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Review Article

Cytosolic sulfotransferases

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Sulfoconjugation (Sulfation or Sulfonation) is an important reaction in the phase II biotransformation of a wide number of endogenous and foreign chemicals, including: drugs, toxic chemicals, hormones, and neurotransmitters. The reaction is catalyzed by the members of the cytosolic sulfotransferase (SULT) superfamily, consisting of ten functional genes in humans. Sulfation reaction in living cells is reversed by sulfatase, which hydrolyses the sulfonated conjugates. It has a major role in regulating the endocrine status of an individual by modulating the activity of steroid hormones, their biosynthesis, and the metabolism of catecholamines. Sulfonation is a key reaction in the body's 'chemical' defense against xenobiotics. Although the primary function of sulfoconjugation is to permit detoxification of the compound, it also results in the activation of chemical procarcinogens, such as certain dietary and environmental agents into carcinogens. In this review, we summarize our current understanding of the structure of mammalian cytosolic sulfotransferases and their role in human steroid associated cancers and in the bioactivation of chemical carcinogens.

Keywords: Breast cancer, Carcinogens Sulfoconjugation, PAPS, Substrate binding region, Sulfotransferases

Introduction

The activation as well as inactivation of numerous xenobiotics, including therapeutics, environmental toxicants and protoxicants occurs via sulfonation. The first observations of the ability of organisms to modify xenobiotic compounds were made in the 19th century when Eugen Baumann, in 1876, found that some compounds could be excreted as their sulfated metabolites in urine[1], yet the field of drug metabolism was not established as such until the late 1960s with the discovery of the cytochrome P450 (CYP450) system in liver preparations.

Currently, we know that sulfoconjugation, catalyzed by sulfotransferases, is an important pathway in the biotransformation of many endogenous and exogenous compounds[2-5]. Chemically, the reactions involve the transfer of a sulfuryl group from 3-phosphoadenosine-5'-phosphosulfate (PAPS) to an acceptor molecule; the products of the sulfation reaction are adenosine 3',5'-diphosphate (PAP) and a sulfuric acid ester[6,7-8] (Fig. 1). This reaction occurs in the cytosol or is associated with the membranes of the Golgi apparatus of the cell. There are two classes of sulfotransferases: (1) cytosolic sulfotransferases (SULTs),* which sulfonate small endogenous and exogenous compounds, such as hormones, bioamines, and xenobiotics, and (2) membrane-associated sulfotransferases, which sulfonate larger biomolecules, such as carbohydrates and proteins. The genes encoding sulfotransferases are classified based on the divergent evolution of a multigene superfamily into several families[5,9]. Members within a family share more than 45% identity in amino acid sequence, whereas members of subfamilies are 60% or more identical in amino acid sequences. About 47 different mammalian cytosolic SULTs, one insect and eight plant isoforms, collectively from nine different families have been identified till now[10]. About 10 functional forms localized on five different chromosomes are evident in humans. While two SULT forms (SULT2B1a, b) are splice variants of the same gene, different genes encode the rest of the human forms. Also, genetic

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*(SULT abbreviation as given in the recent nomenclature guidelines by Blanchard *et al*., 2004.)

Abbreviations: SULT, Sulfotransferases; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; PAP, adenosine 3',5'-diphosphate; DMBA, 7,12-dimethylbenz-[α]-anthracene; 2-AF, 2-aminofluorene; 2-AAF, 2-acetylaminofluorene; N-OH-2AAF, N-hydroxy-2-acetylaminofluorene; 3-MC, 3-methylcholanthrene; 7-HMBA, 7-hydroxymethyl-12-methylbenz-[α]-anthracene.

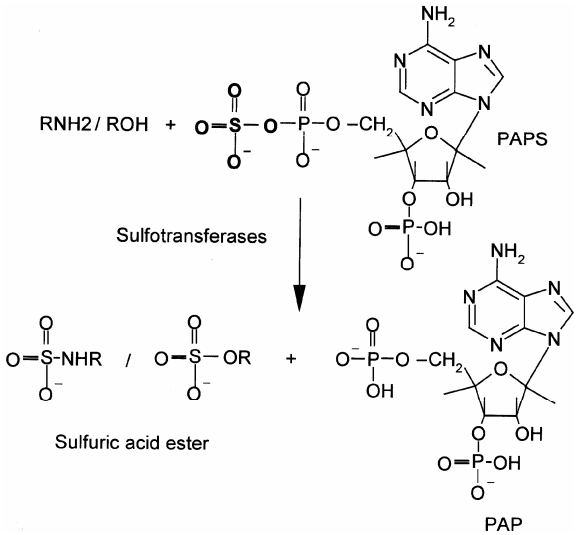


Fig. 1 — General reaction catalyzed by sulfotransferases. PAPS, 3'-phosphoadenosine-5'-phosphosulfate; PAP, adenosine 3',5'-diphosphate. [*Figure generated by MDL ISIS/Draw 2.5*]

polymorphisms, in particular single nucleotide polymorphisms (SNPs), are evident for human SULTs, such as SULT1A1[11-13], SULT1A2[14], SULT1A3[15,16] and SULT2A1[15]. To date, dozens of cDNAs that encode the cytosolic sulfotransferases have been cloned from mammalian tissues to facilitate kinetic and structural characterization studies, and the first enzyme to be characterized at the cDNA level among all the SULTs was the bovine estrogen sulfotransferase[17].

The reactions catalyzed by SULTs often increase the water solubility of substrate compounds including hormones, neurotransmitters, drugs, and carcinogens, thus leading to their enhanced excretion. Sulfation can also contribute to a significant increase in the mutagenicity and carcinogenicity of many pro-mutagens such as 7,12-dimethylbenz-[α]-anthracene (DMBA)[18], 2-aminofluorene (2-AF), 2-acetylamino-fluorene (2-AAF), and N-hydroxy-2-acetylamino fluorene (N-OH-2AAF)[19-21]. It also alters the function of substrate compounds, such as steroid hormones, by alteration of receptor binding capability. Apart from the steroid hormone regulation in non-pathologic human tissues, sulfotransferases also play an important role in the regulation of *in situ* estrogen production in breast and endometrial carcinomas.

Nomenclature for cytosolic sulfotransferases

Until recently, cytosolic sulfotransferases had been named after their substrates. However, since the substrate specificities of the different sulfotransferases are overlapping, attempts have been made to devise a more consistent nomenclature based on their encoded amino acid sequence identity. Guidelines have also been set forth to include a species descriptor and allelic variants of SULT genes. Previously, the human enzymes were designated as **'SULT'**, and rat and mouse isoforms were assigned the prefixes **'ST'** and **'*sult*'** respectively. According to the recent guidelines proposed by Blanchard and colleagues[10], a complete sulfotransferase name would contain species, superfamily, family, isoform, allele, and suballele designations (in ascending order). Also, the prefix **'SULT'** was adopted as the abbreviation for all cytosolic sulfotransferase enzymes. Table 1 gives an outline of the nomenclature of the important cytosolic sulfotransferases from human, rat, and mouse species.

Structure

Till date, the crystal structures of six cytosolic sulfotransferases have been solved and those include mouse SULT1E1 (mEST), human SULT1E1 (hEST)[22,23], human SULT2A3 and SULT2A1 (hHST)[22,24], human SULT1A3[25], SULT1A1[26], and SULT2B1[27]. The first SULT to be characterized was the mouse SULT1E1, where the enzyme was crystallized in presence of the inactive PAP and the substrate, 17β-estradiol[28].

Overall, almost all SULTs are globular proteins with a characteristic α/β fold and 4-5 stranded parallel β-sheet surrounded by α-helices. One exception to this domain arrangement is the retinol dehydratase enzyme[29]. A helical lid makes this enzyme distinct from the other characterized sulfotransferases. All the SULT structures are highly conserved with respect to the PAPS binding site and the catalytic site and also share a structural similarity with nucleotide kinases[28]. Also, there are sequence homologies between the cytosolic and membrane associated sulfotransferases. The largest variation among all SULTs is found in the substrate-binding region. A few of the most important structural aspects are summarized in this section with the information mostly generated based on the crystal structure of the mouse and human SULT1E1.

PAPS-binding region

The ß-sheet constitutes the core of the PAPS binding region and the catalytic site. The PAPS-binding region is highly conserved at the amino acid level in all the SULTs. Three structural motifs are responsible for the PAPS binding: 5'-phosphosulfate-binding (5'-PSB) motif at the N-terminal, the 3'-

phosphate-binding (3'-PB) sequence at the centre of the enzyme, and the ß-strand-loop-helix (also called P-loop) (Fig. 2)[30,31]. The presence of both PSB and PB consenus sequence motifs is used as an important criterion to identify newly cloned SULT cDNAs.

The PSB motif contains the P-loop, which is the phosphate-binding site. The PB motif contains Arg130 and Ser138 (analogous to Ser137 in human SULT1E1), whose side chains interact with the 3'-phosphate group of PAPS. Arg130 is well conserved in both cytosolic and membrane sulfotransferases and Ser138 is totally conserved. These residues help to secure a correct orientation for PAPS binding, and also provide structural constraints for substrate selectivity. From the PAPS bound human SULT1E1 crystal structure[23], it is clear that Lys 47(analogous to Lys48 in mouse SULT1E1) present in the PSB loop, initially interacts with Ser137 and then shifts upon the initiation of the reaction, to interact with the leaving

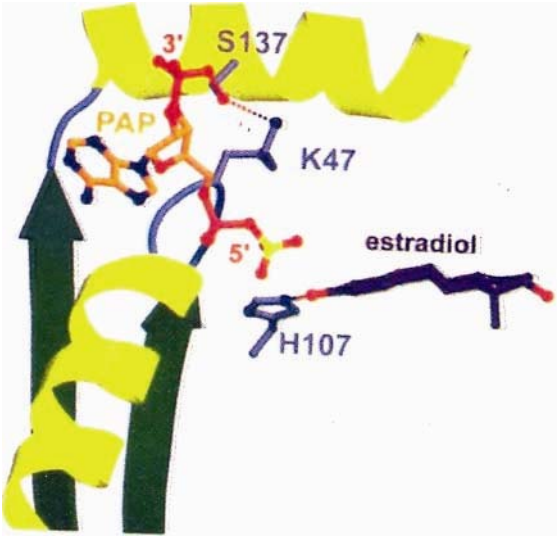


Fig. 2 — PAPS binding region of human SULT1E1. Catalytic site residues shown are Ser, Lys and His*. *Reproduced with permission from Elsevier (Negishi et al, Structure and function of sulfotransferases, Archives of Biochemistry and Biophysics Vol. 390, 2001)* * All aminoacid residues are represented in their three-letter nomenclature

Table 1 — Nomenclature of the cytosolic sulfotransferases[$]

Families	Subfamilies/Isoforms	Principal Substrate/Tissue Expression
HUMAN	SULT1A1	p-nitrophenol/liver, brain, platelets
SULT1	SULT1A2	p-nitrophenol/*
	SULT1A3 (hAST)[@]	dopamine/intestine, brain
	SULT1B1	thyroid hormones/ liver, colon
	SULT1C2	∞/Kidney, stomach, thyroid
	SULT1C4	N-OH-2AAF, p-nitro phenol/ fetal kidney, lung
	SULT1D1	Pseudogene
	SULT1E1 (hEST)[@]	estrone, 17β-estradiol, 17α-ethinylestradiol/ breast, testis, placenta, endometrium
SULT2	SULT2A1 (hHST, DHEA-ST)[@]	dehydroepiandrosterone, bile acids/ adrenal
	SULT2A3	cortex, liver
	SULT2B1'a'	dehydroepiandrosterone
	SULT2B1'b'	pregnenolone/prostate, placenta, trachea
		cholesterol, pregnenolone/lung, prostate, placenta, trachea
RAT	SULT1A1 (AST1V)[@]	β-naphthol/ liver
SULT1	SULT1B1	dopamine, tyrosine isomers/ liver, kidney
	SULT1C1	N-OH-2AAF/Liver
	SULT1D1	Characterization/expression not understood
	SULT1E1	estrogens/male liver
SULT2	SULT2A3	bile acids/liver
MOUSE	SULT1A1 (mPST)[@]	phenolic substrates/liver
SULT1	SULT1B1	dopamine, 3,3', 5-triiodo-L-thyronine/ liver
	SULT1D1	eicosanoids, dopamine/kidney, uterus
	SULT1E1 (mEST)[@]	17β-estradiol/male liver, Testis, placenta
SULT2	SULT2B[#]	dehydroepiandrosterone/Epidydmis, intestine

[$] Only SULT1 and SULT2 families are included in the table.

*Not completely understood, @ Symbols in brackets indicate conventional names, ∞ Substrate unknown, # Isoform not named.

sulfate group (Fig. 3). Further, interaction between Ser137 and Lys 47 protects PAPS from autohydrolysis[32].

In general, the side chain nitrogen of Lys47 forms a hydrogen bond with the oxygen atom of the 5'-phosphate group of PAP while the side chain hydroxyl of Ser137 interacts with an oxygen atom of the 3'-phosphate group. Apart from the serine and lysine residues other conserved residues important for PAP binding include Thr51, Thr52, His 108 (analogous to His107 in human SULT1E1), and Lys 106. In parallel to this, it has also been observed that Lys48, His108 and Lys106 are critical for the sulfuryl transfer reaction in a manner that their mutations nearly abolished the SULT activity[33]. The location of Phe and Trp side chains is such that they form π-stacking interactions with the adenine ring of PAP and stabilize it. Also, the hydrogen bond formed between the Trp residue and the N6 of the adenine moiety stabilizes the structural interactions between the protein and PAP. Coming to the conserved active site, the GxxGxxK motif located near the C-terminus, is necessary not only for the binding of PAPS but also for the interaction with the steroid substrate and it has been shown that mutagenesis of all the three residues produces an inactive enzyme[34].

Thus, the reaction mechanism as proposed for SULTs would involve an SN2 nucleophilic attack by the phenoxide (resulting from extraction of the hydrogen atom of the phenol group by His residue) on the sulfate of PAPS. Other conserved amino acids, including Lys47, are proposed to act in stabilizing the reaction intermediate(s) (Fig. 3)

Substrate binding region

Cytosolic sulfotransferases sulfate small hydrophobic molecules, such as steroid hormones, bioamines (dopamine and catecholamines), and phenols. In contrast to the highly conserved PAPS-binding site, the substrate-binding region of each SULT enzyme reflects structural differences that convey specificity to substrate binding. In general, the

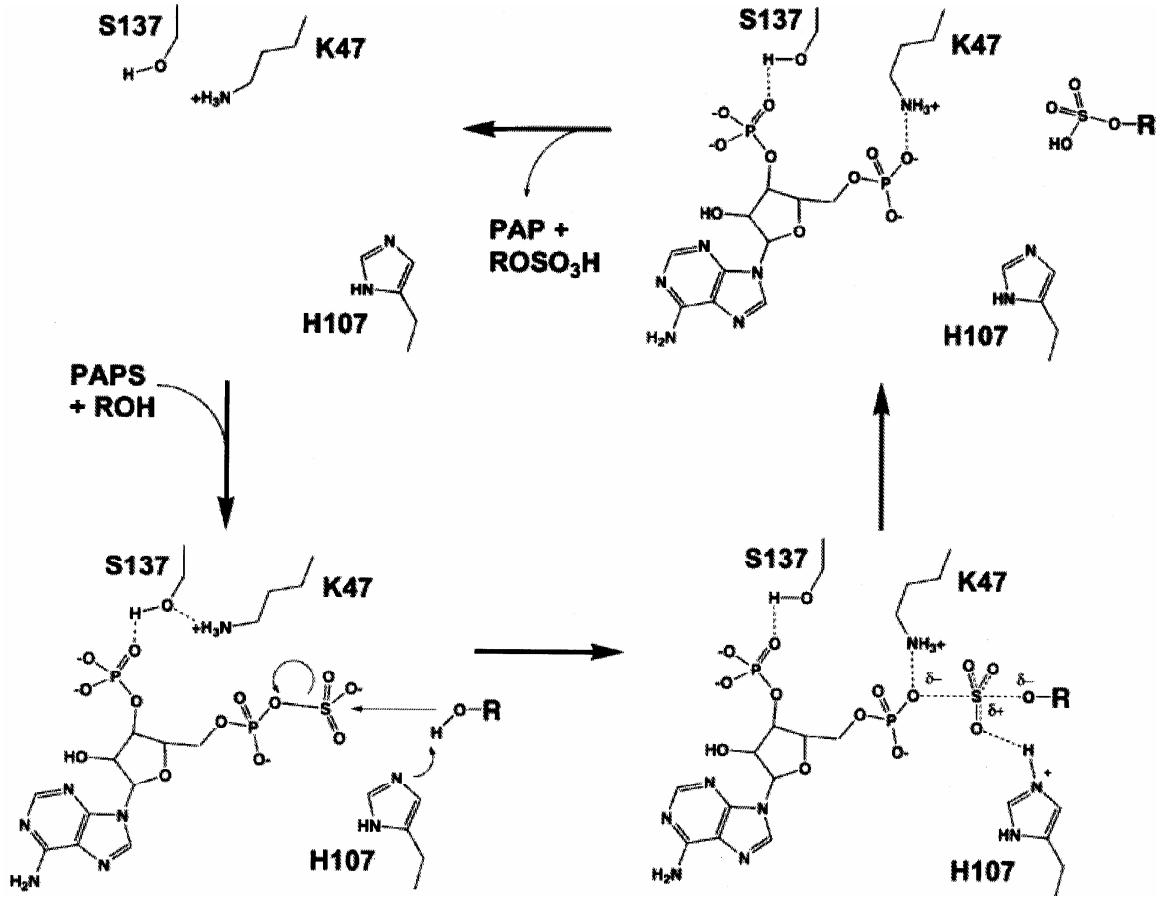


Fig. 3 — Interaction between 5-phosphate group of PAPS and the conserved catalytic residues. Residue numbers are taken from human SULT1E1. See text for details. *Reproduced with permission from Elsevier (Negishi et al, Structure and function of sulfotransferases, Archives of Biochemistry and Biophysics Vol. 390, 2001).*

substrate specificity of sulfotransferases is determined by the binding affinity and positioning of the substrate in the specific site. The binding can also depend on the complementarity of the specific site with the hydrophobic substrates. It is also possible that the chemical nature of the residue at the binding site could be the basis for the enzyme to select its substrate. One of the important characteristics that direct the substrate selectivity is the stereochemistry of the molecule that acts as the sulfuryl group acceptor. This substrate-stereoselectivity has been found in the sulfation of chiral secondary alcohols by rat hydroxysteroid sulfotransferase[35], and in the sulfation of 1- and 2-naphthyl-1-ethanols by human SULT2A1[36].

Various cytosolic SULTs in complex with their substrates, estrogen, dehydroepiandrosterone, cholesterol, and phenol, have been crystallized. Table 2 highlights the substrate binding regions of a few cytosolic sulfotransferases, for which the crystal structures have been solved.

Dimerization-motif

In vitro, cytosolic sulfotransferases appear to form a dimeric structure[42]. Previous studies have indicated that two monomers, homo or hetero can be linked near the C-terminus using a conserved peptide sequence (KXXXTVXXXE) of about 10 residues to form a hydrophobic zipper-like structure enforced by ion pairs at both ends. This zipper has been found in the structures of human SULT1E1 (Fig. 4) and SULT2A3. The main features of the KTVE motif, first identified in SULT2A3 crystal[21], are the hydrophilic interactions through the four backbone hydrogen bonds and the corresponding hydrophobic bonds[22,42]. Though a majority of sulfotransferases are homodimers in their catalytically active forms, the physiological significance of the dimerization in the function of cytosolic SULTs is yet to be defined.

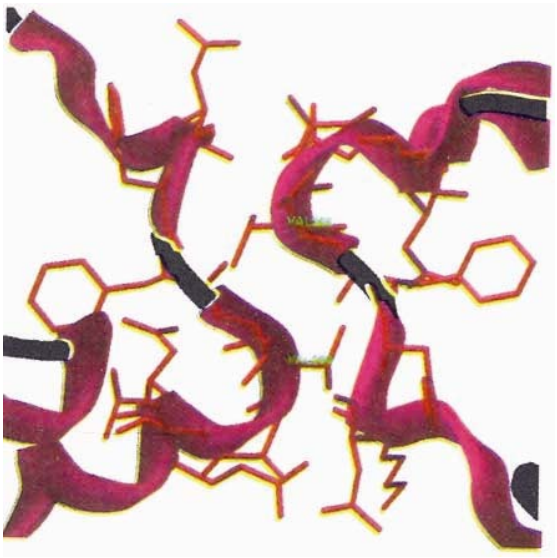


Fig. 4 — Structure of dimerization motif of human SULT1E1. The crystal structure has been taken from PDB (Identification code 1G3M). The dimer interface residues (264-273) are shown in red where as the hEST monomers are represented as ribbon structures. Also shown is the critical residue Valine 269 (in green). *The figure is created using the Sybyl software (version 6.91, Tripos Inc., St Louis, MO) on a Silicon Graphics Fuel workstation*.

Table 2 — Cytosolic sulfotransferases and their substrate binding sites

Enzyme (Crystal structure)	Ligands (Co-crystallized)	Important residues for substrate binding
SULT1A1/Human phenol sulfotransferase	p-nitrophenol	Hydrophobic region residues (84-89)[26,37]
SULT1A3/Human Dopamine sulfotransferase	*	Hydrophobic region residues (142- 148), Glu146[38,39]
SULT1E1/ hEST/Human estrogen sulfotransferase	3,5,3',5'-tetra-chloro biphenyl-4,4'-diol, 17ß- estradiol	Hydrophobic binding pocket[23,40]
sult1e1/mEST/Mouse estrogen sulfotransferase	17ß-estradiol	Hydrophobic binding pocket, Tyr 81[28]
SULT2A3/Human hydroxysteroid sulfotransferase	DHEA	Hydrophobic residues, His99, Trp 77[22,24]
SULT2B1a/Pregnenolone sulfotransferase	*	None identified
SULT2B1b/Cholesterol sulfotransferase	DHEA, pregnenolone	N-terminal peptide (23 residues)[41]

* Indicates that the particular SULT has been crystallized not in complex with its ligand.

Cytosolic sulfotransferases and bioactivation of chemical carcinogens

The superfamily of cytosolic sulfotransferases, together with their substrates, has elicited particular toxicological interest. Various synthetic molecules, including chemical carcinogens, can be sulfated to alter biological activity. This reaction frequently results in the conversion of a procarcinogenic, hydroxyl compound into a more electrophilic and potent carcinogen that is capable of interacting with DNA to form DNA adducts.

Many procarcinogens derived from polycyclic aromatic hydrocarbons can be bioactivated to mutagenic species by hydroxysteroid sulfotransferases[43]. For instance, 7,12-dimethylbenz-[α]-anthracene (DMBA) is metabolized to form 7-hydroxymethyl-12-methylbenz-[α]-anthracene (7-HMBA) (Fig. 5a) by cytochrome P-450, which is further transformed by hydroxysteroid sulfotransferase to the reactive 7-HMBA sulfate[18]. Two additional examples are 2-aminofluorene (2-AF) and its amide derivative, 2-acetylaminofluorene (2-AAF) (Fig. 5b), which were developed in the United States as chemical insecticides during the 1940s and were later found to be procarcinogens that are converted to their carcinogenic form through the action of sulfotransferases. Although the use of both as insecticides has been discontinued, they continue to be used as model compounds for the study of chemical carcinogenesis[44]. The N-hydroxylation of 2-AAF can be catalyzed by cytochrome P-450 to form N-hydroxy-2-acetylaminofluorene (N-OH-2AAF) (Fig. 5c). It has been reported that the sulfation of N-OH-2AAF catalyzed by hepatic sulfotransferases, is an important step leading to a more active form of the chemical carcinogen[19-21,44]. The product molecule of this reaction, N-OH-2AAF sulfate, can spontaneously rearrange to release sulfate and generate a reactive electrophilic cation molecule. Such electrophilic molecules can react with cellular nucleophiles such as DNA to form adducts (Scheme 1). DNA adducts are capable of producing genetic mutations in critical genes, such as proto-oncogenes and tumor suppressor genes that can lead to disease states, such as cancer. Therefore, it is critical to understand the catalysis of enzymatic procarcinogen activation. Previous studies have suggested that SULT1C enzymes have high catalytic activities for the sulfation of N-OH-2AAF[19,45]. Recent experiments have indicated that human SULT1A and SULT2A enzymes are also responsible for the sulfonation, and, thus, further bioactivation of N-OH-2AAF in the liver[46,47]. Recent *in vitro* studies have shown that over expression of human hydroxysteroid sulfotransferase (SULT2A1) enhances the cytotoxicity of N-OH-2AAF in cultured human embryonic kidney cells[48]. Cell viability was significantly reduced when the SULT2A1 expressing embryonic cells were treated with 10μM N-OH-2AAF. Certain non-polycyclic aromatic type benzylic alcohols have also been proposed to undergo sulfotransferase-mediated activation. Rat aryl sulfotransferase (AST1V) activates secondary nitroalkanes such as 2-nitrobutane and 2-nitropentane, and the metabolites were found to be hepatocarcinogenic in rats[49].

Carcinogenic heterocyclic aromatic amines (from dietary sources) undergo metabolic activation by hepatic sulfotransferases to form more potent N-

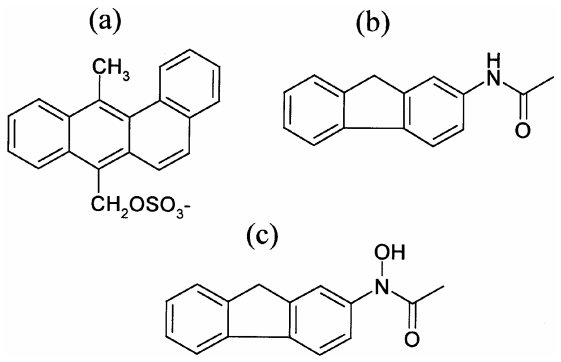
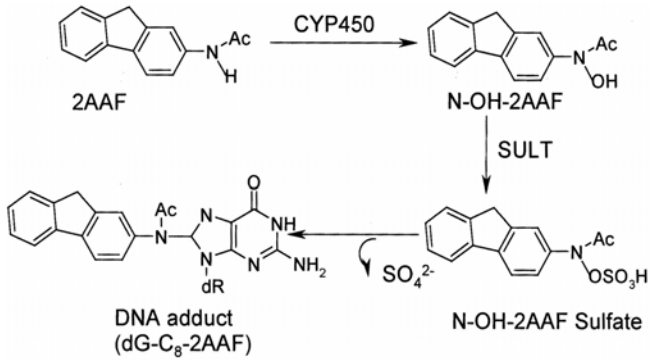


Fig. 5 — General chemical structures of 7-hydroxymethyl-12-methylbenz [α] anthracene (7-HMBA) (a), 2-acetylaminofluorene (2AAF) (b), and N-hydroxy-2-acetylaminofluorene (N-OH-2AAF) (c). The figure was created using ISIS/Draw version 2.0.



Scheme 1 — Representation of the schematic pathway of the SULT-catalyzed biotransformation of the aromatic amides, 2AAF and N-OH-2AAF. 2AAF, 2-acetylaminofluorene; N-OH-2AAF, N-hydroxy-2-acetylaminofluorene; Ac, acetyl group; CYP450, cytochrome 450; and SULT, sulfotransferase. The scheme was created using ISIS/Draw version 2.0.

sulfonyloxy esters that readily react with DNA bases. One such mutagenic amine is N-hydroxy-2-amino-alpha-carboline, which is metabolized by SULT1A1 in the liver, and the metabolite contributes to DNA damage in the human tissues[50]. Dietary chemicals, such as estragole[51] and safrole are dependent on SULT1A3 catalyzed sulfation for generation of DNA-adducting species.

Interestingly, certain procarcinogens are reported to induce a suppression of sulfotransferase-gene expression. For example, 3-methylcholanthrene (3-MC), which is a PAH carcinogen, modulates the mRNA expression of hydroxysteroid sulfotransferase in rats[52]. Certain hepatic SULT isoforms are responsive to phenobarbital with repression of expression observed depending on the isoform studied[53].

Similarly, rat hepatic aryl sulfotransferase (AST1V/Tyrosine ester sulfotransferase) is inactivated by the reactive N-OH-2AAF sulfuric acid ester, probably, due to covalent binding through the formation of cysteine-2AAF adducts[54]. This decrease in the SULT expression acts as a protective mechanism due to which the hepatocytes are able to escape the cytotoxic effects of the highly reactive 2AAF metabolites and progress to cancer[47]. Another detoxification mechanism, which may limit the availability of carcinogenic amines for further activation and DNA adduct formation, is the NADH-dependent reductase system, first identified in human liver microsomes[55].

Determination of the role of sulfotransferases in the bioactivation of the carcinogenic compounds will allow future molecular epidemiological studies to evaluate the impact of exposure on individual susceptibility to human cancers in which the compounds may be implicated.

Cytosolic sulfotransferases and human steroid associated cancers

Reproductive organs are sex steroid-dependent, and thus it is logical to presume that malignancies derived from these tissues develop under abnormal steroidal stimulation. Malignant cellular phenotypes have been classified into two groups according to their response to sex steroids: sex hormone-dependent and sex hormone-independent. For example, DU148 human prostate cancer cells are androgen-independent; while, LNCaP human prostate cancer cells are androgen-dependent. Steroid action is attributed primarily to the regulation of target genes through nuclear receptor binding. This section discusses various aspects of sulfotransferase-mediated steroid signaling in human steroid- associated cancers.

Mechanisms responsible for steroid-associated cancer pathogenesis are diverse and relatively not well defined. Sex hormones have long been considered to play a major role in the growth and function of human reproductive cancers by virtue of their natural role in mediating the differentiation and proliferation of these tissues[56]. Traditional endocrine therapy against steroid-dependent cancers of the reproductive tract has thus aimed to eliminate growth-promoting steroids from the circulation and related tissues. Steroid-associated sulfotransferases could play regulatory roles in human steroid-associated cancer development and progression as mediators of sex-steroid availability and signaling.

Most of the breast cancers in their early stages are hormone-dependent, where the hormone estrogen plays an important role in their progression and development. Previous studies have indicated that sulfoconjugation to inactive steroids reduces the potency of the hormone and thus constitutes a method of regulating the overall bioactive levels of steroids within human cancer cells. Estrogen receptor positive MCF-7 breast cancer cells transfected with SULT1E1 possess the protein at levels similar to normal human mammary epithelial cells and are associated with much lower estrogen-stimulated DNA synthesis or cell proliferation than control MCF-7 cells that do not possess SULT1E1[57-59]. This suggests that loss of estrogen sulfotransferase has resulted in altered estrogen metabolism in the breast cancer cells. Similar clinical studies have also detected SULT1E1 immunoreactivity in 44% of the human breast carcinomas; also, SULT1E1 expression, was inversely correlated with tumor size or lymph node status, and significantly associated with a decreased risk of recurrence or improved prognosis[60]. Apart from SULT1E1, SULT1A1 has also been implicated in steroid sulfation in various breast cancer cell lines[61,62]. Estrogen receptor-positive human breast cancer cell lines, ZR-75-1 and T-47D, have demonstrated SULT1A1 protein expression[62], suggesting that this enzyme could be contributing to the sulfation of estrogens in breast cancer. On the negative side, though SULT1A1 has higher affinity for estrogens, its affinity for estrogen sulfation is 300-fold lower than that of SULT1E1, rendering it less capable to physiologically inactivate estrogen[61,63]. This in turn could contribute to the enhanced proliferation of

breast cancer cells. However, when the breast cancer cells evolve to an estrogen receptor-negative and hormone-independent status, in their later stages (MDA-MB-468 cells), high levels of estrogen sulfates and activity of estrogen sulfotransferase are observed, suggesting that there is a modification of estrogen metabolism in these cells[64,65]. Hence, targeting the estrogen sulfotransferase activity could open new possibilities in the therapy of hormone-independent breast cancer. Increasing estrogen sulfation activity may thus provide a potential mechanism by which stimulation of estrogen-responsive tumors could be abrogated.

Synthetic progestins, such as nomegestrol acetate, promegestone, and Medrogestone in low concentrations stimulate estrogen sulfotransferase expression and also block the proliferation of hormone-dependent breast cancer cells[64,66,67]. Similarly, Tibolone and its metabolites exert a stimulatory effect on sulfotransferase activity in MCF-7 breast cancer cells[68]. This is an important point in the pathophysiology of breast cancer, as it is well known that estrogen sulfates are biologically inactive.

SULTs, such as SULT1A1 and SULT1A2 can catalyze the sulfate conjugation of catecholestrogens (4-hydroxyestrone, 4-hydroxyestradiol)[69], and the common genetic polymorphisms of these enzymes involved in the metabolism of the catecholestrogens could represent a risk factor for estrogen-dependent carcinogenesis. On the other hand, 2-methoxy-estradiol, a non-estrogenic, endogenous metabolite of estrogen, has an anticarcinogenic effect in estrogen-dependent breast tumours. Recent studies have shown that SULT1A1 may have a specific role in reversing the antiproliferative effects of 2-methoxyestradiol by catalyzing its sulfonation[70]. Further exploration of this correlation between SULT1A1 activity and 2-methoxyestradiol's effects can serve to improve clinical applications in breast cancer.

Androgens have a significant role in the pathophysiology of prostate cancer. DHEA, from the adrenal cortex, is an important precursor for androgen biosyntheis in the prostate gland. There are discrepancies concerning sulfotransferase activities in prostate cancer. Some studies have indicated that variation in SULT1A1 alleles and the SULT1A1 catalyzed activation of dietary heterocyclic amines can contribute to prostate cancer risk[71]; whereas, others have supported no such association[72]. Chan and coworkers have reported that transfection with SULT2A1 is able to alter the androgen sensitivity of PC-3 human prostate cancer cells[73]. SULT2B1 and its two transcripts, 2B1a and 2B1b, which catalyze the sulfation of DHEA, an androgen precursor, are highly expressed in the human prostate[74-75]. It is possible that SULT2B1 may be responsible for conjugating DHEA in the prostate cells, thus preventing its conversion to potent androgens. Hence, polymorphisms within the SULT2B1 gene may represent risk factors for the development of androgen-dependent prostate tumors.

More cell biological studies are needed to unveil the regulatory roles of cytosolic sulfotransferases in mediation of the human steroid associated cancer cell growth in response to steroid signaling.

Sulfotransferase inhibitors- Drug targets

Sulfotransferases have been linked with several disease states, prompting investigators to look for specific SULT inhibitors. So far, compounds, such as environmental toxins [Polyhalogenated aromatic hydrocarbons (PHAH) and Polychlorinated biphenols (PCBs)], bisubstrate analogues, which inhibit both PAPS and substrate binding domains of SULTs), ATP derivatives and ADP analogs, which mimic end products of the SULT reaction), and natural products, such as estrogen derivatives have been studied as inhibitors. Most of the compounds were investigated with respect to estrogen sulfotransferase, which is the most widely studied cytosolic SULT.

Hydroxylated metabolites of polyhalogenated aromatic hydrocarbons and polychlorinated biphenols act as endocrine disrupting chemicals by increasing the local bioavailability of estrogen through inhibition of SULT1E1 in target tissues[76,77]. It has been observed that these compounds have a higher affinity (K_m = 4nM) for human SULT1E1 than its cognate substrate, estradiol (K_m = 5nM), and can bind either to the active site or allosteric site on the enzyme. Analogues of adenine and PAPS with covalently bound estrogen groups have high K_i for bovine adrenal estrogen sulfotransferase[78]. These analogues show competitive inhibition for both PAPS and substrate binding sites. Bisubstrate analogues containing covalently bound PAPS and sulfate-acceptor mimetics are also developed as inhibitors of SULT1E1[79,80]; however, these compounds display competitive inhibition only with respect to PAPS but not the substrate. Another class of inhibitors that warrant more robust studies is the ADP analogs, which facilitate the inhibition of

bovine estrogen sulfotransferase[81]. Inhibitors for other SULTs have also been looked upon. For example, flavonoids, such as quercetin cause a specific non-competitive inhibition of SULT1A1[82]; however, the structural requirements of this inhibition are not yet known. Aryl-substituted purine derivatives have been screened for their activity against rat aryl sulfotransferase (AST1V)[83]. The hepatic procarcinogen, N-OH-2AAF, also causes a PAPS-dependent, self-catalyzed, irreversible inactivation of rat hepatic aryl sulfotransferase[84]. Dietary chemicals from red wine, coffee and green tea also act as inhibitors of SULTs, such as SULT1A1 and SULT1A3 in the gastrointestinal tract, and may be function as natural chemoprotectants[85].

Closing remarks

Interest in the field of sulfotransferases is expanding because of the potential regulatory importance of this class of phase II enzymes. Although *in vitro,* molecular, crystallization studies have provided a great deal of insight, further studies are still required if potent and specific inhibitors are to be designed for therapeutic use. Also the current approach to the design of SULT inhibitors has to change as it is focuses mostly on the mechanism of the reaction rather than on drug development. More investigation is required in order to understand the relation between cytosolic and membrane-associated sulfotransferases. Also understanding SULT gene regulation is an important future goal- this would elucidate valued information on the physiological function of SULTs, and may serve as a window to establish the role of SULTs in disease processes in humans.

It is hence likely that as additional functions of SULTs will continue to emerge, their prominence as targets of therapeutic interest will increase further.

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