C, and 65 s at 72 °C. The final reaction mixtures were applied onto a 0.9% agarose gel, separated by electrophoresis, and visualized by ethidium bromide staining.

2.5. Miscellaneous methods

PAP[^{35}S] was synthesized from ATP and carrier-free [^{35}S]sulfate using the bifunctional human ATP sulfurylase/APS kinase and its purity determined as previously described (Yanagisawa et al., 1998; Lin and Yang, 2000). The PAP[^{35}S] synthesized was adjusted to the required concentration and specific activity by the addition of cold PAPS. SDS-PAGE was performed on 12% polyacrylamide gels using the method of Laemmli (1970). Protein determination was based on the method of Bradford (1976) with bovine serum albumin as the standard.

3. Results

3.1. Molecular cloning of the zebrafish SULT1 ST7 and SULT ST8

By searching the GenBank database, two zebrafish sequences (GenBank Accession# XM_688954 (SULT1 ST7) and AI384974 (SULT1 ST8)) encoding putative SULTs were identified. The cDNAs encoding these two putative SULTs were amplified by RT-PCR, cloned into the pGEX-2TK prokaryotic expression vector, and subjected to nucleotide sequencing for authenticity (Sanger et al., 1977). The nucleotide sequences obtained were submitted to the GenBank database under the Accession number EU502841 for SULT1 ST7 and EU502842 for SULT1 ST8. Fig. 1 shows the alignment of the deduced amino acid sequences of the two newly identified zebrafish SULT1 STs. The open reading frames of SULT1 ST7 and SULT1 ST8 both encompass 906 nucleotides and code for 301-amino acid polypeptides. Similar to other SULTs, these two new zebrafish SULT1 STs contain sequences resembling the so-called "signature sequences" (YPKSGTxW in the N-terminal region and RKGxxGDWKNxFT in the C-terminal region; as underlined) (Weinshilboum et al., 1997). Of these two sequences, YPKSGTxW has been demonstrated by X-ray crystallography to be responsible for binding to the 5′-phosphosulfate group of PAPS, a co-substrate for SULT-catalyzed sulfation reactions (Lipmann, 1958), and thus

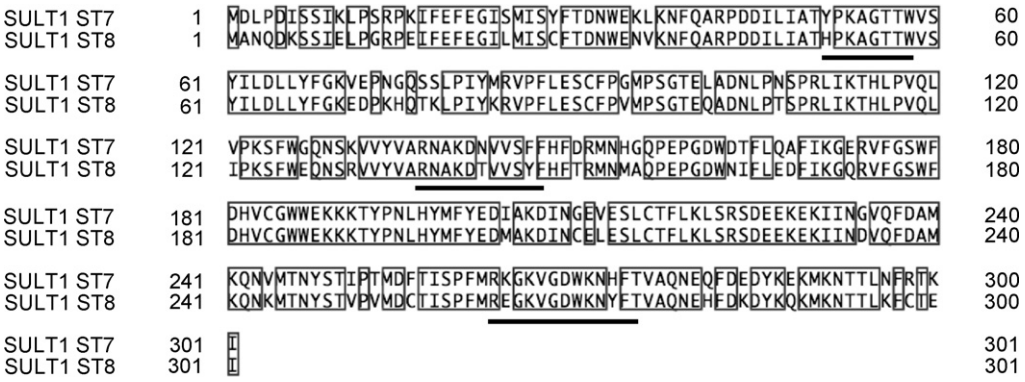

Fig. 1. Alignment of deduced amino acid sequences of the zebrafish SULT1 ST7 and SULT1 ST8. Residues conserved among these two SULT1 STs enzymes are in shaded boxes. Two "signature sequences" located, respectively, in the N-terminal and C-terminal regions, as well as a conserved sequence in the middle region are underlined.

has been designated the "5′-phosphosulfate binding (5′-PSB) motif" (Negishi et al., 2001). The two zebrafish SULT1 STs also contains the "3′-phosphate binding (3′-PB) motif" (amino acid residues 137–147; as underlined) that has been proposed to be responsible for the binding to the 3′-phosphate group of PAPS (Negishi et al., 2001). It is generally accepted that members of the same SULT gene family share at least 45% amino acid sequence identity, and members of subfamilies within each SULT gene family are greater than 60% identical in amino acid sequence (Weinshilboum et al., 1997; Nagata and Yamazoe, 2000; Blanchard et al., 2004). Sequence analysis based on a BLAST pairwise search revealed that the deduced amino acid sequence of the zebrafish SULT1 ST7 and SULT1 ST8 display 45–76% and 36–40% amino acid sequence identity to, respectively, the six zebrafish SULT1 STs (Sugahara et al., 2003a,b; Liu et al., 2005; Yasuda et al., 2005a,b) and the three SULT2 STs (Sugahara et al., 2003c; Yasuda et al., 2006) previously reported. Between the two newly identified zebrafish SULTs identified, 83% amino acid sequence identity was observed. Based on these criteria, these two zebrafish SULTs appear to belong to the SULT1 gene family, and are therefore designated the zebrafish SULT1 ST7 and SULT1 ST8 in accordance with the nomenclature used in ZFIN database (cf. the dendrogram shown in Fig. 2).

3.2. Expression, purification, and characterization of recombinant zebrafish SULT1 ST7 and SULT1 ST8

pGEX-2TK harboring zebrafish SULT1 ST7 or ST8 cDNA was transformed into BL21 (DE3) cells for the expression of the recombinant protein. As shown in Fig. 3, the GST fusion protein form of recombinant zebrafish SULT1 ST7 or ST8, purified from the *E. coli* extract, migrated at ca. 60 kDa position upon SDS-PAGE. Upon thrombin digestion to cut out the GST moiety, the free zebrafish

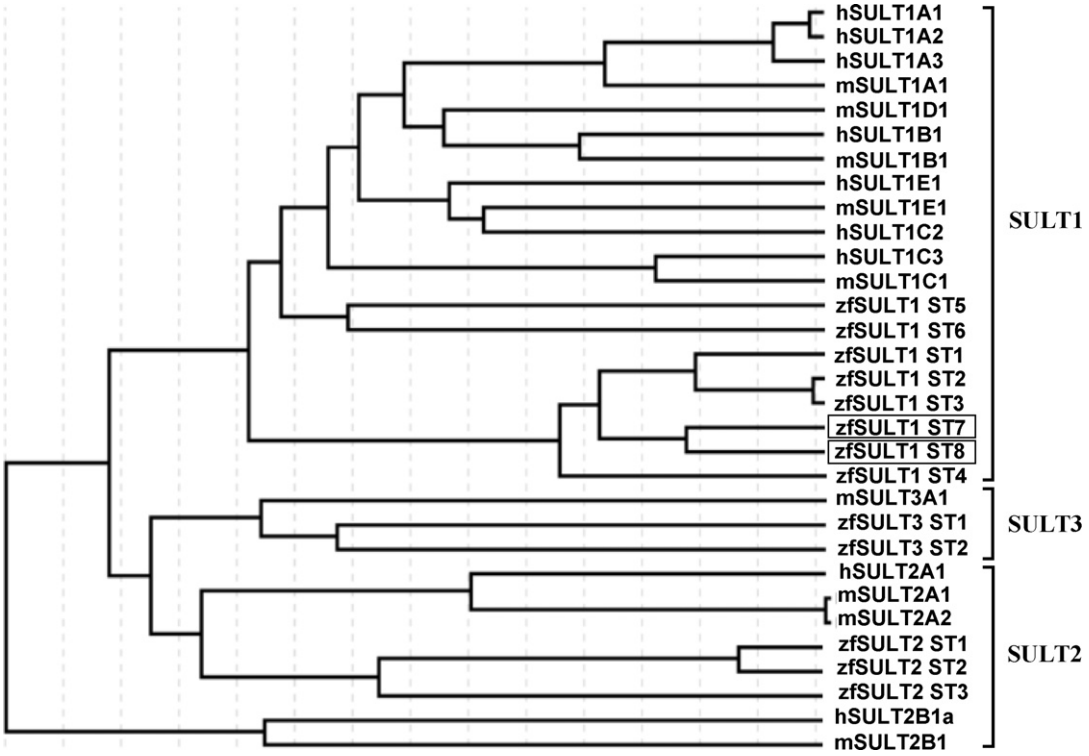

Fig. 2. Classification of the zebrafish SULT1 ST7 and SULT1 ST8 on the basis of their amino acid sequences. The dendrogram shows the degree of amino acid sequence homology among different SULTs. For references for individual SULTs, see the review by Blanchard et al. (2004). h, human; m, mouse; and zf, zebrafish. The dendrogram was generated based on Greedy algorithm (Brodskiĭ et al., 1995; Nikolaev et al., 1997).

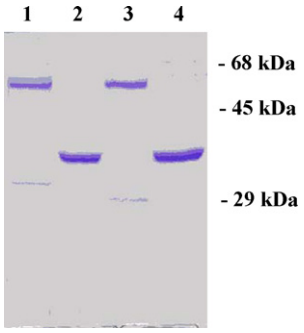

Fig. 3. SDS gel electrophoretic pattern of the purified recombinant zebrafish SULT1 ST7 and ST8. Purified zebrafish SULT samples were subjected to SDS-PAGE on a 12% gel, followed by Coomassie blue staining. Samples analyzed in lane 1 and 2 were, respectively, GST-fusion protein and thrombin-digested forms of SULT1 ST7. Samples in lane 3 and 4 were GST-fusion protein and thrombin-digested forms of SULT1 ST8. Positions of the protein molecular weight markers co-electrophoresed were: carbonic anhydrase (M_r = 29,000), ovalbumin (M_r = 45,000), and bovine serum albumin (M_r = 68,000).

SULT1 ST7 and ST8 both migrated as ~35 kD proteins. Preliminary experiments showed that the thrombin-digested SULT1 ST7 and ST8 exhibited lower and unstable sulfating activity in comparison with the GST-fusion protein form of these two enzymes (data not shown). Therefore, the GST-fusion protein form of both zebrafish SULT1 ST7 and ST8 was used for the enzymatic characterization. A pilot experiment first revealed that the SULT1 ST7 and ST8 exhibited strong activities toward chlorogenic acid. pH-dependence experiments subsequently performed showed that pH optima of SULT1 ST7 and SULT1 ST8 with chlorogenic acid as substrate were, respectively, 6.5 and 8.0 (Fig. 4). A number of endogenous and xenobiotic compounds were tested as substrates for these two enzymes, and the activity data obtained are compiled in Table 2. Based on the molecular mass of the GST moiety (25,499 Daltons) and those of the GST-SULT1 fusion proteins (60,531 Daltons for ST7 and 60,892 Daltons for ST8), correction factors of 1.728 and 1.720 were used in the calculation of specific activities of SULT1 ST7 and ST8, respectively. Among the compounds we tested, the zebrafish SULT1 ST7 and ST8 displayed strongest sulfating activities toward catechin (at 9.89 ± 1.07 nmol/min/mg enzyme) and chlorogenic acid (at 1.59 ± 0.11 nmol/min/mg enzyme), respectively. In addition, SULT1 ST7 also showed sulfating activities toward kaempferol, genistein, β-naphthol, caffeic acid, daidzein, quercetin, *n*-propyl gallate, β-naphthylamine, acetaminophen, epigallocatechin gallate, chlorogenic acid; and SULT1 ST8 displayed activities toward kaempferol, genistein, β-naphthol, caffeic acid, daidzein, quercetin, *n*-propyl gallate, epicatechin, epigallocatechin gallate. Interestingly, neither SULT1 ST7 nor SULT1 ST8 exhibited detectable activities toward endogenous compounds including 3,3′5-triiodo-L-thyronine (L-T_3), L-thyroxine (L-T_4), D-Dopa, L-Dopa, dopamine, 17β-estradiol, estrone, 4-androstene-3, 17-dione, chlolesterol, corticosterone, DHEA, hydrocortisone, 17α-hydroxypregnenolone, 17α-hydroxyprogesterone, pregnenolone, progesterone, allopregnanolone, and other exogenous compounds including gallic acid, butylated hydroxylanisole, butylated hydroxytoluene, myricetin, *p*-nitrophenol, mestranol, and minoxidil. SULT1 ST7 and SULT1 ST8 were also assayed for sulfating activity towards some environmental estrogens including bisphenol A, *n*-octylphenol, *n*-nonylphenol, diethylstilbestrol, 17α-ethynylestradiol, 17β-estradiol and hydroxylated PCBs. The results compiled in Table 3 indicated that

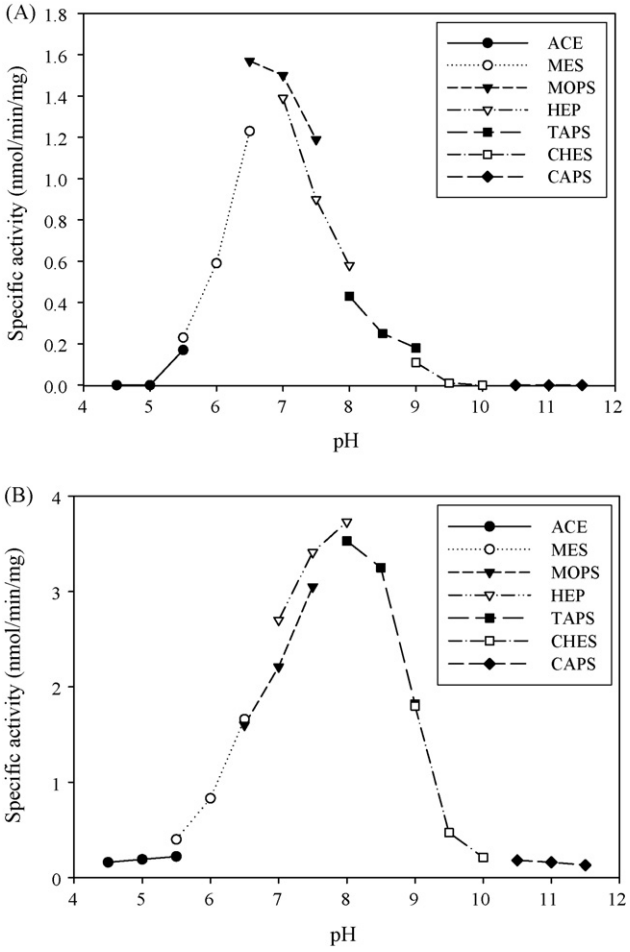

Fig. 4. pH dependency of the sulfating activity of the zebrafish SULT1 ST7 (A) and SULT1 ST8 (B) with chlorogenic acid as substrate. The enzymatic assays with 50 μM chlorogenic acid as substrate were carried out under standard assay conditions as described under Section 2, using different buffer systems as indicated. The data represent calculated mean values derived from three experiments.

Table 2
Specific activities of zebrafish SULT1 ST7 and SULT1 ST8 with xenobiotics as substrates

Compound tested as substrate	Specific activity (nmol min^{-1} mg^{-1})[a]	
	SULT1 ST7	SULT1 ST8
Acetaminophen	1.09 ± 0.02	ND[b]
Butylated hydroxylanisole	ND	ND
Butylated hydroxytoluene	ND	ND
Caffeic acid	0.46 ± 0.04	ND
Catechin	9.89 ± 1.07	0.65 ± 0.11
Chlorogenic acid	1.50 ± 0.27	1.59 ± 0.11
Daidzein	0.99 ± 0.03	0.11 ± 0.03
Epicatechin	ND	0.51 ± 0.10
Epigallocatechin gallate	2.17 ± 0.88	1.32 ± 0.09
Gallic acid	ND	ND
Genistein	0.71 ± 0.04	0.16 ± 0.01
Kaempferol	0.74 ± 0.12	0.79 ± 0.08
Mestranol	ND	ND
Minoxidil	ND	ND
Myricetin	ND	ND
β-Naphthol	1.38 ± 0.27	0.17 ± 0.02
β-Naphthylamine	0.12 ± 0.01	ND
p-Nitrophenol	ND	ND
n-Propyl gallate	4.55 ± 0.11	0.16 ± 0.01
Quercetin	1.50 ± 0.27	0.78 ± 0.06

[a] Specific activity refers to nmol substrate sulfated min^{-1} mg^{-1} purified enzyme. Data represent means ± S.D. derived from three experiments.

[b] ND, specific activity determined is lower than the detection limit (estimated to be ≈0.01 nmol min^{-1} mg protein^{-1}).

Table 3
Specific activities of the zebrafish SULT1 ST7 and SULT1 ST8 with representative environmental estrogens as substrates

Environmental estrogen	Specific activity (nmol min^{-1} mg^{-1})[a]	
	SULT1 ST7	SULT1 ST8
Bisphenol A	ND[b]	ND
n-Octylphenol	0.20 ± 0.06	ND
n-Nonylphenol	ND	ND
Diethylstilbestrol	ND	ND
17α-Ethynylestradiol	ND	ND
3-Chloro-4-biphenylol	1.85 ± 0.21	3.65 ± 0.14
3,3′,5,5′-Tetrachloro-4,4′-biphenyldiol	1.82 ± 0.04	2.81 ± 0.04
17β-Estradiol	ND	ND
Dehydroepiandrosterone	ND	ND

[a] Data represent means ± S.D. derived from three experiments.
[b] ND, specific activity determined is lower than the detection limit (estimated to be ~0.01 nmol min^{-1} mg protein^{-1}).

both SULT1 ST7 and SULT1 ST8 exhibited the strongest sulfating activities toward the two representative hydroxylated PCBs (3-chloro-4-biphenylol and 3,3′,5,5′-tetrachloro-4,4′-biphenyldiol) tested as substrates.

To further investigate the enzymatic characteristics of SULT1 ST7 and ST8, the kinetic parameters of these two enzymes in catalyzing the sulfation of an environmental estrogen, 3-chloro-4-biphenylol, and two xenobiotics compounds, catechin and chlorogenic acid were examined. In these experiments, varying concentrations of these compounds were used in the assays. Data obtained were processed using the SigmaPlot to generate the best fitting trend-lines for the Lineweaver-Burk double-reciprocal plots (Fig. 5). The kinetic constants shown in the figure revealed that, while the V_{max} values of SULT1 ST7 and ST8 toward the substrates tested were comparable, the K_m values of SULT1 ST8 were an order of magnitude higher than those of SULT1 ST7. Based on calculated V_{max}/K_m values, both SULT1 ST7 and SULT1 ST8 appeared to be catalytically more efficient with 3-chloro-4-biphenylol as substrate than with catechin or chlorogenic acid as substrate.

3.3. Developmental stages-dependent expression of the zebrafish SULT1 ST7 and SULT1 ST8

The developmental stages-dependent expression of the zebrafish SULT1 ST7 and ST8 were examined. As shown in Fig. 6A, the mRNA encoding SULT1 ST7 was not detected in unfertilized eggs and in embryos during the early phase of embryonic development. An initial expression of the SULT1 ST7 mRNA was observed at the hatching period (48 h) and selectively expressed in the larval stages (1–2-week-old larvae). Throughout the post-larvae stage onto early maturity, however, no message encoding SULT1 ST7 could be detected. Interestingly, a significant level of its coding message was again expressed in adult female, but not male zebrafish. For SULT1 ST8, no expression was detected in unfertilized eggs and during entire embryogenesis. A significant level of expression appeared in 1-week-old larvae and, intriguingly, gradually decreased in 2- and 3-week-old larvae, and disappeared

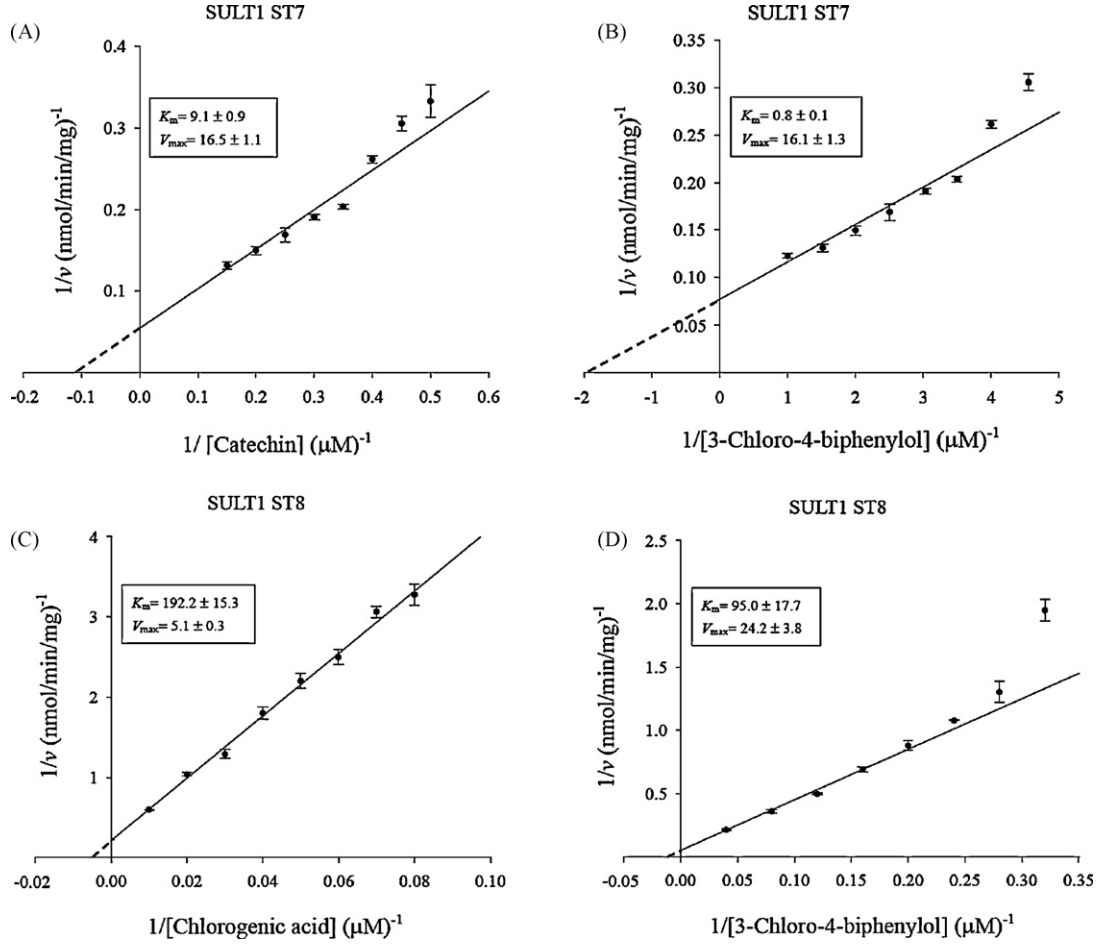

Fig. 5. Lineweaver-Burk double-reciprocal plots of (A) the sulfation of catechin by SULT1 ST7, (B) the sulfation of 3-chloro-4-biphenyl by SULT1 ST7, (C) the sulfation of chlorogenic acid by SULT1 ST8, and (D) the sulfation of 3-chloro-4-biphenyl by SULT1 ST8. Concentrations of the substrates used are expressed in μM and velocities are expressed as nmol of product formed/min/mg enzyme. Each data point represents the mean value derived from three determinations.

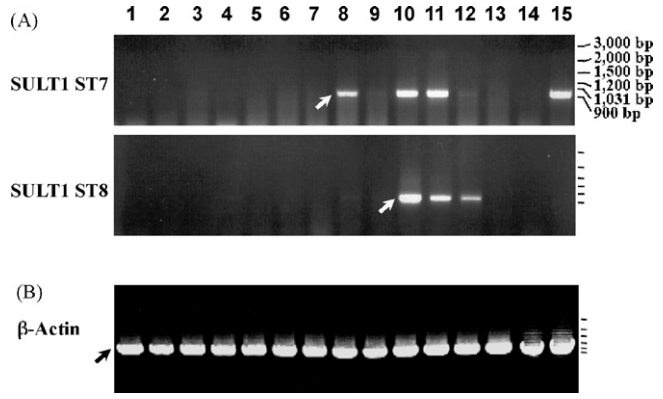

Fig. 6. Developmental stage-dependent expression of zebrafish SULTs. (A) RT-PCR analysis of the expression of mRNAs encoding SULT1 ST7 and SULT1 ST8 at different stages during embryogenesis and larval development onto maturity. Final PCR mixtures were subjected to 2% agarose electrophoresis. Samples analyzed correspond to unfertilized zebrafish eggs (lane 1), zebrafish embryos during the zygote period (0-h post-fertilization (pf); lane 2), cleavage period (1-h pf; lane 3), blastula period (3-h pf; lane 4), gastrula period (6-h pf; lane 5), neurula/segmentation period (12-h pf; lane 6), pharyngula period (24-h pf; lane 7), and hatching period (48- and 72-h pf; lane 8, 9), 1, 2, 3, 4-week-old zebrafish larvae (lane 10, 11, and 12, 13), and 3-month-old adult male or female zebrafish (lane 14, 15). Positions of the DNA size markers co-electrophoresed are indicated on the right. The PCR products corresponding to the zebrafish SULT1 ST7 or ST8 cDNAs, visualized by ethidium bromide staining, are marked by arrows. (B) RT-PCR analysis of the expression of the zebrafish β-actin at the same developmental stages as those described in (A). The figure is illustrative of three independent repetitions.

thereafter throughout the juvenile unto maturity. In contrast to the developmental stage-dependent expression of the SULT1 ST7 and ST8, β-actin, a house keeping protein, was found to be expressed throughout the entire developmental process (Fig. 6B).

4. Discussion and conclusion

The current study is part of an overall effort to establish a zebrafish model for systematic studies on the ontogeny, cell type/tissue/organ-specific distribution, as well as the physiological involvement of the SULTs, a group of Phase II detoxifying enzymes commonly found among vertebrates (Nowell and Falany, 2006). A prerequisite for using zebrafish in these studies is the identification of the various SULTs and their biochemical characterization. We have recently embarked on the molecular cloning of zebrafish SULTs (Sugahara et al., 2003a,b,c,d; Liu et al., 2005; Yasuda et al., 2005a,b, 2006). Sequence analysis via BLAST search revealed that the zebrafish SULTs we have cloned display sequence homology to mammalian SULTs. Of the ten zebrafish SULTs that have been cloned, six fall within the SULT1 gene family (Sugahara et al., 2003a,b; Liu et al., 2005; Yasuda et al., 2005a,b), three belongs to the SULT2 gene family (Sugahara et al., 2003c; Yasuda et al., 2006), and one appears to be independent from all known SULT gene families (Sugahara et al., 2003d). In this study, we have identified two new SULT1 STs, SULT1 ST7 and SULT1 ST8, and demonstrated that both SULT1 ST7 and SULT1 ST8 displayed sulfating activities toward xenobiotic compounds, particularly hydroxylated PCBs and other environmental chemicals that have been reported to be capable of exerting estrogenic effects in humans as well as wildlife (Safe, 1994). In the enzymatic characterization, SULT1 ST7 and SULT1 ST8, with representative substrates, displayed pH optima at 6.5 and 8.0, respectively. Kinetic parameters of the two enzymes in catalyzing the sulfation of catechin and chlorogenic acid as well as 3-chloro-4-biphenylol were determined. It should be pointed out that SULT1-like enzymes have also been reported to be present in other fish species (Tong and James, 2000; Assem et al., 2006; Martin-Skilton et al., 2006; Wang and James, 2007). These SULT1-like enzymes exhibited differential sulfating activities toward various endogenous as well as xenobiotic compounds. For some of them, endocrine disruptors including PCBs have been shown to exert inhibitory effects on their sulfating activities (van den Hurk et al., 2002; Wang and James, 2007).

As mentioned in Section 1, environmental-estrogen-like chemicals have been implicated in the developmental abnormality of wildlife (Guillette et al., 1995; Fry, 1995), as well as pathophysiologic conditions of humans (Carlsen et al., 1992; Auger et al., 1995; Falck et al., 1992; Rogan, 2007). Prominent among these environmental estrogens are the PCBs (Ulbrich and Stahlmann, 2004). PCBs are a group of the halogenated aromatic hydrocarbons which are among the most persistent and widespread environmental estrogens (Safe, 1994; Pocar et al., 2006). Because of their chemical stability, lipophilic property and resistance to degradation, PCBs are making their way into all levels of food chain and preferentially bioaccumulate and biomagnify in wildlife as well as humans (McFarland and Clarke, 1989; Buckman et al., 2006). Several studies have demonstrated that the accumulation of PCBs may affect the reproductive system in fish populations (Örn et al., 1998; Mac et al., 1993; Hansen et al., 1985). Exposure of high level of PCBs in aquatics have been shown to lead to decreased hatching success, impairment of egg development, a decline of the larvae survival, and inhibition of spermatogenesis and various testicular abnormalities (Freeman et al., 1982; Sangalang et al., 1981). Although the PCB-induced toxicity has been extensively investigated, both the metabolic mechanism and ultimate elimination of PCB and its metabolites in aquatic species remain poorly understood. It should be pointed out that PCBs have been shown to be subjected to biotransformation by cytochrome P-450-mediated hydroxylation in vertebrates (Borlakoglu and Wilkins, 1993; Henriksen et al., 2000; Buckman et al., 2006). Hydroxylated PCBs have also been found in a number of fish species and may be derived from the biotransformation of individual PCB congeners by the Phase I enzymes, particularly cytochrome P-450 (Vega-López et al., 2007; Mortensen et al., 2007). In the present study, two new zebrafish SULTs, SULT1 ST7 and SULT1 ST8, were identified and shown to exhibit strong sulfating activities toward the two representative hydroxylated PCBs, 3-chloro-4-biphenylol and 3,3′,5,5′-tetrachloro-4,4′-biphenyldiol. That both enzymes displayed sulfating activities toward hydroxychlorobiphenyls may imply the utilization of sulfation as a means for the inactivation/disposal of hydroxylated PCBs in zebrafish. It is worthwhile pointing out that, in our previous study, two other zebrafish SULTs, SULT1 ST1 and SULT1 ST2, also exhibited differential activities toward hydroxylated PCBs (Sugahara et al., 2003a). In that study, metabolic sulfation of representative hydroxylated PCBs was also demonstrated using cultured zebrafish liver cells (Sugahara et al., 2003a). Whether sulfation truly poses a physiological involvement with regard to the metabolic elimination of hydroxylated PCBs in zebrafish will be an interesting and important issue to clarify.

The adverse effects caused by the halogenated aromatic hydrocarbons such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and PCBs may depend on the developmental stage of the affected animal (Elonen et al., 1998; Ankley and Johnson, 2004). These environmental contaminants pose particularly hazardous effects to fish, especially during the early stage of their development (Matta et al., 1997; Powell et al., 2000; Elonen et al., 1998; Toomey et al., 2001; Walker et al., 1991). Embryonic exposure to PCBs or complex mixtures of congeners had been shown to result in edema, hemorrhage, craniofacial deformity, pathological alterations, and significant mortality in newly hatched fish (Spitsbergen et al., 1991; Walker et al., 1991; Walker and Peterson, 1991; Walker et al., 1992; Toomey et al., 2001).

An increasing volume of evidence has demonstrated that PCB-induced toxicity is highly dependent on the developmental stage and structural and functional maturation of fish species (Matta et al., 1997; Örn et al., 1998; Powell et al., 2000; Mac et al., 1993; Monosson et al., 1994). Whether developing fish embryos or larvae are equipped with mechanisms, in particular detoxifying enzymes such as SULTs, for counteracting or eliminating PCBs, however, had remained unresolved. The developmental stage-dependent expression of zebrafish SULT1 ST7 and ST8 as revealed in this study may imply the use of sulfation as a mechanism for the inactivation and/or elimination of PCBs, following their cytochrome P-450-mediated hydroxylation, by developing zebrafish. An initial expression of SULT1 ST7 was observed during the hatching period when primary organogenesis including early maturation of organ rudiments, protrusion of mouth, and cartilage development in the jaw primordia are nearly complete. This suggested the capacity of hatched larvae in metabolizing PCBs before the exogenous feeding commences and before the gut and endodermal organs are fully developed (Falk-Petersen, 2005). During larval development, the prominent abundance of SULT1 ST7 and ST8 were observed, which then disappeared thereafter throughout the juvenile onto maturity. Interestingly, a significant level of SULT1 ST7-coding message was again expressed in adult female, but not male, zebrafish. The significance of this latter finding awaits further clarification. Collectively, these data may nevertheless imply the physiological involvement of SULT1 ST7 and ST8 in counteracting PCB-induced adverse effects in developing zebrafish embryo/larva and in maintaining the normal functioning of reproductive system of female zebrafish in adulthood (Danzo, 1998; Toppari, 2002).

In conclusion, we have identified two novel xenobiotic-sulfating SULT1 STs, SULT1 ST7 and ST8, which may play a role in the metabolism of environmental estrogens including hydroxylated PCBs. This study is part of an overall effort to obtain a complete repertoire of the SULT enzymes present in zebrafish. As pointed out earlier, the identification of the various SULTs and their biochemical characterization is a prerequisite for using the zebrafish as a model for a systematic investigation on the physiological relevance of SULTs in the detoxification of environmental xenobiotics. More work is warranted in order to achieve this goal.

Acknowledgements

This work was supported in part by a grant (#0542235) from the National Science Foundation and a startup fund from College of Pharmacy, The University of Toledo.

References

Allali-Hassani, A., Pan, P.W., Dombrovski, L., Najmanovich, R., Tempel, W., Dong, A., Loppnau, P., Martin, F., Thonton, J., Edwards, A.M., Bochkarev, A., Plotnikov, A.N., Vedadi, M., Arrowsmith, C.H., 2007. Structural and chemical profiling of the human cytosolic sulfotransferases. PLoS Biol. 5, 1063–1097.

Ankley, G.T., Johnson, R.D., 2004. Small fish models for identifying and assessing the effects of endocrine-disrupting chemicals. ILAR J. 45, 469–483.

Assem, F.L., Kirk, C.J., Chipman, J.K., 2006. Substrate characterisation of a recombinant sulfotransferase SULT1 and mRNA expression in chub (*Leuciscus cephalus*) tissues. Biochem. Biophys. Res. Commun. 349, 900–905.

Auger, J., Kunstmann, J.M., Czyglik, F., Jouannet, P., 1995. Decline in semen quality among fertile men in Paris during the past 20 years. N. Engl. J. Med. 332, 281–285.

Blanchard, R.L., Freimuth, R.R., Buck, J., Weinshilboum, R.M., Coughtrie, M.W., 2004. A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. Pharmacogenetics 14, 199–211.

Borlakoglu, J.T., Wilkins, J.P., 1993. Metabolism of di-, tri-, tetra-, penta- and hexachlorobiphenyls by hepatic microsomes isolated from control animals and animals treated with Aroclor 1254, a commercial mixture of polychlorinated biphenyls (PCBs). Comp. Biochem. Physiol. C. 105, 95–106.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brodskiĭ, L.I., Ivanov, V.V., Kalaĭdzidis, Ia. L., Leontovich, A.M., Nikolaev, V.K., Feranchuk, S.I., Drachev, V.A., 1995. GeneBee-NET: Internet-based server for analyzing biopolymers structure. Biochemistry (Moscow) 60, 923–928.

Buckman, A.H., Wong, C.S., Chow, E.A., Brown, S.B., Solomon, K.R., Fisk, A.T., 2006. Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish. Aquat. Toxicol. 78, 176–185.

Carlsen, E., Giwercman, A., Keiding, N., Skakkebaek, N.E., 1992. Evidence for decreasing quality of semen during past 50 years. BMJ 305, 609–613.

Coughtrie, M.W., 2002. Sulfation through the looking glass—recent advances in sulfotransferase research for the curious. Pharmacogenom. J. 2, 297–308.

Danzo, B.J., 1998. The effects of environmental hormones on reproduction. Cell. Mol. Life Sci. 54, 1249–1264.

Elonen, G.E., Spehar, R.L., Holcombe, G.W., Johnson, R.D., Fernandez, J.D., Tietge, J.E., Cook, P.M., 1998. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to seven freshwater species during early-life-stage development. Environ. Toxicol. Chem. 17, 472–483.

Falany, C., Roth, J.A., 1993. Properties of human cytosolic sulfotransferase involved in drug metabolism. In: Jeffery, E.H. (Ed.), Human Drug Metabolism: From Molecular Biology to Man. CRC Press, Boca Raton, pp. 101–115.

Falck, F., Ricci, A., Wolff, M.S., Godbold, J., Deckers, P., 1992. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. Arch. Environ. Health 47, 143–146.

Falk-Petersen, I.B., 2005. Comparative organ differentiation during early life stages of marine fish. Fish Shellfish Immunol. 19, 397–412.

Freeman, H.C., Sangalang, G., Flemming, B., 1982. The sublethal effects of a polychlorinated biphenyl (Aroclor 1254) diet on the Atlantic cod (*Gadus morhua*). Sci. Tot. Environ. 24, 1–11.

Fry, D.M., 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ. Health Perspect. 103, 165–171.

Gamage, N., Barnett, A., Hempel, N., Duggleby, R.G., Windmill, K.F., Martin, J.L., McManus, M.E., 2006. Human sulfotransferases and their role in chemical metabolism. Toxicol. Sci. 90, 5–22.

Guillette, L.J., Gross, T.S., Gross, D., Rooney, A.A., Percival, H.F., 1995. Gonadal steroidogenesis in vitro from juvenile alligators obtained from contaminated and control lakes. Environ. Health Perspect. 103, 31–36.

Hansen, P.D., von Westernhagen, H., Rosenthal, H., 1985. Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. Mar. Environ. Res. 15, 59–76.

Henriksen, E.O., Gabrielsen, G.W., Trudeau, S., Wolkers, J., Sagerup, K., Skaare, J.U., 2000. Organochlorines and possible biochemical effects in glaucous gulls (*Larus hyperboreus*) from Bjørnøya, the Barents Sea. Arch. Environ. Contam. Toxicol. 38, 234–243.

Kari, G., Rodeck, U., Dicker, A.P., 2007. Zebrafish: an emerging model system for human disease and drug discovery. Clin. Pharmacol. Ther. 82, 70–80.

Kester, M.H.A., Bulduk, S., Tibboel, D., Meinl, W., Glatt, H., Falany, C.N., Coughtrie, M.W.H., Bergman, A., Safe, S.H., Kuiper, G.G., Schuur, A.G., Brouwer, A., Visser, T.J., 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: A novel pathway explaining the estrogenic activity of PCBs. Endocrinology 141, 1897–1900.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lieschke, G.J., Currie, P.D., 2007. Animal models of human disease: zebrafish swim into view. Nat. Rev. Genet. 8, 353–367.

Lin, E.S., Yang, Y.S., 2000. Nucleotide binding and sulfation catalyzed by phenol sulfotransferase. Biochem. Biophys. Res. Commun. 271, 818–822.

Lipmann, F., 1958. Biological sulfate activation and transfer. Science 128, 575–580.

Liu, M.C., Lipmann, F., 1984. Decrease of tyrosine-*O*-sulfate-containing proteins found in rat fibroblasts infected with Rous sarcoma virus or Fujinami sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. 81, 3695–3698.

Liu, M.Y., Yang, Y.S., Sugahara, T., Yasuda, S., Liu, M.C., 2005. Identification of a novel zebrafish SULT1 cytosolic sulfotransferase: cloning, expression, characterization, and developmental expression study. Arch. Biochem. Biophys. 437, 10–19.

Mac, M.J., Schwartz, T.R., Edsall, C.C., Frank, M.F., 1993. Polychlorinated biphenyls in Great Lakes lake trout and their eggs: relations to survival and congener composition 1979–1988. J. Great Lakes Res. 19, 752–765.

Martin-Skilton, R., Coughtrie, M.W., Porte, C., 2006. Sulfotransferase activities towards xenobiotics and estradiol in two marine fish species (*Mullus barbatus* and *Lepidorhombus boscii*): characterization and inhibition by endocrine disrupters. Aquat. Toxicol. 79, 24–30.

Matta, M.B., Cairncross, C., Kocan, R.M., 1997. Effect of a polychlorinated biphenyl metabolite on early life stage survival of two species of trout. Bull. Environ. Contam. Toxicol. 59, 146–151.

McFarland, V.A., Clarke, J.U., 1989. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ. Health Perspect. 81, 225–239.

Monosson, E., Fleming, W.J., Sullivan, C.V., 1994. Effects of the planar PCB 3,38,4,48-tetrachlorobiphenyl (TCB) on ovarian development, plasma levels of sex steroid hormones and vitellogenin, and progeny survival in the white perch (*Morone americana*). Aquat. Toxicol. 29, 1–19.

Mortensen, A.S., Braathen, M., Sandvik, M., Arukwe, A., 2007. Effects of hydroxy-polychlorinated biphenyl (OH-PCB) congeners on the xenobiotic biotransformation gene expression patterns in primary culture of Atlantic salmon (*Salmo salar*) hepatocytes. Ecotoxicol. Environ. Saf. 68, 351–360.

Mulder, G.J., Jakoby, W.B., 1990. Sulfation. In: Mulder, G.J. (Ed.), Conjugation Reactions in Drug Metabolism. Taylor and Francis, London, pp. 107–161.
Nagata, K., Yamazoe, Y., 2000. Pharmacogenetics of sulfotransferase. Annu. Rev. Pharmacol. Toxicol. 40, 159–176.
Negishi, M., Pedersen, L.G., Petrotchenko, E., Shevtsov, S., Gorokhov, A., Kakuta, Y., Pedersen, L.C., 2001. Structure and function of sulfotransferases. Arch. Biochem. Biophys. 390, 149–157.
Nikolaev, V.K., Leontovich, A.M., Drachev, V.A., Brodsky, L.I., 1997. Building multiple alignment using iterative analyzing biopolymers structure dynamic improvement of the initial motif alignment. Biochemistry (Moscow) 62, 578–582.
Nowell, S., Falany, C.N., 2006. Pharmacogenetics of human cytosolic sulfotransferases. Oncogene 25, 1673–1678.
Örn, S., Andersson, P.L., Förlin, L., Tysklind, M., Norrgren, L., 1998. The impact on reproduction of an orally administered mixture of selected PCBs in zebrafish (*Danio rerio*). Arch. Environ. Contam. Toxicol. 35, 52–57.
Pocar, P., Brevini, T.A., Antonini, S., Gandolfi, F., 2006. Cellular and molecular mechanisms mediating the effect of polychlorinated biphenyls on oocyte in vitro maturation. Reprod. Toxicol. 22, 242–249.
Powell, W.H., Bright, R., Bello, S.M., Hahn, M.E., 2000. Developmental and tissue-specific expression of AHR1, AHR2, and ARNT2 in dioxin-sensitive and -resistant populations of the marine fish Fundulus heteroclitus. Toxicol. Sci. 57, 229–239.
Ridgway, T.J., Wiseman, H., 1998. Removal of oestrogens and oestrogen mimics from the environment. Biochem. Soc. Trans. 26, 675–680.
Rogan, E., 2007. Xenoestrogens, biotransformation, and differential risks for breast cancer. Altern. Ther. Health Med. 13, S112–121.
Roy, D., Palangat, M., Chen, C.W., Thomas, R.D., Colerangle, J., Atkinson, A., Yan, Z.J., 1997. Biochemical and molecular changes at the cellular level in response to exposure to environmental estrogen-like chemicals. J. Toxicol. Environ. Health 50, 1–29.
Safe, S.H., 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit. Rev. Toxicol. 24, 87–149.
Sangalang, G.B., Freeman, H.C., Crowell, R., 1981. Testicular abnormalities in cod (*Gadus morhua*) fed Aroclor 1254. Arch. Environ. Contam. Toxicol. 10, 617–626.
Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74, 5463–5467.
Spitsbergen, J.M., Walker, M.K., Olson, J.R., Peterson, R.E., 1991. Pathological alterations in early life stages of lake trout, Salvelinus namaycush, exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin as fertilized eggs. Aquat. Toxicol. 19, 41–72.
Sugahara, T., Liu, C.C., Pai, T.G., Collodi, P., Suiko, M., Sakakibara, Y., Nishiyama, K., Liu, M.C., 2003a. Sulfation of hydroxychlorobiphenyls. Molecular cloning, expression, and functional characterization of zebrafish SULT1 sulfotransferases. Eur. J. Biochem. 70, 2404–2411.
Sugahara, T., Liu, C.C., Pai, T.G., Collodi, P., Suiko, M., Sakakibara, Y., Nishiyama, K., Liu, M.C., 2003b. cDNA cloning, expression, and functional characterization of a zebrafish SULT1 cytosolic sulfotransferase. Arch. Biochem. Biophys. 414, 67–73.
Sugahara, T., Yang, Y.S., Liu, C.C., Pai, T.G., Liu, M.C., 2003c. Sulphonation of dehydroepiandrosterone and neurosteroids: molecular cloning, expression, and functional characterization of a novel zebrafish SULT2 cytosolic sulphotransferase. Biochem. J. 375, 785–791.
Sugahara, T., Liu, C.C., Pai, T.G., Liu, M.C., 2003d. Molecular cloning, expression, and functional characterization of a novel zebrafish cytosolic sulfotransferase. Biochem. Biophys. Res. Commun. 300, 725–730.
Tong, Z., James, M.O., 2000. Purification and characterization of hepatic and intestinal phenol sulfotransferase with high affinity for benzo[a]pyrene phenols from channel catfish, *Ictalurus punctatus*. Arch. Biochem. Biophys. 376, 409–419.
Toomey, B.H., Bello, S., Hahn, M.E., Cantrell, S., Wright, P., Tillitt, D., DiGiulio, R.T., 2001. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces apoptotic cell death and cytochrome P4501A expression in developing Fundulus heteroclitus embryos. Aquat. Toxicol. 53, 127–138.
Toppari, J., 2002. Environmental endocrine disrupters and disorders of sexual differentiation. Semin. Reprod. Med. 20, 305–312.
Ulbrich, B., Stahlmann, R., 2004. Developmental toxicity of polychlorinated biphenyls (PCBs): a systematic review of experimental data. Arch. Toxicol. 78, 252–268.
van den Hurk, P., Kubiczak, G.A., Lehmler, H.J., James, M.O., 2002. Hydroxylated polychlorinated biphenyls as inhibitors of the sulfation and glucuronidation of 3-hydroxy-benzo[a]pyrene. Environ. Health Perspect. 110, 343–348.
Vega-López, A., Martínez-Tabche, L., Martínez, M.G., 2007. Toxic effects of waterborne polychlorinated biphenyls and sex differences in an endangered goodeid fish (*Girardinichthys viviparus*). Environ. Int. 33, 540–545.
Walker, M.K., Spitsbergen, J.M., Olson, J.R., Peterson, R.E., 1991. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). Can. J. Fish Aquat. Sci. 48, 875–883.
Walker, M.K., Peterson, R.E., 1991. Potencies of polychlorinated dibenzo-p-dioxin, dibenzofuran, and biphenyl congeners, relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin, for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 21, 219–238.
Walker, M.K., Hufnagle, L.C., Clayton, M.K., Peterson, R.E., 1992. An egg injection method for assessing early life stage mortality of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 22, 15–38.
Wang, L.Q., James, M.O., 2007. Sulfonation of 17beta-estradiol and inhibition of sulfotransferase activity by polychlorobiphenylols and celecoxib in channel catfish, Ictalurus punctatus. Aquat. Toxicol. 81, 286–292.
Weinshilboum, R.M., Otterness, D.M., 1994. Sulfotransferase enzymes. In: Kaufmann, F.C. (Ed.), Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity. Springer-Verlag, Berlin, pp. 45–78.
Weinshilboum, R.M., Otterness, D.M., Aksoy, I.A., Wood, T.C., Her, C., Raftogianis, R.B., 1997. Sulfation and sulfotransferases. 1: Sulfotransferase molecular biology: cDNAs and genes. FASEB J. 11, 3–14.
Yanagisawa, K., Sakakibara, Y., Suiko, M., Takami, Y., Nakayama, T., Nakajima, H., Takayanagi, K., Natori, Y., Liu, M.C., 1998. cDNA cloning, expression, characterization of the human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase enzyme. Biosci. Biotechnol. Biochem. 62, 1037–1040.
Yasuda, S., Kumar, A.P., Liu, M.Y., Sakakibara, Y., Suiko, M., Chen, L., Liu, M.C., 2005a. Identification of a novel thyroid hormone-sulfating cytosolic sulfotransferase, SULT1 ST5, from zebrafish. FEBS J. 272, 3828–3837.
Yasuda, S., Liu, C.C., Takahashi, S., Suiko, M., Chen, L., Snow, R., Liu, M.C., 2005b. Identification of a novel estrogen-sulfating cytosolic SULT from zebrafish: molecular cloning, expression, characterization, and ontogeny study. Biochem. Biophys. Res. Commun. 330, 219–225.
Yasuda, S., Liu, M.Y., Yang, Y.S., Snow, R., Takahashi, S., Liu, M.C., 2006. Identification of novel hydroxysteroid-sulfating cytosolic SULTs, SULT2 ST2 and SULT2 ST3, from zebrafish: cloning, expression, characterization, and developmental expression. Arch. Biochem. Biophys. 455, 1–9.