ACCELERATED COMMUNICATION

A Single Amino Acid, Glu146, Governs the Substrate Specificity of a Human Dopamine Sulfotransferase, SULT1A3

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ABSTRACT

Sulfation, catalyzed by members of the sulfotransferase (SULT) superfamily, exerts considerable influence over the biological activity of numerous endogenous and xenobiotic chemicals. In humans, catecholamines such as dopamine are extensively sulfated, and a SULT isoform (SULT1A3 or the monoamine-sulfating form of phenolsulfotransferase) has evolved with considerable selectivity for dopamine and other biogenic amines. To investigate the molecular basis for this selectivity, we identified a region of SULT1A3, which, we hypothesized, contributes to its preference for biogenic amines, and mutated two amino acids within this domain to the corresponding residues in a closely related but functionally distinct phenol sulfotransferase, SULT1A1 (H143Y and E146A). The change of a single amino acid, E146A, was sufficient to transform the catalytic properties and substrate preference of SULT1A3, such that they closely resembled those of SULT1A1. These experiments confirm the functional role of Glu146 in the selectivity of SULT1A3 for biogenic amines and suggest that this region is a key determinant of sulfotransferase substrate specificity.

Sulfation is a major contributor to the homeostasis and regulation of numerous biologically potent endogenous chemicals such as catecholamines, steroids, and iodothyronines, as well as to the detoxication of xenobiotics (Coughtrie et al., 1998). Sulfation reactions require PAPS as the sulfuryl donor (Klaassen and Boles, 1997) and are catalyzed by members of the SULT enzyme family (Falany, 1997). These enzymes are widely expressed in human tissues including liver, intestine, and brain, and may be classified on the basis of their substrate specificity and amino acid sequence into two subfamilies, SULT1 (phenol sulfotransferases) and SULT2 (steroid sulfotransferases) (Falany, 1997; Weinshilboum et al., 1997; Coughtrie et al., 1998). At least six homodimeric enzymes make up the human SULT1 family, with amino acid sequence identities ranging from 47–96%. Humans differ from rodents and many other animal species in that they rely heavily on sulfation as a means of modulating the activity and facilitating the transport of catecholamines and other biogenic amines. For example, up to 98% of circulating dopamine in humans exists in the sulfated form (Goldstein et al., 1995; Eisenhofer et al., 1997; Dousa and Tyce, 1988). This biological function is reflected in the existence of a SULT isoform (called SULT1A3 or alternatively the monoamine-sulfating or thermolabile form of PST) which exhibits strong substrate preference for endogenous catecholamines, such as dopamine, and which is highly expressed in the gastrointestinal tract (Rubin et al., 1996), where most circulating catecholamines and their sulfates are now believed to originate (Goldstein et al., 1995; Eisenhofer et al., 1997). To date, a SULT isoform with such a distinct substrate preference for catecholamines has not been conclusively identified or characterized in other species. The SULT1A3 enzyme shares 93.2% amino-acid sequence identity (i.e., 20 amino acid differences out of 295) with the major hepatic phenol sulfotransferase, SULT1A1 (also known as the phenol-sulfating or thermostable form of PST), but the two enzymes may be distinguished in vitro on the basis of their substrate preference with SULT1A1 selectively sulfating 4-nitrophenol at low micromolar concentrations whereas at similar concentrations SULT1A3 preferentially sulfates dopamine. These enzymes thus provide an excellent basis for identifying key

ABBREVIATIONS: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, sulfotransferase; mEST, mouse estrogen sulfotransferase; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PST, phenolsulfotransferase.
amino acids defining the substrate specificity of human SULTs.

Amino-acid sequence alignment analysis reveals a number of domains and amino acids that are common to all SULTs (Rikke and Roy, 1996; Weinshilboum et al., 1997). The spatial arrangement in three dimensions of these conserved regions has recently been visualized for the first time after solution of the X-ray crystal structure of a monomeric mEST, a member of the SULT1 family that shares 46% amino-acid sequence identity with human SULT1A3 (Kakuta et al., 1997). Examination of the mEST crystal structure, and consideration of data from experiments in which amino acids in a number of the conserved regions have been mutated (Komatsu et al., 1994; Driscoll et al., 1995; Tamura et al., 1997), shows that several of these domains and amino acids are involved in binding of the sulfuryl donor, PAPS. Little is known, however, of the amino acids that influence the substrate specificity of these important enzymes. Comparison of the amino acid sequences of SULTs 1A1 and 1A3 with mEST reveals a region, at the end of a conserved sequence of amino acids forming the α-6 helix of mEST (Kakuta et al., 1997), which harbors four of the 20 amino-acid differences between these two human sulfotransferases (Fig. 1). This α-helix is predicted to be located over the top of the substrate binding pocket of members of the phenol SULT family (Kakuta et al., 1997). In light of the substrate preference of SULT1A3 for basic biogenic amines, such as dopamine and tyramine, we hypothesized that charged amino acids in this region of the molecule may be important in substrate recognition. We chose two charged amino acids (His143 and Glu146) as mutagenic targets in an attempt to define the role of this region of the molecule.

Cloning of wild-type human SULT1A1. We have previously cloned and expressed the cDNA coding for an allelic variant of human SULT1A1 (Jones et al., 1995); however, for the kinetic analysis reported here, it was necessary to use wild-type SULT1A1. This was isolated from human liver cDNA by PCR, using the sense primer 5′-AAAGACTCAGGACATGGAG-3′ and the anti-sense primer 5′-CCCTCTTCAGCTCAGAGC-3′. cDNA (1 ng) was mixed with primers (0.2 μM each), 1 mM MgCl2, 0.2 mM dNTPs, 4 units of Bio-X-Act DNA polymerase, and reaction buffer provided with the enzyme. PCR conditions of 94° for 5 min, followed by 30 cycles of 30 sec at 94°, 1 min at 56°, 2 min at 72°, and finally 10 min at 72°, produced the expected product of 0.9 kb. This PCR product was directly ligated into the vector pCR2.1 and sequenced (Li-Cor 4000 automated sequencer; MWG Biotech, Milton Keynes, UK). To generate restriction sites for ligation into the expression vector pET-17b, the cDNA was PCR-amplified from pCR2.1 using the sense primer 5′-CTTAAAGCTCAGGACATGGAGCTGCC-3′, which inserts an NdeI site, and the anti-sense primer 5′-CTGGAACTCGAGTTC-3′, which inserts an XhoI site. The PCR reaction was performed as above but using 100 ng of pCR2.1/SULT1A1 DNA. The product was ligated into pCR2.1, after which it was excised using NdeI and XhoI and ligated into pET-17b linearized with NdeI and XhoI. The final expression construct was resequenced to ensure no errors had been introduced, and the pET-17b/SULT1A1 construct was transformed into E. coli expression strain BL21(DE3).

Preparation of SULT1A3 E. coli expression construct. Our SULT1A3 cDNA (in the vector pTBlue) (Jones et al., 1995) was amplified by PCR using the sense primer 5′-CATATGGAAGCTTACGATCAGGCAC-3′, which inserts an NdeI restriction site, and the anti-sense primer 5′-GGATCCGGATCC-3′, which inserts a BamHI restriction site. pTBlue/SULT1A3 (100 ng) was mixed with primers (0.2 μM each), 0.2 mM dNTPs, 1 mM MgCl2, 5 units of BioTaq, and reaction buffer provided with the polymerase.}

### Materials and Methods

**Materials.** Oligonucleotides were purchased from Genosys Biotechnologies, Cambridge, UK. Vectors pT7Blue, pET-11a, pET-17b, and pCR2.1 were from Invitrogen, Leek, The Netherlands. Human liver cDNA (Quickclone) was from Clontech (Palo Alto, CA). All enzymes were purchased from Promega (Southampton, UK), except BioTag and Bio-X-Act (Bioline, London, UK) and BSA (New England Biolabs, Hitchin, UK). Protein purification columns and media were from Amersham Pharmacia Biotech (Little Chalfont, UK). PAPS (>99% pure) was from H. Glatt and R. Landried, German Institute for Human Nutrition (Potsdam, Germany), and PAP35S was from DuPont/NEN (Stevenage, UK). All SULT substrates were purchased from Sigma-Aldrich (Poole, UK), and all other chemicals were obtained from commonly used local suppliers. The product was amplified by PCR using the sense primer 5′-AAAGACTCAGGACATGGAG-3′ and the anti-sense primer 5′-CTGGAACTCGAGTTC-3′, which inserts an NdeI site, and the anti-sense primer 5′-CTGGAACTCGAGTTC-3′, which inserts an XhoI site. The PCR reaction was performed as above but using 100 ng of pCR2.1/SULT1A1 DNA. The product was ligated into pCR2.1, after which it was excised using NdeI and XhoI and ligated into pET-17b linearized with NdeI and XhoI. The final expression construct was resequenced to ensure no errors had been introduced, and the pET-17b/SULT1A1 construct was transformed into E. coli expression strain BL21(DE3).

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enzyme. PCR conditions of 94° for 3 min, followed by 30 cycles of 94° for 30 sec, 65° for 30 sec, 72° for 2 min, and finally 72° for 10 min, yielded the desired 0.9-kilobase-pair fragment. This PCR product was ligated into pcR2.1, excised with NdeI and BamHI and ligated into pET-11a linearized with NdeI and BamHI. The pET-11a/SULT1A3 construct was sequenced to ensure that no errors had been introduced and was transformed into E. coli strain BL21(DE3)plysS for expression.

**Mutagenesis procedure.** A method based on that described by Kunkel (Kunkel, 1985) was used. An Apal-KpnI fragment of SULT1A3 containing the mutagenesis target region (nucleotides 89–519) was excised from the pET-11a/SULT1A3 construct and ligated into pBluescript II SK(+) linearized with Apal and KpnI. This construct was transformed into E. coli C323 (‘cut ung’). The transformants were grown in 2-YT broth (16 g/liter SELECT peptone 140, 10 g/liter yeast extract, 5 g/liter NaCl, supplemented with 34 μg/ml chloramphenicol) and infected with helper phage VCS-M13 to produce single stranded template wild-type DNA in which uracils had been incorporated. Oligonucleotides used for mutagenesis were as follows: for H143Y, 5′-CTCTAATGTTTCTACGGAGGAAA-GGCCGACC-3′; for E146A, 5′-CTCTAATGTTTCTACCGAGGAAA-GGCCGACC-3′; for E146A, 5′-CTCTAATGTTTCTACCGAGGAAA-GGCCGACC-3′; for the double mutant, H143Y/E146A, 5′-CTCTAATGTTTCTACCGAGGAAA-GGCCGACC-3′. A silent mutation inserting a BsuRI restriction site was included in each of the mutagenic oligonucleotides to facilitate mutant screening. Mutagenic oligonucleotides were phosphorylated using T4 polynucleotide kinase, and 200 ng of purified, single-stranded, uracil-containing template DNA was annealed to 2 ng of phosphorylated primer by heating the mixture to 65° and allowing the temperature to cool slowly to below 30°. The synthesis of complementary DNA strand primed with the mutagenic oligonucleotide was achieved by adding the annealing mixture to T4 DNA polymerase, T4 DNA ligase, 0.5 mM dNTPs, 1 mM ATP, 10 mM Tris-HCl, pH 7.4, 2 mM MgCl₂, and 2 mM dithiothreitol and incubating the reaction at 37°. The synthesis reaction mixture was transformed into E. coli XL1-Blue, which selects against the uracil-containing wild-type strand and replicates the mutant strand, resulting in double-stranded, mutated DNA. DNA sequencing was performed to confirm the mutagenesis. The mutated Apal-KpnI SULT1A3 fragment was excised from the pBluescript II SK(+) and re-ligated into the pET-11a/SULT1A3 expression construct.

**Expression in E. coli and purification of recombinant proteins.** All pET/SULT/E. coli transformants were grown in L-Broth (supplemented with 100 μg/ml ampicillin and, for the BL21(DE3)plysS strain, 34 μg/ml chloramphenicol) at 30°. Cultures were inoculated from an overnight culture and expression induced with 1 mM isopropyl β-D-thiogalactoside (when cells had reached an A₆₀₀ of 0.5–0.6) for 16 hr at 30°. Cells were pelleted by centrifugation at 7,000 × g for 10 min at 4°. Lysis of cells was achieved by freezing and thawing the cell pellet and homogenizing in 40 mM Tris-HCl buffer, pH 8.0, with the exception of pET-17b/SULT1A1/BL21(DE3), for which the homogeneous was incubated in the presence of 0.5 mg/ml lysozyme for 15 min at room temperature. Cell extracts were centrifuged at 100,000 × g at 4° for 45 min and the resulting supernatants (cell-free extracts) were subjected to 30–55% ammonium sulfate fractionation. The precipitated protein was collected by centrifugation, dissolved in 50 mM Tris-HCl buffer, 1 mM mercaptoethanol, pH 7.4 (buffer A), and dialysed overnight against two changes of buffer A. For the wild-type enzymes, protein was applied to a column (2.6 × 35 cm) of DEAE-Sepharose equilibrated with buffer A. After washing the column with buffer A, recombinant proteins were eluted with buffer A containing 75 mM NaCl. This fraction was concentrated, exchanged into buffer A, and applied to a 3′-5′-ADP agarose affinity column (1.6 × 8 cm). The column was washed with buffer A and bound protein eluted with 40 ml of 100 μM PAPS. For the three recombinant SULT1A3 mutants, the ammonium sulfate fractionated protein was applied to HiTrap Q Sepharose (two 5-ml columns connected in series), and the protein eluted using buffer A containing 125 mM NaCl. Affinity chromatography was performed as above.

To assess purity, proteins were resolved on SDS-polyacrylamide gels (11% acrylamide monomer) according to the procedure originally described by Laemmli (Laemmli, 1970), and stained with Coomasie blue. Protein concentrations were estimated by the Bradford method (Bradford, 1976), with bovine serum albumin as standard.

**SULT enzyme activity.** Kinetic parameters for purified wild-type SULTs 1A3 and 1A1 and the three mutant proteins were determined with the substrates dopamine and 4-nitrophenol, using PAP35S as originally described by Foldes and Meek (Foldes and Meek, 1973). Assays were carried out at 37° in a final volume of 150 μl with 10 mM potassium phosphate buffer, pH 7.4, and 0.04 μCi of PAP35S. Control incubations contained no substrate. Assays were optimized with respect to incubation time and protein content for dopamine and 4-nitrophenol as substrates and carried out using saturating concentrations of either PAPS or substrate, where appropriate. Details of substrate concentration ranges used for kinetic parameter determination are given in the legends to the tables. Additional substrates were also used to determine substrate specificity/preference of the five proteins. For estimation of Kₘ and Vₘₐₓ values, enzyme activity data were plotted using hyperbolic regression analysis with the Hyper.exe software package (v. 1.1s; Dr. J. S. Easterby, University of Liverpool).

**Results**

**Design of mutants.** We hypothesized, based on sequence alignments and examination of the X-ray crystal structure of mEST, that amino acids located between residues 140 and 150 in the human catecholamine sulotransferase SULT1A3 might play an important role in acceptor substrate recognition by this enzyme (Fig. 1). This was based on the following observations: (a) four differences (out of the 20) between SULTs 1A3 and 1A1 reside in this region, (b) two of these involve changes from charged to uncharged amino acids (His143 to Tyr and Glu146 to Ala), and (c) both amino acids were found almost exclusively in SULT1A3 sequence or amino acids 133–156 showed that, other than SULT1A3, no sulotransferase whose sequence is currently known has Glu146 in an equivalent position. Only two other sulotransferase-related GenBank/EMBL entries (Accession numbers AF026074 and Z97055, neither of which have been characterized with respect to their substrate specificity) have an equivalent of His143. We suspected Glu146 in particular may play an important role in dopamine recognition by SULT1A3, because it will carry a negative charge at physiological pH, and because SULT1A1 contains a hydrophobic, uncharged amino acid (Ala146) at the same position.

**Cloning of wild-type SULT1A1.** At least two members of the human phenol SULT family exist as one or more allelic variants (Jones et al., 1995; Zhu et al., 1996; Raftogianis et al., 1997), and the allozymes encoded by these alleles display markedly different kinetic properties (Zhu et al., 1997), and the allozymes encoded by these alleles display markedly different kinetic properties (Zhu et al., 1997), and the allozymes encoded by these alleles display markedly different kinetic properties (Zhu et al., 1997), and the allozymes encoded by these alleles display markedly different kinetic properties (Zhu et al., 1997), and the allozymes encoded by these alleles display markedly different kinetic properties (Zhu et al., 1997), and the allozymes encoded by these alleles display markedly different kinetic properties (Zhu et al., 1997). Thus, to reliably compare the properties of mutant and wild-type SULT1A3 proteins with SULT1A1, it was necessary to isolate the cDNA coding for wild-type SULT1A1. PCR amplification from human liver cDNA using SULT1A1-specific primers resulted in a 925-base-pair fragment that was ligated into pCR2.1 and sequenced (GenBank/EMBL Accession number AJ007418). This sequence was 100% identical.
within the coding region to a SULT1A1 cDNA sequence (GenBank/EMBL Accession number X78283) reported by Ozawa et al. (1995). Comparison of our sequence with other SULT1A1 cDNA and gene sequences, and with the various allelic variants known to exist in the human population, strongly suggests this is the wild-type sequence.

**Expression and purification of recombinant proteins.** Wild-type SULTs 1A1 and 1A3 and the three mutant SULT1A3 cDNAs (H143Y, E146A, and H143Y/E146A) were expressed in *E. coli* and the recombinant proteins purified using a simple three-step process. Similar levels of expression of each protein were observed, and up to 30 mg of purified protein per liter of bacterial culture were obtained. Fig. 2 shows SDS-PAGE analysis of the five purified proteins. Initial SULT enzyme assays (data not shown) using 5 μM dopamine (SULT1A3-specific) and 4-nitrophenol (SULT1A1-specific) revealed that the H143Y mutant of SULT1A3 sulfated principally dopamine, whereas the E146A and H143Y/E146A mutants sulfated principally 4-nitrophenol.

**Kinetic properties of wild-type and site-directed mutant enzymes.** We carried out extensive kinetic analysis of all five recombinant proteins by determining apparent *K*<sub>m</sub> and *V*<sub>max</sub> values (and calculating *V*<sub>max</sub>/*K*<sub>m</sub> ratios) for the sulfonyl donor PAPS and for the substrates dopamine and 4-nitrophenol. Because of the very different substrate affinities exhibited by SULTs 1A3 and 1A1 toward dopamine and 4-nitrophenol, it was necessary to employ different substrate concentration ranges to determine *K*<sub>m</sub> and *V*<sub>max</sub> values with wild-type SULT1A3 and SULT1A3 H143Y than with SULT1A1 and the SULT1A3 E146A and SULT1A3 H143Y/E146A mutants.

Table 1 shows that the wild-type SULT1A1 and SULT1A3 enzymes demonstrated a similar *K*<sub>m</sub> value for PAPS, and that neither the single mutations H143Y and E146A nor the double mutation H143Y/E146A substantially affected the *K*<sub>m</sub> value of SULT1A3 for PAPS with dopamine as substrate. The E146A substitution did reduce the *V*<sub>max</sub> values and *V*<sub>max</sub>/*K*<sub>m</sub> ratios, consistent with a reduced ability to sulfate dopamine. These results support the idea that this particular region of the phenol SULT enzymes is not directly involved in PAPS recognition or binding.

**Substrate specificity profiles of wild-type and mutant enzymes.** Whereas SULTs 1A3 and 1A1 display marked selectivity (at low micromolar concentrations) for dopamine and 4-nitrophenol, respectively, there is considerable substrate specificity overlap between the two enzymes (Jones et al., 1995; Coughtrie et al., 1998). We therefore examined the effect the generated mutations had on the specificity of SULT1A3 toward other substrates. SULT enzyme activity was determined with the five recombinant enzymes using three different concentrations (1, 10, and 100 μM) of 1-naphthol, tyramine, phenol, vanillin, sesamol, and 4-methylphenol, in addition to dopamine and 4-nitrophenol (Fig. 3). As with dopamine and 4-nitrophenol, the H143Y mutation had little effect on SULT1A3 activity or selectivity for the other substrates.

**Kinetic parameters for the substrates dopamine and 4-nitrophenol were determined with the five enzymes and are shown in Table 2. As expected, wild-type SULT1A3 displayed a low *K*<sub>m</sub> value for dopamine (*K*<sub>m</sub> = 2.2 μM), whereas wild-type SULT1A1 exhibited a low *K*<sub>m</sub> value for 4-nitrophenol (*K*<sub>m</sub> = 3.8 μM). Conversely, wild-type SULTs 1A3 and 1A1 displayed high *K*<sub>m</sub> values for 4-nitrophenol (*K*<sub>m</sub> = 1392 μM) and dopamine (109 μM), respectively. The *V*<sub>max</sub> value of wild-type SULT1A1 with 4-nitrophenol was comparable with that of wild-type SULT1A3; however, when dopamine was used as substrate, the *V*<sub>max</sub> value of wild-type SULT1A1 was only about 7% of that obtained with wild-type SULT1A3. The SULT1A3 H143Y mutant did not differ substantially in *K*<sub>m</sub> value or reaction rate from wild-type SULT1A3 when measured with either dopamine or 4-nitrophenol as substrate. In stark contrast, the E146A mutant displayed a dramatic (60-fold) increase in *K*<sub>m</sub> value for dopamine compared with wild-type SULT1A3, whereas its kinetic properties with 4-nitrophenol strongly resembled those of wild-type SULT1A1. The *V*<sub>max</sub>/*K*<sub>m</sub> ratio (which, with purified enzymes that have almost exactly the same molecular weight, equates to the specificity constant *K*<sub>s</sub>/*K*<sub>m</sub>) for dopamine was reduced by 150-fold in the E146A mutant compared with the wild-type SULT1A3 enzyme. The E146A mutant had a *K*<sub>m</sub> value similar to that of wild-type SULT1A1 with 4-nitrophenol as substrate, but a reduced (to 40%) *V*<sub>max</sub> value. In all cases, the double mutant H143Y/E146A behaved similarly to the E146A mutant. Together, these results demonstrate clearly that amino acid 146 is a key determinant of the ability of SULT1A3 to accept dopamine as a substrate, and that mutation of this amino acid from glutamate to the corresponding residue in SULT1A1 (alanine) alters its kinetic properties dramatically. Thus, the E146A mutation turns SULT1A3 into a SULT1A1-like enzyme in its substrate specificity toward dopamine and 4-nitrophenol.

![Fig. 2. SDS-PAGE analysis of purified recombinant wild-type and site-directed mutant enzymes.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Recombinant Enzyme</th>
<th><em>K</em>&lt;sub&gt;m&lt;/sub&gt; (μM)</th>
<th><em>V</em>&lt;sub&gt;max&lt;/sub&gt; (nmol/min/mg)</th>
<th><em>V</em>&lt;sub&gt;max&lt;/sub&gt;/<em>K</em>&lt;sub&gt;m&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type SULT1A3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6</td>
<td>665</td>
<td>145</td>
</tr>
<tr>
<td>SULT1A3 H143Y&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0</td>
<td>334</td>
<td>167</td>
</tr>
<tr>
<td>SULT1A3 E146A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0</td>
<td>153</td>
<td>51</td>
</tr>
<tr>
<td>SULT1A3 H143Y/E146A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8</td>
<td>280</td>
<td>58</td>
</tr>
<tr>
<td>Wild-type SULT1A1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3</td>
<td>35</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Data are the average of duplicate determinations performed with either 11 μM<sup>(a)</sup> or 1 μM<sup>(b)</sup> dopamine as acceptor substrate and with PAPS concentrations between 0.7 and 67 μM.
toward the compounds tested. However, the E146A mutation (and the H143Y/E146A double mutation) again transformed the properties of SULT1A3 into those of an enzyme strongly resembling SULT1A1. The main exception was vanillin, which is a very potent inhibitor of SULT1A1 at micromolar concentrations but is also a good substrate for SULT1A3 (Bamforth et al., 1993; Coughtrie et al., 1998).

Discussion

Human SULT1A3 is the only sulfotransferase characterized to date that selectively sulfates dopamine at low micromolar concentrations in preference to simple phenols such as 4-nitrophenol. Humans extensively sulfate endogenous biogenic amines (including catecholamines); presumably, this has a detoxication function, because the sulfate conjugates are in the main biologically inactive. Sulfated biogenic amines may also function as transport intermediates, because the free compounds can be reactivated in target cells and tissues through enzymatic hydrolysis by sulfatase(s) (Yoshizumi et al., 1995). Dogs also have high levels of circulating sulfated dopamine (Doussa and Tyce, 1988; Hashizume et al., 1989), and there is some evidence (as yet inconclusive) that a “dopamine” sulfotransferase exists also in this species (Roimain et al., 1982; Oddy et al., 1997). Strong evidence indicates that mesenteric organs, in particular the gastrointestinal tract, are the major site for production of dopamine and dopamine sulfate in humans (Eisenhofer et al., 1997), and the upper gastrointestinal tract is also the major site of SULT1A3 expression (Rubin et al., 1996). There is, therefore, a physiological and presumably evolutionary basis for having a sulfotransferase enzyme with high selectivity toward dopamine and other biogenic amines. Despite its important biological function, little is known about this enzyme, and nothing is known about the features that define its substrate specificity.

We identified a region of the SULT1A3 enzyme that we believed was involved in substrate discrimination; to test the hypothesis, amino acids His143 and Glu146 in SULT1A3 were targeted for site-directed mutagenesis to the corresponding residues in the closely related human phenol sulfotransferase SULT1A1. Our results clearly demonstrate that the amino acid Glu146 critically determines the sulfation of dopamine (at low micromolar concentrations) by SULT1A3, because mutation to Ala146 essentially turned SULT1A3 into a SULT1A1-like enzyme. The H143Y mutation had little or no effect on the substrate specificity or kinetic properties of SULT1A3, and the double mutant H143Y/E146A behaved in a manner that was strikingly similar to E146A. This is noteworthy because His143 is not commonly found in other sulfotransferases. During the course of the experimentation reported here, Sakakibara et al. (1998) reported production of recombinant chimeric proteins formed by exchanging various regions of SULTs 1A1 and 1A3 and showed that a large central segment of the proteins, which included amino acids 143 and 146, was involved in substrate recognition. These data are in accord with our results. There are examples from other enzyme families of individual amino acids conferring a striking degree of substrate selectivity; such studies provide valuable insight into enzyme mechanisms and biological function. For instance, two members of the mouse cytochrome P450 2A family (2A4 and 2A5) can be induced to change their substrate specificity from Δ4,3-ketosteroids to Δ5,3-hydroxysteroids by mutation of a single amino acid (A117V for 2A4 and F209N for 2A5, respectively) (Iwasaki et al., 1994), and it was recently reported that mutating two amino acids in a guanylyl cyclase (Tucker et al., 1998) changed the substrate specificity of this enzyme into that of an adenylyl cyclase. Another interesting example is provided by the rat liver enzymes thiosulfate sulfurtransferase (rhodanese) and mercaptopyruvate sulfurtransferase, which share 66% amino-acid sequence identity but catalyze different enzyme reactions. The respective enzyme activities can be interconverted by replacement of arginine and lysine residues in the respective active sites (Nagahara et al., 1995; Nagahara and Nishino, 1996). Sulfotransferases obviously have equally precise mechanisms for influencing their substrate specificity, as demonstrated by our results.

Biogenic amines such as dopamine and tyramine are basic; consequently, they carry a positive charge at physiological pH. Exchanging the acidic glutamic acid for the hydrophobic, uncharged alanine was sufficient to dramatically alter the ability of SULT1A3 to sulfate dopamine and tyramine. This suggests that an interaction between positively charged substrates and a negatively charged amino acid in the substrate-binding pocket of SULT1A3 is central to the reaction specificity associated with these compounds. Although SULT1A3 has marked selectivity for dopamine and tyramine, it does have a broad substrate specificity; indeed, it shares many common substrates with SULT1A1 and other SULTs (Ganguly et al., 1995; Coughtrie et al., 1998), therefore it is likely that additional amino acids perform key functions in the SULT1A3 active site. Residue 146 in SULT family 1 mem-

### Table 2

Kinetic properties of wild-type SULTs 1A3 and 1A1 and site-directed mutant SULT1A3 enzymes toward the substrates dopamine and 4-nitrophenol

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dopamine</th>
<th>4-Nitrophenol</th>
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<tbody>
<tr>
<td></td>
<td>$K_m$ (μM)</td>
<td>$V_{max}$ (nmol/min/mg)</td>
</tr>
<tr>
<td>Wild-type SULT1A3</td>
<td>2.2</td>
<td>501</td>
</tr>
<tr>
<td>SULT1A3 H143Y</td>
<td>5.6</td>
<td>455</td>
</tr>
<tr>
<td>SULT1A3 E146A</td>
<td>127</td>
<td>187</td>
</tr>
<tr>
<td>SULT1A3 H143Y/E146A</td>
<td>87</td>
<td>211</td>
</tr>
<tr>
<td>Wild-type SULT1A1</td>
<td>109</td>
<td>37</td>
</tr>
</tbody>
</table>

Data shown are the average of duplicate determinations performed using PAPS at a concentration of 13 μM. Enzyme activities of wild-type SULT1A3 and SULT1A3 H143Y were measured with dopamine concentrations between 0.13 and 26.7 μM, whereas for SULT1A3 E146A and H143Y/E146A mutants and wild-type SULT1A1, the concentration range used was 0.015–1.3 μM. Enzyme activities of wild-type SULT1A3 and SULT1A3 H143Y were measured with 4-nitrophenol concentrations between 0.013 and 1.3 mM, whereas for SULT1A3 E146A and H143Y/E146A mutants and wild-type SULT1A1, the concentration range used was 0.13–26.7 μM.
bers is likely to be an important determinant of substrate specificity since amino acids at position 146 (or its equivalent) are conserved within the various SULT1 subfamilies. For example, all SULT1A1 orthologs identified so far (human, monkey, dog, cow, rat, and mouse) have alanine at residue 146, whereas in SULT1B and SULT1C family enzymes from human, rat, and mouse, asparagine is found at this position. Estrogen sulfotransferase isoforms (SULT1E) from human, cow, rat, and mouse have valine and sometimes isoleucine or methionine (which may be considered functionally equivalent) in this position. It is not clear whether the region we have studied here, which forms the predicted α-6 helix in members of the SULT family 1, performs a similar role in SULT family 2 proteins. The SULT2 enzymes share

![Substrate specificity profiles of wild-type SULTs 1A3 and 1A1 and site-directed mutants. SULT enzyme activity was determined using seven substrates at concentrations of 1 μM (■), 10 μM (□), and 100 μM (△) and PAP35S at 13 μM. Data represent the mean of duplicate determinations using the same amount of each recombinant protein (200 ng).](image-url)
only about 30% amino acid sequence identity with SULT1 proteins, although sequence alignments suggest the proposed α-6 helix region may be conserved in SULT2 proteins. The residue in SULT2 enzymes which is equivalent to 146 in the SULT1 family is not conserved either within SULT2 members or between the SULT1 and SULT2 families, but this may reflect the very distinct substrate specificities observed between members of the SULT2 family. Therefore the amino acid at position 146 or equivalent may be class-specific for mammalian sulphotransferases.

Clearly, further studies are required to identify the structural features and key amino acids that are necessary for the sulfuryl transfer reaction per se, as well as those that are responsible for determining the substrate specificities of other SULT isoforms. Our data indicate that residue 146 (or its equivalent) is a key amino acid influencing the substrate specificity of SULT1A3, and probably many other sulphotransferases. Molecular modeling of the active site of SULT1A3 and related proteins will be possible when the X-ray crystal structures of the enzymes are available. When this information is coupled to further site-directed mutagenesis experiments, it will doubtless provide the answers to many of these questions.

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References


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