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MINIREVIEW

Structure and Function of Sulfotransferases

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Sulfotransferases (STs) catalyze the transfer reaction of the sulfate group from the ubiquitous donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an acceptor group of numerous substrates. This reaction, often referred to as sulfuryl transfer, sulfation, or sulfonation, is widely observed from bacteria to humans and plays a key role in various biological processes such as cell communication, growth and development, and defense. The cytosolic STs sulfate small molecules such as steroids, bioamines, and therapeutic drugs, while the Golgi-membrane counterparts sulfate large molecules including glucosaminylglycans and proteins. We have now solved the X-ray crystal structures of four cytosolic and one membrane ST. All five STs are globular proteins composed of a single α/β domain with the characteristic five-stranded β-sheet. The β-sheet constitutes the core of the Paps-binding and catalytic sites. Structural analysis of the PAPS-, PAP-, substrate-, and/or orthovanadate (VO_4^{3-})-bound enzymes has also revealed the common molecular mechanism of the transfer reaction catalyzed by sulfotransferses. The X-ray crystal structures have opened a new era for the study of sulfotransferases. © 2001 Academic Press

Key Words: **sulfotransferases; sulfuryl transfer; sulfation; sulfonation; steroids; glucosaminylglycans; drug metabolism.**

3′-Phosphoadenosine 5′-phosphosulfate (PAPS)[2] is a biologically active form of inorganic sulfate that serves as the sulfate donor in various biological processes (1, 2) (Fig. 1). A naturally-occurring defect in PAPS synthesis is lethal in humans (3). By action of a large family of enzymes known as sulfotransferases (STs), the sulfate group of PAPS is transferred to numerous endogenous as well as exogenous chemicals. For example, a Golgi-membrane ST sulfates glucosaminoglycans and thereby converts the common polysaccharides to unique binding sites that can be recognized by a biological signal molecule essential for cell growth and/or development (4, 5). *N*-Sulfation of the GlcNAc moiety by heparan sulfate *N*-deacetylase/*N*-sulfotransferases (NDSTs) is the first step in the biosynthesis of heparan/heparin sulfates, followed by 2O-, 3O-, and 6O-sulfations. An NDST1-null mouse is nonviable (6). Likewise, mice lacking the heparan sulfate 2O-sulfotranferase gene die from defective kidney development (7). Mucin-type Sialyl Lewis X oligosaccharides are specifically sulfated to provide the capacity of homing lymphocytes to roll on endothelial venules (8). These are only a small fraction of the roles played by the Golgi membrane STs in various biological processes. Collectively, the large number of membrane STs produce numerous biological substances essential for life.

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[2] Abbreviations used: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; ST, sulfotransferase; NDST, *N*-deacetylase/*N*-sulfotransferase; EST, estrogen sulfotransferase; mEST, mouse EST; PAP, 3′-phosphoadenosine-5′-phosphate; HST, hydroxysteroid sulfotransferase; hHST, human HST; hEST, human EST; NST, *N*-sulfotransferase; ADP, adenosine diphosphate; F3ST, flavonol 3-sulfotransferase; DHEA, dehydroxyepiandrosterone; 17β-HSD1, estrogenic 17β-dehydrogenase.

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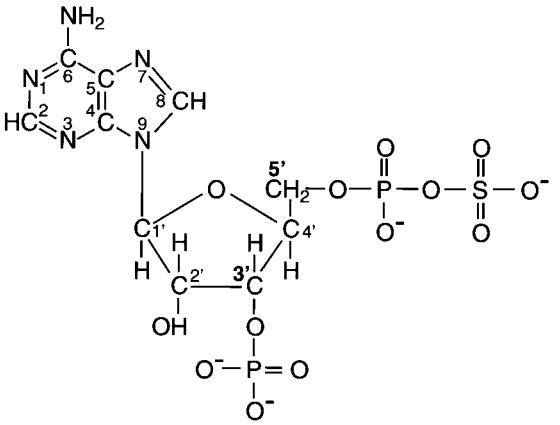

FIG. 1. Chemical structure of the sulfate donor 3′-phosphoadenosine 5′-phosphosulfate, PAPS.

Cytosolic STs are involved in the inactivation of endogenous signal molecules such as steroids, thyroids, and neurotransmitters (9). The sulfated products can be removed from cells or can be stored for possible reactivation by sulfatases (10). Dehydroxyepiandosterone sulfate is the major source of estrogen in pregnant women, while estradiol sulfate is a risk factor for hormone-dependent tumors such as breast cancer in women after menopause (11). The cytosolic enzymes also play an important role in the second-phase metabolism of xenochemicals such as therapeutic drugs, synthetic and naturally occurring toxins, and carcinogens. Since the sulfated metabolites are readily eliminated, the sulfation can be considered to be a cellular defense mechanism against toxicity and/or carcinogenicity of xenochemicals (12). In some cases, however, procarcinogenic and protoxic xenochemicals are sulfated to form active metabolites that attack macromolecules such as DNA (13). Since the same cytosolic STs can sulfate both endogenous and exogenous chemicals, defining a specific biological role for a given enzyme is often problematic.

Since the late 1980s, nearly a hundred ST cDNAs have been cloned, including cytosolic and membrane enzymes (5, 14). One difficulty soon realized was the lack of overall amino acid sequence homology between the cytosolic and membrane STs. A question arose as to whether the two groups belong to the same family of enzymes. Homology alignments of the deduced amino acid sequences from these cDNAs identified various conserved residues (15, 16). Also cDNA-based site-directed mutagenesis studies demonstrated that some of the residues, in fact, play critical roles in ST activity (17–19). Despite this progress, the opportunity to study STs based on the same structural features has only recently become possible with the solution of the first X-ray crystal structure of a ST (20). A large gap in time had passed since sulfation of glucosaminoglycans was first reported in the early 1950s and urinary phenyl sulfate was first discovered in 1876 (Ref. 21 for these histories). In this minireview, we describe the structural features and the common reaction mechanism that can be applied to all STs, including both the cytosolic and the membrane enzymes. We will also discuss a structural principle that might determine the substrate specificity of a given ST enzyme.

OVERALL STRUCTURE

STs are a single α/β globular protein with a characteristic five-stranded parallel β-sheet (Fig. 2). α-Helices flank both sides of the sheet. Estrogen sulfotransferase (EST) was originally described as the activity to form estrone sulfate in rat liver by Nose and Lipmann (22). Mouse EST (mEST), complexed with the inactive sulfate donor 3′-phosphoadenosine-5′-phosphate (PAP) and the acceptor substrate estradiol (E2), became the first X-ray crystal structure solved for the ST enzyme family (20). This structure revealed the PAP binding site and the E2 molecule buried deeply in the hydrophobic substrate pocket. Subsequently, the X-ray crystal structures of three more cytosolic enzymes have been determined: human dopamine/catecholamine sulfotransferase (SULT1A3) (23, 24), human hydroxysteroid sulfotransferase (hHST) (25), and human estrogen sulfotransferase (hEST) (26). By removing the amino-terminal membrane-binding region and the *N*-deacetylase domain from the Golgi membrane heparan sulfate *N*-deacetylase/*N*-sulfotransferase 1, only the *N*-sulfotransferase domain (NST1) was expressed in *E. coli* cells and cocrystallized with PAP (19, 27). Similar to cytosolic mEST, hEST, SULT1A3, and hHST, the membrane NST1 structure is composed of a single α/β domain and displays the characteristic five-stranded parallel β-sheet (Fig. 2). The β-sheet constitutes the PAPS-binding site and the core of the catalytic site, both of which are conserved in cytosolic as well as membrane STs. As a result, all STs appear to be members of a single gene superfamily.

Interestingly, STs share a similar structural resemblance to the nucleotide kinases with their secondary structures conserved not only in position but also in connectivity (20). The 5′-phopshate of the PAP molecule in the mEST structure superimposes with the β-phosphate of the ADP molecule in the structure of the uridylate kinase–ADP–AMP complex. Not only the leaving phosphate group, but also the acceptor group of the E2 molecule superimposes with the acceptor group of the AMP molecule. Considering the fact that sulfatase and phosphatase are also similar in their core structures, the sulfate and phosphate metabolisms may have coevolved by sharing structural and functional similarities. One of the major differences separating the sulfate donor PAPS from the phosphate donor ATP is the presence of the 3′-phosphate group. It is of interest to determine whether the 3′-phosphate

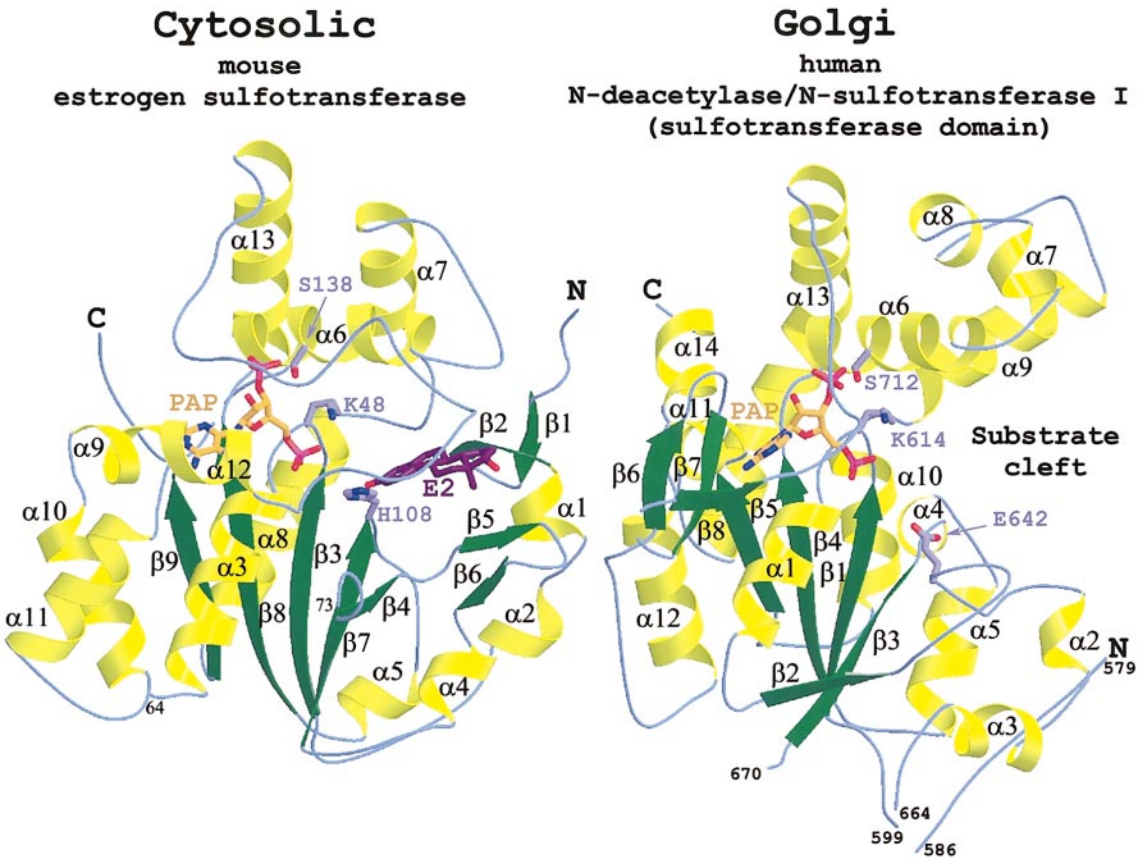

FIG. 2. Ribbon representation of X-ray crystal structures of ST enzymes. The structure of mEST is the ternary complex with PAP and E2, while the NST-1 structure contains only the PAP. Region between residues 586 and 599 is disordered in the NST-1 structure. This figure is created using Molscript (44) and Raster3D (45).

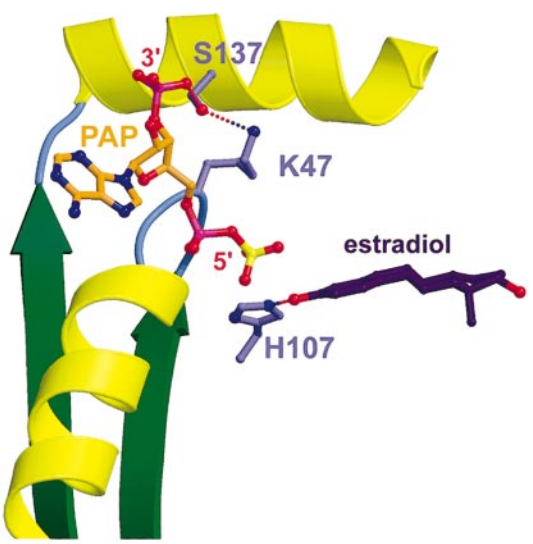

FIG. 3. The conserved strand–loop–helix and strand–turn–helix structure constituting the catalytic core of all ST enzymes. This structural representation is depicted from the hEST structures. The hydrogen bonding interaction between Lys[47] and Ser[137] is depicted from the PAPS bound hEST structure, while that of His[107] with the 3-hydroxyl is observed in the PAP/E2 bound hEST structure. This figure is created using Molscript (44) and Raster3D (45).

might play a unique role in sulfuryl transfer reaction catalyzed by ST enzymes.

In sharp contrast to the well-conserved PAP binding site and catalytic core in all STs, the substrate-binding site is totally different in the membrane and cytosolic enzymes. The binding site of NST1 is a large open cleft with a hydrophilic surface running perpendicular to the 5′-phosphate of the PAP molecule, while a deep hydrophobic pocket provides the substrate-binding site in the cytosolic STs. The structural differences are consistent with the binding of chemically and structurally unique substrates by these enzymes.

PAPS BINDING SITE

The strand–loop–helix and strand–turn–helix motifs constitute the core PAPS binding site, providing the majority of the enzyme interactions with the PAP molecule in all five structures of ST enzymes. The loop (named PSB-loop) interacts with the 5′-phosphate of the PAP molecule, whereas helix 6 of the strand–turn–helix unit that runs parallel to the PSB-loop provides interaction with the 3′-phosphate (Fig. 3). Not only the structures, but also the amino acid sequences of the phosphate-binding sites are conserved in all STs in-

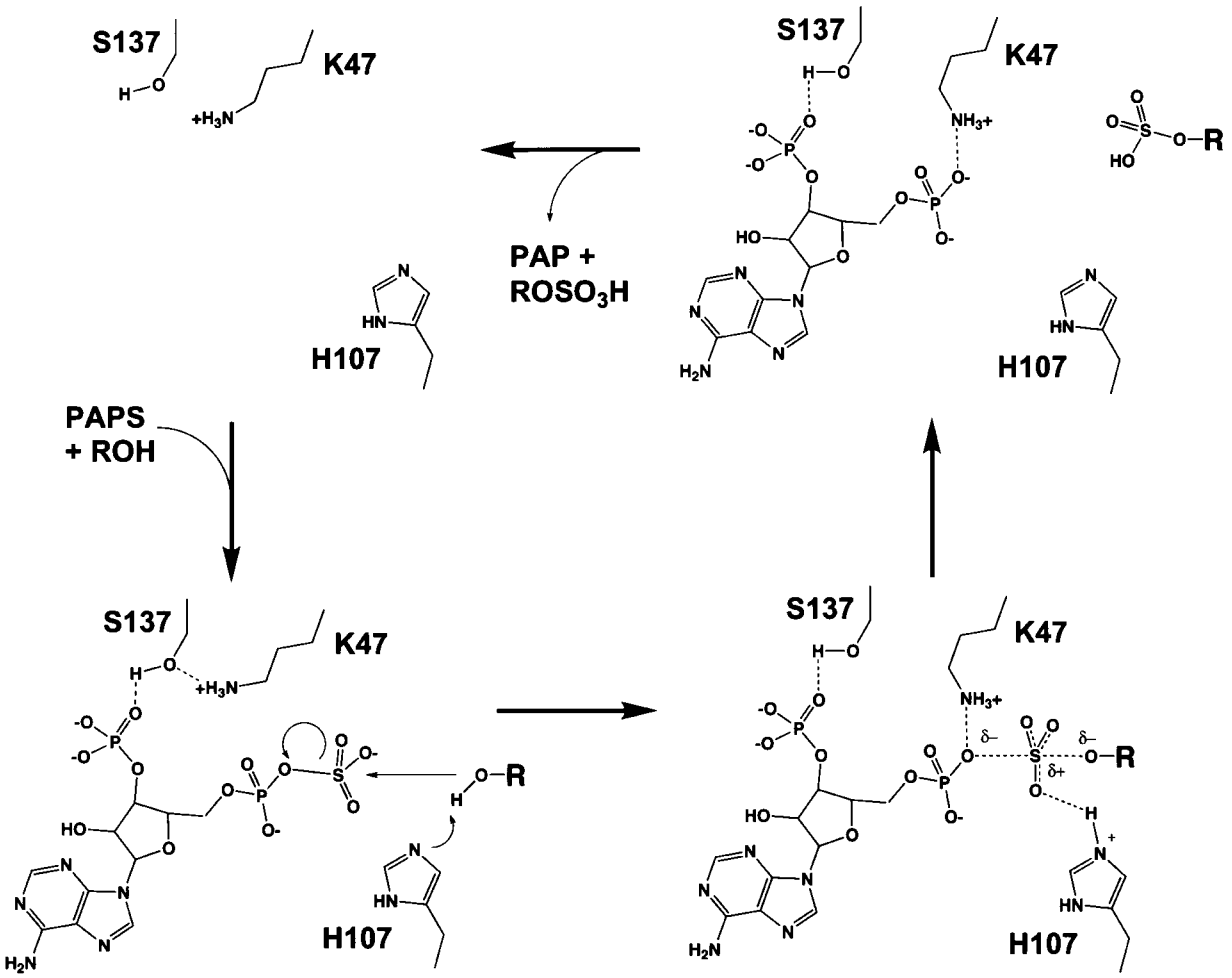

FIG. 4. The proposed reaction mechanism of sulfuryl transfer catalyzed by STs. Residues' numbers are taken from hEST.

cluding both the cytosolic and membrane enzymes. The consensus sequence motifs PKT/SGTTW/AL and IT/YV/I/LLRNPA/KDR/VL/AVSYYY/Q for the 5′- and 3′-phosphate binding sites, respectively, are designated as 5′PSB and 3′PB (28). The presence of these motifs has been used as a key criterion for identifying newly cloned ST cDNAs. The lysine residue within the 5′PSB motif, [Lys^{48} (in mEST) and Lys^{614} (in NST1) for example], is conserved in virtually all ST enzymes. The side-chain nitrogen of the lysine residue forms a hydrogen bond to an oxygen atom of the 5′-phosphate group in the PAP bound structures of STs. From the 3′PB motif, the serine residue, Ser^{138} (in mEST) and Ser^{725} (in NST1), for instance, is totally conserved in all STs. Its side-chain hydroxyl interacts with an oxygen atom of the 3′-phosphate group. These lysine and serine residues play critical roles not only in the binding of PAP but also in the catalysis, which will be discussed in the later sections of this minireview.

In the membrane NST-1, a three-stranded anti-parallel β-sheet and following random coil near the C-terminus provide additional interactions with the PAP molecule (Fig. 2). The side chain of Lys^{833} directly interacts with the 5′-phosphate of the PAP molecule, while that of Phe^{816} on strand 7 is in position to form a parallel ring-stacking interaction with the adenine moiety. The β-sheet bears a single disufide bond between strands 7 and 8. The presence of the bond appears to be essential since mutation of the cysteines was reported to inactivate NDST1 (29). Since disulfide bond can be formed only in an acidic environment, the presence of the disulfide bond indicates that the NST1 domain is located in the lumen of the Golgi sack.

REACTION MECHANISM

Earlier kinetic studies showed that cytosolic STs catalyze sequential transfer reactions with the formation of a ternary complex between enzyme, PAPS, and acceptor substrate (30, 31). Whether the reaction proceeds through a Bi Bi or ordered mechanism remains controversial. Site-directed mutagenesis studies have demonstrated that residues Lys^{59} and His^{118} are determining factors for the activity of flavonol 3-sulfotrans-

ferase (F3ST) (15, 32). These lysine and histidine residues are conserved in practically all ST enzymes and the corresponding residues were critical for the activity of other STs [Lys^{48} and His^{108} (in mEST), Lys^{47} and His^{107} (in hEST), Lys^{614} (in NST1), His^{98} (in rat ST-40)]. Recently, the conserved Ser^{197} (in HNK-1ST, respectively) was subjected to mutational analysis, with the finding that these serine residues also regulate ST activity (33). Consequently, we have addressed the question as to how the X-ray crystal structures consolidate these preexisting experimental considerations so as to provide the mechanism underlying the sulfuryl transfer reaction catalyzed by STs (Fig. 4).

The X-ray crystal structures of the mEST–PAP–E2 and mEST–PAP–vanadate complexes make it possible to provide a reasonable speculation about the catalytic mechanism (35). Given the caveat that PAP is not the active sulfate donor, these structures have helped us visualize the ternary complex of the ST with donor and acceptor substrates. Some heavy metal oxoanions such as orthovanadate (VO_4^{3-}) have been used to model a transition state of phosphoryl transfer reaction catalyzed by nucleotide kinases (34). Since Na_3VO_4 inhibits the activity of mEST, we soaked mEST–PAP crystals in Na_3VO_4 solution to produce the structure of the mEST–PAP–vanadate complex (35). This complex mimicks the transition state for the sulfuryl transfer reaction. The vanadium atom is on a line 2.1 Å to the leaving oxygen of the 5′-phosphate group and 2.3 Å to the water molecule that superimposes with the acceptor 3-phenol of the E2 molecule. Most recently, the active sulfate donor PAPS has been experimentally defined in hEST (Fig. 3), providing further supporting evidence for the transition state conformation adopted by the vanadate molecule (26). These structural features indicate that an in-line transfer mechanism for the catalysis. This finding is consistent with the previously proposed sequential transfer reaction.

What do the structures tell us about the possible roles of the conserved residues in catalysis? The side-chain nitrogen of the conserved Lys is coordinated to both leaving oxygen of the 5′-phosphate of the PAP molecule in all known ST structures. Superposition of the mEST–PAP–E2 with the mEST–PAP–vanadate structures showed that the conserved Lys^{48} also coordinates to an equatorial oxygen of the vanadate molecule. The superimposition also shows the coordination ligands of the conserved His^{108} to the 3-phenol of the E2 molecule and a different equatorial oxygen of the vanadate molecule. Thus, the conserved histidine and lysine residues appear to be catalytic residues. His^{108} can be a catalytic base that removes the proton from the acceptor 3-phenol group, thereby converting the 3-phenol to a strong nucleophile. Once formed, the nucleophile attacks the sulfur atom of PAPS, which in turn leads to an accumulation of negative charge at the bridging oxygen (i.e., leaving oxygen) between the 5′-phosphate and sulfate. On the other hand, the Lys may donate its proton to the bridging oxygen, thereby assisting in the dissociation of the sulfate group from PAPS. Thus, the structures have revealed that the conserved histidine and lysine residues likely play essential roles in catalysis.

What is a possible role of the conserved serine in the reaction? The side chain of the serine directly interacts with the 3′-phosphate in the known ST structures. Since mutation of the conserved serine increased $K_{m,\mathrm{PAPS}}$ or decreased ST binding to PAP–agarose, the serine appears to be critical for the binding of enzyme to PAPS. The function of the serine became evident, however, when the X-ray crystal structure of the hEST in the presence of the active sulfate donor PAPS complex was solved (26). In addition to the interaction to the 3′-phosphate, the side chain of the conserved Ser^{137} was also found to interact with the side chain of the conserved Lys^{47}. This side-chain interaction moves the side-chain nitrogen of the lysine away from the bridging oxygen of the PAPS molecule, preventing the nitrogen interaction with the oxygen. Apparently, the serine decreases PAPS hydrolysis when substrate is not present in the active site. Consistent with this idea, the $hEST_{S137A}$ mutant markedly increases PAPS hydrolysis (26). The conserved serine may thus regulate the sulfuryl transfer reaction through its interaction with the catalytic lysine.

A question now arises as to how the conserved serine, lysine, and histidine residues work in concert to advance the sulfuryl transfer reaction. A structure of SULT1A3 was solved without donor or acceptor substrates (23), in which the side chain of the conserved Ser^{139} was not in position to form a hydrogen bond with Lys^{51}. This finding implies that the side chain coordination of Ser^{137} to Lys^{47} occurs subsequent to the binding of the 3′-phosphate to Ser^{137}. Whereas Ser^{137} interacts with Lys^{47} to decrease the PAPS hydrolysis, the side chain nitrogen of the lysine must be coordinated with the bridging oxygen to play a role as the catalytic acid and/or the stabilization of transient state in aiding dissociation of the sulfate from the PAPS. It is necessary that the side chain switches from the Ser^{137} to the bridging oxygen during the reaction. How does this side chain switch occur? In this case, the conserved His may play the major role in the switch as the catalytic base. Following the substrate binding, the histidine removes the proton from the acceptor group, making it the nucleophile that subsequently attacks the sulfur atom of the PAPS molecule. Negative charge accumulates on the bridging oxygen. Finally, the developing negative charge forces the side-chain nitrogen to switch from the serine to the bridging oxygen and the sulfate dissociation occurs. In support of this concept, the mutation of His^{107} to asparagine abrogated not only estrogen sulfotransferase activity but also PAPS hydrolysis activity of hEST (26). Thus, the molecular

mechanism underlying the sulfuryl transfer reaction appears to be the dynamic charge redistribution on the chain that links the 3′-phopshate to the histidine residue by way of the serine and lysine residues, the bridging oxygen, the sulfur atom, and the acceptor group.

In the Golgi-membrane NST-1 structure, Glu^{642} occupies the position of the histidine residue in the cytosolic ST structures so that the glutamic acid can be a catalytic base. Molecular dynamics simulation was employed on the model structure of the NST-1/PAPS/heparan sulfate precursor (36). Interestingly, Lys^{614} moved near the sulfate group and a water molecule (not present in the X-ray crystal structure) became a bridge between the acceptor-NH_3^+ group of the precursor and the carboxylate group of Glu^{642}. The Lys^{614} thus appears to play a role in the charge and spatial stabilization of an associative transition state.

Is the sulfuryl transfer reaction a Bi Bi or ordered mechanism? The donor and acceptor substrates bind and their products release in random fashion in the former mechanism. Our present structural and mutational studies favor the latter mechanism in which the binding and release are ordered. The serine residue appears to be conserved so as to prevent PAPS hydrolysis in the absence of substrate. Moreover, the 3′-phosphate of PAPS is positioned to assist the serine in attracting the catalytic Lys from the bridging oxygen so as to prevent PAPS hydrolysis. In retrospect, the 3′-phopshate is a defining functional group that separates the reactivity of the sulfate donor PAPS from that of the phosphate donor ATP. These findings are consistent with the conclusion that the binding of the cosubstrates is ordered: the donor substrate may bind first and the binding of acceptor substrate follows. The introduction of the 3′-phosphate group on the sulfate donor might be an important force that has evolved sulfotransferases.

SUBSTRATE SPECIFICITY

Collectively, cytosolic STs are capable of sulfating virtually unlimited numbers of substrates. Although the ST enzymes display broad substrate specificity, a given enzyme can often be characterized by a specific substrate. The underlying principle that regulates the characteristic substrate specificity is not well developed. In light of the crystal structure containing substrate, we have chosen mEST to review how its specificity may be determined. The structural complementary of the substrate with the hydrophobic surface of the binding pocket appears to provide a principal determinant for the substrate specificity.

EST sulfates E2 effectively while exhibiting low activity toward hydroxysteroids such as dehydroxyepiandrosterone (DHEA). The acceptor 3-phenolic group of E2 is directed toward the 5′-phsophate of the PAP molecule, while the 17β-hydroxyl group is near the entrance of the pocket. The 3-phenol and 17β-hydroxyl groups are in position to form hydrogen bonds with His^{108} and Asn^{86}, respectively. As discussed in a previous section, the interaction between the 3-phenol and His^{108} may play an important role in catalysis as well as in acceptor substrate binding. On the other hand, mutation of Asn^{86} to alanine did not affect estrogen sulfotransferase activity of either mEST or hEST (25, 37). Thus, the major factor for the E2 binding is the hydrophobic interaction that is provided by the complimentary surface of the pocket. Superposition of DHEA with E2 in the structure of the mEST–PAP–E2 complex reveals that the 19-methyl group of the DHEA molecule is sterically hindered with Tyr^{81} of mEST (Fig. 5). The side chains of $Tyr^{81}$and Phe^{142} block the active site. We hypothesized that these residues may prevent DHEA from locating its acceptor 3-hydroxyl group near the active site (38). To test this idea, Tyr^{81} of mEST was replaced with a smaller hydrophobic residue. Subsequently, the E2 and DHEA sulfotransferase activities were measured, demonstrating a decrease in the former and increase in the latter, respectively. The $mEST_{Y81L}$ mutant exhibited an increased $K_{m,E2}$ and a decreased $k_{cat,E2}$. $K_{m,DHEA}$ was decreased and $k_{cat,DHEA}$ increased greatly, respectively, compared with wild-type mEST. Assuming that the k_{cat} value is a direct reflection of the distance and orientation between the acceptor group and the transferring sulfate group in a given ST enzyme, the larger increase of $k_{cat,DHEA}$ suggests that removing the block of the large residues allows the DHEA molecule to penetrate deeper into the substrate-binding pocket, and thereby place the 3β-hydroxyl group near the active site. Thus, it is reasonable to suggest that the estrogen specificity of mEST depends on the principle of surface complementarity of a substrate-binding pocket to the structure of a substrate. Whether this principle can be extended to other ST enzymes remains an interesting question for future investigation.

Estrogenic 17β-dehydrogenase (17β-HSD1) exhibits high dehydrogenase activity toward estrogens relative to 19-methyl steroids such as DHEA. The side-chain interaction of Leu^{149} with the 19-methyl group is a major structural factor for discriminating the 19-methyl steroids from the active site, since substitution of Leu^{149} with the small hydrophobic residue Ala resulted in the loss of the steroid selectivity in 17β-HSD1 (39). Interestingly, Leu^{149} is located near the surface of the substrate pocket, whereas the $Tyr^{81}$of mEST is closer to the active site, consistent with the different binding orientations of the 19-methyl group in these two enzymes. Thus, the interaction of the enzyme with the 19-methyl group likely provides a structural principle that determines the substrate specificity of various steroid-metabolizing enzymes.

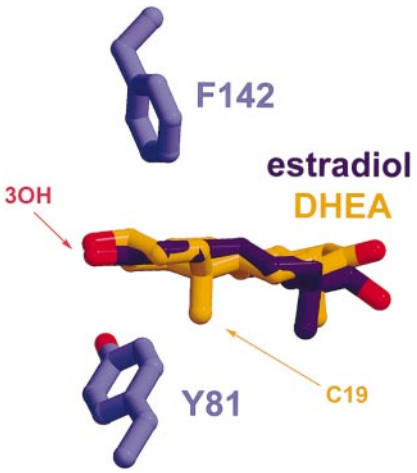

FIG. 5. The substrate access gate in mEST. DHEA molecule superimposed with the E2 molecule in the mEST–PAP–E2 structure. The C-19 methyl group is in steric hindrance with Tyr[81] that forms a stricture-like gate with Phe[142]. This figure is created using Molscript (44) and Raster3D (45).

A phenol ST SULT1A1 sulfates *p*-nitrophenol, whereas SULT1A3, which has 93% amino acid sequence identity to SULT1A1, sulfates preferentially bioamines such as dopamine and catecholamine. When the Glu146 of SULT1A3 was mutated to the corresponding Ala in SULT1A1, the activity of the mutated enzyme was altered to that of SULT1A1 (40, 41). Since the substrate-bound structure of SULT1A3 is not available, dopamine was modeled into the presumed substrate-binding pocket of the SULT1A3-PAP structure by positioning the acceptor group at the position of 3-phenolic group of the E2 molecule in the mEST pocket. The side chain of Glu[146] was found to form a direct charge interaction with the amino group of dopamine. The substitution Glu[146] with alanine should result in loss of this charge interaction. In this case, therefore, the specific charge interaction may be essential for conferring high activity toward dopamine (24, 42).

Understanding the subtle substrate specificity of the Golgi-membrane STs is particularly challenging since a given membrane ST catalyzes very specific sulfation of polysaccharide. Likewise, it is extremely important because a specific sulfation may produce a unique signal molecule essential for life. Although the structure of the membrane NST1 reveals a large open cleft for a putative substrate-binding site (27), the lack of an acceptor substrate in the structure hampers our effort to elucidate the specificity. To search for possible residues that might bind to the acceptor substrate, we modeled a hexasaccharide chain $(GlcA\text{-}GlcN)_3$ into the PAP bound NST 1 structure and mutated residues that interact with the chain (Kakuta *et al.*, unpublished). The chain runs perpendicular to the 5′-phosphate of the PAP molecule in the cleft with the trisaccharide unit sandwiched between helix 6 and a hydrophilic stretch of amphipathic random coil (residues 640 to 647, named Sweet Hill motif). The helix and Sweet Hill motif constitute the core of the putative substrate-binding site. Residues Phe[640], Glu[641], Glu[642], and Gln[644] (from the Hill) and Trp[713], His[716], and His[720] (on helix 6) are in position to interact with the trisaccharide moiety. Mutations of these residues to Ala either abolish or severely decrease NST-1 activity. These conserved res-

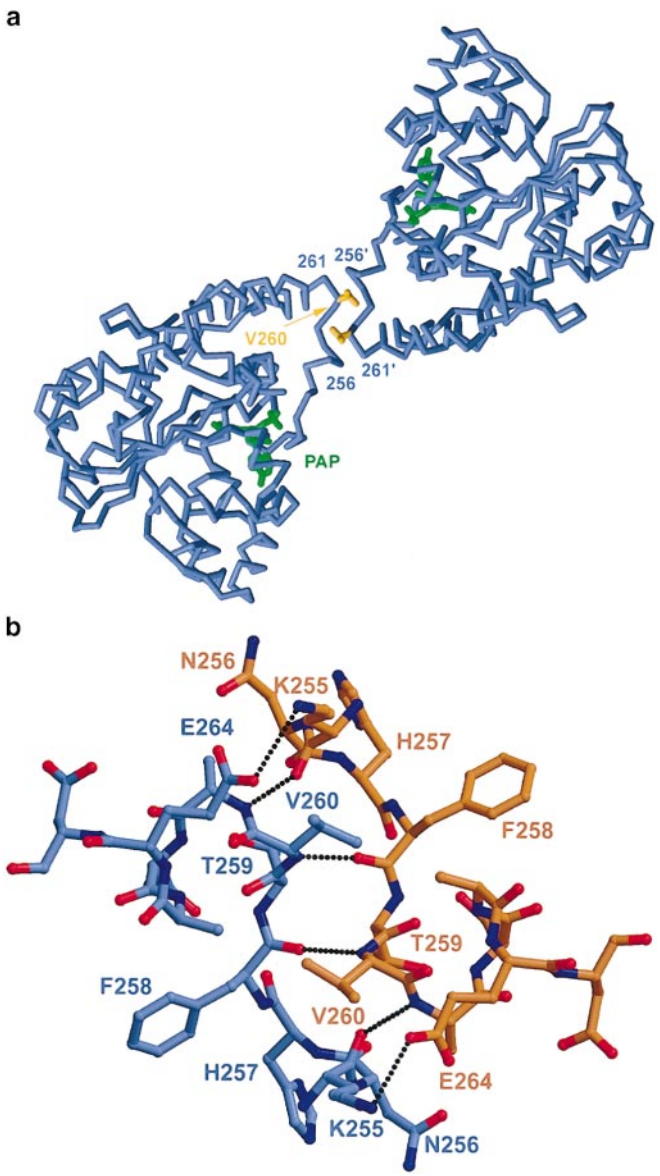

FIG. 6. The dimer interface (a) and dimerization motif (b) of hHST. A loop near the C-terminus forms a small (approximately 3% of the total surface) lattice contact between two monomers. The dimerization motif is a short Zipper-like sructure. The main hydrophobic interaction comes from residue Val[260] of one molecule extending into a hydrophobic pocket comprised with the carbon side chain of residues of the other molecule. The charge interaction at each end of the loop enforces the Zipper structure. This figure is created using Molscript (44) and Raster3D (45).

idues will surely be investigated for understanding the structural basis that confers the substrate specificity of various heparan sulfate sulfotransferases.

DIMERIZATION MOTIF

Cytosolic STs are generally homodimers in solution. The conserved dimerization motif is found in the cytosolic enzymes (43). Multiple amino acid sequence alignments show that the motif consists of ten residues near the C-terminus and is represented by the consensus sequence KXXXTVXXXE. An exception, mEST is monomeric with the central Thr and Val substituted with Phe^{269} and Glu^{270}, respectively. Replacing Phe^{269} and Glu^{270} with threonine and valine, respectively, resulted in dimerization of mEST. Likewise, hEST can be induced into a monomeric form by a single mutation of Val^{260} to glutamic acid. Similarly, the corresponding mutation of Val^{260} to glutamic acid converted hHST into a monomer. The KXXXTVXXXE sequence appears to be a dimerization motif, designated the KTVE motif. It is conserved in nearly all cytosolic ST enzymes.

The conserved KTVE motif is located on a loop region consisting of residues from Trp^{254} to Glu^{264} near the C-terminus of the hHST molecule (Fig. 6). This loop is involved in a small lattice contact between two monomers. Despite the small area of the interface, the overall interactions within this region are extensive. The hydrophilic interactions are composed of four backbone hydrogen bond interactions that are flanked at both ends by side chain charge interactions between Lys^{255} and Glu^{264} of both molecules. Consequently, the side chains from two hHST monomers form a hydrophobic zipper-like structure that is enforced by ion pairs at each end of the loop. This hydrophobic zipper-like structure is also found in the structures of hAST and hEST. The physiological significance of the dimerization in the function of cytosolic STs is yet to be defined.

CONCLUSION

It has been more than 3 years since the initial X-ray crystal structure was solved for the ST family (20). Supplemented by molecular biological and biochemical studies, the crystal structures of STs have provided the basis for answering various long-standing questions about the structure and function of ST enzymes. The structures suggest that all STs, including both the cytosolic and Golgi membrane enzymes, belong to a single gene superfamily. Further, the structures have revealed a conserved catalytic core and have led to a proposal for a common mechanism for sulfuryl transfer reactions. Most interestingly, the structures have shown the unique characteristics of sulfotransferases, one of which is the role of the 3′-phosphate of PAPS in the catalysis. The 3′-phosphate regulates the function of the catalytic lysine through its interaction with the conserved serine. The structures have uncovered a coordination chain that connects the 3′-phosphate with the conserved catalytic histidine in ST enzymes. The binding of donor and acceptor substrates apparently leads to charge redistribution of the chain. The underlying feature of the reaction mechanism appears to be the manner in which the conserved residues serine, lysine, and histidine act in concert to advance the reaction. The ST structures have implicated a necessary structural compatibility of the acceptor substrate with the surface of binding pocket for determining substrate specificity. Identification of the dimer interface will aid our future investigations for clarifying the significance of homo (as well as hetero-)-dimerization, for ST activity, and for physiology in general.

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