Identification of Amino Acid Residues Critical for Catalysis and Cosubstrate Binding in the Flavonol 3-Sulfotransferase*

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The comparison of the deduced amino acid sequences of plant and animal sulfotransferases (ST) has allowed the identification of four well conserved regions, and previous experimental evidence suggested that regions I and IV might be involved in the binding of the cosubstrate, 3'-phosphoadenosine 5'-phosphosulfate (PAPS). Moreover, region IV is homologous to the glycine-rich phosphate binding loop (P-loop) motif known to be involved in nucleotide phosphate binding in several protein families. In this study, the function of amino acid residues within these two regions was investigated by site-directed mutagenesis of the plant flavonol 3-ST. In region I, our results identify Lys59 as critical for catalysis, since replacement of this residue with arginine resulted in a 300-fold decrease in specific activity, while a 15-fold reduction was observed after the conservative replacement with arginine. Photoaffinity labeling of K59R and K59A with [35S]PAPS revealed that Lys59 is not required for cosubstrate binding. However, the K39A mutant had a reduced affinity for 3'-phosphoadenosine 5'-phosphate (PAP)-agarose, suggesting that Lys59 may participate in the stabilization of an intermediate during the reaction. In region IV, all substitutions of Arg276 resulted in a marked decrease in specific activity. Conservative and unconservative replacements of Arg276 resulted in weak photoaffinity labeling with [35S]PAPS and the R276A/T73A and R276E enzymes displayed reduced affinities for PAP-agarose, suggesting that the Arg276 side chain is required to bind the cosubstrate. The analysis of the kinetic constants of mutant enzymes at residues Lys59, Gly281, and Lys284 allowed to confirm that region IV is involved in cosubstrate binding.

Sulfotransferases catalyze the transfer of a sulfonate group from an activated nucleotide donor, 3'-phosphoadenosine 5'-phosphosulfate (PAPS), to the appropriate alcoholic or phenolic hydroxyl groups of acceptor substrates. In contrast with plant tissues in which STs have yet to be assigned a particular function, in mammals these enzymes play an important role in the detoxification of xenobiots and endogenous metabolites, as the presence of a sulfate group increases water solubility of hydrophobic molecules and facilitates their excretion. In addition, STs are involved in the metabolic pathways of biologically active molecules, such as steroid hormones and neurotransmitters. In that case, it is generally well established that sulfate conjugation of such compounds is important to modulate their biological activity (1). Research conducted to elucidate the role of flavonoid sulfation in plants has resulted in the isolation and biochemical characterization of four position-specific STs which are involved in the stepwise formation of flavonol polysulfates (2, 3). The plant flavonol 3- and 4'-STs exhibit strict specificity for position 3 of flavonol aglycones and 4' of flavonol 3-sulfates, and cDNA clones encoding these two enzymes were isolated and characterized (4). In a recent investigation, we constructed a series of hybrid enzymes by the substitution of protein segments between the flavonol 3- and 4'-STs. Analysis of substrate preference of the resulting chimeric proteins allowed the identification of a domain located in the central portion of these enzymes that is responsible for both substrate and position specificities (5).

Progress in understanding the structure-function relationship of STs has been limited by the fact that their three-dimensional structure has not yet been resolved. However, a large number of cDNA clones coding for STs of different specificities have been isolated from various organisms. The comparison of the deduced amino acid sequences of ST enzymes of plant and animal origin has revealed significant homology, and four well conserved regions have been identified (4, 6). These conserved regions could participate in shared functions of these enzymes, such as cosubstrate binding or specifying the proper folding for catalysis.

Two of the conserved regions of STs represent almost uninterrupted blocks of sequence identity. The conserved region I is located in the N-terminal portion of STs and its sequence is YPKG[S/T/N]W (Fig. 1). It is interesting to note that this motif is also present in two bacterial STs which, otherwise, exhibit very weak general homology with their eukaryotic functional homologs (14, 15). Recently, affinity labeling experiments with a nucleotide analog allowed the identification of two labeled amino acid residues located in the N-terminal portion of the rat hepatic aryl ST IV (16). However, it is unlikely that these amino acid residues are involved in PAPS binding, since they are not conserved among all cloned STs, but their proximity to the amino acids of region I suggests that the latter may interact with the cosubstrate.

The conserved region IV, on the other hand, is located in the C-terminal portion of STs and its sequence is XR[K/G][A]XXG-DW/K(N/T)XXFT. Regions sharing homology with this motif have been identified in the nonhomologous, membrane-bound N-heparan sulfate ST (17) and in adenosine phosphosulfate kinases (18). The motif GXGXXX present in region IV has been
proposed to act as a “PAPS-binding site” because of its homology with the consensus sequence GXXXXXGK, as described the glycine-rich phosphate binding loop (P-loop) known to be involved in nucleotide phosphate binding in a number of enzymes (19, 20). Crystal structures of adenylate kinase, p21ras, and F1-ATPase bound to substrate analogs have revealed that the P-loop wraps around the phosphate groups of the nucleotide and that the side chain of the invariant lysine is positioned to make contact with the ϕ- and γ-phosphates of ATP or GTP (21–23). The critical role of the lysine residue in substrate binding has been confirmed by the results of affinity labeling and site-directed mutagenesis studies of several enzymes (20). In addition, it has been suggested that the lysine side chain is directly involved in transition state stabilization of adenylate kinase (24, 25).

The involvement of region IV in PAPS binding has recently been suggested by the results of a site-directed mutagenesis study of the guinea pig estrogen ST (26). However, since this study made use of triple mutants within the region, the contribution of discrete positions to substrate binding could not be evaluated. In order to further characterize the structure and function relationship of STs, we have modified amino acid residues located in the conserved regions I and IV of the flavonol 3-ST by site-directed mutagenesis. In this paper, we describe the results of experiments which allowed to identify residues present in these two regions that are important for substrate binding and catalysis.

MATERIALS AND METHODS

Site-directed Mutagenesis—Site-directed mutagenesis was performed according to the method of Kunkel (27). The pST3 cDNA, which encodes the flavonol 3-ST, was digested with EcoRI and religated into the EcoRI site of the phage M13mp19. This construct was used to produce single-stranded template for mutagenesis experiments. Oligonucleotides used for mutagenesis were for K59R: 5'-GGATCCGAGTTGG(A/G)(G/C)(G/C)GGGTAA-3'; for G281A: 5'-GAAGTGGCAC-3'; and for K59A: 5'-GAAGTGGCA-3'. The amplified DNA fragment containing the desired mutation was cut with either the appropriate restriction enzyme or with the restriction enzyme M13-reverse primer. The amplified fragment was cloned into the plasmid pBR322 using the bacterial expression vector pQE30 (Qiagen, Chatsworth, CA) in order to obtain reproducible results, chromatography on nickel-agarose, aliquots of the purified wild-type and mutant recombinant STs were subjected to 12% polyacrylamide gel electrophoresis according to the method of Laemmli (31). The proteins were visualized by Coomassie Blue staining.

Affinity Chromatography on PAP-agarose—Immediately after purification on nickel-agarose, the enzyme preparations were desalted on a PD-10 column (Pharmacia, Uppsala, Sweden) preequilibrated with buffer A (25 mM bis-Tris, pH 6.8, 14 mM ϕ-mercaptoethanol). The eluted proteins were chromatographed on a PAP-agarose column (approximately 2 ml) (Sigma) preequilibrated with buffer A and washed with 3 column volumes of the same buffer. The bound proteins were eluted with a linear gradient of 0.0 to 1 M NaCl in buffer A, at a flow rate of 0.5 ml/min, and fractions of 0.5 ml each were collected. Protein absorbance was monitored at 280 nm with a Waters 460 tunable absorbance detector. In order to obtain reproducible results, chromatography on PAP-agarose was performed with a Waters 625 LC HPLC system and a Waters PAP minicolumn.

Immunodetection—Aliquots of the PAP-agarose affinity-purified fractions were applied onto a nitrocellulose membrane, and the dot blots were developed with polyclonal antibodies raised against the flavonol 3-ST, as described previously (30). Immunodetection was performed with an alkaline phosphate-conjugated anti-rabbit antibody as secondary antibody and nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrate.

Photoaffinity Labeling with [35S]PAPS—Photoaffinity labeling with [35S]PAPS was performed according to the method of Otterness et al. (32) with minor modifications. The reaction mixture (50 μl) contained 50 pmol of [35S]PAPS and approximately 20 μg of E. coli-soluble protein extracted in 50 mM sodium phosphate, pH 7.5. In control experiments, 5 nmol of PAP was added as a competitor for the covalent binding of [35S]PAPS to proteins. The samples were irradiated for 10 min at 4°C in quartz microcuvettes held at a distance of 1 cm from the top plate of a UV transilluminator (model T5–36, Ultra-Violet Products, San Gabriel, CA). Aliquots of the reaction mixture were diluted with SDS sample buffer, boiled for 5 min, and submitted to SDS-PAGE electrophoresis. After electrophoresis, the proteins were electrotransferred onto a nitrocellulose membrane according to the Bio-Rad semi-dry transfer apparatus protocol and autoradiographed. To measure the migration of the recombinant STs, replicas of the gels were stained with Coomassie Blue.

DNA Sequencing and Sequence Analysis—Nucleotide sequences were determined by the dideoxy chain-termination method (33). The samples were irradiated for 10 min at 4°C in quartz microcuvettes held at a distance of 1 cm from the top plate of a UV transilluminator (model T5–36, Ultra-Violet Products, San Gabriel, CA). Aliquots of the reaction mixture were diluted with SDS sample buffer, boiled for 5 min, and submitted to SDS-PAGE electrophoresis. After electrophoresis, the proteins were electrotransferred onto a nitrocellulose membrane according to the Bio-Rad semi-dry transfer apparatus protocol and autoradiographed. To measure the migration of the recombinant STs, replicas of the gels were stained with Coomassie Blue.

RESULTS

In order to identify amino acids involved in cosubstrate binding and catalysis, we modified the amino acid Lys59 within the
conserved region I, and Arg^{276}, Lys^{277}, Gly^{281}, and Lys^{284} within the conserved region IV by site-directed mutagenesis of the flavonoid 3-ST (Fig. 1).

Conservative replacement of basic amino acids was sought, as well as nonconservative replacements with either alanine, in order to minimize structural alterations and to prevent the formation of hydrogen bonds (35), or with glycine, which eliminates side chain interactions. The recombinant wild-type and mutant enzymes were expressed in E. coli and were ready purified from bacterial supernatants by affinity chromatography on nickel-agarose. The recombinant proteins migrated at a distance corresponding to the invariant lysine in the P-loop motif, had no significant effect on the $k_{cat}$ or the $K_m$ for both substrates. Nonconservative replacement of Lys^{277} and Lys^{284} with glycine gave rise to mutant proteins having a similar decrease in $k_{cat}$ (approximately 2–4-fold) and increase of the $K_m$ for PAPS (approximately 6–9-fold) (Table I). The $K_m$ for quercetin for both mutants was similar to that of the wild-type recombinant enzyme. The effect on the kinetic constants of replacing Gly^{281} with alanine was comparable with that observed for mutants K277G and K284G (Table I).

The binding properties of the inactive or very weakly active mutants toward the sulfate donor were characterized by photoaffinity labeling with [35S]PAPS. UV irradiation of a crude soluble protein extract of E. coli harboring pFST3 in the presence of 1 $\mu$M [35S]PAPS resulted in the labeling of a protein migrating at a position corresponding to that of the recombinant wild-type flavonoid 3-ST (Fig. 2, A and B). As expected, the addition of a 100-fold molar excess of unlabeled PAP completely prevented labeling of the recombinant wild-type enzyme. Photoaffinity labeling of the K59A mutant was similar to that of the K59R and recombinant wild type enzymes (Fig. 2, A and B), indicating that PAPS binding is not impaired in this mutant. These results strongly suggest that although conservative and nonconservative replacements of Lys^{59} have an impact on catalytic activity, this residue is not required for cosubstrate binding. In contrast, photoaffinity labeling of R276K, R276E, and R276A/T73A resulted in similar bands of very weak intensity, supporting the role of Arg^{276} in PAPS binding (Fig. 2, A and B). The intensities of the photoaffinity labeled products of K277G, G281A, and K284G were intermediate between those of the Arg^{276} mutants and recombinant wild-type enzyme (Fig. 2, C and D). These results are consistent with the 5–9-fold increases of the $K_m$ for PAPS observed for these three mutants.

To further characterize mutants of Lys^{59} and Arg^{276} that did not have a sufficient level of catalytic activity for reliable kinetic analysis, they were submitted to affinity chromatography on PAP-agarose. The plant flavonoid 3-ST is sensitive to product inhibition by PAP, a competitive inhibitor of PAPS for the active site of the enzyme, with a $K_i$ (0.1 $\mu$M) slightly lower than the $K_m$ for PAPS (0.18 $\mu$M) (30). As expected, the recombinant wild-type flavonoid 3-ST bound strongly to the PAP-agarose affinity matrix and was eluted with 0.78 M NaCl with good reproducibility between individual experiments. The activity profile of the recombinant wild-type enzyme coincided with the elution profiles determined by monitoring the absorbance at 280 nm (Fig. 3) and by immunodetection of the purified fractions (Fig. 4). The strong interaction of the recombinant wild-type flavonoid 3-ST with the PAP affinity matrix is similar to that previously observed with the plant enzyme (30).

The mutant proteins retained the ability to bind to PAP-agarose, although significant differences were observed in the salt concentration required for their elution. Mutant K59A eluted at 0.56 M NaCl, indicating a weaker affinity for PAP than the recombinant wild-type flavonoid 3-ST (Figs. 3 and 4). The elution profile of the K59R mutant was similar to the recombinant wild-type enzyme, reflecting the fact that it displays only a minor change in the $K_m$ for PAPS. Mutant R276K also eluted at the same salt concentration as the recombinant wild-type enzyme (Figs. 4 and 5). In contrast, mutants R276E and R276A/T73A eluted at a lower salt concentration of 0.64 and 0.66 M, respectively. The reduction in affinity for PAP-
agarose observed with mutant R276E, as compared with R276A/T73A, could be due to a charge repulsion between the phosphate groups of PAP and the carboxyl group of the glutamyl side chain. These results strongly suggest that both Lys59 and Arg276 are involved in PAP binding through ionic interactions.

Valid interpretation of the affinity chromatography data requires a prior demonstration that the affinity for PAP-agarose is specific. The following evidence suggests that the interaction with PAP-agarose is highly specific: 1) PAP-agarose affinity chromatography has already been applied to the purification of several STs, and it has been shown that they can be specifically eluted from the support by the addition of PAP or PAPS at a concentration of 1 mM or less (36–38). 2) To test whether nonspecific ionic interactions could contribute in a significant way to the affinity for PAP-agarose, control experiments were performed with mutants involving a change to the net charge of the enzyme. Mutant E101K, that displays no change in kinetic constants (data not shown), eluted at the same salt concentration as the recombinant wild-type enzyme, indicating that the introduction of a positive charge did not enhance binding to the negatively charged chromatographic support. 3) Mutants K277R and K277G, having 6–9-fold increases of the \( K_m \) for PAPS, showed only slight reductions of affinity for PAP-agarose compared to the recombinant wild-type flavonol 3-ST, eluting respectively at 0.75 and 0.77 M NaCl. Since these reductions are much smaller than those observed for K59A, R276A/T73A, and R276E, they cannot be interpreted only by the loss of a positive charge on the mutant proteins. Taken together, these results support the hypothesis that the interaction of the en-

### Table I

| Enzyme form | \( K_m \) quercetin\( ^a \) | \( K_m \) PAPS\( ^b \) | \( k_{cat} \) | \( k_{cat}/K_m \) (PAPS) \( ^c \) | Specific activity | Relative activity |
|-------------|-----------------|-----------------|-------------|-----------------------------|-----------------|-----------------|
| F3ST        | 0.20            | 0.18            | 1.86        | 10.3                       | 276             | 100             |
| rF3ST       | 0.45            | 0.22            | 1.43        | 6.5                        | 0.06            | 0.02            |
| Region I    |                 |                 |             |                             |                 |                 |
| K59R        | 0.43            | 0.10            | 0.07        | 0.7                        | 18              | 6.5             |
| K59A        |                 |                 |             |                             | 0.8             | 0.3             |
| Region IV   |                 |                 |             |                             |                 |                 |
| R276K       |                 |                 |             |                             | 0.06            | 0.02            |
| R276E       |                 |                 |             |                             | 0.6             | 0.2             |
| R276A       |                 |                 |             |                             | 0.06            | 0.02            |
| K277R       | 0.57            | 0.32            | 1.17        | 3.7                        | 199             | 72              |
| K277G       | 0.43            | 1.34            | 0.34        | 0.2                        | 81              | 29              |
| G281A       | 0.56            | 1.17            | 0.52        | 0.4                        | 97              | 35              |
| K284R       | 0.06            | 0.18            | 0.30        | 1.2                        | 239             | 87              |
| K284G       | 0.04            | 0.18            | 0.30        | 1.2                        | 113             | 41              |

\( ^a \) Maximum S.D. was 7% with \( n = 3 \).

\( ^b \) Maximum S.D. was 12% with \( n = 3 \).

\( ^c \) Maximum S.D. was 18% with \( n = 3 \).

**FIG. 2.** [\( ^35 \)S]PAPS photoaffinity labeling of the recombinant wild-type and mutant flavonol 3-STs. A, SDS-PAGE of the protein extracts of the recombinant wild-type enzyme and of the Lys59 and Arg276 mutants after the photoaffinity labeling reaction. B, autoradiograph obtained with the same protein preparations shown in A. C, SDS-PAGE of the protein extracts of the recombinant wild type flavonol 3-ST and of the K277G, G281A, and K284G mutants after the photoaffinity labeling reaction. D, autoradiograph obtained with the same protein preparations shown in C. The protein band corresponding to the flavonol 3-ST is indicated by an arrow. rF3ST, histidine-tagged recombinant wild type flavonol 3-sulfotransferase.

**FIG. 3.** Elution profile of the recombinant wild-type, K59R, and K59A enzymes following chromatography on PAP-agarose. rF3ST, histidine-tagged recombinant wild type flavonol 3-sulfotransferase.

Lys59 and Arg276 are involved in PAP binding through ionic interactions.

Valid interpretation of the affinity chromatography data requires a prior demonstration that the affinity for PAP-agarose is specific. The following evidence suggests that the interaction with PAP-agarose is highly specific: 1) PAP-agarose affinity chromatography has already been applied to the purification of several STs, and it has been shown that they can be specifically eluted from the support by the addition of PAP or PAPS at a concentration of 1 mM or less (36–38). 2) To test whether nonspecific ionic interactions could contribute in a significant way to the affinity for PAP-agarose, control experiments were performed with mutants involving a change to the net charge of the enzyme. Mutant E101K, that displays no change in kinetic constants (data not shown), eluted at the same salt concentration as the recombinant wild-type enzyme, indicating that the introduction of a positive charge did not enhance binding to the negatively charged chromatographic support. 3) Mutants K277G and K284G, having 6–9-fold increases of the \( K_m \) for PAPS, showed only slight reductions of affinity for PAP-agarose compared to the recombinant wild-type flavonol 3-ST, eluting respectively at 0.75 and 0.77 M NaCl. Since these reductions are much smaller than those observed for K59A, R276A/T73A, and R276E, they cannot be interpreted only by the loss of a positive charge on the mutant proteins. Taken together, these results support the hypothesis that the interaction of the en-
In view of the proximity of the affinity labeled amino acids, but is important for the thermal stability of the enzyme, revealed that it is not involved in substrate binding or catalysis. Site-directed mutagenesis of the corresponding conserved among the members of the phenol and estrogen ST families, and site-directed mutagenesis of the corresponding cysteine residue to serine in the human liver phenol ST has revealed that it is not involved in substrate binding or catalysis, but is important for the thermal stability of the enzyme (39). In view of the proximity of the affinity labeled amino acids to region I, Zheng et al. (16) proposed that the latter might be involved in the interaction with the cosubstrate. Our results identify Lys59 within this region as critical for catalysis, since replacement of this amino acid with alanine produces a pronounced decrease in specific activity. The results of photoaffinity labeling studies clearly indicate that Lys59 is not required for PAPS binding, since K59A is labeled to a similar extent as the K59R and recombinant wild-type enzymes, suggesting that this residue acts as a catalyst in the flavonol 3-ST. In the enzyme-PAPS complex, the Lys59 side chain may be too distant to interact with the cosubstrate, but when a longer arginine side chain is introduced at this position, it may interact weakly with the sulfate donor. This is consistent with the results of the affinity labeling experiments, and the small but significant reduction of the Km for PAPS of the K59R mutant, was reproduced in several independent experiments. On the other hand, the reduced affinity of K59A for PAP-agarose compared with that of the K59R and recombinant wild-type enzymes suggests that Lys59 binds a phosphate group of PAP through an ionic interaction, indicating that this residue may stabilize the leaving group of the reaction. In the absence of a proposed catalytic mechanism for STs, we can only speculate that the role of Lys59 may be to stabilize an intermediate and/or to lower the activation energy of a transition state.

The region IV of STs shares sequence homology with the phosphate binding loop involved in nucleotide phosphate binding in several protein families. However, a distinctive feature of region IV is the presence of additional conserved residues on both sides of the segment homologous to the P-loop motif. The function of residues specific to the ST motif (Arg276 and Lys277) and of residues homologous to those of the P-loop motif (Gly281 and Lys284) was investigated. All substitutions of Arg276 resulted in a dramatic decrease in specific activity, and the results of photoaffinity labeling studies suggest that this residue is involved in the formation of the enzyme-PAPS complex. Furthermore, the interaction is specific for the arginine side chain as demonstrated by the drastic reduction in catalytic activity of the R276K mutant. In addition, Arg276 is also involved in the binding of the product of the reaction as suggested by the reduction in affinity for PAP-agarose of mutants R276E and R276A/T73A. The participation of Arg276 in product binding suggests that it may also be involved in catalysis. These results are in agreement with the previous finding by chemical modification with phenylglyoxal that one arginine residue is required for catalysis in the rat liver phenol ST (40). We cannot exclude the possibility that structural alterations are induced by the substitutions at Lys59 and Arg276, but the normal affinities for PAPS of the Lys59 mutants and the normal affinity for PAP-agarose observed for R276K suggest that there is no major change of the tertiary structure in these mutants.

The results of the kinetic analysis and photoaffinity labeling of mutants K277G, G281A, and K284G support the involvement of region IV in cosubstrate binding. Although mutations at these positions have a moderate impact on the formation of the enzyme-PAPS complex and on the catalytic activity of the enzyme, they may participate with Arg276 in the binding of the cosubstrate. In a recent mutational study of the guinea pig estrogen ST, it was found that the replacement with alanine of the amino acids corresponding to Gly281, Gly284, and Lys284 of the flavonol 3-ST resulted in a triple mutant with no catalytic activity that could not be photoaffinity labeled with [35S]PAPS (26). Our results on Gly281 and Lys284 suggest that the absence of catalytic activity in this mutant is due to the cumulative effects of the three substitutions on cosubstrate binding. Alternatively, these residues may play a role in maintaining the proper conformation of the loop region. The effects on the enzyme with PAP-agarose is specific. However, the technique does not allow the accurate detection of small differences in affinity for the immobilized ligand.

**DISCUSSION**

In this study, the function of conserved residues within regions I and IV of STs was investigated (Fig. 1). Several important features justify their choice as targets for site-directed mutagenesis. First, they represent almost uninterrupted blocks of sequence identity present in all eukaryotic cytoplasmic STs. Also, a secondary structure algorithm predicts that both regions form loop structures frequently associated with the formation of active sites. Finally, region IV is homologous to the P-loop involved in nucleotide phosphate binding in several enzymes (17).

The involvement of region I in cosubstrate binding is suggested by the recent affinity labeling of the amino acids Lys59 and Cys56 of the rat hepatic aryl ST IV by the nucleotide analog ATP dialdehyde (16). However, these amino acids are only conserved among the members of the phenol and estrogen ST families, and site-directed mutagenesis of the corresponding cysteine residue to serine in the human liver phenol ST has revealed that it is not involved in substrate binding or catalysis, but is important for the thermal stability of the enzyme (39). In view of the proximity of the affinity labeled amino acids
catalytic constants observed with mutants K277G, G281A, and K284G may result from subtle structural changes affecting the position of the Arg276 side chain.

This study represents an important step toward an understanding of catalysis in STs. Our results confirm that the conserved region IV of STs and the P-loop motif are functionally related in both that are involved in the binding of nucleotide cosubstrates. In view of the absolute conservation of the amino acids Lys59 and Arg276 in all clonedeukaryotic cytoplasmic STs, it is likely that the results presented here can be extended to all members of this class of enzymes. Other electrophilic loci may be needed in addition to Arg276 to stabilize the negatively charged groups of the cosubstrate, especially since PAPS is not bound by STs as a chelate complex with a divalent cation, and a residue acting as a base catalyst may be required to abstract a proton from the hydroxyl group of the acceptor substrate to activate it for nucleophilic attack at the sulfuryl group. To address these aspects of catalysis by the sulfotransferases, the construction and analysis of site-directed mutants at other conserved residues of the flavonol 3-ST are presently under progress.

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