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Review

Cytosolic Sulfotransferases and Environmental Estrogenic Chemicals

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Over the past three decades, a substantial body of evidence has accumulated on the estrogenic activities of numerous environmental compounds. These "environmental estrogens," consisting of pesticides and a variety of industrial chemicals and their by-products, are becoming ubiquitous in the environment and are making their way into the food chain. An important issue is whether vertebrate animals are equipped with mechanisms for the inactivation and/or disposal of environmental estrogens. This review attempts to summarize the currently available data concerning the sulfation of environmental estrogenic compounds by the cytosolic sulfotransferases in vertebrate animals. © Pesticide Science Society of Japan

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INTRODUCTION

The sulfotransferases (SULTs), which are ubiquitous in both plants and animals, catalyze the sulfation of hydroxyl or amino groups on a variety of target acceptor molecules.[1,2] While the membrane-bound SULTs use proteins, glycolipids, and other macromolecules as acceptor substrates, the cytosolic SULTs sulfate smaller molecules and are part of the Phase II detoxification pathway for the biotransformation/excretion of drugs and xenobiotics.[1,2] Increasingly, the cytosolic SULTs have also been shown to be important in regulating the levels and/or activities of endogenous compounds such as thyroid/steroid hormones, and catecholamine neurotransmitters/hormones.[3,4] It is conceivable that perturbation of the normal functioning of the cytosolic SULTs may lead to endocrinological and/or neurological problems. The current review will focus on an emerging issue concerning the role of the cytosolic SULTs in the metabolism of environmental estrogenic chemicals and the effects of these latter compounds on the normal functioning of certain cytosolic SULTs.

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OVERVIEW OF ENVIRONMENTAL ESTROGENS AND THEIR ADVERSE EFFECTS

Over the past three decades, a substantial body of evidence has accumulated on the estrogenic activities of numerous environmental compounds. These "environmental estrogens," consisting of a group of structurally diverse compounds, include pesticides and a variety of industrial chemicals and their by-products.[5] Among the environmental estrogens are bisphenol A (used widely in various plastics and polycarbonate resins including those present in food packaging materials and dental fillings), 4-*n*-octylphenol and 4-*n*-nonylphenol (alkyl phenols which are widely used as surfactants and plastic additives), the insecticide 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT), and polychlorinated biphenyls (PCBs; industrial chemicals used extensively in the past).[6] These compounds are becoming (or already are) ubiquitous in the environment and are making their way into the food chain. Another group of environmental estrogens that are increasingly being recognized are the ones used in some medical applications. Examples are 17α-ethynylestradiol and mestranol (the synthetic estrogen component of oral contraceptives), diethylstilbestrol (a potent synthetic estrogen applied in some clinical applications), and replacement estrogen therapeutics (conjugated estrogen, 17β-estradiol, and progestogens).[7–9] It is generally thought that environmental estrogens may exert their effects by binding directly to the estrogen receptor[6] or

Table 1. List of known human cytosolic SULTs and their possible functions[a]

Enzyme	Proposed functional role	Reference
SULT1A1 (P-form PST)	Detoxification of xenobiotic compounds in general	[30]
SULT1A2 (P-form PST)	Detoxification of xenobiotic compounds in general	[31]
SULT1A3 (M-form PST)	Homeostasis of dopamine; detoxification of dietary monoamines	[32]
SULT1B1 (TH ST)	Metabolism of thyroid hormones	[33]
SULT1C2 (SULT1C #1)	Metabolism of thyroid hormones?	[34]
SULT1C4 (SULT1C #2)	Unknown	[35]
SULT1E1 (EST)	Inactivation and/or transport of endogenous estrogens	[36]
SULT2A1 (DHEA ST)	Transport of dehydroepiandrosterone	[37]
SULT2B1a	Sulfation of pregnenolone	[38,39]
SULT2B1b	Sulfation of cholesterol	[38,39]
SULT4A1 (Brain ST)	Unknown	[40,41]

[a] M-form PST, monoamine-form phenol ST; P-form PST, simple phenol-form phenol ST; TH ST, thyroid hormone ST; EST, estrogen ST; DHEA ST, dehydroepiandrosterone ST.

by interfering with the action of enzymes that are involved in regulating the level of endogeneous estrogens.[10] Whether environmental estrogens, like endogenous estrogens, can exert their estrogenic activity through non-genomic signalling pathways[11,12] remains to be determined. Exposure to high levels of synthetic estrogen mimics has been associated with harmful effects for humans as well as wildlife.[13–16] Environmental estrogens are not only suspected of causing developmental abnormalities in wildlife but are also being cited as a serious hazard for human health.[5] A worldwide debate on a decline in sperm quality in men[17,18] and an increased incidence of breast cancer in women[19–21] implicate environmental estrogens as the putative cause for these epidemiological observations. An important issue is whether vertebrate animals are equipped with mechanisms for the inactivation and/or disposal of environmental estrogens. Previous studies have revealed sulfation and glucuronidation as two major pathways for the detoxification of drugs and other xenobiotics.[22] Of these two detoxification pathways, sulfation as catalyzed by the cytosolic SULTs has received an increasing amount of attention in recent years.[23–25]

BIOCHEMICAL PROPERTIES AND FUNCTIONAL ROLES OF THE CYTOSOLIC SULTs

The cytosolic SULTs have been detected in all vertebrates examined to date.[1,2] These 33–35 kDa enzymes all utilize 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the sulfonyl group donor[26] and share sequences responsible for PAPS-binding.[27] All members of the cytosolic SULTs constitute a gene superfamily which can be further subdivided, based on amino acid sequence homology, into gene families and subfamilies.[28,29] In humans, eleven distinct cytosolic SULTs have been reported. The systematic/trivial names and their proposed functional roles, as well as the corresponding references are compiled in Table 1. Cytosolic SULTs have been classified into five families based on their amino acid sequence homology. All the cytosolic SULTs are members of same SULT gene superfamily. A workshop held in 1995 first recommended using a SULT systematic nomenclature.[42] Figure 1 shows a dendrogram encompassing the five mammalian SULT gene families.

The various human cytosolic SULTs exhibit distinct tissue-

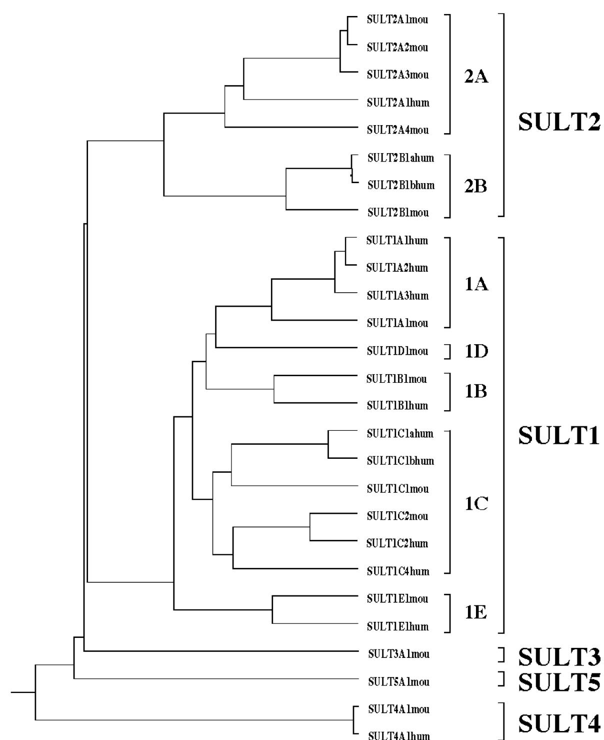

Fig. 1. Classification of human and mouse cytosolic SULTs on the basis of their amino acid sequences.

specific expression in the body. The M-form PST (SULT1A3) has been found in the upper gastro-intestinal tract and brain,[43] and the P-form PST (SULT1A1) in the adrenal gland, lung, and liver.[44] SULT1A2 P-form PST, which is 95.9% identical to SULT1A1 P-form PST at the amino acid sequence level,[45] was found in liver and some bladder tumors.[46] TH ST (SULT1B1) has the highest expression in colon and is also expressed in liver, small intestine, and leukocytes.[33,47] SULT1C #1 (SULT1C2) has been detected in kidney, stomach, thyroid gland, and fetal liver,[34] while SULT1C #2 (SULT1C4) was found in ovary, spinal cord, and fetal lung, kidney, and heart.[35] SULT1E1 was shown to be expressed in liver, endometrium, jejunum, adrenal gland, mammary gland, and fetal kidney, lung, and liver.[48,49] DHEA ST (SULT2A1) was found in liver, adrenal gland and jejunum.[50,51] Both SULT2B1a and SULT2B1b have been detected in placenta, prostate, and jejunum.[38] The brain SULT4A1 was found to be present in brain and spinal cord.[40,41] Different human cytosolic SULTs may exert their sulfating activities locally in a tissue-specific manner toward their respective endogenous substrates and xenobiotic compounds. Sulfate conjugation in general leads to the inactivation and/or facilitated excretion of these compounds.[1,2]

Mouse cytosolic SULTs have also been extensively studied. At least 14 distinct cytosolic SULTs exist in mouse (see Fig. 1). Like human cytosolic SULTs, the 14 mouse cytosolic SULTs are classified into five gene families (Fig. 1). The mouse SULT1 family consists of five subfamilies (SULT1A, 1B, 1C, 1D and 1E subfamily). SULT1A1 is a major detoxification enzyme, previously called PST or Stp.[52] It catalyzes the sulfation of simple phenols and dopamine. SULT1B1 was originally cloned from a mouse liver cDNA library and designated as the dopa/tyrosine SULT.[53] It catalyzes the sulfation of dopa and tyrosine, as well as 4-nitrophenol. SULT1C1 was demonstrated as an olfactory-specific enzyme[54] that catalyzes the sulfation of, among other substrates, *N*-hydroxy-acetylaminofluorine (N-OH-AAF), a carcinogenic compound capable of binding DNA, RNA and proteins. SULT1C2 catalyzes the sulfation of 4-nitrophenol and triiodothyronine (Sakakibara *et al.*, unpublished data). SULT1D1 represents a SULT1 subfamily unique to mouse and rat, but not humans. This SULT displays a characteristic substrate specificity for naphthylamine.[55] Mouse SULT1E1 specifically catalyzes the sulfation of two endogenous estrogens, β-estradiol and estrone. Mouse SULT2 family comprises SULT2A1, 2A2, 2A3, 2A4 and SULT2B1, which is more complicated than the human SULT2 family. Members of mouse SULT2 family can catalyze the sulfation of DHEA, pregnenolone and/or cholesterol (Sakakibara *et al.,* unpublished data). The mouse SULT4A1, like its human counterpart, was found to be specifically present in brain.[41] In contrast to human cytosolic SULTs, mouse has two additional SULT families, designated SULT3A1 and SULT5A1. SULT3A1 catalyzes the sulfation of aromatic amines such as naphthylamine and pyridine, whereas the substrate specificity of SULT5A1 is currently unknown.

Table 2. Tissue-specific expression of mouse cytosolic SULTs[a]

Enzyme	Sites of expression
SULT1A1	Heart, Kidney, Lung, Liver, Muscle
SULT1B1	Stomach, Liver, Intestine
SULT1C1	N.D.[c]
SULT1C2	Kidney, Stomach, Liver
SULT1D1	Kidney, Lung, Stomach, Liver, Intestine
SULT1E1	Testis
SULT2A1, 2A2, 2A3[b]	Liver
SULT2A4	Liver
SULT2B1	Skin, Stomach, Intestine
SULT3A1	Liver
SULT4A1	Brain
SULT5A1	Kidney, Thymus, Lung, Skin

[a] Sakakibara *et al.*, unpublished data. [b] As SULT2A1, 2A2, 2A3 have high homology, the same PCR primers are used. [c] N.D. :not detectable amplification by RT-PCR using all templates.

Table 2 shows the expression profiles of the 14 mouse cytosolic SULTs (Sakakibara *et al.*, unpublished data). Liver, as major organ involved in the detoxification and metabolism of endogenous hormones and other bioactive compounds, represents a major site of the expression of SULT1A1, 1B1, 1C2, 1D1, 2A1-3, 2A4, 3A1. Some of these SULT, as described above, can catalyze the sulfation of xenobiotics such as simple phenols and aromatic amines. SULT1E1 was detected in testis, where it may catalyze the sulfation of endogenous estrogens thereby preventing the accumulation of excess estrogens that may interfere with the testicular development. SULT4A1, which displays an unusually high sequence homology (∼98% at amino acid sequence level) to human SULT4A1, was found to be present in brain, where it may play an important physiological role in the nervous system. The functional roles of other mouse (and human) cytosolic SULTs identified by reverse genetics still remains unknown.

SULT-MEDIATED SULFONATION AS A MEANS FOR THE INACTIVATION AND/OR DISPOSAL OF ENVIRONMENTAL ESTROGENIC CHEMICALS

In vertebrate animals, host defense (detoxifying) mechanisms may operate in the gastro-intestinal (GI) tract, lung, blood (specifically platelets and leukocytes) and liver (the major organ involved in detoxification). The GI tract, lung, blood platelets and leukocytes, and liver are known to express a number of detoxifying enzymes including the cytosolic SULTs and glucuronosyltransferases, and may serve as frontline defenses for detoxifying the xenoestrogens, among other xenobiotic compounds. These protective mechanisms appear

Table 3. Specific activities of human cytosolic sulfotransferases with their typical substrates and with environmental xenoestrogens[a]

	M-PST	P-PST	THST	DHEA ST	EST	SULT1C ST#1	SULT1C ST#2	SULT2B1a ST	SULT2B1b ST	NST
Bisphenol A	ND[b]	1490±29	93±10	209±6	ND	ND[b]	790±46	7±1	13±2	3.3±0.1
4-*n*-Octylphenol	847±21	2429±53	110±11	127±4	594±29	ND	1294±45	22±21	10±2	8.9±2.9
4-*n*-Nonylphenol	98±21	1929±39	161±15	210±8	88±2	ND	976±24	11±6	9±2	5.8±2.9
Diethyl stilbestrol	85±10	1876±66	45±4	246±6	85±3	ND	753±18	11±2	16±4	3.5±0.9
17α-Ethynylestradiol	85±7	1670±26	58±2	538±18	1089±16	ND	57±4	17±8	14±1	2.7±0.4
4-Nitrophenol	1526±8	2378±30	1358±8	101±7	160±7	71±2	1311±35	163±5	22±3	24.9±2.2
Dopamine	2684±14	948±25	43±4	134±4	ND	ND	307±4	ND	ND	ND
DHEA	ND	507±10	84±10	1649±41	40±22	ND	86±5	1443±50	1018±34	ND
Estrone	254±16	827±31	80±3	427±17	1592±103	ND	272±34	14±2	22±4	ND

[a] The specific activities in the table are in units of pmole/min/mg enzyme. The assay mixture contained 1 mM DTT, 14 μM PAPS, 50 μM substrate and phosphate buffer, pH 7.0. The assay temperature was 37°C. Data are the mean±S.D from three determinations.
[b] Activity not detected.

to keep the xenobiotics away from more sensitive organs such as brain, kidney, breast, endocrine organs (*e.g.*, adrenals and thyroid), and the reproductive organs (such as ovary and testis). Several recent studies have indeed demonstrated the sulfation of environmental estrogens by some human cytosolic SULTs.[23–25] In a systematic investigation, ten of the eleven known human cytosolic SULTs were bacterially expressed, purified, and assayed for their activities toward representative environmental estrogens. Activity data (Table 3) showed that SULT1A1 displayed the highest activity toward the compounds tested, followed by SULT1C2, indicating that these two enzymes may play a more important role in detoxification of environmental estrogens. SULT1C4, SULT2B1a, SULT2B1b and SULT4A1 showed negligible or undetectable activity toward these compounds. The other four enzymes, SULT1A3, SULT1B1, SULT2A1 and SULT1E showed intermediate levels of activity toward some of these compounds. Data from the kinetic experiments (Table 4) using SULT1A1 showed that the catalytic efficiency, as reflected by V_{max}/K_m, for the sulfation of environmental estrogens appeared to be comparable to that for the sulfation of an endogenous substrate, 17β-estradiol. It seems therefore possible that sulfation as catalyzed by the above-mentioned human cytosolic SULTs may play a significant role in the metabolism of environmental estrogens. Once intruding upon the susceptible organs, however, the environmental estrogens may exert an indirect effect on the homeostasis of important biologically active compounds such as endogenous estrogens, steroid and thyroid hormones, and neurotransmitters.[1,2,56] Entry of environmental estrogens may disrupt the homeostasis of these important endogenous compounds by interfering with the normal functioning of the cytosolic SULTs that play important roles in the homeostatic regulation of these endogenous compounds. For example, estrogen-responsive cells/tissues, such as mammary epithelial cells, have been shown to contain the SULT1E1, which is involved in the inactivation and homeostasis of estrogens *via* sulfation.[57] The intruding environmental estrogens may exert their pathophysiologic effects, in addition to the direct binding to the estrogen receptor, by interfering with the action of SULT1E1. Indeed, recent studies have demonstrated that hydroxylated polyhalogenated aromatic hydrocarbons, a group of environmental estrogens, may exert their estrogenic effects at least in part by inhibiting SULT1E1-catalyzed sulfation of 17β-estradiol.[10] Elevated levels of endogenous estrogens (estrone and 17β-estradiol) resulting from the inhibition of their metabolism through sulfation may potentially lead to the carcinogenesis within estrogen-responsive tissues.[58,59] In support of these hypothetical events, MCF-7 breast cancer cells were shown to lack the expression of SULT1E1, and the absence of this enzyme has been suggested be critical to the growth of MCF-7 cells in the presence of estrogens.[57] Interestingly, expression of SULT1E1 in MCF-7 cells by cDNA

Table 4. Kinetic constants of human SULT1A1 with environmental estrogens and 17β-estradiol as substrates[a]

Substrate	K_m (μM)	V_{max} (nmol/min/mg)	V_{max}/K_m
Bisphenol A	6.69	38.6	5.77
4-*n*-Octylphenol	5.87	119	20.3
4-*n*-Nonylphenol	21.2	90.1	4.24
Diethylstilbestrol	4.53	47.4	10.5
17α-Ethynylestradiol	1.03	20.5	19.9
17β-Estradiol	3.76	24.9	6.62

[a] Data shown represent the mean derived from three determinations.

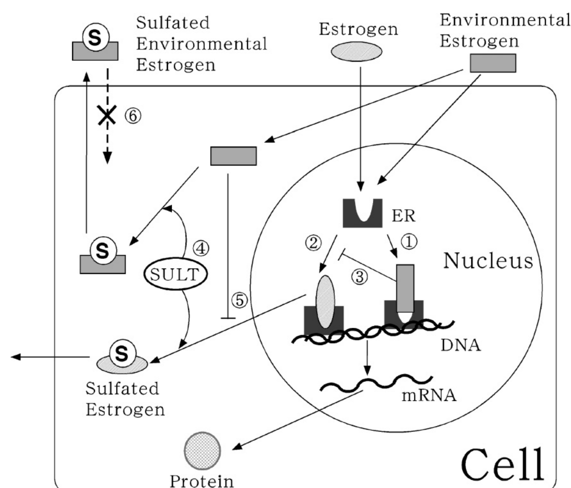

Fig. 2. A schematic diagram illustrating the involvement of sulfation, as catalyzed by cytosolic SULTs in the metabolism and adverse functioning of environmental estrogens in estrogen-responsive cells. ① Environmental estrogen binding to estrogen receptor, ② Endogenous estrogen binding to estrogen receptor, ③ Inhibition of receptor binding by environmental estrogen, ④ Sulfation by cytosolic sulfotransferase, ⑤ Inhibition of estrogen sulfation by environmental estrogen, ⑥ Release of sulfated environmental estrogen for excretion.

transfection was found to suppress the estrogen response and inhibit cell growth, indicating the important role of SULT1E1 in regulating the levels of estrogens and, therefore, the estrogen-dependent growth of breast epithelial cells.[60,61] Figure 2 shows a diagram illustrating the possible events involving the sulfation of environmental estrogens in an estrogen-responsive cell. On entering such a cell, environmental estrogens (Step 1), like endogenous estrogens (Step 2), may bind estrogen receptors. The binding of environmental estrogen to estrogen receptor can *i*) simply tie up the receptor and block the normal functioning of endogenous estrogens (Step 3), *ii*) exert the same estrogenic effect, as do the endogenous estrogens, leading to the expression of the same set of genes, or *iii*) elicit a different estrogenic response leading to the expression of a different set of genes. In the cytosol, environmental estrogens may also compete with endogenous estrogens (Step 4) for use as substrates for the SULT enzyme(s) involved in the homeostasis of endogenous estrogens under normal circumstances. While sulfation of environmental estrogens may lead to their own inactivation and disposal, it may at the same time block the sulfation of endogenous estrogens (Step 5), resulting in elevated levels of these compounds and therefore enhanced/prolonged estrogenic response. In this regard, it may be noted that normal mammary epithelial cells contain predominantly SULT1E1 which has a K_m in the nM range for estrogen and may thus metabolize it at lower thresholds. In the case of MCF-7 breast cancer cells, SULT1E1 seems absent[57] and appears to be replaced by SULT1A1 which has a K_m in the μM range for estrogens and may only metabolize them at higher levels. At issue also is whether sulfated environmental estrogens represent metabolic wastes or are capable of exerting estrogenic effects. Our previous studies[23] have demonstrated that sulfated environmental estrogens may not be capable of crossing the cell membrane to enter the cell (Step 6). However, the sulfated environmental estrogens generated inside the cell may still have access to the estrogen receptor prior to their removal from the cell. It is worthwhile pointing out that a recent study suggested that two biocides, tributyltin and triphenyltin, may competitively inhibit the sulfation of endogenous estrogens, estrone and 17β-estradiol, by human SULT1E1.[62]

DEVELOPMENT OF THE ZEBRAFISH AS A MODEL FOR INVESTIGATING THE ROLE OF THE CYTOSOLIC SULTs IN THE METABOLISM OF ENVIRONMENTAL ESTROGENIC COMPOUNDS

Primarily due to the limitations in using mammalian animal models, there is relatively little information currently available concerning the ontogeny, cell type/tissue/organ-specific expression, and physiological involvement of the cytosolic SULTs and their roles in the metabolism and adverse functioning of environmental estrogens. To comprehensively investigate these important issues, a suitable experimental model is required. The zebrafish (*Danio rerio*) has in recent years emerged as an indispensable vertebrate animal model.[63,64] Compared with alternative mouse, rat, or other animal models, the zebrafish offers several important advantages, including the small size, short generation time, availability of relatively large number of eggs laid at weekly intervals, rapid embryonic development (with all major organs formed within 2–4 days) and the transparency of the zebrafish embryo, *etc*.[65] These unique characteristics make zebrafish an excellent model for a systematic study on the ontogeny, cell type/tissue/organ-specific expression, and physiological involvement of the cytosolic SULTs. As an aquatic vertebrate, the zebrafish may serve as a particularly useful model for studying the adverse effects of polluting environmental estrogens. By searching the zebrafish expressed sequence tag database, a number of putative cytosolic SULT cDNAs have been identified. Based on the sequence information obtained, oligonucleotides corresponding to 5′- or 3′-regions of the putative cytosolic SULT cDNAs were designed and synthesized. These primers, in addition to those designed and synthesized based on the sequences derived from 5′- or 3′-rapid amplification of cDNA ends (RACE) experiments, were used in the reverse transcriptase-polymerase chain reaction (RT-PCR)-cloning of cytosolic SULT cDNAs. Table 5 shows a list of seven full-length zebrafish cytosolic SULT cDNAs that have been cloned. BLAST sequence comparisons revealed considerable homology between the zebrafish SULTs and human cytosolic SULTs, in support of the view that cytosolic SULTs are conserved between zebrafish and humans.

Table 5. List of cloned zebrafish cytosolic SULT cDNAs

Designated name	Method of cloning	Reference
1. SULTX	Obtained Commercially	[66]
2. SULT1 ST1	Obtained Commercially	[67]
3. SULT1 ST2	Obtained Commercially	[67]
4. SULT1 ST3	RT-PCR Cloning	[68]
5. SULT1 ST4	RT-PCR Cloning	[69]
6. SULT2 ST1	RT-PCR Cloning	[70]
7. SULT4A1	RACE/RT-PCR Cloning	[a]

[a] Liu *et al.*, unpublished data.

The seven zebrafish SULT cDNAs listed in Table 2 have been subcloned into prokaryotic expression vector (pGEX-2TK or pET23c) and used to transform BL21 or BL21(DE3) *E. coli* cells. The recombinant zebrafish cytosolic SULTs were expressed, purified, and characterized with regard to their enzymatic properties including substrate specificity, pH optimum, and temperature stability. Table 6 shows the activity data obtained using a number of endogenous compounds and xenobiotics as substrates. Among the seven enzymes, SULT1 ST2 displayed the highest activity toward estrone. SULT1 ST3 showed a relatively high activity toward triiodothyronine and dopamine. SULT1 ST1 and ST4 showed in general low activities toward endogenous substrates tested, but high activities toward some xenobiotics. Interestingly, SULT2 ST1, like mammalian SULT2 enzymes, exhibited a strong activity toward DHEA. On the other hand, SULTX and SULT4A1 displayed low or undetectable activities with all of the endogenous and xenobiotic compounds tested.

The seven zebrafish cytosolic SULTs were tested for their sulfating activities toward representative environmental estro-

Table 6. Specific activities of purified zebrafish cytosolic SULTs with endogenous and xenobiotic compounds as substrates[a]

Substrate	SULTX	SULT1 ST1	SULT1 ST2	SULT1 ST3	SULT1 ST4	SULT2 ST1	SULT4 A1
Triiodothyronine (T3)	0.7±0.1	8.0±0.7	17.4±1.4	22.9±0.9	1.0±0.1	0.1±0.1	ND
Thyroxine (T4)	1.2±0.2	0.3±0.1	3.2±0.5	3.5±0.9	0.4±0.1	ND	ND
Dopamine	3.9±0.9	3.0±1.2	0.3±0.1	11.2±1.0	ND	ND	ND
Estrone	ND[b]	0.4±0.1	84.0±3.8	3.0±0.5	1.5±0.5	ND	ND
DHEA	ND	0.2±0.1	0.9±0.1	0.5±0.1	0.2±0.1	5.9±0.1	ND
Gallic acid	6.0±0.6	2.7±1.1	4.0±0.8	17.7±0.9	ND	ND	ND
β-Naphthol	ND	122±4	155±4	110±4	152±5.0	ND	ND
4-Nitrophenol	4.6±0.6	10.1±1.3	60.5±4.4	15.0±0.6	0.5±0.2	ND	ND
β-Naphthylamine	2.6±0.4	16.9±1.0	18.0±0.4	17.4±2.1	5.0±0.2	ND	ND
Catechin	3.3±0.4	58.8±3.3	45.2±4.2	308±6	9.1±0.6	ND	ND

[a] Specific activity refers to nmol substrate sulfated /min/mg purified enzyme. Data represent means±S.D. derived from three experiments.
[b] Activity not detected.

Table 7. Specific activities of zebrafish cytosolic SULTs with representative environmental estrogens as substrates[a]

Substrate	Specific activity (nmol/min/mg)						
	SULTX	SULT1 ST1	SULT1 ST2	SULT1 ST3	SULT1 ST4	SULT2 ST1	SULT4A1
Bisphenol A	ND[a]	0.1±0.2	12.8±1.7	ND	0.3±0.1	ND	ND
4-*n*-Octylphenol	0.6±0.1	16.9±1.5	73.1±0.5	ND	0.4±0.1	ND	ND
4-n-Nonylphenol	0.2±0.1	7.1±0.7	51.0±2.4	ND	0.6±0.1	ND	ND
Diethylstilbestrol	0.3±0.1	0.9±0.1	51.3±0.2	ND	0.3±0.1	ND	ND
17α-Ethynylestradiol	1.3±0.1	1.6±0.7	64.3±2.7	ND	0.3±0.1	ND	ND
3-Chloro-4-biphenylol	2.2±0.1	205±1	30.3±0.1	ND	46.3±0.8	ND	ND
Tetrachlorobiphenyldiol	0.7±0.1	108±5	22.3±0.7	ND	0.6±0.1	ND	ND
17β-Estradiol	ND	0.7±0.5	104±0.7	ND	ND	0.4±0.1	ND

[a] Data shown represent the mean derived from three determinations. ND, activity not detected.

Table 8. Kinetic constants of zebrafish SULT1 ST1 and ST2 with chlorohydroxybiphenyls and 3,3′,5-triiodo-L-thyronine as substrates[a)]

Substrate	SULT1 ST1			SULT1 ST2		
	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m
	(μM)	(nmol/min/mg)		(μM)	(nmol/min/mg)	
3-Chloro-4-hydroxybiphenyl	76.0	435	5.7	1.3	66.7	49.8
3,3′,5,5′-Tetrachloro-4,4′-dihydroxybiphenyl	8.1	145	17.8	1.1	18.1	16.8
3,3′,5-Triiodo-L-thyronine	64.4	5.4	0.08	9.4	8.3	0.9

[a)] Data shown represent the mean derived from three determinations.

gens. Data shown in Table 7 illustrate that three of the seven zebrafish SULTs were inactive. The other four zebrafish SULTs (SULTX, and SULT1 ST1, ST2, and ST4) exhibited differential activities toward the environmental estrogens tested, with SULT1 ST1 and ST2 being highly active. Table 8 shows the kinetic constants of the zebrafish SULT1 ST1 and ST2 with 3-Chloro-4-hydroxybiphenyl (3-Cl-4-OH-BP), 3,3′,5,5′-Tetrachloro-4,4′-dihydroxybiphenyl (3,3′,5,5′-Cl_4-4,4′-$(OH)_2$-BP) or triiodothyronine (an endogenous compound) as substrate. Compared with SULT1 ST2, SULT1 ST1 demonstrated a greater K_m and yet higher V_{max}. That these two enzymes displayed sulfating activities toward the two hydroxylated PCBs, as well as other environmental estrogens, suggests sulfation as a means for the inactivation/disposal of environmental estrogens in zebrafish. In a follow-up metabolic labeling experiment, [^{35}S]sulfated 3-Cl-4-OH-BP or 3,3′,5,5′-Cl_4-4,4′-$(OH)_2$-BP were indeed generated and released by zebrafish liver cells that had been incubated in medium containing [^{35}S]sulfate and 3-Cl-4-OH-BP or 3,3′,5,5′-Cl_4-4,4′-$(OH)_2$-BP (Fig. 2). These findings demonstrate that zebrafish cells, like mammalian cells, are equipped with SULT enzymes capable of sulfating environmental estrogens.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Collectively, the currently available data summarized above indicate unambiguously a significant role of sulfation in the metabolism and adverse functioning of environmental estrogens. There remains, however, many unanswered questions that need to be addressed in future research. One area is the genetic polymorphisms of the SULT enzymes that are capable of catalyzing the sulfation of environmental estrogens. It will be important to clarify to what extent the genetic polymorphisms leading to differential gene expression and/or catalytic activity of environmental estrogen-sulfating SULTs may influence individual differences in the metabolism of environmental estrogens through sulfation. How environmental factors, such as contaminating divalent metal cations that have been demonstrated to be inhibitors or enhancers of certain SULTs, may affect the activity of environmental estrogen-sulfating SULTs poses also an interesting issue. To gain a complete understanding of the involvement of sulfation in environmental estrogen metabolism in vertebrates other than human and mouse, it is essential to identify in them the complete repertoire of the cytosolic SULTs and characterize their activity toward and sensitivity to environmental estrogens.

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