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Review

Human sulfatases: A structural perspective to catalysis

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Abstract. The sulfatase family of enzymes catalyzes hydrolysis of sulfate ester bonds of a wide variety of substrates. Seventeen genes have been identified in this class of sulfatases, many of which are associated with genetic disorders leading to reduction or loss of function of the corresponding enzymes. Amino acid sequence homology suggests that the enzymes have similar overall folds, mechanisms of action, and bivalent metal ion-binding sites. A catalytic cysteine residue, strictly conserved in prokaryotic and eukaryotic sulfatases, is post-translationally modified into a formylglycine. Hydroxylation of the formylglycine residue by a water molecule forming the activated hydroxylformylglycine (a formylglycine hydrate or a gem-diol) is a necessary step for the enzyme's sulfatase activity. Crystal structures of three human sulfatases, arylsulfatases A and B (ARSA and ARSB), and estrone/dehydroepiandrosterone sulfatase or steroid sulfatase (STS), also known as arylsulfatase C, have been determined. While ARSA and ARSB are water-soluble enzymes, STS has a hydrophobic domain and is an integral membrane protein of the endoplasmic reticulum. In this article, we compare and contrast sulfatase structures and revisit the proposed catalytic mechanism in light of available structural and functional data. Examination of the STS active site reveals substrate-specific interactions previously identified as the estrogen-recognition motif. Because of the proximity of the catalytic cleft of STS to the membrane surface, the lipid bilayer has a critical role in the constitution of the active site, unlike other sulfatases.

Keywords. Sulfatase, steroid sulfatase, aryl sulfatase, three-dimensional structure, crystal structure, catalysis, membrane protein, estrone sulfate.

Introduction

The sulfatase family of enzymes catalyzes the hydrolysis of sulfate ester bonds of a wide variety of substrates ranging from sulfated proteoglycans to conjugated steroids and sulfate esters of small aromatics. Seventeen human sulfatase proteins and their genes have been identified [1, 2]. Several of them are associated with genetic disorders leading to reduction or loss of function of corresponding enzymes. The sequence homology among the members of the sulfatase family ranges between 20 % and 60 %. This is reflected in similarity of the tertiary structures as well as of the active site architectures. The catalytically active residues, mostly from the N-terminal half of the polypeptide chain, are highly conserved, indicating a common catalytic mechanism shared by the members of the family. One particular active site amino acid that is strictly conserved in prokaryotic and eukaryotic sulfatases is a cysteine, which is post-translationally modified into a formylglycine (FG) [3]. Hydroxylation of FG by a water molecule forming the activated hydroxylformylglycine (HFG; an FG

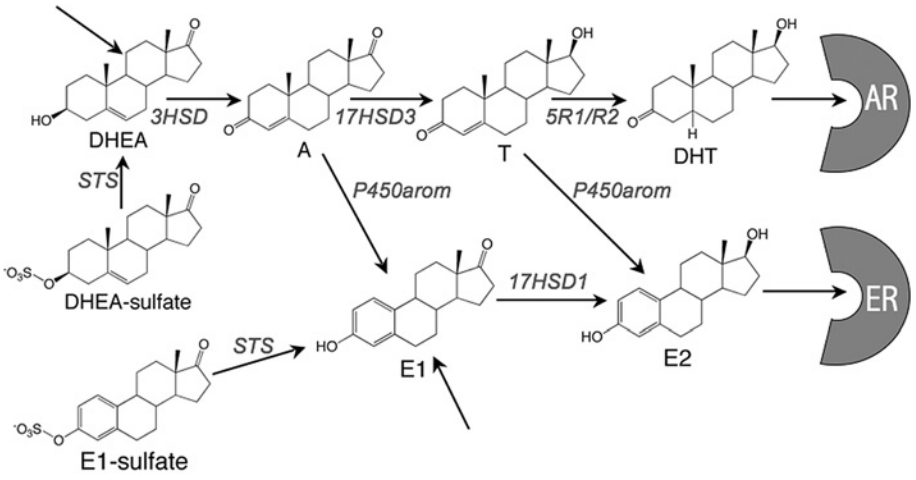
hydrate or a gem-diol) is a necessary step for the enzyme's sulfatase activity [3, 4].

Human sulfatases are active at either acidic or neutral pH, based on their subcellular localization. The lysosomal sulfatases have pH optima in the acidic range, while those localized in the endoplasmic reticulum (ER), Golgi apparatus and cell surface are most active at neutral pH. Human arylsulfatases, such as arylsulfatses A (ARSA) and B (ARSB), are lysosomal and represent soluble forms of the enzyme. Mutations in genes of these lysosomal enzymes lead to diseases such as metachromatic leukodystrophy and mucopolysaccharidoses [5]. Estrone (E1)/dehydroepiandrosterone (DHEA) sulfatase (steroid sulfatase; STS), also known as arylsulfatase C, is a microsomal enzyme and is an integral membrane protein of the ER [6, 7]. It is most active at or near neutral pH and can be solubilized only in the presence of detergents [7, 8]. STS is expressed in several tissues including human placenta, skin fibroblasts, breasts, and fallopian tubes [6, 7, 9–16]. Mutations in the STS gene and inactive enzyme have also been associated with X-linked ichthyosis, a disease related to scaling of the skin [17–19]. Localization of STS in the smooth and rough ER was demonstrated by immunohistochemical labeling [9]. Arylsulfatases D, E, F, G, H, J and K are reported to be localized in ER or Golgi compartments [1, 2]. Iduronate 2-sulfatase, sulfamidase, galactose 6-sulfatase, *N*-acetyl galactosamine-4-sulfatase, and glucosamine sulfatase, other members of the human sulfatase family, are localized in lysosomes. Mutations in these genes, causing deficiency of one or more of these enzymes necessary for normal cell metabolism, are commonly referred to as lysosomal storage disorders, known collectively as mucopolysaccharidosis, such as hunter, sanfilippo and morquio syndromes [2]. Two new sulfatases, recently reported, Hsulf1 and Hsulf2 are heparin sulfate endosulfatases that release sulfate groups on the C6 position of GlcNAc from an internal subdomain in intact heparin and are localized on the cell surface [2, 20]. The cause of multiple sulfatase deficiency (MSD) has been attributed to mutations in the gene encoding the enzyme known as FG generating enzyme that activates all human sulfatases by catalyzing the conversion of catalytic cysteine to FG [21–23].

Crystal structures of three members of the human sulfatase family, human placental STS [8], ARSA [24], ARSB [25] and one homologous bacterial arylsulfatase from *Pseudomonas aeruginosa* (PAS) [26] have been determined. The structure of the native, full-length human placental STS provides the first direct evidence of membrane integration of these enzymes, suggesting roles for the lipid bilayer, and possibly for the ER membrane, in catalysis [8]. The overall three-dimensional structures of all three soluble sulfatases exhibit a high degree of homology for the domain of STS that scaffolds the catalytic residues. Furthermore, the spatial arrangement of amino acids responsible for hydrolysis of sulfate esters is virtually identical in all four sulfatases, demonstrating the high degree of similarity of their catalytic mechanism. Nonetheless, subtle differences in the sequences of the substrate-binding cleft result in differences in the active site architecture that account for the variation in substrate specificity. Having a membrane-spanning domain bordering the lipid bilayer and partially contributing to the architecture of the active site makes STS uniquely different from the other three known structures of the sulfatase family. In this article, we describe the commonality of the sulfatase structures in relation to their function with special emphasis on STS, and probe issues related to catalytic mechanism and substrate specificity from examination and analysis of their molecular structures.

STS in hormonal breast and prostate cancers

In addition to adrenal sources, hydrolysis of conjugated steroids catalyzed by STS is an alternative source of sex-steroid precursors for the local biosynthesis of active estrogens and androgens. STS catalyzes the hydrolysis of E1-sulfate to unconjugated E1, which is subsequently reduced to 17β-estradiol (E2) by 17β-hydroxysteroid dehydrogenase 1 (17HSD1) (Fig. 1). Androstenedione (A) to E1 and testosterone (T) to E2 aromatization steps are catalyzed by aromatase (P450arom). However, local biosynthesis of E2 from E1-sulfate has been proposed to be the major cause of high levels of active estrogens in the breast for post-menopausal women [27, 28]. The presence of STS in breast carcinomas and STS-dependent proliferation of breast cancer cells have been demonstrated [27, 29]. STS immunoreactivity was detected in 84 out of 113 breast carcinoma cases and was significantly associated with their mRNA levels as well as enzymatic activities [30]. Immunoreactivity was also found to correlate with tumor size and increased risk of recurrence. Higher mRNA levels were associated with poor prognosis in patients with estrogen receptor-positive breast cancer [31]. Additionally, STS has also been found in LNCaP prostate cancer cells [32]. STS hydrolyzes DHEA-sulfate to DHEA, which is then converted to dihydrotestosterone (DHT), the most potent agonist of the androgen receptor, by the actions of 3HSD, 17HSD3, and 5α-reductases 1 and 2 (5R1/R2) (Fig. 1). Recently published transcriptional data showing increased expres-



DHEA: dehydroepiandrosterone; A: androstenedione; T: testosterone; DHT: dihydrotestosterone; AR: androgen receptor; ER: estrogen receptor
STS: steroid sulfatase; 3HSD: 3β-hydroxysteroid dehydrogenase; 17HSD3: 17β-HSD type 3; 5R1: 5α-reductase type 1; 5R2: 5α-reductase type 2 P450arom: aromatase; 17HSD1: 17β-HSD type1

Figure 1. Enzymes involved in last steps of biosynthesis of hormonal sex steroids in the prostate and the breast. In addition to the adrenal sources, dehydroepiandrosterone (DHEA) and E1 are produced locally through the hydrolysis of their sulfate conjugates catalyzed by steroid sulfatase (STS).

sion of STS in adipose tissue complementing DHEA-sulfate uptake [33] supports a key role of the enzyme in intracrine biosynthesis of active sex hormones. In addition, STS immunoreactivity was detected in 65 of 76 cases (86 %) of human endometrial carcinoma, and immunoreactivity was significantly correlated with enzyme activity and semi-quantitative mRNA analysis [34]. Higher levels of STS transcription have been detected in breast cancer tissues of patients with developed progressive disease and found to be an important predictor of clinical outcome [35]. STS mRNA was confirmed in LNCaP, DU-145 and PC-3 prostate cancer cell lines, as well as the synthesis of E1 and E2 [36]. Furthermore, STS immunoreactivity was detected in 44 of 52 human prostate cancer tissues [36].

Tertiary structures of human sulfatases

Human sulfatases consist of single polypeptide chains of lengths between 500 and 800 amino acids, share considerable sequence homologies and have roughly the same overall tertiary fold. The sequence homology for the N-terminal 150 or so residues that include catalytic and many active site residues is higher than the rest, suggesting conservation of the bivalent cation binding site and the catalytic mechanism across this family of genes. However, as these enzymes exhibit differential substrate specificities, substrate-binding interactions in the active site must be different. The sequence identities of sulfatases with known tertiary structures, namely STS, ARSA, ARSB and PAS, range between 20 % and 32 %. As mentioned earlier, the overall three-dimensional structures of all three soluble sulfatases exhibit good homology with each other and with the structure of STS for the domain that scaffolds the catalytic residues. The spatial arrangement of amino acids responsible for hydrolysis of sulfate esters is virtually identical in all four sulfatases.

The three-dimensional structure of the full-length STS from human placenta as determined by X-ray crystallography is shown in Figure 2a. In Figure 2b, a least squares superposition of all four structures (PDB ID codes are ARSA: 1AUK, ARSB: 1FSU and PAS: 1HDH) is shown. The tertiary structure of STS consists of two domains – a globular (55×60×70 $Å^3$), polar domain containing the catalytic site, and the putative transmembrane domain consisting of two antiparallel hydrophobic alpha helices, nearly 40 Å long. As shown in Figure 2b, all four sulfatases have the catalytic polar domain that superimpose on each other very well.

The topologies of the overall fold of STS *versus* ARSA, as a representative of the soluble arylsulfatases, are given in Figures 3a and b, respectively. The major polar domain consists of two subdomains with the α/β sandwich fold. Subdomain 1 (SD1) winds around a central 11-stranded (strands 1, 2, 4–11, 17) mixed β sheet flanked by 13 α-helices/helical turns (helices 1–7, 10–15) in STS and contains the catalytic core. Subdomain 2 (SD2), consisting of roughly 110 C-terminal residues, winds around a four-stranded antiparallel β sheet (strands 13–16) flanked by α helix 16, packs against turn and loop regions of the β sheet of

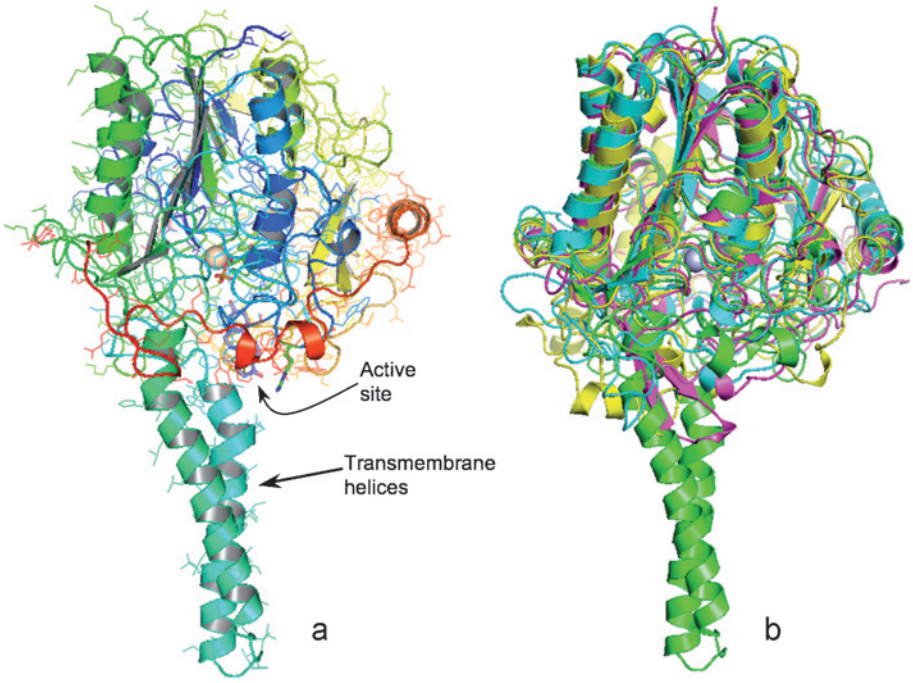


Figure 2. (*a*) Crystal structure of human placental STS. The polypeptide chain is color-coded: N terminus is blue and the C terminus is red. (*b*) Superposition of four sulfatase structures: STS (green), arylsulfatases A (ARSA) (blue), ARSB (magenta) and arylsulfatase from *Pseudomonas aeruginosa* (PAS) (yellow). The Ca^{2+} ion is shown as a sphere in both figures.

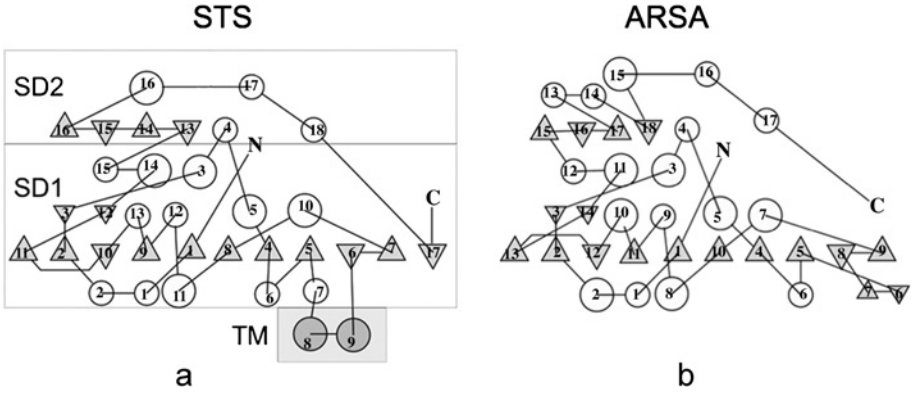


Figure 3. Overall folds of human sulfatases (*a*) STS and (*b*) ARSA. The α-helices are represented by circles and β-strands by shaded triangles. SD1: subdomain 1, SD2: subdomain 2, TM: transmembrane domain. The secondary structure elements are numbered.

SD1. The two putative transmembrane helices α8 and α9 protrude on one side of the catalytic polar domain. Despite the overall similarity, however, each of the sulfatases has distinct differences in its three-dimensional structure, clearly seen in Figure 3. The central β-sheet in the SD1 of STS has 11 strands as opposed to 10 for other sulfatases. In the ARSB structure, the missing transmembrane helices 8 and 9 of STS are compensated by the formation of a pair of antiparallel β-strands. The ARSA structure also has a similar loop region, but in an entirely different conformation with only a hint of antiparallel strands. Additionally, the loop regions of the three human enzymes have significant differences, both in lengths and conformations. Some of the loop regions in STS that have proposed membrane association [8], such as loops between α4 and α5, and between β9 and α13 that approach the lipid bilayer, have 4–7-residue peptide insertions when compared with the structures of the soluble ARSA and ARSB. Nevertheless, the overall fold of the catalytic domain of STS, the location and composition of two β-sheets of SD1 and SD2, as well as the locations, number and lengths of the flanking helices closely resemble those of the three soluble sulfatases.

Both STS and ARSA contain 12 cysteines and 6 disulfide bonds, whereas ARSB has 8 cysteines and 4 disulfides. In contrast, PAS, being a cytoplasmic enzyme, has no cysteine residues. All of the cysteine residues in STS are distributed in two catalytic subdomains, with the Cys170–Cys242 disulfide "zipper-lock" near the lipid-protein interface serving to stabilize the putative transmembrane helices. The presence of disulfides and four glycosylation sites in STS suggests that the catalytic domain is located on the lumen side of the ER.

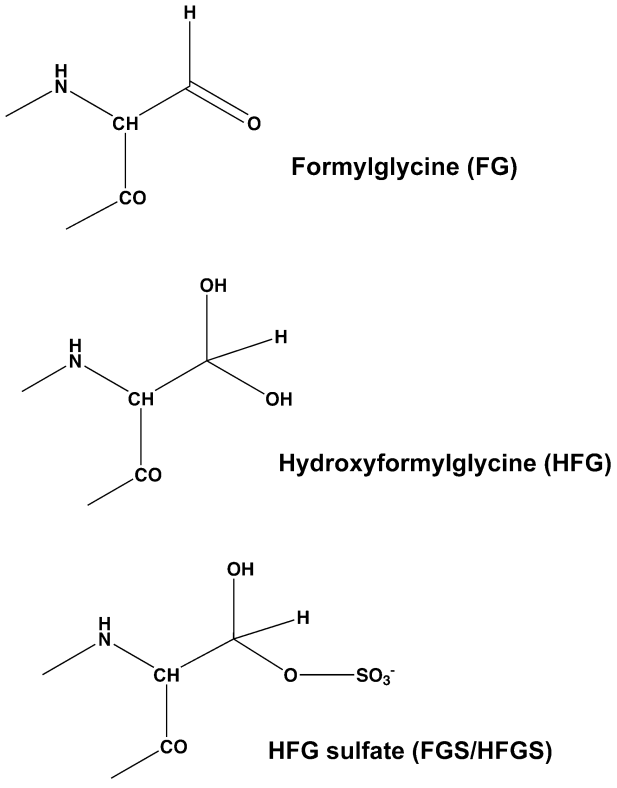


Figure 4. Three states of the catalytic residue in sulfatases.

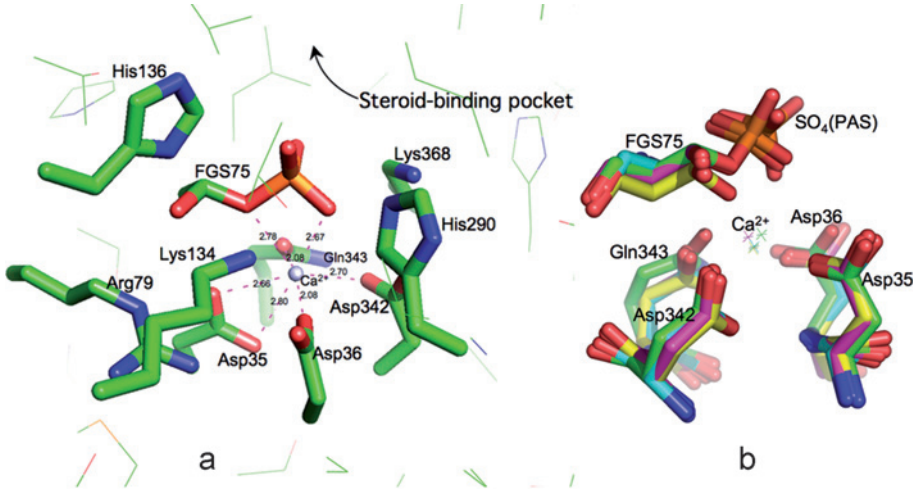


Figure 5. (*a*) Catalytic site of STS. Important catalytic residues and Ca^{2+} coordination (distances range between 2.08 Å and 2.80 Å) are shown. Other side chains are in thin lines. (*b*) Superposition of the bivalent cation binding sites in STS (green), ARSA (blue), ARSB (magenta) and PAS (yellow) demonstrates their similarity. Four ligand side chains, formylglycine (FG) sulfate (FGS) moieties, and metal centers (X) are shown. The sulfate in PAS is not covalently linked to FG. The residues numbering is after STS sequence.

Catalytically important amino acids in human sulfatases

As described in the introduction, the catalytic cysteine in sulfatases is post-translationally modified to an FG residue, which is further activated to an HFG by a water molecule (Fig. 4). In the STS structure, the catalytic residue HFG75 was found covalently linked to a sulfate moiety (*i.e.*, as a sulfate ester, FGS/HFGS; Fig. 4). The metal ion found at the center of the catalytic site near the FGS75 side chain was interpreted to be a Ca^{2+} [8]. The metal ions in the catalytic sites of ARSB and PAS were also proposed to be Ca^{2+} ions [25, 26]; but it was an Mg^{2+} in the wild-type ARSA [24], as well as in the C69A mutated complex of ARSA with *p*-nitrocatechol sulfate [37].

Figure 5a is a close-up view of the catalytically important residues in STS. While the oxygen atoms of the Asp35, Asp36, Asp342, Gln343 and FGS75 side chains serve as ligands for the bivalent cation (Ca^{2+} …O distances range between 2.1 and 2.8 Å) in STS, Lys134, Lys368 and Arg79 are involved in neutralization of negative charges of the carboxylic moieties. The positively charged amino groups of the Lys134 and Lys368 side chains are also within contact distances (2.7–3.1 Å) of the sulfate oxygen atoms of FGS75 (Fig. 5a). In addition, two sulfate oxygen atoms are within coordination distances of Ca^{2+} (~2.7 Å). Several histidine residues in the immediate vicinity may play important roles in catalysis as well. The imidazole ring of His136 is situated within a hydrogen-bonding distance (2.6 Å) of the hydroxyl of FGS, and His 290 $N_{\varepsilon 2}$ is 2.6 Å away from a sulfate oxygen of FGS. Also, the His346 side chain is linked to Lys368 and the Thr291 side chain *via* a bridging water molecule. The main chain NH groups of FGS75 and Thr76 point towards the sulfate-binding cavity and may thus be responsible for the formation of an oxyanion hole.

The catalytic end of the active site in STS is highly homologous to those in ARSA, ARSB and PAS. Nine of the ten catalytically important residues, namely Asp35, Asp36, FGS75, Arg79, Lys134, His136, His290, Asp342, and Lys368 are strictly conserved in all four enzymes. Most of these residues are conserved in all 17

human sulfatases as well [2]. When these nine α-carbon atom positions were superimposed by least squares minimization, the root mean square deviation (rmsd) was 0.4 Å. The tenth residue, Gln343, a ligand to the cation in STS, is an asparagine in ARSA, ARSB and PAS. Shown in Figure 5b is a close-up view of superimposed active sites of the four sulfatase structures by least square fitting of the alpha carbon atoms (rmsd ~0.2 Å) of four side chains (Asp35, Asp36, Asp342 and Gln343 in STS) that provide ligand oxygen atoms to the bivalent cation. The rmsd of the metal cation positions resulting from the superposition was 0.3 Å. The positions of the HFGS esters including the sulfate moieties in STS and ARSB are nearly identical, as shown in Figure 5b. Although the position of the HFG side chain in the bacterial PAS is also quite similar to that in STS, the sulfate moiety being non-covalently bound in PAS is 1.0 Å away from the superimposed sulfate position in STS and ARSB. In ARSA, the FG residue was originally modeled as a glycine and the sulfate moiety as a chloride ion [24]. However, in two more recently determined structures of human placental ARSA with covalently bound phosphate moieties [38], the metal ion is proposed to be a bivalent Ca^{2+} instead of an Mg^{2+}, and the catalytic FG69 is found to be esterified with a phosphate group. It is, thus, likely that all human sulfatases use Ca^{2+} as the bivalent metal ion in the sulfate hydrolysis. Whether it is exchangeable with other cations without disrupting the enzyme activity is not yet known.

Catalytic mechanism

The mechanism of hydrolysis of the sulfate ester is similar to that of other hydrolytic enzymes, with a few exceptions, namely the utilization of the non-standard side chain FG gem-diol as the catalytic residue, and the presence of a metal ion and several electrophilic and nucleophilic side chains that interact specifically with the sulfate moiety. However, the evidence of a gem-diol as a reaction intermediate has been noted before for other classes of hydrolytic enzymes [39, 40]. Owing to the conservation of residues at the catalytic end of the substrate binding site, the proposed mechanism, shown schematically in Figure 6 for STS, should generally be applicable to all human sulfatases, including ARSA and ARSB. Positively charged side chains Lys134, Lys368 and Arg79, as well as His136 and His290 participate in catalysis, in addition to their role in charge neutralization inside the active site cavity. Step I is the activation of HFG75 by a water molecule, forming the gem-diol. In step II, a nucleophilic attack on the sulfur atom by one of the hydroxyls of the HFG follows its activation by Ca^{2+}, while the other hydroxyl is deprotonated by His136. This causes the sulfate moiety to covalently link with the FG side chain and release the unconjugated substrate. The free hydroxyl is involved in a nucleophilic attack on the ester bond in step III. In step IV, the HSO_4^- moiety is released, and the FG side chain is regenerated.

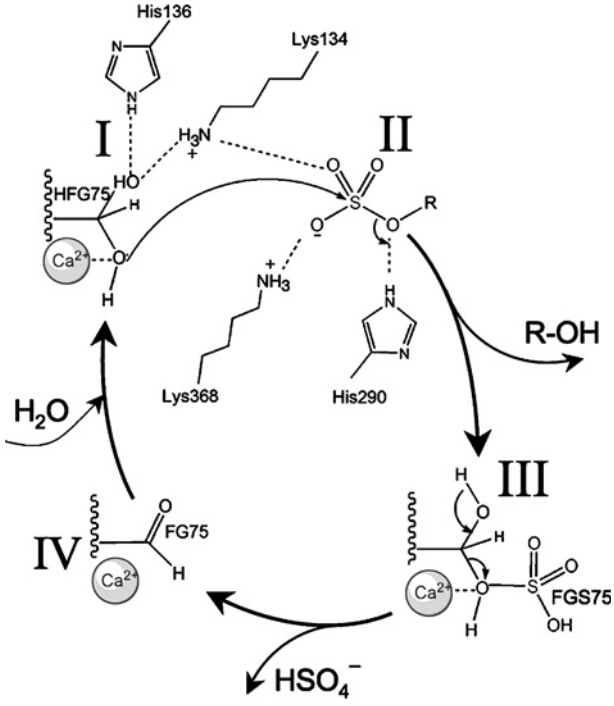


Figure 6. Proposed catalytic mechanism of STS [41].

The scheme, however, does not explain the observation of a sulfate covalently linked to HFG in the crystal structures of ARSA, ARSB and STS, suggesting that the sulfated form of HFG from the last substrate hydrolyzed is the resting state of human sulfatases. The presence of this covalently linked sulfate in STS has recently been confirmed at a higher 2.10 Å resolution (Fig. 7; D. Ghosh et al., unpublished data). It is possible that an additional water molecule is necessary as a nucleophile to effectively perform the de-esterification reaction, in accordance with the scheme proposed in Figure 8. This molecule of water is possibly transported to the catalytic cavity by the solvated sulfate group of an incoming substrate, shielding the charged group during its passage through the hydrophobic substrate-entry path.

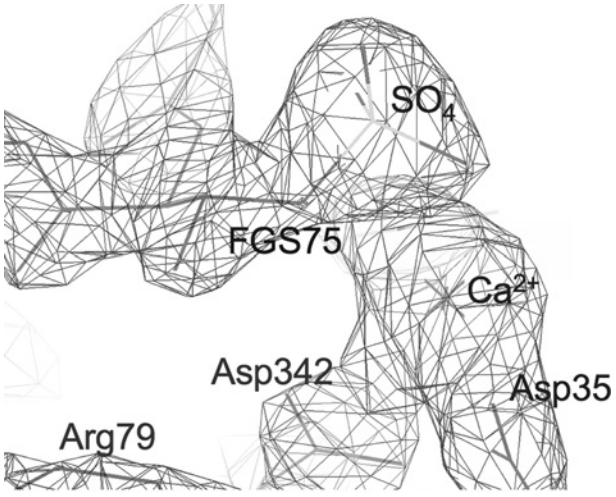


Figure 7. Electron density (contoured at three times the standard deviation) of the catalytic residue FGS75 in STS at 2.10 Å resolution (D. Ghosh et al., unpublished data).

Substrate recognition at the active site

While the sequence and structural conservation among human sulfatases ARSA, ARSB and STS are strong at the catalytic ends, those at the opposite ends of the catalytic clefts, responsible for substrate recognition and binding, are significantly different. The difference at these ends is even more pronounced between STS and soluble sulfatases ARSA and ARSB. While these ends are open to the exterior in ARSA and ARSB, transmembrane helices 8 and 9, each roughly 40 Å long (Figs 2 and 9) and situated between residues 179 and 241, border the catalytic cleft in STS, thereby positioning the active site on the surface of the lipid bilayer [8]. Owing to the presence of several disulfide groups and four glycosylation sites, it is highly likely that the polar catalytic domain rests on the lumen side of the lipid bilayer.

In STS, therefore, the opening to the active site cleft is embedded into what is presumed to be the lipid-protein interface. Near the opening, a constellation of large hydrophobic side chains, Phe178, Phe182, Phe187, Phe230, Phe233, Tyr236, and Phe237 from the transmembrane domain, and Phe104, Tyr493, Trp550, Phe553, Leu554, Trp555, and Trp558 of the catalytic domain line the surface of a hydrophobic "tunnel" leading to the active site [8] (Fig. 9). The Arg98 and Thr99 side chains position themselves as gatekeeprs to the "tunnel". A bound steroid sulfate substrate covers the entire length of the catalytic cleft up to the lipid interface [41]. The number and distribution of hydrophobic residues around the substrate-binding cleft and the path to the putative lipid protein interface are distinctly different in STS from those in ARSA and ARSB. The interactions of the hydrophobic residues with a steroidal substrate in STS, such as the steroid A-ring recognition by a Leu103–Val486 sandwich (Fig. 9), is also reminiscent of previously observed recognition motifs in another steroidogenic enzyme [41–43]. Although STS specifically uses both E1-sulfate and DHEA-sulfate as substrates, highly purified enzyme exhibits higher specific activity with the former (~8.5 μmol/min/mg) than with the latter (~1.5 μmol/min/mg) substrate [7]. These data suggest a preference of STS for an estrogenic substrate, which is consistent with the presence of an aromatic A-ring-specific recognition motif in the steroid-binding site.

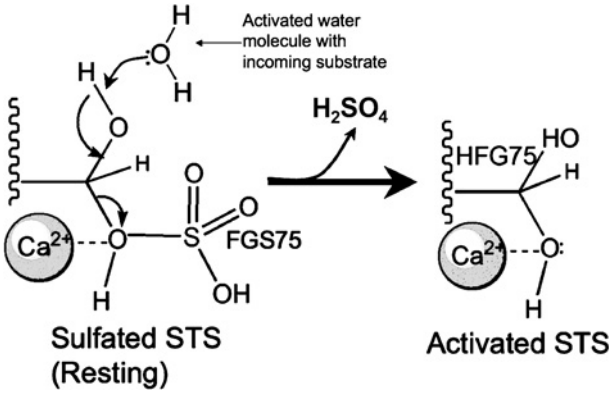


Figure 8. A proposed mechanism for hydrolysis of the covalently bound sulfate group and activation of STS by an incoming water molecule shielding the sulfate moiety of an incoming substrate.

As has been proposed, in addition to helices 8 and 9 (residues 179–241), other loop and helical turn regions that the above residues belong to (residues 468–500, 550–559) associate with the lipid bilayer [8]. Therefore, participation of the lipid bilayer in maintenance of the integrity of the active site and passage of the substrate and the product can be envisioned. The crystal structure of STS is indicative of functional roles for the lipid bilayer with which it associates.

Therapeutic relevance of STS and other sulfatases

As noted earlier, in recent years STS has emerged as a key player in the maintenance of high intratumoral estrogen and androgen levels through intracrine biosynthesis of active hormonal steroids. STS is, thus, a valuable drug target for estrogen and androgen deprivation therapies in hormonal diseases, especially in postmenopausal hormone-dependent breast cancer. Many inhibitors of STS have been reported and reviewed [44, 45]. In general, STS inhibitors can be divided into two classes: Irreversible arylsulfamate inhibitors and reversible non-sulfamate inhibitors.

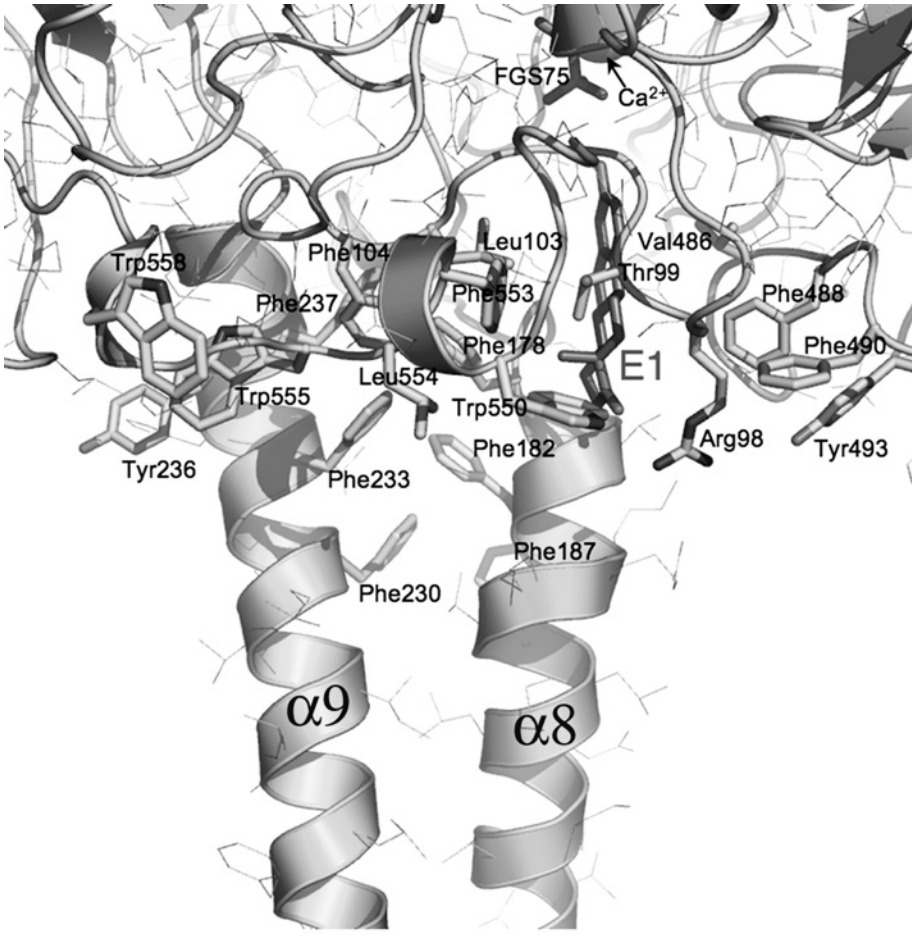


Figure 9. Clusters of large hydrophobic residues at the putative protein-lipid interface in STS. The location of transmembrane helix pair α8 and α9 is such that the substrate end of the catalytic cleft (shown with a bound E1 molecule) rests on the lipid bilayer. The opposite end of the cleft marks the location of the catalytic residue FGS75.

The former category includes the vast majority of STS inhibitors reported to date. Irreversible arylsulfamate inhibitors are suicide inhibitors of STS in which the S-O bond is first hydrolyzed by the enzyme, resulting in the release of the phenolic portion of the inhibitor and sulfamic acid, which then irreversibly inhibits STS [44, 45]. The earliest example of this class of inhibitors is EMATE [46]. However, EMATE is estrogenic due to the release of estrone during the inhibition process. Non-steroidal STS inhibitors, such as coumarin [47], also known as 667-COUMATE, and chromenone [48] have been developed to circumvent this problem. All of these compounds are effective STS inhibitors in cellular assays. A Phase I clinical trial of 667-COUMATE has been completed successfully [49].

A variety of reversible, non-sulfamate STS inhibitors have been developed. Early studies focused on replacing the sulfate group of estrone or estradiol with O-, N-, or S-linked sulfate surrogates [44]. Efforts were made to design reversible inhibitors that are non-hydrolyzable analogs of E1-sulfate [50, 51]. E1-sulfate analogs with a boronic acid substitution at the 3-position in lieu of the sulfate group were found to be good competitive inhibitors of STS [52]. 17α-Benzyl-substituted estradiol derivatives were shown to be reversible inhibitors of STS [44, 53, 54]. It is possible that the high affinity of these compounds can be attributed to additional hydrophobic interactions between the hydrocarbon moiety at the 17-position and the hydrophobic transmembrane helices of STS. A few other steroidal inhibitors of STS, such as sulfamate analogs of E1-sulfate derivatives KW-2581 and STX213, have shown promise in preclinical studies [55, 56]. Dual target agents have also been developed, such as SR16157, which are sulfamate analogs of E1-sulfate derivatives that first inhibit STS; then, upon hydrolysis, the steroidal moieties act as estrogen receptor antagonists [57]. Following a similar idea, estrogen-3-sulfamate analog-based compounds have been proposed as effective inhibitors for both STS and P450arom activities [58].

Attempts have been made to treat sulfatase deficiencies in humans with functional recombinant enzymes. Galsulfase, a recombinant form of human *N*-acetylgalactosamine 4-sulfatase, was approved in the United States for the treatment of mucopolysaccharidosis, a lysosomal storage disorder causing severe skeletal abnormalities [59]. Similar approaches could be

adopted for the treatment of diseases caused by deficiencies in other sulfatases as well.

Structure-guided rational design of inhibitors could be an effective means where overactivation of the enzyme is an issue. Guided by the atomic structures of STS and its complexes, it may be possible to design high-affinity enzyme inhibitors that have high specificity for STS.

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1 Parenti,G., Germana,M. and Ballabio,A. (1997) The sulfatase gene family. Curr. Opin. Genet. Dev. 7, 386 – 391.
2 Diez-Roux,G. and Ballabio, A. (2005) Sulfatases and human diseases. Annu. Rev. Genomics Hum. Genet. 6, 355 – 379.
3 Schmidt, B., Selmer, T., Ingendoh, A. and von Figura, K. (1995) A novel amino acid modification in sulfatases that is defective in multiple sulfatase deficiency. Cell 82, 271 – 278.
4 Recksiek, M., Selmer, T., Dierks, T., Schmidt, B. and von Figura, K. (1998) Sulfatases, trapping of the sulfated enzyme intermediate by substituting the active site formylglycine. J. Biol. Chem. 273, 6096 – 6103.
5 Neufeld, E. F. and Muenzer, J. (2001) The mucopolysaccharidoses. In: The Metabolic and Molecular Bases of Inherited Disease, 8th edn, vol. 3, pp. 3421 – 3452, Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Childs, B., Kinzler, K. W. and Vogelstein, B. (eds.), McGraw-Hill, New York.
6 Vaccaro, A. M., Salvioli, R., Muscillo, M. and Renola, L. (1987) Purification and properties of arylsulfatase C from human placenta. Enzyme 37, 115 – 126.
7 Hernandez-Guzman, F. G., Higashiyama, T., Osawa, Y. and Ghosh, D. (2001) Purification, characterization and crystallization of human placental estrone/dehydroepiandrosterone sulfatase, a membrane-bound enzyme of the endoplasmic reticulum. J. Steroid Biochem. Mol. Biol. 78, 441 – 450.
8 Hernandez-Guzman, F. G., Higashiyama, T., Pangborn, W., Osawa, Y. and Ghosh, D. (2003) Structure of human estrone sulfatase suggests functional roles of membrane association. J. Biol. Chem. 278, 22989 – 22997.
9 Noel, H., Plante, L., Bleau, G., Chapdelaine, A. and Roberts, K. D. (1983) Human placental steroid sulfatase: Purification and properties. J. Steroid Biochem. 19, 1591 – 1598.
10 Dibbelt, L. and Kuss, E. (1986) Human placental sterylsulfatase: Enzyme purification, production of antisera, and immunoblotting reactions with normal and sulfatase-deficient placentas, Biol. Chem. Hoppe-Seyler 367, 1223 – 1229.
11 Burns, G. R. J. (1983) Purification and partial characterization of arylsulphatase C from human placental microsomes. Biochim. Biophys. Acta 759, 199 – 204.
12 Yanaihara, A., Yanaihara, T., Toma, Y., Shimizu, Y., Saito, H., Okai, T., Higashiyama, T. and Osawa, Y. (2001) Localization and expression of steroid sulfatase in human fallopian tube. Steroids 66, 87 – 91.
13 Stein, C., Hille, A., Seidel, J., Rijnbout, S., Waheed, A., Schmidt, B., Gueze, H. and von Figura, K. (1987). Cloning and expression of human steroid sulfatase. J. Biol. Chem. 264, 13865 – 13872.
14 Suzuki, T., Hirato, K., Yanaihara, T., Kodofuku, T., Sato, T., Hoshino, M. and Yanaihara, N. (1992) Purification and properties of steroid sulfatase from human placenta. Endocrinol. Jpn. 39, 93 – 101.
15 Van der Loos, C. M., Van Breda, A. J., Van den Berg, F. M., Walboomers, J. M. M. and Jobsis, A. C. (1983) Human placental steroid sulfatase purification and monospecific antibody production in rabbits. J. Inherit. Metab. Dis. 7, 97 – 103.
16 Purohit, A., Potter, B. V. L., Parker, M. G. and Reed, M. J. (1998) Steroid sulfatase: expression, isolation and inhibition for active site identification studies. Chem. Biol. Interact. 109, 183 – 193.
17 Alperin, E. S. and Shapiro, L. J. (1997) Characterization of point mutations in patients with X-linked ichthyosis. J. Biol. Chem. 272, 20756 – 20763.
18 Hernandez-Martin, A., Gonzalez-Sarmiento, R. and De Unamuno, P. (1999) X-linked ichthyosis: an update. Br. J. Dermatol. 141, 617 – 627.
19 Ghosh, D. (2004) Mutations in X-linked ichthyosis disrupt the active site structure of Estrone/DHEA sulfatase. Biochim. Biophys. Acta 1739, 1 – 4.
20 Uchimura, K., Morimoto-Tomita, M. and Rosen, S. D. (2006) Measuring the activities of the Sulfs: two novel heparin/heparan sulfate endosulfatases. Methods Enzymol. 416, 243 – 253.
21 Cosma, M. P., Pepe, S., Annunziata, I., Newbold, R. F., Grompe, M., Parenti, G. and Ballabio, A. (2003) The multiple sulfatase deficiency gene encodes an essential and limiting factor for the activity of sulfatases. Cell 113, 445 – 456.
22 Dierks, T., Dickmanns, A., Preusser-Kunze, A., Schmidt, B., Mariappan, M., von Figura, K., Ficner, R. and Rudolph, M. G. (2005) Molecular basis for multiple sulfatase deficiency and mechanism for formylglycine generation of the human formylglycine-generating enzyme. Cell 121, 541 – 552.
23 Dierks, T., Schmidt, B., Borissenko, L. V., Peng, J., Preusser, A., Mariappan, M. and von Figura, K. (2003) Multiple sulfatase deficiency is caused by mutations in the gene encoding the human C(alpha)-formylglycine generating enzyme. Cell 113, 435 – 444.
24 Lukatela, G., Krauss, N., Theis, K., Selmer, T., Gieselmann, V., von Figura, K. and Saenger, W. (1998) Crystal structure of human arylsulfatase A: the aldehyde function and the metal ion at the active site suggest a novel mechanism for sulfate ester hydrolysis. Biochemistry 37, 3654 – 3664.
25 Bond, C. S., Clements, P. R., Ashby, S. J., Collyer, C. A., Harrop, S. J., Hopwood, J. J. and Guss, J. M. (1997) Structure of human lysosomal sulfatase. Structure 5, 277 – 289.
26 Boltes, I., Czapinska, H., Kahnert, A., von Bülow, R., Dierks, T., Schmidt, B., von Figura, K., Kertesz, M. A. and Usón, I. (2001) 1.3 Å structure of arylsulfatase from *P. aeruginosa* establishes the catalytic mechanism of sulfate ester cleavage in the sulfatase family. Structure 9, 483 – 491.
27 Pasqualini, J. R. and Chetrite, G. S. (2002) The selective estrogen enzyme modulators in breast cancer. In: Breast Cancer. Prognosis, Treatment and Prevention, pp. 187 – 249, Pasqualini, J. R. (ed.), Marcel Dekker, New York.
28 Reed, M. J. and Purohit, A. (1997) Breast cancer and the role of cytokines in the regulating estrogen synthesis: an emerging hypothesis. Endocr. Rev. 18, 701 – 715.
29 Billich, A., Nussbaumer, P. and Lehr, P. (2000) Stimulation of MCF-7 breast cancer cell proliferation by estrone sulfate and dehydroepiandrosterone sulfate: inhibition by novel non-steroidal steroid sulfatase inhibitors. J. Steroid Biochem. Mol. Biol. 73, 225 – 235.
30 Suzuki, T., Nakata, T., Miki, Y., Kaneko, C., Moriya, T., Ishida, T., Akinaga, S., Hirakawa, H., Kimura, M. and Sasano, H. (2003) Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. Cancer Res. 63, 2762 – 2770.
31 Miyoshi, Y., Ando, A., Hasegawa, S., Ishitobi, M., Taguchi, T., Tamaki, Y. and Noguchi, S. (2003) High expression of steroid sulfatase mRNA predicts poor prognosis in patients with estrogen receptor-positive breast cancers. Clin. Cancer Res. 9, 2288 – 2293.
32 Selcer, K. W., Kabler, H., Saraf, J., Xiao, Z. and Li, P.-K. (2002) Inhibition of steryl sulfatase activity in LNCaP human prostate cancer cells. Steroids 67, 821 – 826.
33 Dalla Valle, L., Toffolo, V., Nardi, A., Fiore, C., Bernante, P., Di Liddo, R., Parnigotto, P. P. and Colombo, L. (2006) Tissue specific transcriptional inhibition and activity of steroid sulfatase complementing dehydroepiandrosterone sulfate up-

take and intracrine steroid activation in human adipose tissue. J. Endocrinol. 190, 129 – 139.
34 Utsunomiya, H., Ito, K., Suzuki, T., Kitamura, T., Kaneko, C., Nakata, T., Niikura, H., Okamura, K., Yaegashi, N. and Sasano, H. (2004) Steroid sulfatase and estrogen sulfotransferase in human endometrial carcinoma. Clin. Cancer Res. 10, 5850 – 5856.
35 Al Sarakbi, W., Mokbel, R., Salhab, M., Jiang, W. G., Reed, M. J. and Mokbel, K. (2006) The role of STS and OATP-B mRNA expression in predicting clinical outcome in human breast cancer. Anticancer Res. 26, 4985 – 4990.
36 Nakamura, Y., Suzuki, T., Fukuda, T., Ito, A., Endo, M., Moriya, T., Arai, Y. and Sasano, H. (2006) Steroid sulfatase and estrogen sulfotransferase in human prostate cancer. Prostate 66, 1005 – 1012.
37 von Bulow, R., Schmidt, B., Dierks, T., von Figura, K. and Uson, I. (2001) Crystal structure of an enzyme-substrate complex provides insight into the interaction between human arylsulfatase A and its substrates during catalysis. J. Mol. Biol. 305, 269 – 277.
38 Chruszcz, M., Laidler, P., Monkiewicz, M., Ortlund, E., Lebioda, L. and Lewinski, K. (2003) Crystal structure of a covalent intermediate of endogenous human arylsulfatase A. J. Inorg. Biochem. 96, 386 – 392.
39 Fleming, S. M., Robertson, T. A., Langley, G. J. and Bugg, T. D. H. (2000) Catalytic mechanism of a C-C hydrolase enzyme: evidence for a *gem*-diol intermediate, not an acyl enzyme. Biochemistry 39, 1522 – 1531.
40 Li, J.-J., Li, C., Blindauer, C. A. and Bugg, T. D. H. (2006) Evidence for a gem-*diol* reaction intermediate in bacterial C-C hydrolase enzymes BphD and MhpC from ^{13}C NMR spectroscopy. Biochemistry 45, 12461 – 12469.
41 Ghosh, D. (2005) Three-dimensional structures of sulfatases. Methods Enzymol. 400, 273 – 293.
42 Ghosh, D. and Vihko, P. (2002) Molecular mechanism of estrogen recognition and 17-keto reduction by 17β-hydroxysteroid dehydrogenase type 1. Chem. Biol. Interact. 130 – 132, 637 – 650.
43 Sawicki, M., Erman, M., Puranen, T., Vihko, P. and Ghosh, D. (1999) Structure of the ternary complex of human 17β-hydroxysteroid dehydrogenase type 1 with equilin and $NADP^+$. Proc. Natl. Acad. Sci. USA 96, 840 – 845.
44 Nussbaumer, P. and Billich, A. (2004) Steroid sulfatase inhibitors. Med. Res. Rev. 24, 529 – 576.
45 Reed, M. J., Purohit, A., Woo, L. W., Newman, S. P. and Potter, B. V. (2005) Steroid sulfatase: molecular biology, regulation and inhibition. Endocr. Rev. 26, 171 – 202.
46 Purohit, A., Potter, B. V. L., Williams, G. J., Howarth, N. M., Potter, B. V. and Reed, M. J. (1995) Inactivation of steroid sulfatase by an active site-directed inhibitor, estrone-3-O-sulfamate. Biochemistry 34, 11508 – 11514.
47 Woo, L. W. L., Purohit, A., Malini, B., Reed, M. J. and Potter, B. V. L. (2000) Potent active site-directed inhibition of steroid sulfatase by tricyclic coumarin-based sulfamates. Chem. Biol. 7, 773 – 791.
48 Nussbaumer, P., Lehr, P. and Billich, A. (2002) 2-Substituted 4-(thio)chromenone 6-O-sulfamates: potent inhibitors of human steroid sulfatase. J. Med. Chem. 45, 4310 – 4320.
49 Stanway, S. J., Purohit, A., Woo, L. W., Sufi, S., Vigushin, D., Ward, R., Wilson, R. H., Stanczyk, F. Z., Dobbs, N., Kulinskaya, E., Elliott, M., Potter, B. V., Reed, M. J. and Coombes, R. C. (2006) Phase I study of STX 64 (667 Coumate) in breast cancer patients: the first study of a steroid sulfatase inhibitor. Clin. Cancer Res. 12, 1585 – 1592.
50 Lapierre, J., Ahmed, V., Chen, M.-J., Ispahany, I., Guillemette, J. G. and Taylor, S. D. (2004) The difluoromethylene group as a replacement for the labile oxygen in steroid sulfates: A new approach to steroid sulfatase inhibitors. Bioorg. Med. Chem. Lett. 14, 151 – 155.
51 Liu, Y., Ahmed, V., Hill, B. and Taylor, S. D. (2005) Synthesis of a non-hydrolyzable estrone sulfate analogue bearing the difluoromethanesulfonamide group and its evaluation as a steriod sulfatase inhibitor. Org. Biomol. Chem. 3, 3329 – 3335.
52 Ahmad, V., Liu, Y., Silvestro, C. and Taylor, S. D. (2006) Boronic acids as inhibitors of steroid sulfatase. Bioorg. Med. Chem. 14, 8564 – 8573.
53 Poirier, D. and Boivin, R. P. (1998) 17alpha-alkyl- or 17alpha-substituted-17beta-estradiols. A new family of estrone-sulfatase inhibitors. Bioorg. Med. Chem. Lett. 8, 1891 – 1896.
54 Boivin, R. P., Luu-The, V., Lachance, R., Labrie, F. and Poirier, D. (2000) Structure-activity relationships of 17-alpha derivatives of estradiol as inhibitors of steroid sulfatase. J. Med. Chem. 43, 4465 – 4478.
55 Ishida, H., Nakata, T., Suzuki, M., Shiotsu, Y., Tanaka, H., Sato, N., Terasaki, Y., Takebayashi, M., Anazawa, H., Murakata, C., Li, P. K., Kuwabara, T. and Akinaga, S. (2007) A novel steroidal selective steroid sulfatase inhibitor KW-2581 inhibits sulfated-estrogen dependent growth of breast cancer cells *in vitro* and in animal models. Breast Cancer Res. Treat. (Epub ahead of print).
56 Foster, P. A., Newman, S. P., Chander, S. K., Stengel, C., Jhalli, R., Woo, L. L., Potter, B. V., Reed, M. J. and Purohit, A. (2006). *In vivo* efficacy of STX213, a second-generation steroid sulfatase inhibitor, for hormone-dependent breast cancer therapy. Clin. Cancer Res. 12, 5543 – 5549.
57 Rasmussen, L. M., Zaveri, N. T., Stenvang, J., Peters, R. H. and Lykkesfeldt, A. E. (2007) A novel dual-target steroid sulfatase inhibitor and anti-estrogen: SR 16157, a promising agent for the therapy of breast cancer. Breast Cancer Res. Treat. (Epub ahead of print).
58 Numazawa, M., Tominaga, T., Watari, Y. and Tada, Y. (2006) Inhibition of estrone sulfatase by aromatase inhibitor-based estrogen-3-sulfamates. Steroids 71, 371 – 379.
59 Hopwood, J. J., Bate, G. and Kirkpatrick, P. (2006) Galsulfatase. Nat. Rev. Drug Discov. 5, 101.