Analysis of the Substrate Specificity of Human Sulfotransferases SULT1A1 and SULT1A3: Site-Directed Mutagenesis and Kinetic Studies†

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ABSTRACT: Sulfonation is an important metabolic process involved in the excretion and in some cases activation of various endogenous compounds and xenobiotics. This reaction is catalyzed by a family of enzymes named sulfotransferases. The cytosolic human sulfotransferases SULT1A1 and SULT1A3 have overlapping yet distinct substrate specificities. SULT1A1 favors simple phenolic substrates such as *p*-nitrophenol, whereas SULT1A3 prefers monoamine substrates such as dopamine. In this study we have used a variety of phenolic substrates to functionally characterize the role of the amino acid at position 146 in SULT1A1 and SULT1A3. First, the mutation A146E in SULT1A1 yielded a SULT1A3-like protein with respect to the Michaelis constant for simple phenols. The mutation E146A in SULT1A3 resulted in a SULT1A1-like protein with respect to the Michaelis constant for both simple phenols and monoamine compounds. When comparing the specificity of SULT1A3 toward tyramine with that for *p*-ethylphenol (which differs from tyramine in having no amine group on the carbon side chain), we saw a 200-fold preference for tyramine. The kinetic data obtained with the E146A mutant of SULT1A3 for these two substrates clearly showed that this protein preferred substrates without an amine group attached. Second, changing the glutamic acid at position 146 of SULT1A3 to a glutamine, thereby neutralizing the negative charge at this position, resulted in a 360-fold decrease in the specificity constant for dopamine. The results provide strong evidence that residue 146 is crucial in determining the substrate specificity of both SULT1A1 and SULT1A3 and suggest that there is a direct interaction between glutamic acid 146 in SULT1A3 and monoamine substrates.

Sulfonation is an important reaction involved in the biotransformation of various endogenous compounds and xenobiotics (*1*). Generally, sulfonation leads to the detoxification and excretion of the chemicals, but it can also cause activation as in the case of *N*-hydroxy heterocyclic amines, arylamines, and polycyclic aromatic hydrocarbons (*2, 3*). When these compounds are sulfonated they become highly reactive electrophiles that are both mutagenic and carcinogenic (*2, 3*). The sulfonation reaction involves transfer of a sulfuryl group from the donor substrate 3′-phosphoadenosine 5′-phosphosulfate (PAPS)[1] to the acceptor substrate and is catalyzed by a superfamily of membrane bound and cytosolic enzymes called sulfotransferases (SULTs) (*4, 5*). To date more than 30 different cytosolic SULT cDNAs have been cloned from species ranging from plants to human (*5*). In humans two major subfamilies of cytosolic sulfotransferases have been described: phenol sulfotransferases (humSULT1A) and hydroxysteroid sulfotransferases (humSULT2A) (*5*). Two of the human phenol SULT isoforms, SULT1A1 and SULT1A3, have been cloned in this laboratory (previously named HAST1 and HAST3, respectively) (*6, 7*). SULT1A1 and SULT1A3 are more than 93% identical at the amino acid level and have overlapping substrate specificities, but with very marked differences. SULT1A1 prefers simple phenolic substrates such as *p*-nitrophenol whereas SULT1A3 prefers catecholamines such as dopamine (*8*). Thus SULT1A1 and SULT1A3 provide an ideal model to investigate the amino acids involved in determining the differing substrate specificities of these two isoforms.

Chimeric proteins of human SULT1A1 and SULT1A3 have previously been characterized in order to address this issue (*9, 10*). In the study by Sakakibara *et al.* (*9*) two distinct regions spanning amino acids 84−89 and 143−148 were shown to determine the substrate specificity of SULT1A1 and SULT1A3 (*9*), which is in general agreement with the recent findings from our laboratory (*10*). Similarly, chimeric constructs of rat hydroxysteroid and plant SULTs have indicated that regions 102−164 and 92−194, respectively, contain the residues that are involved in determining the substrate specificity of these SULTs (*11, 12*). To date the

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[1] Abbreviations: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, sulfotransferase; LB, Luria−Bertani; IPTG, isopropyl thiogalactopyranoside; Ni−NTA, nickel−nitrilotriacetic acid; BSA, bovine serum albumin; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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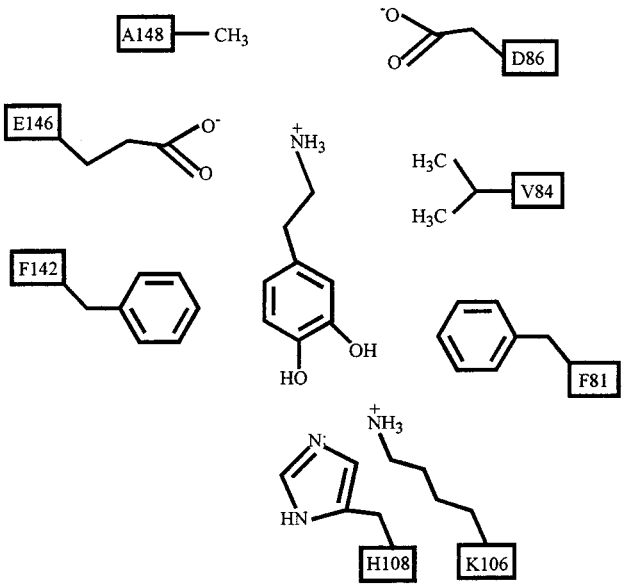

FIGURE 1: Schematic diagram of dopamine in the substrate binding pocket of human SULT1A3. The model is based on the crystal structure of the mouse estrogen SULT (Brookhaven Protein Data Bank ID code 1AQU; *13*).

only published SULT crystal structure is that of the mouse estrogen SULT (mouSULT1E1) (*13*). This structure led to the identification of several amino acids (positions 81, 84, 86, 106, 108, 142, 146, 149, 243, 247, and 248) that are found near β-estradiol, which is the preferred substrate of mouSULT1E1. Alignment of these amino acids with the sequences of SULT1A1 and SULT1A3 reveals that only four (84, 86, 146, and 247) correspond to residues differing between the two phenol SULT isoforms. Interestingly, two of these amino acids (86 and 146) also reside within the substrate binding regions determined with chimeric proteins (*9*) of SULT1A1 and SULT1A3. Residues 84, 86, 146, and 247 have been investigated by site-directed mutagenesis for their involvement in determining the substrate specificity of SULT1A1 and it was found that amino acids 84 and 86 were not involved in *p*-nitrophenol specificity but have a significant influence on the affinity for both dopamine and the cosubstrate PAPS (*10*). Amino acid 247 had no effect on determining substrate preference (unpublished results), but we recently reported that amino acid 146 in the human phenol sulfotransferase SULT1A1 was a major determinant of substrate specificity (*10*). The corresponding amino acid in SULT1A3 has also recently been reported to have an important function in the substrate preferences of this isoform (*14*). On the basis of the mouSULT1E1 structure (Brookhaven Protein Data Bank ID code 1AQU; *13*) we have modeled dopamine into the substrate binding pocket of SULT1A3 (Figure 1). This model illustrates the close proximity of residue 146 to the positively charged amine group of dopamine.

In this study we have used a variety of phenolic substrates to functionally characterize the amino acid at position 146 in both SULT1A1 and SULT1A3. Furthermore, we have constructed and functionally characterized two additional mutants of the human catecholamine sulfotransferase SULT1A3 in order to investigate whether a charge interaction is involved in the binding of positively charged monoamines by SULT1A3.

EXPERIMENTAL PROCEDURES

Materials. [^{35}S]PAPS was obtained from Amrad Pharmacia Biotech (Boronia, Victoria, Australia). PAPS, *p*-nitrophenol, *p*-cresol, *p*-aminophenol, *p*-nitrocatechol, *m*-nitrophenol, *p*-ethylphenol, tyramine, and dopamine were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

Subcloning of SULT cDNAs. Full-length cDNAs encoding SULT1A1 and SULT1A3, previously cloned in our laboratory (*6*, *7*), were subcloned into the bacterial expression vector pET28a(+) (Novagen, Madison, WI). The pET28a-(+) vector enables expression of the recombinant protein bearing an N-terminal six-residue histidine tag, which was used to readily and effectively purify the expressed protein by nickel-affinity chromatography. The mutant SULT1A1-A146E was subcloned from the previously described mammalian expression vector (SULT1A1-A146E/pCMV5) construct (*10*) using the internal *Pst*I restriction site.

Site-Directed Mutagenesis. Site-directed mutagenesis was performed with *Pfu* DNA polymerase and the QuikChange site-directed mutagenesis method (Stratagene, La Jolla, CA). The three mutants SULT1A3-E146A, SULT1A3-E146Q, and SULT1A3-E146D were constructed with the SULT1A3 cDNA inserted into the pET28a(+) vector as the original DNA template. Oligonucleotides used for mutagenesis are shown below. The mutated codon is underlined, while lowercase letters indicate a base change from wild type: SULT1A3-E146A, 5′-CCATTTCCACCGTATGGcAAAG-GCGCACCCTGAG-3′; SULT1A3-E146Q: 5′-CCATTTC-CACCGTATGcAAAAGGCGCACCCTG-3′; SULT1A3-E146D, 5′-CCATTTCCACCGTATGGAcAAGGCGCACC-CTG-3′. *Escherichia coli* cells (HB101 strain) were transformed with the resultant products (i.e., amplified plasmids with mutated cDNAs). Single colonies were isolated and screened for the desired mutation by DNA sequencing.

Verification of cDNA Sequences by DNA Sequencing. The mutated cDNA sequences were confirmed by automated sequencing with the ABI Prism dye terminator cycle sequencing ready reaction sequencing protocol (Perkin-Elmer, Foster City, CA).

Bacterial Expression and Purification of Recombinant His-Tagged Wild-Type and Mutant SULTs. A single BL21(DE3) *E. coli* colony transformed with the pET28a(+) SULT plasmid of interest was used to inoculate 5 mL of LB medium containing kanamycin (30 μg/mL) and incubated overnight at 37 °C. The culture was then diluted 1:100 in 100 mL of SOB medium (20 g/L bactotryptone, 5 g/L bactoyeast extract, 10 mM NaCl, and 10 mM $MgCl_2$) containing 30 μg/mL kanamycin and left shaking at 37 °C for 16 h (in the absence of IPTG). All subsequent steps were performed at 4 °C unless otherwise stated. The bacterial cells were harvested by centrifugation at 5000*g* for 15 min and the pellet was resuspended in 15 mL of 0.1 M Tris-acetate pH 7.5, and 17.2% sucrose. Lysozyme solution (0.2 mg/mL) was added and the mixture was incubated on ice for 30 min. Cells were then reharvested by centrifugation at 5000*g* for 15 min. Sonication buffer [100 mM potassium phosphate, pH 7.4, 6 mM magnesium acetate, and 20% (v/v) glycerol] was added and the cells were frozen at −70 °C for a minimum of 1 h. As the cells were thawed, the protease inhibitor phenylmethanesulfonyl fluoride (PMSF) was added

(1 mM). Samples were sonicated with 3 × 20 s bursts, with cooling on ice between each burst, and cell debris was removed by centrifugation at 10000*g* for 30 min. The SULT protein in the supernatant (cell extract) has a six-residue histidine tag attached to the N-terminus of the SULT protein, which allowed the enzyme to be purified by selective binding to a Ni−NTA spin column (Qiagen, Clifton Hill, Victoria, Australia). Column preparation, washing, and protein elution were undertaken according to the manufacturer's instructions. Before the protein was stored at −70 °C, glycerol was added to a final concentration of 20% (v/v).

Protein Analysis. Protein concentration of the purified preparation was determined by the method of Lowry et al. (*15*) with BSA as the standard. Proteins were separated by SDS−PAGE (*16*) and visualized by Coomassie blue staining.

Sulfotransferase Assays. Substrate specificity of the wild-type and mutant proteins toward PAPS and various acceptor substrates was measured as described by Foldes and Meek (*17*). The final incubation mixtures contained 10 mM phosphate buffer, pH 7.0, 0.01−0.06 μg of purified protein, an acceptor substrate, and 50 nM−8 μM PAPS, of which 1.5 nM−0.24 μM was [^{35}S]PAPS. The acceptor substrates were used at the following concentrations: *p*-nitrophenol (0.15 μM−4.5 mM), *p*-cresol (0.2 μM−8 mM), *p*-aminophenol (2.5 μM−3.2 mM), *p*-nitrocatechol (0.25 nM−0.2 mM), *m*-nitrophenol (0.1 μM−2 mM), *p*-ethylphenol (0.25 μM−8 mM), tyramine (35 μM−24 mM), and dopamine (0.8 μM−6.4 mM). Reactions were initiated by the addition of enzyme and incubated for 30 min at 37 °C. Assays were performed in duplicate and corrected for background activity with controls that contained no substrate. Linearity of product formation with respect to time and protein concentration was determined for all proteins, and optimum assay conditions were used in all kinetic studies.

Analysis of Kinetic Data. The apparent kinetic constants for PAPS and the above-mentioned acceptor substrates were determined by fitting the Michaelis−Menten equation to the data. This was achieved by nonlinear regression with the program GraphPad Prism (AMPL Software Pty Ltd, Turramurra, NSW, Australia).

RESULTS AND DISCUSSION

Expression of Recombinant Wild-Type and Mutant SULT1A1 and SULT1A3 Proteins. SDS−PAGE analysis of the purified SULT wild-type and mutant proteins (1 μg) is shown in Figure 2. A similar level of expression was observed for all recombinant proteins. The SULT1A1 and SULT1A1-A146E proteins exhibited a slightly lower apparent molecular weight compared to wild-type SULT1A3 and the SULT1A3 mutants (Figure 2), as observed previously (*8, 10*). A single, unidentified, high molecular weight impurity was observed by SDS−PAGE in all of the protein preparations.

Sulfotransferase Activities of Recombinant Wild-Type and Mutant SULT1A1 and SULT1A3 Proteins toward PAPS, p-Nitrophenol and Dopamine. The K_m values and specificity constants (V_{max}/K_m) for the wild-type SULT1A1 and SULT1A3 and the mutants SULT1A1-A146E and SULT1A3-E146A toward PAPS were determined with saturating concentrations of *p*-nitrophenol while varying the PAPS concentration was varied. The SULT1A1-A146E mutant compared to the wild-type SULT1A1 showed no marked differences in the K_m values (1.2 μM and 1.8 μM, respectively) and specificity constants (259 and 111, respectively) toward PAPS. The K_m value for the mutant SULT1A3-E146A (0.3 μM) was slightly lower than that of wild-type SULT1A3 (1.5 μM). Since the V_{max}/K_m values for these enzymes were similar (340 and 336, respectively), we conclude that residue 146 has no major effect on the binding of the cosubstrate PAPS.

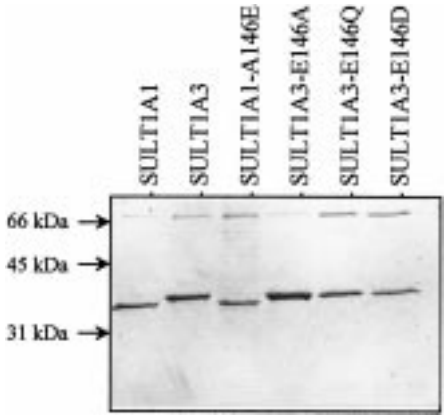

FIGURE 2: SDS−PAGE of human phenol sulfotransferases SULT1A1 and SULT1A3 and the mutants SULT1A1-A146E, SULT1A3-E146A, SULT1A3-E146Q, and SULT1A3-E146D. Each lane contains 1 μg of Ni−NTA spin column-purified protein. The migration of molecular mass markers is indicated by arrows.

SULT1A1 and SULT1A3 have overlapping but very different substrate specificities. SULT1A1 prefers *p*-nitrophenol whereas SULT1A3 prefers the catecholamine dopamine. We have previously shown, using COS cell-expressed protein, that mutation of A146E in SULT1A1 has a pronounced effect on the substrate specificity of this enzyme. The mutant protein is more like SULT1A3, especially with respect to the substrate *p*-nitrophenol, but also to some extent with dopamine (*10*). In the present study, these results for wild-type and mutant SULT1A1-A146E have been confirmed and extended by using Ni−NTA column-purified protein, which allows for the determination of the specificity constant V_{max}/K_m, for more reliable comparisons of their overall substrate affinities.

The complementary mutant in SULT1A3, changing the glutamic acid at position 146 to an alanine (which is present at the corresponding position in SULT1A1) was also constructed (SULT1A3-E146A) to further investigate the role of amino acid 146 in determining the substrate preferences of SULT1A3. The results (Table 1) showed that the SULT1A3-E146A mutant, compared to wild-type SULT1A3, had a very strong preference (low K_m value and high specificity constant) for *p*-nitrophenol and discriminated against dopamine (high K_m value and low specificity constant).

Sulfotransferase Activities of Recombinant Wild-Type and Mutant SULT1A1 and SULT1A3 Proteins toward Various Phenolic Substrates. To further elucidate the substrate preferences of SULT1A1, SULT1A3, SULT1A1-A146E, and SULT1A3-E146A, we kinetically characterized these proteins with *p*-nitrophenol, dopamine, and another six phenolic or catechol substrates: *m*-nitrophenol, *p*-nitrocatechol,

Table 1: Kinetic Analysis of Wild-Type and Mutant Human Sulfotransferases (SULTs) with Various Substrates[a]

	SULT1A1			SULT1A1-A146E			SULT1A3			SULT1A3-E146A		
	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1})	V_{max}/K_m	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1})	V_{max}/K_m	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1})	V_{max}/K_m	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1})	V_{max}/K_m
p-nitrophenol	1.1 ± 0.2	581 ± 67	528	389 ± 74	279 ± 16	0.7	1024 ± 127	437 ± 19	0.4	5.4 ± 0.1	112 ± 0.7	21
p-cresol	0.5 ± 0.1	315 ± 20	630	2291 ± 207	1140 ± 38	0.5	1536 ± 122	471 ± 15	0.3	27 ± 3.4	323 ± 17	12
p-aminophenol	12 ± 2.8	85 ± 8	7	~ 6000[b]	~16[b]	0.003	107 ± 19	13 ± 1.0	0.1	64 ± 13	24 ± 1.8	0.4
p-nitrocatechol	<0.001[c]	4[c]	>4000	35 ± 7	112 ± 7	3.2	49 ± 9	99 ± 7	2	<0.00025[c]	13[c]	>52000
m-nitrophenol	0.7 ± 0.3	364 ± 83	520	295 ± 20	1401 ± 43	4.7	697 ± 21	615 ± 8	0.9	17 ± 0.9	126 ± 2.4	7.4
p-ethylphenol	1.4 ± 0.1	296 ± 15	211	12920 ± 1546	1105 ± 92	0.1	3254 ± 181	391 ± 11	0.1	34 ± 2.4	153 ± 5	4.5
tyramine	6483 ± 1017	197 ± 13	0.03	~ 3000[b]	~ 1[b]	0.0003	35 ± 3	691 ± 17	20	871 ± 54	170 ± 4	0.2
dopamine	9.7 ± 1.4	22 ± 1.0	2.3	~ 1000[b]	~ 3[b]	0.003	3.7 ± 0.3	799 ± 30	216	29 ± 0.5	99 ± 0.7	3.4

[a] Values represent best fit ± standard error of the estimate obtained from nonlinear regression analysis. [b] Low rates were observed in these experiments and only approximate values of the kinetic constants could be obtained. [c] Low rates were observed with apparent saturation at the lowest substrate concentration used.

FIGURE 3: Chemical structures of various phenol sulfotransferase substrates.

tyramine, *p*-ethylphenol, *p*-cresol, and *p*-aminophenol (Figure 3). These last six additional substrates were chosen because they are intermediate in structure between *p*-nitrophenol and dopamine. It was anticipated that the kinetic data obtained would help us to understand more about the type of interaction(s) occurring between these substrates and amino acid 146 in the SULT proteins.

Wild-type SULT1A1 showed a strong preference for the simple phenolic substrates (*p*-nitrophenol, *p*-cresol, *m*-nitrophenol, and *p*-ethylphenol) as shown by the low K_m values and high specificity constants (Table 1). The substrates with an amine group attached (*p*-aminophenol, tyramine, and dopamine) were poor substrates (Table 1).

The A146E mutant of SULT1A1 showed a high K_m value for the simple phenolic substrates *p*-nitrophenol, *p*-cresol, *m*-nitrophenol, and *p*-ethylphenol and even higher K_m values for *p*-aminophenol, tyramine, and dopamine (Table 1). These

results indicate that the SULT1A1-A146E protein is more SULT1A3-like with respect to the simple phenolic substrates. However, this mutation abolishes nearly all of the activity with substrates that have an amine group attached. This is consistent with the result obtained with the recombinant SULT1A1-A146E mutant expressed in COS cells, which showed SULT1A3-like affinity toward *p*-nitrophenol but no apparent activity toward dopamine (*10*). The above results show that a glutamic acid residue at position 146 will not allow amine-containing substrates to be sulfonated by the A146E mutant protein. This may be a result of steric hindrance, as glutamic acid is a considerably larger than alanine in the wild-type SULT1A1.

Wild-type SULT1A3 preferred the substrates with an amine group attached to a two-carbon side chain, such as dopamine and tyramine. In contrast, the substrate *p*-aminophenol, in which the amine group is attached directly to the phenol ring, was a poor substrate for SULT1A3. This finding suggests that the lack of the side chain results in the amine group of the substrate being too far from the site of interaction on the enzyme to enable efficient catalysis. Alternatively, this finding may be explained by differences in charge between *p*-aminophenol (primarily uncharged) and dopamine/tyramine (charged) at physiological pH. Comparison of the specificity of SULT1A3 toward tyramine and *p*-ethylphenol reveals that tyramine is a 200-fold better substrate than *p*-ethylphenol (Table 1). These substrates differ only in that tyramine has an amine group attached to the alkyl side chain (Figure 3). This result in itself indicates the importance of the amine group on the substrate for efficient SULT1A3 catalysis. The K_m value of SULT1A3 for the substrates *p*-nitrophenol, *p*-cresol, and *m*-nitrophenol were all high, confirming that this enzyme discriminates against small phenolic substrates (Table 1).

The data obtained with the SULT1A3-E146A protein showed that *p*-nitrophenol, *p*-cresol, *m*-nitrophenol, and *p*-ethylphenol were all relatively good substrates for SULT1A3-E146A, whereas tyramine and dopamine were poor substrates compared to wild-type SULT1A3. We could therefore conclude that the E146A mutation of SULT1A3 changed the substrate specificity to that of a more SULT1A1-like protein. Interestingly, the kinetic properties of the SULT1A3-E146A mutant toward tyramine and *p*-ethylphenol clearly showed that the enzyme no longer preferred the substrate with an amine group attached. This illustrates that removal of the negatively charged side chain of glutamic acid at position 146 in SULT1A3 (SULT1A3-E146A) abolishes the preference for a substrate with a positively charged amine group. This observation provides strong evidence that there is a direct interaction between glutamic acid 146 in SULT1A3 and the amine group of substrates such as tyramine and dopamine.

The behavior of *p*-nitrocatechol was quite unusual. For example, SULT1A1 exhibited only a slow rate of *p*-nitrocatechol utilization that was at its maximum rate at the lowest substrate concentration used (1 nM). If this result is accepted at face value, it implies a K_m that is about 500-fold lower than that of any other substrate and a specificity constant 6-fold higher. In contrast, SULT1A3 behaved in a conventional manner toward this substrate with values for K_m and V_{max}/K_m within the range of the values for other substrates. Conversion of the alanine at position 146 in SULT1A1 to a glutamic acid resulted in kinetics that are almost indistinguishable from those of SULT1A3. The reverse change in SULT1A3 (E146A) resulted in the enzyme acquiring the unusual behavior typical of SULT1A1. Although these results might suggest that residue 146 is recognizing the hydroxylated end of the substrate, other data tend to rule this out. For example, comparison of the results obtained with dopamine and tyramine shows that SULT1A1, SULT1A3, SULT1A1-A146E, and SULT1A3-E146A all have higher specificity constants for dopamine than tyramine. This indicates that amino acid 146 is not involved in determining the affinity for the hydroxy end of the substrates, presumably because this end of the substrate molecule is pointing away from amino acid 146 in the substrate binding pocket.

The importance of the phenolic hydroxyl group of a substrate being at the *meta* or *para* position was investigated by comparing the kinetic values of *m*-nitrophenol and *p*-nitrophenol (Table 1). These data showed that the position of the hydroxyl group had relatively little effect on the kinetics of the proteins tested.

Effect of Amino Acid 146 Mutants of SULT1A3 toward Dopamine Binding. The preferred substrate for SULT1A3 is dopamine. This substrate contains an amine group that is positively charged at physiological pH. When the negatively charged glutamic acid residue at position 146 was replaced with the neutral amino acid alanine in SULT1A3 (SULT1A3-E146A), a marked decrease in substrate preference was observed, with the K_m value increasing 8-fold and the specificity constant decreasing 64-fold (Table 1). This led us to hypothesize that a negatively charged amino acid at position 146 was crucial for recognition of dopamine as a substrate. A similar suggestion was made recently by Dajani et al. (*14*). We therefore constructed two additional SULT1A3 mutants: one with the glutamic acid changed to a glutamine, SULT1A3-E146Q (removing the negative charge without interfering with the size of the amino acid), and another mutant with an aspartic acid at position 146, SULT1A3-E146D (retaining the negative charge but with a shorter chain length than glutamic acid).

The K_m values and specificity constants for the mutants SULT1A3-E146Q and SULT1A3-E146D toward PAPS were determined with saturating concentrations of dopamine while the PAPS concentration was varied. The K_m values for these mutants were both slightly lower than the value obtained with wild-type SULT1A3 (0.2 and 0.8 μM, respectively, compared to 1.5 μM); however, the specificity constants were similar (210 and 438, respectively, compared to 336). These results confirm the conclusion stated earlier that residue 146 in these proteins does not affect the binding of the cosubstrate PAPS.

The SULT1A3-E146Q mutant showed a marked decrease in its ability to catalyze the sulfonation of dopamine, as seen by the 20-fold increased K_m value and a 360-fold decrease of the specificity constant in comparison to the wild type (Table 2). The SULT1A3-E146D mutant also showed an increased K_m value (10-fold) and a decreased specificity constant (8−fold) toward dopamine, compared to the SULT1A3 wild type (Table 2). These results together confirm the hypothesis that a negatively charged amino acid

Table 2: Kinetic Analysis of Wild-Type and Mutants of Human Sulfotransferase SULT1A3 with the Substrate Dopamine[a]

	dopamine		
	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1})	V_{max}/K_m
SULT1A3	3.7 ± 0.3	799 ± 30	216
SULT1A3-E146Q	81 ± 18	51 ± 4	0.6
SULT1A3-E146D	35 ± 8	903 ± 97	26

[a] Values represent best fit ± standard error of the estimate obtained from nonlinear regression analysis.

at position 146 in SULT1A3 is crucial for the recognition of dopamine, and furthermore, that the side chain length of the negatively charged amino acid also influences the affinity toward this substrate.

In this study we have shown the importance of amino acid 146 in the substrate preference of SULT1A1 and SULT1A3. The mutation of A146E in SULT1A1 yielded a SULT1A3-like protein with respect to the affinity toward simple phenols. The mutation of E146A in SULT1A3 resulted in a SULT1A1-like protein toward both simple phenols and monoamine compounds. When comparing the specificity of SULT1A3 toward tyramine (positively charged at physiological pH) with the substrate *p*-ethylphenol (which lacks the side chain amine group), we saw a 200-fold decrease in affinity (as indicated by the specificity constant). The kinetic data obtained with the E146A mutant of SULT1A3 toward tyramine and *p*-ethylphenol clearly showed that this protein no longer preferred the substrate with the amine group. Another SULT1A3 mutant showed that changing the glutamic acid at position 146 to a glutamine, and thereby neutralizing the negative charge at this position, resulted in a 360-fold decrease in the specificity constant for dopamine. In conclusion, these results provide strong evidence that residue 146 is crucial in determining the substrate specificity of both SULT1A1 and SULT1A3 and suggest that there is a direct interaction between glutamic acid 146 in SULT1A3 and monoamine substrates.

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