Arginine Residues in the Active Site of Human Phenol Sulfotransferase (SULT1A1)[*]

Guangping Chen[‡] and **Xinrong Chen**
Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma 74078

Abstract

Cytosolic sulfotransferases (STs) catalyze the sulfation of hydroxyl containing compounds. Human phenol sulfotransferase (SULT1A1) is the major human ST that catalyzes the sulfation of simple phenols. Because of its broad substrate specificity and lack of endogenous substrates, the biological function of SULT1A1 is believed to be an important detoxification enzyme. In this report, amino acid modification, computer structure modeling, and site-directed mutagenesis were used for studies of Arg residues in the active site of SULT1A1. The Arg-specific modification reagent, 2,3-butanedione, inactivated SULT1A1 in an efficient, time- and concentration-dependent manner, suggesting Arg residues play an important role in the catalytic activity of SULT1A1. According to the computer model, Arg^{78}, Arg^{130}, and Arg^{257} may be important for SULT1A1 catalytic activity. Site-directed mutagenesis results demonstrated that the positive charge on Arg^{78} is not critical for SULT1A1 because R78A is still active. In contrast, a negative charge at this position, R78E, completely inactivated SULT1A1. Arg^{78} is in close proximity to the site of sulfuryl group transfer. Arg^{257} is located very close to the 3′-phosphate in adenosine 3′-phosphate 5′-phosphosulfate (PAPS). Site-directed mutagenesis demonstrated that Arg^{257} is critical for SULT1A1: both R257A and R257E are inactive. Although Arg^{130} is also located very close to the 3′-phosphate of PAPS, R130A and R130E are still active, suggesting that Arg^{130} is not a critical residue for the catalytic activity of SULT1A1. Computer modeling suggests that the ionic interaction between the positive charge on Arg^{257}, and the negative charge on 3′-phosphate is the primary force stabilizing the specific binding of PAPS.

Sulfotransferases (STs)[1] catalyze the sulfation of hydroxyl-containing molecules. The substrate specificities of STs are very broad. Most hydroxyl groups in phenols, alcohols, and *N*-substituted hydroxylamines are substrates for one of the ST isoforms. The co-substrate for sulfation of all STs is adenosine 3′-phosphate 5′-phosphosulfate (PAPS). Sulfation (sulfuryl transfer) is widely observed in various biological processes. Various biological signaling molecules, including hormones, neurotransmitters, peptides, and proteins, can be sulfated to alter biological activity. STs also catalyze the sulfation of a broad range of xenobiotics. Sulfation of drugs and xenobiotics is mainly associated with detoxification:

[‡]To whom correspondence should be addressed264 McElroy Hall, Dept. of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078. Tel.: 405-744-2349; Fax: 405-744-8263; cguang@okstate.edu.

*Note Added in Proof—*The authors sincerely apologize that our statement in the introduction "The crystal structure of the human simple phenol ST (P-PST, SULT1A1) has not been shown" is not true. This crystal structure complexed with PAP and *p*-nitrophenol (Protein Data Bank entry 1LS6) has been solved and published (Gamage, N. U., Duggleby, R. G., Barnett, A. C., Tresillian, M., Latham, C. F., Liyou, N. E., McManus, M. E., and Martin, J. L. (2003) *J. Biol. Chem.* **278,** 7655–7662).

[1]The abbreviations used are: ST, sulfotransferase; PAP, 3′-phosphoadenosine-5′-phosphate; PAPS, 3′-phosphate 5′-phosphosulfate; EST, expressed sequence tag; DA, dialdehyde; BD, 2,3-butadione; 4PP, 4-phenylphenol; 2NP, 2-naphthol; PNPS, *p*-nitrophenyl sulfate; PNP, *p*-nitrophenol.

biotransformation of a relatively hydrophobic xenobiotic into a more water-soluble sulfuric ester that is readily excreted. However, there are numerous important exceptions wherein the formation of chemically reactive sulfuric esters is an essential step in the metabolic pathways leading to toxic or carcinogenic responses. Detoxification or bioactivation is highly dependent on the electrophilic reactivity of the individual sulfuric ester products formed. Most sulfation products are stable enough for excretion, while other sulfuric ester products can be reactive toward nucleophilic sites on DNA, RNA, and protein, and so become involved in the initiation of carcinogenesis and other toxic responses.

Structure-activity relationship studies of STs started in the 1980s. Protein sequence alignments of the different STs have revealed two highly conserved regions, one (PKSGTTW) in the N-terminal region and one (RKGXXGDWK) in the C-terminal region (1). Since all STs use the same sulfuryl donor, it was speculated that these two regions were involved in PAPS binding (2, 3). Affinity labeling of rat liver aryl ST-IV identified a peptide sequence in the PAPS-binding site, which is in close proximity to the highly conserved sequence in the N-terminal region (4). Site-directed mutagenesis and [^{35}S]PAPS affinity labeling studies of flavonol 3-ST have supported that the two regions mentioned above are involved in PAPS binding (5). Affinity chromatography and ^{31}P NMR studies of flavonol 3-ST demonstrated the involvement of Lys^{59}, Arg^{11}, and Arg^{277} in PAPS binding (6). Point mutations and [^{35}S]PAPS affinity labeling studies of guinea pig EST have also strongly supported the C-terminal conserved region mentioned above as being part of the PAPS-binding site (7). The x-ray crystal structure of mouse EST supports the idea that these two regions, residues 259–265 and 45–51 for mouse estrogen ST, are directly involved in PAPS binding (8). In addition to these two regions, Thr^{227}, Trp^{53}, and Phe^{229} of mouse EST have also been demonstrated to be involved in PAPS binding.

Limited information is available on the structural determinants of the ST substrate-binding site. Of the four regions proposed by Varin's group for flavonol STs, regions I and IV are the same two regions mentioned above for the PAPS-binding site, while domain II, spanning amino acids 92–194 of the flavonol 3-ST sequence, is believed to contain all the determinants for substrate binding (2, 9). Subsequent mutational analysis of domain II of flavonol 3-ST did not identify amino acids critical for substrate binding; however, Leu^{95} may be in direct contact with the flavonol B ring (10). *N*-Bromoacetyl-4-hydroxyphenylamine has been used for affinity labeling of the substrate-binding site in rat liver aryl ST-IV (11). Four modified amino acids, Cys^{232}, Cys^{283}, Lys^{286}, and Cys^{289} have been identified. Sakakibara *et al.* (3) reported localization and a functional analysis of the substrate specificity and catalytic domains of the human M-PST and P-PST-1. By comparing the kinetic parameters of a series of expressed chimeric PSTs, where the M-form and P-form coding regions were exchanged, it was concluded that amino acid residues 84–148 contain the structural determinants for substrate specificity in both forms. They also demonstrated the differential roles of the two highly variable regions (amino acid residues 84–89 and 143–148) in substrate binding, catalysis, and sensitivity to inhibition by 2,6-dichloro-4-nitrophenol. Similar studies on human aryl-STs (HAST1, HAST3, and HAST4) suggested that the two highly divergent regions, region A (amino acids 44–107) and region B (amino acids 132–164), determine the substrate specificity of human aryl STs (12, 13). A single amino acid change in HAST1 (A146E) altered its activity for *p*-nitrophenol (PNP) to that of HAST3. Studies on guinea pig 3-hydroxysteroid ST isoforms revealed that the amino acid residue at position 51 plays a fundamental role in determining the stereospecificity exhibited by the α- and β-isoforms. Mutational studies on mouse EST demonstrated that Tyr^{81} determines the substrate specificity of this enzyme (14). The x-ray crystal structure of mouse EST showed that residues Phe^{142}, Ile^{146}, Tyr^{149} contribute to binding of steroid 17β-estradiol. Asn^{86} is believed to be in a position to form a hydrogen bond with the 17β-

hydroxyl group, while Lys[106] and His[108] are within hydrogen bonding distance of the 3α-phenol group of the steroid (8).

Studies of structure-function relationships on human STs are relatively limited. The work on human phenol STs has revealed two regions and one specific amino acid (position 146) as important for the determination of substrate specificity, as mentioned above (3, 12, 13). To our knowledge, studies on the identification of specific residues in the active site of STs by amino acid modification reagents are limited. Borchardt *et al.* (16, 17) used 2,3-butanedione and phenylglyoxal to identify the arginyl residues in the active site of rat liver PST. *N*-Ethylmaleimide has also been used for the identification of essential sulfhydryl residues in rat PST (17). Ribonucleotide dialdehydes (ATPDA, ADPDA, AMPDA, and APSDA) have been characterized as affinity labeling reagents for rat liver PST, and it was speculated that the dialdehydes inactivated the ST by possible formation of a Schiff's base adduct with an active site lysine residue (17, 18). ATPDA has been used as an affinity labeling reagent for the identification of a peptide sequence in the PAPS-binding site of rat liver AST-IV (4). We have studied the carboxyl amino acid residues in the active site of SULT1A1 (19). Our results indicated that the conserved residues Asp[134] and Asp[263] are critical for the binding of PAPS. Glu[83] and Glu[246] may be important for the binding of substrate, and Glu[83] may be involved in the catalysis reaction.

Since the crystallization of the mouse estrogen ST (8), a few human STs have also been crystallized and their crystal structures partially solved. They include human monoamine ST (M-PST, SULT1A3) (20, 21), human estrogen ST (EST, SULT1E1) (22), and hydroxysteroid ST (DHEA-ST, SULT2B1) (23). The crystal structure of the human simple phenol ST (P-PST, SULT1A1) has not been shown. We built the computer model of SULT1A1 based on the other known ST crystal structures (19). Even with the known crystal structure, the roles of amino acid residues in the active site still need to be studied using experimental methods.

In this report, we studied Arg residues in the active site of human phenol sulfotransferase (SULT1A1) using amino acid modification, computer modeling, and site-directed mutagenesis. Arginine is one of the two amino acids that carry a positive charge. It may be very important for the catalytic activity of STs. Arg-specific modification reagents have been used to study the importance of Arg for the catalytic activity of SULT1A1. Our results demonstrate that the positive charge of Arg[78] does not contribute to the catalytic activity of SULT1A1. A negative charge at this position inactivated SULT1A1. Although both Arg[257] and Arg[130] are in close proximity to the 3′-phosphate on PAPS, only Arg[257] appears to be critical for the catalytic activity of SULT1A1.

EXPERIMENTAL PROCEDURES

Material

2,3-Butanedione (BD), 4-phenylphenol (4PP), 2-naphthol (2NP), 3′-phosphoadenosine-5′-phosphate (PAP), PAPS, isopropyl-1-thio-β-D-galactopyranoside, ampicillin, and dithiothreitol were purchased from Sigma. Amylose affinity resin and maltose were purchased from New England Biolabs (Beverly, MA). All other chemicals and solvents were of the highest grade available.

Enzymatic Assay

Methylene Blue Assay—This method is used for determining activity of SULT1A1 and its mutants and for SULT1A1 mutant protein kinetic parameter determinations. This assay method was developed by Nose and Lipmann (24). SULT1A1 activity was determined in

the assay mixture containing 0.125 M phosphate buffer, pH 6.2, 8 μM PAPS, 2–10 μg mutant protein, and different concentrations of substrate in a total volume of 400 μl. After incubating at 37.0 °C for 30 min, the reaction was stopped by adding 0.5 ml of methylene blue solution (250 mg of methylene blue, 10 ml of H_2SO_4, 50 g of Na_2SO_4 in 1 liter of water) and 1 ml of chloroform. After vortexing, the tubes were centrifuged in a benchtop centrifuge for 1 min. The chloroform phase was then transferred to tubes containing 20–50 mg of dehydrated Na_2SO_4 and read at 651 nm. Kinetic constants were calculated according to the Michaelis-Menten equation.

p-Nitrophenyl Sulfate (PNPS) Assay—This method utilizes PNPS to regenerate PAPS from product PAP. At the same time, the color reagent PNP is generated for colorimetric measurement (25–27). This method was used for the determination of time- and concentration-dependent inactivation, kinetic parameter determination for partially inactivated SULT1A1, and PAP and substrate protection for BD inactivation of SULT1A1.

Purification of SULT1A1—Human SULT1A1 cDNA (and its mutants) was expressed using the pMAL-c2 expression system as described previously (26, 28). Briefly, the maltose-binding fusion protein (MBSULT1A1 and its mutants) was expressed in XL1-Blue cells containing the pMAL-SULT1A1 vector. The cytosol of fusion protein was loaded onto an amylose affinity column (New England Biolabs) pre-washed with 5 mM phosphate buffer, pH 7.4. After washing out other proteins, the fusion protein (mutants or wild type) was eluted using a 0–1 mM maltose gradient in the same buffer. The final purified SULT1A1 was apparent homogenous according to SDS-PAGE analysis.

SULT1A1 Inactivation by 2,3-Butanedione

SULT1A1 (0.1 mg/ml) was incubated in 0.05 M borate, pH 7.5, containing 1 M NaCl at room temperature. Different concentrations of 2,3-butanedione (in ethanol) were added to start the inactivation reaction. Aliquots (10 μl) were taken at different times for standard assay of SULT1A1 activity. Arginine (0.5 M) was included in the enzyme assay reaction mixture (for time- and concentration-dependent inactivation) or used to stop the reaction (for partially inactivation of SULT1A1). For substrate or PAP protection experiments, phenol substrates (2-naph-thol or 4-phenylphenol) or PAP were added to the SULT1A1 solution before the addition of 2,3-butanedione.

Modeling of the SULT1A1 Structure

The molecular coordinates of mSULT1E1 were obtained from the Protein Data Bank file 1AQU. A Silicon Graphics Iris work station was used for modeling. Since the primary sequence of SULT1A1 and mSULT1E1 could be aligned with no gaps, a significant amino acid similarity (93%) existed between the two proteins, and each had the same number of amino acids. Biosym's Homology program was used to build the backbone of SULT1A1 based on the coordinates of mSULT1E1. Side chain bumps of 0.1% overlap of the Van der Walls radii were relieved manually. The structure was then minimized using steepest descents (2000 steps) followed by conjugate gradients (1000 steps) using Biosym's Discover program, version 2.9. No constraints were used. The program RasMol, version 2.6, was used for display of the SULT1A1 structure.

Site-directed Mutagenesis of the cDNA Encoding SULT1A1

The cDNA encoding SULT1A1 in the pKK233-3 vector was from Dr. Charles Falany, and this construct has been described previously (29, 30). All mutant cDNAs were created with the QuikChange mutagenesis kit (Stratagene, La Jolla, CA), and all primers for mutagenesis were obtained from Integrated DNA Technologies Inc. (Coralville, IA). The primers used

for the mutations are list in Table I. The primers were designed using the Gene Fisher primer designing and Multialignment software. Mutant cDNA was generated and selected through a series of three steps consisting of primer extension/thermocycling, digestion of parental DNA, and transformation as outlined in the manufacturer's protocol. Colonies resulting from the transformation were grown in sterile medium by standard protocols and plasmid was isolated from cells with the QIAprep spin mini-prep kit (Qiagen Inc., Valencia, CA). The presence of the desired mutations was confirmed by DNA sequencing which was performed by The Oklahoma State University, Department of Biochemistry and Molecular Biology, Recombinant DNA/Protein Resource Facility.

RESULTS AND DISCUSSION

Chemical Inactivation of SULT1A1 by Arginine Residue-specific Modification Reagent 2,3-Butadione

BD is a reagent specifically reactive toward the Arg residues of a protein (Fig. 1). BD has been used for the characterization of Arg residues in various enzymes (31–34). Because borate is necessary to form a stable adduct (Fig. 1), the inactivation reactions were done in 50 mM borate buffer, pH 7.5.

BD inactivated SULT1A1 in an efficient, time- and concentration-dependent manner (Fig. 2*A*). In the concentration range from 0.0625 to 2 mM, BD effectively inactivated SULT1A1. At 2 mM, BD inactivated 95% of SULT1A1 activity within 30 s. At all BD concentrations, log(percent activity) is linear *versus* time. These facts indicate that the inactivation is specific through active site Arg residues. According to the equation $d[E]/dt = K_{app}[E]$ or $\log[E] = 2.3K_{app}t$ (where E represents active SULT1A1, t is time, K_{app} is apparent first order rate constant), K_{app} can be calculated from the slopes of Fig. 2*A*. According to equation $K_{app} = K[BD]^n$ or $\log K_{app} = \log K + n\log[BD]$ (where K is the rate constant and n is the reaction order for BD), when $\log K_{app}$ is plotted against the $\log[BD]$, a straight line results (Fig. 2*B*). From the data in Fig. 2*B*, $n = 1.07$ and $K = 450$ min^{-1} M^{-1}. This further demonstrates that BD is a specific reagent for the inactivation of critical Arg residues in the active site of SULT1A1. The BD reaction order, n, is ~1. This suggests that there may be only one Arg residue in the active site whose modification could lead to complete inactivation of SULT1A1.

SULT1A1 was partially inactivated using 0.25 mM BD in 50 mM borate buffer, pH 7.5, containing 1 M NaCl for 1 min at room temperature. The inactivation was stopped by addition of an equal volume of 1 M arginine solution at pH 7.5. The partially inactivated SULT1A1 was used to determine kinetic parameter changes by varying either PAPS or substrate 4PP concentrations. Ethanol (BD stock solution was prepared in ethanol) was used for the control. Fig. 3, *A* and *B*, demonstrate that the partially inactivated SULT1A1 did not change K_m for either PAPS or substrate 4PP. This suggests that the SULT1A1 completely lost its catalytic activity when BD modified the active site Arg residues. If any of the BD modifications to SULT1A1 had lead to partial loss of catalytic activity, the above partially inactivated SULT1A1 would have changed in K_m for either PAPS or 4PP.

Fig. 4 demonstrates that both PAP and phenol substrates (4-phenylphenol and 2-naphthol) protected the BD inactivation of SULT1A1. This further suggests that the inactivation is due to the modification of Arg residues in the active site. Because the inactivation is irreversible and substrate or PAP binding is reversible, the protection cannot be 100%.

Computer Modeling Structure of SULT1A1

The computer model of human SULT1A1 was generated (19) based on the published crystal structure of mouse estrogen ST (mSULT1E1) (Protein Data Bank file 1AQU) (15).

According to the computer SULT1A1 model, Arg^{78}, Arg^{130}, and Arg^{257} may be important for the catalytic activity of SULT1A1 (Fig. 5). Arg^{257} and Arg^{130} are in close proximity to the 3′-phosphate group on PAPS. Arg^{78} is in close proximity to both the 5′-phosphosulfate on PAPS and the hydroxyl group on substrate, 2-naphthol. Arg^{78} may affect the catalytic activity of SULT1A1. All other Arg residues are located on the surface of the protein structure. Table II lists the shortest calculated distances of these Arg residues to different functional groups.

Site-directed Mutagenesis of Important Arg Residues

According to the BD inactivation results and computer modeling structure, Arg^{78}, Arg^{130}, and Arg^{257} were selected for site-directed mutagenesis studies. Arg^{213} (located on the surface of SULT1A1) was selected for control mutation. Arg^{78}, Arg^{130}, and Arg^{257} were mutated to either the neutral amino acid alanine or the negatively charged amino acid glutamic, acid. Arg^{213} was only mutated to glutamic acid. All the mutated SULT1A1s were purified. Pure proteins of the mutants were used for kinetic enzyme activity measurements. Fig. 6 shows the SDS-PAGE for the purified mutant proteins. All mutant proteins were pure based on the SDS-PAGE. Fig. 7 shows the enzymatic specific activities of different mutants at 100 μM 2-naphthol, 8 μM PAPS, pH 6.2. Fig. 7 demonstrates that R213E (control mutant) did not change SULT1A1 catalytic activity. R257A and R257E almost completely inactivated SULT1A1. This suggests that a positive charge at the Arg^{257} position is critical for the catalytic activity (or PAPS binding) of SULT1A1. Although R130 is also in close proximity to the 3′-phosphate of PAPS, neither R130A nor R130E significantly changed the catalytic activity of SULT1A1. Arg^{130} may not play a critical role for the binding of PAPS or the catalytic activity of SULT1A1.

R78A retained activity (Fig. 7 and Table III). This suggests that the positive charge on Arg^{78} is not critical for the catalytic activity of SULT1A1. R78E was inactive, suggesting that a negative charge at position 78 will inactivate SULT1A1. Based on the computer model of SULT1A1 (Fig. 8), Lys^{106} (the other amino acid carrying a positive charge on its side chain) is in closer proximity than Arg^{78} to the location of sulfuryl group transfer (Table II). The positive charge on Arg^{78} is neutralized by Asp^{59} (negatively charged residue). Therefore the positive charge at position 78 is not critical for catalytic activity of SULT1A1. If position 78 is mutated to a negatively charge amino acid, Lys^{106} may be affected, thus inactivating SULT1A1. We are currently investigating the role of Lys residues on the catalytic activity of SULT1A1.

The kinetic parameters for the active mutants were determined (Table III). The results in Table III indicate that R213E, R78A, R130A, and R130E have catalytic characteristics similar to wild type SULT1A1. K_m and $V_{\max}$ for these mutants did not change significantly compared with the wild type.

CONCLUSION

The effect of specific Arg residues on SULT1A1 activity has not been previously studied. To the best of our knowledge, the only report on the study of Arg residues in STs was published by Borchardt and Schasteen in 1977 (16). They studied 2,3-butandione inactivation of a rat liver phenol ST. Their results indicated that BD inactivated this rat enzyme in a time- and concentration-dependent manner. They suggested a possible role for arginyl residues as anionic recognition sites for sulfate transfer reactions. In this work, we focused on the study of Arg residues in the active site of human SULT1A1 using the combination of amino acid modification, computer modeling, and site-directed mutagenesis methods. Our results consistently suggest that among the Arg residues, only Arg^{257} is critical for the catalytic activity of SULT1A1. The inactivation reaction order for BD (*n*) is

~1. This suggests that there is only one Arg modification that can completely inactivate SULT1A1. Because R78A is active, the BD modification of R78 should not inactivate SULT1A1. Because both R130A and R130E are active, the BD modification of Arg[130] also should not inactivate SULT1A1. BD partially inactivated SULT1A1 did not change the K_m values for either substrate or PAPS (Fig. 3), suggesting that when BD modified Arg[257], SULT1A1 would completely lose its activity. When other Arg residues were modified, SULT1A1 was still active. These findings agree with the site-directed mutagenesis results.

Mutational analysis of substrate-binding domains in SULT1A1 (P-PST) and SULT1A3 (M-PST) demonstrated that the amino acid at position 146 (Ala[146] in SULT1A1 and Glu[146] in SULT1A3) determine the substrate specificity of SULT1A1 and SULT1A3 (12, 13, 35). Our SULT1A1 model indicates that Ala[146] is in direct contact with substrate 2-naphthol. Mutation of His[108] in both SULT1A1 and SULT1A3 resulted in complete loss of activity suggesting His[108] is a critical residue in SULT1A1 and SULT1A3 (35). Our recent amino acid modification experimental results demonstrated that His[108] is critical for catalysis in both SULT1A1 and SULT1A3 but not for the binding of substrate or PAPS.[2] According to our SULT1A1 model, His[108] is located at the site of sulfuryl group transfer. The imidazole group in His[108] may act as a base catalyst for the sulfuryl transfer. These results demonstrate critical residues for catalysis and substrate binding.

In this work, Arg[257] was found to be critical for SULT1A1 catalytic activity. Arg[257] is located near the 3′-phosphate but not the site of sulfuryl group transfer. This suggests that Arg residues in SULT1A1 do not contribute to the transfer of the sulfuryl group but do contribute to the specific binding of PAPS. A mutational study on rat liver phenol ST demonstrated that Ser[134] plays a key role in PAPS binding but not in catalysis (15). This work also demonstrated the roles of Arg[126] and Arg[253] (corresponding to Arg[130] and Arg[257], respectively, in human SULT1A1) in the specific binding of PAP or AMP. Our work demonstrates only Arg[257], and not Arg[130], to be critical for human SULT1A1 activity. This is in agreement with the commonly recognized fact that the two regions, PKSGTTK (47–53 for SULT1A1) and RKG*XX*GDWK*XX*FT (257–269 for SULT1A1), are the primary sequences in the specific binding of PAPS to STs (1–8, 15). Arg[257] is in the latter conserved region, whereas Arg[130] is in neither. Arg[130] in SULT1A1 and Arg[126] in rat phenol ST may have different roles in the two different enzymes.

Fig. 9 shows PAPS and both positively and negatively charged residues to be within 5 Å of 3′-phosphate. Both Arg[257] and Arg[130] are in close proximity to the 3′-phosphate. Lys[258] is also close to the 3′-phosphate (within 2 Å), but its positively charged ammonium ion lies more than 8 Å away. There are two negatively charged residues, Asp[134] and Asp[263], within 5 Å distance. Asp[134] is near Arg[130] (2 Å), neutralizing its positive charge. Asp[263] is near Lys[258] (2 Å), neutralizing its positive charge. Arg[257] is not neutralized by any neighboring residues. The ionic interaction between the positive charge on Arg[257] and the negative charge on 3′-phosphate is the primary force stabilizing the specific binding of PAPS. Arg[257] is conserved in all STs and is one of the most important residues for specific binding of PAPS.

Acknowledgments

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[2]G. Chen, manuscript in preparation.

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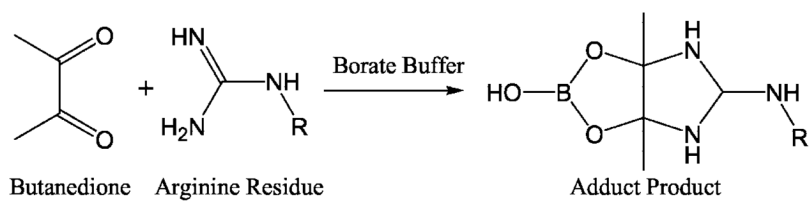35. Liu MC, Suiko M, Sakakibara Y. J Biol Chem. 2000; 275:13460–13464. [PubMed: 10788459]

Fig. 1.
Chemical reaction for 2,3-butanedione inactivation of Arg residues in a protein.
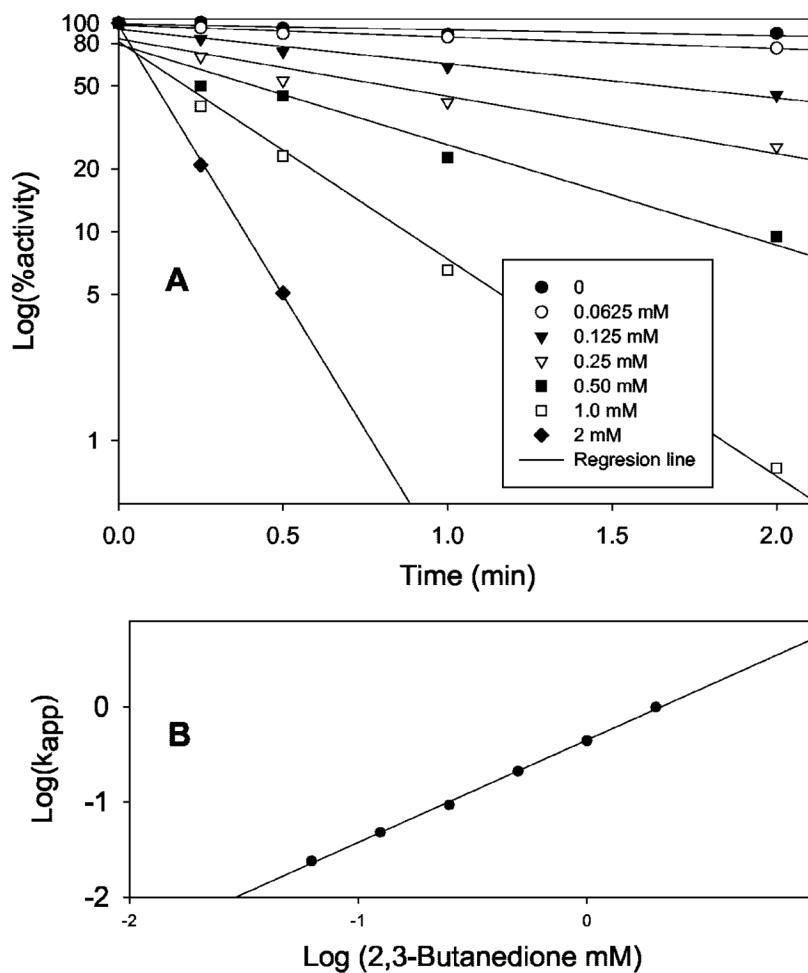
Fig. 2. Time- and concentration-dependent inactivation of SULT1A1 by 2,3-butanedione
A, log(percent activity) *versus* time. SULT1A1 (0.1 mg/ml) was incubated with different concentrations of 2,3-butanedione at room temperature in 50 mM borate, 1 M NaCl, pH 7.5. Aliquots were taken at different time points. SULT1A1 remaining activity was assayed using the PNPS assay method in a mixture containing 0.5 M arginine. *B*, log(K_{app}) *versus* log(BD).
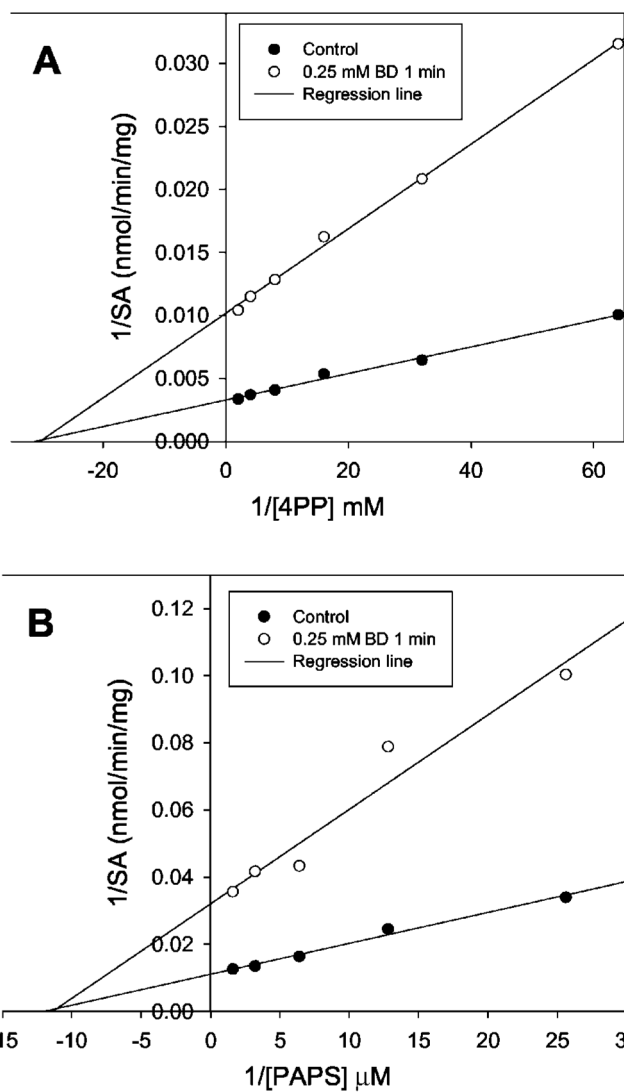
Fig. 3. Kinetic parameter determination of partially inactivated SULT1A1
The PNPS assay method was used for the determination of enzyme activity. *A*, varied
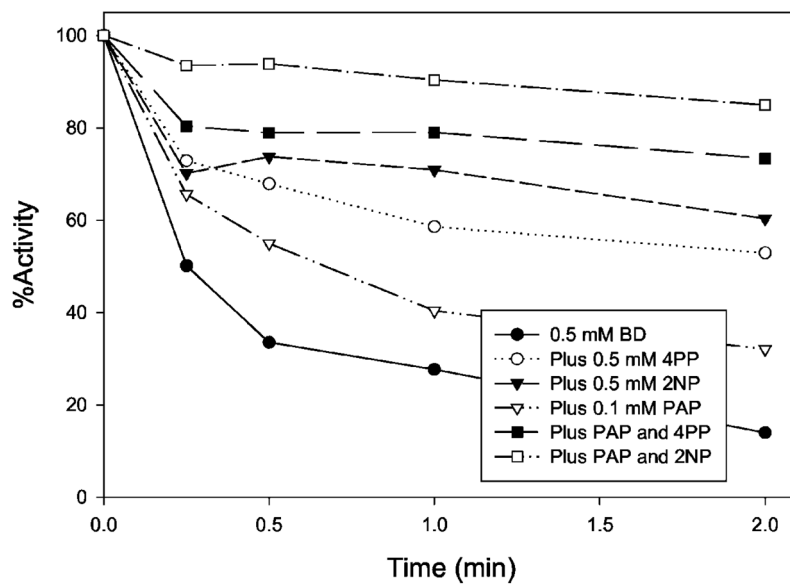substrate 4-phenylphenol concentration. *B*, varied PAPS concentration.

Fig. 4. PAP and substrate protection of 2,3-butanedione inactivation of SULT1A1
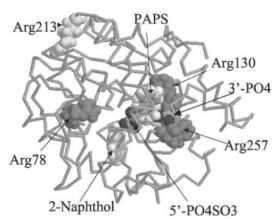The PNPS assay method was used to determine the remaining enzyme activity.

Fig. 5. Computer modeling structure of SULT1A1
The structure was depicted by RasMol 2.6. The whole structure was displaced by
"Backbone." PAPS, 2-naphthol, Arg[78], Arg[130], Arg[257], and Arg[213] were displaced by
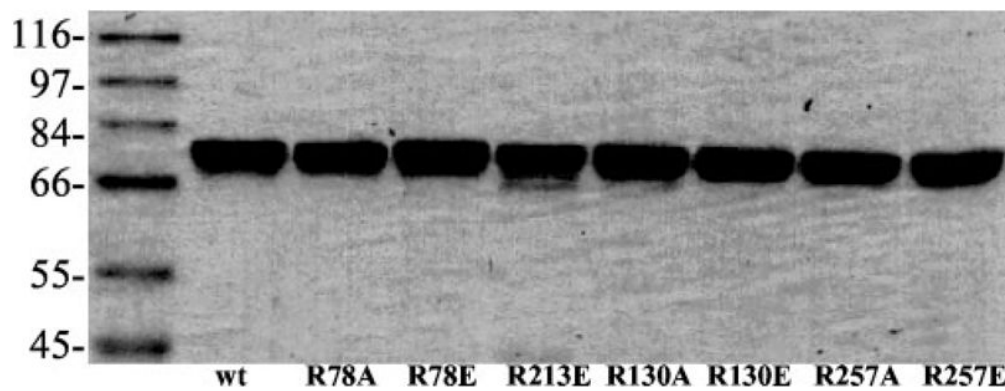"Spacefill."

Fig. 6. SDS-PAGE of the purified SULT1A1 mutant proteins
12% acrylamide gel was used for the separation of proteins. The gel was run at 200 V.
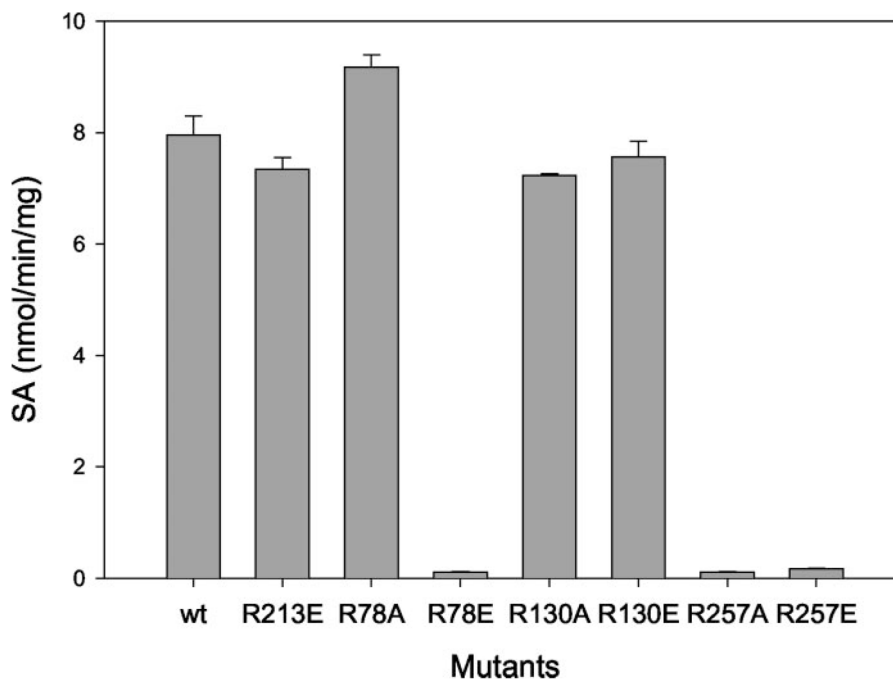
Fig. 7. 2-Naphthol sulfation activity of SULT1A1 mutant proteins
The methylene blue assay method was used for enzyme activity determinations. 0.1 M 2-naphthol was used as substrate for the assay at 8 μM PAPS, pH 6.2.
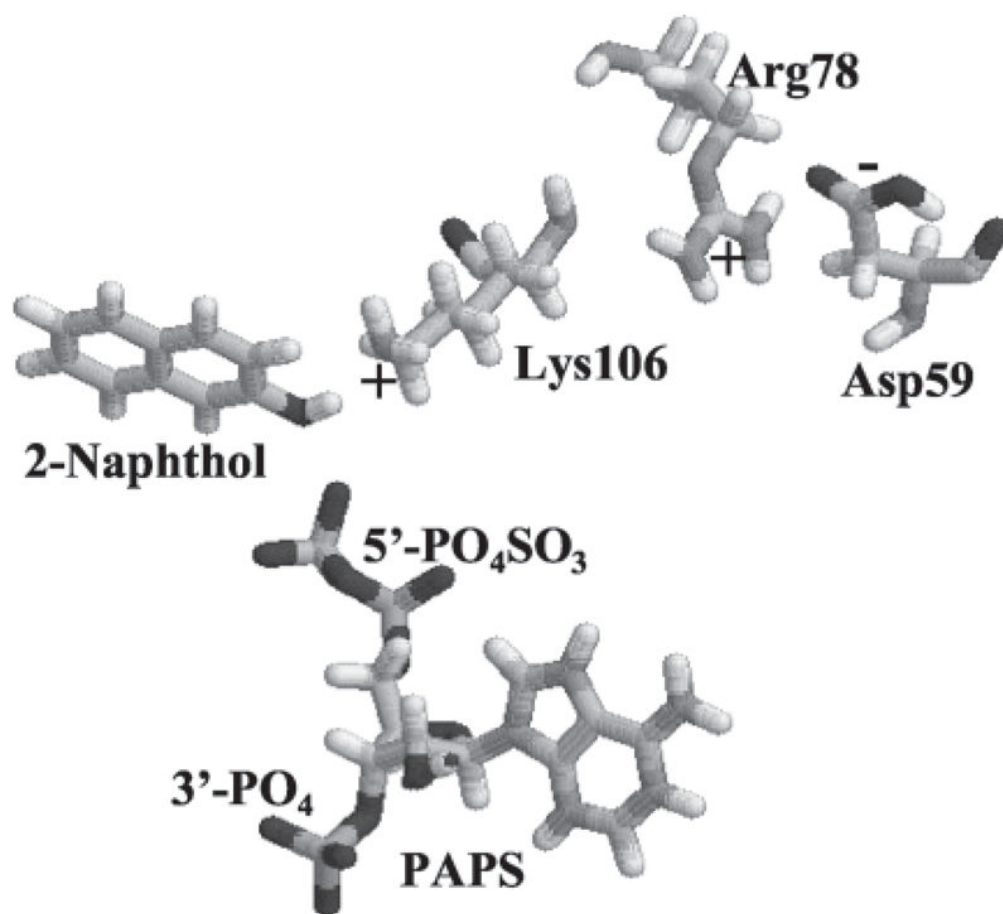
Fig. 8. Computer modeling structure of SULT1A1 showing Arg[78] and related residues and ligands

The model structure of SULT1A1 was depicted by RasMol 2.6. Only Arg[78], PAPS, 2-naphthol, Lys[106], and Asp[59] are displaced.
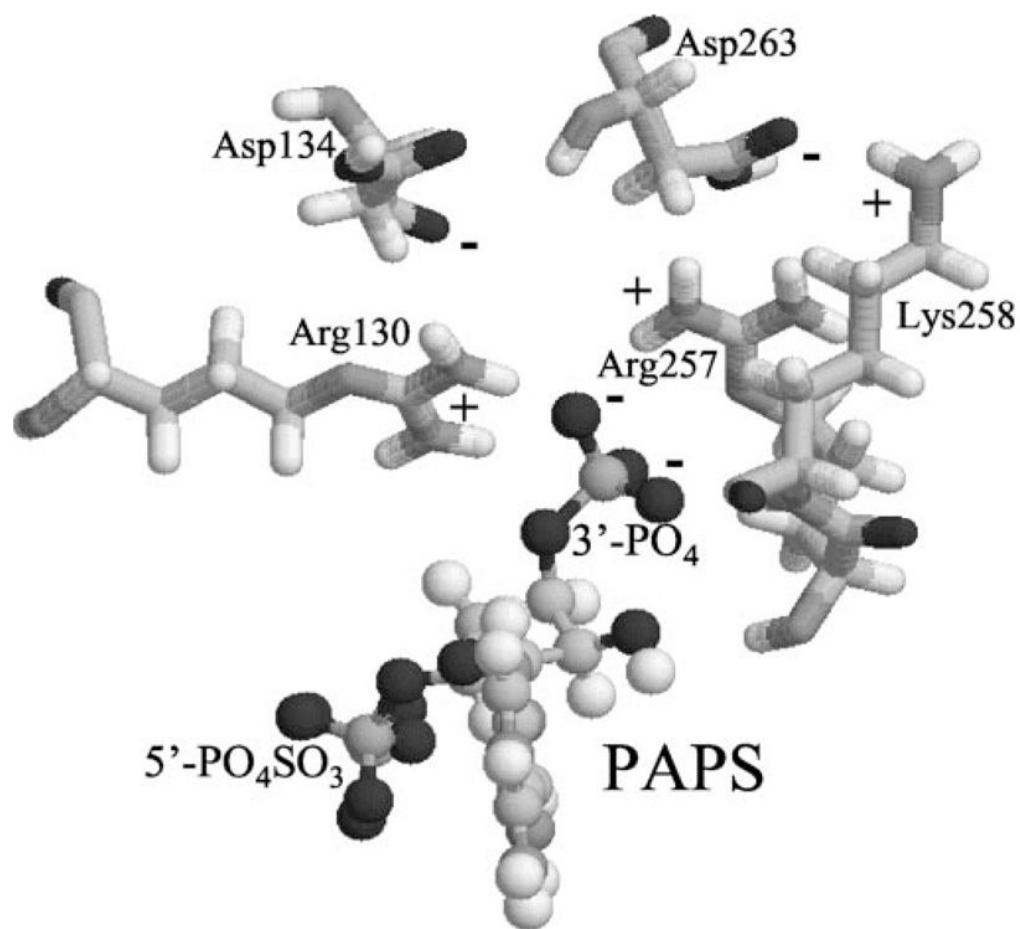
Fig. 9. Computer modeling structure of SULT1A1 showing charged residues in close proximity to 3′-phosphate of PAPS
The model structure of SULT1A1 was depicted by RasMol 2.6. Only PAPS and charged residues within 5 Å to 3′-phosphate are displaced.

Table I

Primer pairs for SULT1A1 mutants

Mutants[a]	Primers
R78A	5′-GCTCCCATCTTCATGGCCGTGCCCTTCC-3′
R78E	5′-GCTCCCATCTTCATGGAGGTGCCCTTCC-3′
R78r	5′-GGAAACTGCCACATCCTTTGCGTTGCGG-3′
R130A	5′-GGTCTATGTTGCCGCCAACGCAAAGGATGTGC-3′
R130E	5′-GGTCTATGTTGCCGAGAACGCAAAGGATGTGC-3′
R130r	5′-GGTAGAGAACAGGGTGGGTCCGGCTCAGCTCC-3′
R257A	5′-GCATCTCCCCCTTCATGGCCAAAGGCATGGCT-3′
R257E	5′-GCATCTCCCCCTTCATGGAGAAAGGCATGGCT-3′
R257r	5′-CGCTTGGTCAGGTTTGATTCGCACACTCCCTC-3′
R213E	5′-CCTGGAGTTTGTGGGGCAATCCCTGCCAGAC-3′
R213r	5′-GGACCTCAAACACCCCGTTAGGGACGGTCTC-3′

[a]Note: two different mutants in the same position used the same reverse primer (r).

Table II

Calculated shortest distance between the related residues to the functional groups

	Arg[78]	Arg[78]	Arg[130]	Arg[213]	Arg[213]	Arg[257]	Lys[106]	Lys[106]
Functional group	PAPS (5′-sulfate)	2NP (OH)	PAPS (3′-phosphate)	PAPS (5′-sulfate)	2NP (OH)	PAPS (3′-phosphate)	PAPS (5′-sulfate)	2NP (OH)
Distance (Å)	11.3	11.1	2.8	23.8	23.3	2.0	3.7	2.0

Table III

2-Naphthol sulfation kinetic parameters for SULT1A1 mutants

	wt	R213E	R78A	R130A	R130E
V_{max} (nmol/min/mg)	13.2	14.4	13.2	14.0	16.4
K_m (mM)	16.0	20.9	18.2	18.4	18.4
V_{max}/K_m	0.84	0.68	0.72	0.76	0.88