X-ray Crystal Structure of Human Dopamine Sulfotransferase, SULT1A3

MOLECULAR MODELING AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS DEMONSTRATE A MOLECULAR BASIS FOR SULFOTRANSFERASE SUBSTRATE SPECIFICITY*

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Humans are one of the few species that produce large amounts of catecholamine sulfates, and they have evolved a specific sulfotransferase, SULT1A3 (M-PST), to catalyze the formation of their conjugates. An orthologous protein has yet to be found in other species. To further our understanding of the molecular basis for the unique substrate selectivity of this enzyme, we have solved the crystal structure of human SULT1A3, complexed with 3′-phosphoadenosine 5′-phosphate (PAP), at 2.5 Å resolution and carried out quantitative structure-activity relationship (QSAR) analysis with a series of phenols and catechols. SULT1A3 adopts a similar fold to mouse estrogen sulfotransferase, with a central five-stranded β-sheet surrounded by α-helices. SULT1A3 is a dimer in solution but crystallized with a monomer in the asymmetric unit of the cell, although dimer interfaces were formed by interaction across crystallographic 2-fold axes. QSAR analysis revealed that the enzyme is highly selective for catechols, and catecholamines in particular, and that hydrogen bonding groups and lipophilicity (cLogD) strongly influenced Km. We also investigated further the role of Glu146 in SULT1A3 using site-directed mutagenesis and showed that it plays a key role not only in defining selectivity for dopamine but also in preventing many phenolic xenobiotics from binding to the enzyme.

Sulfation is a ubiquitous process in nature. In eukaryotic organisms, the enzymatic formation of sulfate conjugates from small endogenous and xenobiotic molecules is catalyzed by members of the cytosolic sulfotransferase (SULT)1 superfamily (1, 2), all of which utilize PAPS as the sulfuryl donor for the reaction (3). In mammals, sulfation functions in the detoxification of therapeutic, dietary, and environmental xenobiotics as well as contributing to the homeostasis and regulation of numerous biologically active endogenous chemicals such as steroids, iodothyronines, bile acids, and neurotransmitters (1, 4). In addition, for a large number of procarcinogens sulfation is the terminal step in the bioactivation pathway and is necessary to reveal their mutagenic/carcinogenic activity (5, 6). In humans there are at least 10 distinct SULT enzymes, which may be distinguished on the basis of their substrate specificity and/or amino acid sequence identity, which ranges from 30 to 96%. On this basis, two subfamilies have been defined: the phenol sulfotransferases (SULT1) and the steroid sulfotransferases (SULT2), and seven of the human SULTs belong to the SULT1 family (1, 2). One of the most important SULT isomers in humans (known as SULT1A3 or M-PST) is the enzyme responsible for the sulfation of amine neurotransmitters such as dopamine, adrenaline, noradrenaline, and 5-hydroxytryptamine as well as certain iodothyronines, drugs, and dietary xenobiotics (7). In adult humans, SULT1A3 expression is extremely low in liver and is predominant in the upper gastrointestinal tract (8), although substantial expression is also found in other extrahepatic tissues such as brain, lung, and platelets. Unlike all other known members of the human SULT family, SULT1A3 exhibits a high degree of selectivity for dopamine, and interestingly, orthologs of the SULT1A3 enzyme have yet to be identified in other mammalian species. This probably reflects the important role the enzyme plays in producing sulfated catecholamines, a process that is relatively specific to humans (9).

A number of advances in our understanding of the sulfuryl transfer mechanism have been made recently (10), aided considerably by the first x-ray crystal structure of a mammalian cytosolic sulfotransferase, that of a mouse estrogen SULT (mEST) (11, 12). These studies have defined the amino acids in this SULT (and, by deduction, other members of the SULT enzyme family) that are important for binding of the universal sulfuryl donor PAPS and stabilization of the transition state. Furthermore, a histidine residue (His108) was identified and hypothesized to function as the catalytic base in the reaction; this amino acid is conserved in all cytosolic sulfotransferases...
from plants to man. However, the nature of the amino acids that define the substrate specificities of individual sulfotransferases is still unclear. We recently demonstrated, using site-directed mutagenesis, that a single amino acid in SULT1A3 (Glu146) governs the ability of the enzyme to sulfate dopamine selectively (13). McManus and co-workers (14) also showed that substituting the alanine residue at position 146 in SULT1A1 with glutamate affected the kinetic properties of the enzyme, which shares 93% amino acid sequence identity with SULT1A3. To understand more about the properties of SULT1A3 that define its unique substrate specificity, we have determined the x-ray crystal structure of the protein and applied quantitative structure-activity relationship (QSAR) analysis and molecular modeling approaches to the problem. Here we present, for the first time, the crystal structure of a human cytosolic SULT, and we begin to identify the molecular properties that define the substrate specificity of SULT1A3.

EXPERIMENTAL PROCEDURES

Materials—PAPS (>-99% pure) was purchased from H. Flatt and R. Landsiedel, German Institute for Human Nutrition, Potsdam, Germany. Human SULT1A1/PAPS and human SULT1A3/PAPS from DuPont/NEN, Stevenage, United Kingdom. All SULT substrates were purchased from Sigma-Aldrich, Poole, UK, and all other chemicals were obtained from commonly used local suppliers.

Protein Expression and Purification—cDNAs encoding human SULT1A1, SULT1A3, and the E146A mutant of SULT1A3 were expressed in Escherichia coli, and the recombinant proteins were purified and characterized as described previously (13, 15).

Crystallization and Structure Determination—Crystals of SULT1A3 were initially grown using the sitting drop method, with the best crystals growing at 20 °C. Drops were prepared by mixing 2 μl of protein solution (10 mg/ml in 50 mM Tris/HCl, pH 7.4, 1 mM 2-mercaptoethanol, and 4 mM PAP) with 2 μl of well buffer comprising 650 mM ammonium sulfate, 100 mM sodium citrate, pH 5.1, and 10 mM 2-mercaptopetanol. Crystals usually appeared after 4 days. They were rhombohedral in shape with a length of up to 0.6 mm and a cross-section of up to 0.2 mm. X-ray diffraction data were collected from these crystals at room temperature. Crystals of a morphology similar to those previously described were also obtained from hanging drops containing 1 μl of protein (10 mg/ml in 50 mM Tris/HCl, pH 7.4, 1 mM 2-mercaptoethanol, and 4 mM PAP) and 1 μl of well solution comprising 12–14% (w/v) polyethylene glycol 6000, 0.1 mM sodium citrate, pH 4.7, 10 mM MgCl2, and 10 mM 2-mercaptoethanol. These crystals grew to a size of 0.15 x 0.05 x 0.02 mm at 20 °C. They usually appeared overnight, grew to full size within 1 week, and were of the same space group (P3221) and unit cell dimensions as the crystals grown from ammonium sulfate.

In the case of the crystals from ammonium sulfate, x-ray diffraction data were collected at room temperature on the Daresbury synchrotron (Daresbury, Cheshire, UK) from 6 crystals and processed using DENZO and SCALPEPACK (16). X-ray diffraction data for the pH 6.0 PAP-soaked crystals were collected in-house using a R-AXIS IIIe system (Molecular Structure Corp., The Woodlands, TX) and at Daresbury from single crystals at 100 K. These were processed in the same way as for the previous data set. Initial phasing for the SULT1A3 structure was obtained from a molecular replacement solution. A search model was constructed from mEST (Brookhaven entry 1qaq) in which sequence differences were truncated to alanine. This model was used to obtain a solution to the rotation and translation functions using Amore (17). Refinement of the model was carried out using REFMAC (18) and including data between 20.0 and 2.5 Å. The structure of the pH 6.0 PAP-soaked crystal form was then solved by refining the previously solved SULT1A3 structure (ammonium sulfate crystal form) against the pH 6.0 PAP-soaked x-ray diffraction data and calculating difference Fourier maps.

Sulfotransferase Enzyme Assays—Enzyme activities of purified wild type SULTs 1A3 and 1A1 and the E146A mutant of SULT1A3 were determined using PAP-35S as originally described by Poesl and Meek (19) and as reported recently (13). Assays were carried out at 37 °C in a final volume of 150 μl with 10 mM potassium phosphate buffer, pH 6.8, and 0.04 μCi of PAP-35S. Control incubations contained no substrate. Assays were performed in duplicate, optimized with respect to incubation time and protein content, and carried out using saturating concentrations of PAPS. For estimation of Km and Vmax, enzyme activity data were plotted and analyzed using hyperbolic regression analysis with the Hyper.exe software package (version 1.1 s, J. S. Esterby, University of Liverpool).

RESULTS AND DISCUSSION

X-ray Crystal Structure of Human SULT1A3—Crystals were of space group P321, with 1 molecule in the asymmetric unit, and cell dimensions of a = b = 57.14 Å, c = 192.7 Å for the data set collected at room temperature, and a = b = 56.92 Å, c = 191.82 Å for the 100K data set. Statistics for each data set are shown in Table I. The solution to the rotation and translation function for the ammonium sulfate form was obtained using Amore (17). This resulting crystal model packed into the SULT1A3 unit cell with some overlap of residues; therefore residues 59–78 and 230–243 were deleted for refinement, giving a starting model with an R factor of 48.4%, between 8.0 and

| Table I  | Data collection and refinement statistics |
|----------|------------------------------------------|
| Data set | PAP-free form | PAP-bound form |
| Temperature of data | Room temperature | 100 K |
| Resolution (Å) | 2.5 | 2.5 |
| Rcryst | 0.063 | 0.098 |
| Number of reflections | 12,399 | 13,472 |
| IoverF | 8.7 (2.4) | 10.2 (1.26) |
| Completeness (%) | 95.8 (94.2) | 95.5 (93.8) |
| Reff | 20.0–2.5 | 20.0–2.5 |
| Rfree | 0.215 | 0.242 |
| Number of waters | 200 | 200 |
| Root mean square bond length (Å) | 0.012 | 0.017 |
| Root mean square bond angles (°) | 2.2 | 2.5 |
| Mean B value (Å²) | 66.7 | 64.1 |

* Rcryst = Σ|Io − |Fo|/ΣIo, where Io is the intensity of the ith observation and |Fo| is the mean intensity of the reflection.
4.0 Å. The $R$ factor for the refined structure is 21.1% ($R_{free} = 27.1\%$). The refined model comprises residues 7–67, 76–228, and 259–292, 200 water molecules, and 1 sulfate ion. The electron density maps reveal distinct regions of disorder in the structure; specifically residues 229–258 cannot be traced, although some uninterpretable density is present, and the same is true for residues 68–75. Residues equivalent to 68–75 were also disordered in the mEST structure (11). The temperature factors on all the atoms are high, with an average $B$ factor for the whole molecule of 66.7 Å$^2$.

Clear electron density corresponding to a sulfate ion was apparent in the active site but there was no evidence for the presence of PAP, although PAP was present during crystallization. It was proposed that the high concentration of ammonium sulfate present in the crystallization buffer led to competition between PAP and sulfate ions for the binding site. Consequently, crystallization conditions avoiding high salt concentrations were screened, and crystals were obtained using polyethylene glycol 6000 at pH 4.7 in the presence of PAP. X-ray diffraction data collected from these crystals showed that neither sulfate nor PAP was present in the active site, and we postulated that PAP may not be bound because of the low pH of the crystallization conditions. We therefore tested crystals under different harvesting conditions at pH 6.

The in-house data set for the pH 6.0 PAP-soaked crystals diffracted to only 3.2 Å, but strong electron density for PAP was clearly visible in the active site. The synchrotron data set, which was processed to 2.5 Å, showed severe anisotropy, with clearly visible in the active site. The synchrotron data set, diffracted to only 3.2 Å, but strong electron density for PAP was different harvesting conditions at pH 6.

The structural topology of SULT1A3 (Fig. 1) is very similar to that of mEST (11), which was expected given the degree of sequence identity between the two proteins (46%). The overall structure is a central five-stranded β-sheet surrounded by α-helices, and the structure superposes very closely on the mEST molecule with a root mean square deviation of 0.81 Å on the Ca atoms of the secondary structural elements of molecule A in the mEST dimer. The shape of the SULT1A3 monomer as crystallized here is roughly spherical with a slight depression on one side. The active site lies at the bottom of this depression, and it is postulated that the missing residues are located in this region. A cis-peptide bond was observed for Pro$^{102}$, a feature that was also present in the mEST structure. The level of amino acid sequence identity between SULT1A3 and other SULT1 family enzymes (47–96%) means it is almost certain that other SULT1 isoforms will also adopt this fold.

The major differences between the mEST and SULT1A3 structures are in the dimer interface region. Although mEST exists as a monomer in solution, it crystallized as a dimer (11), with a well defined interface; the equivalent residues cannot be seen in the SULT1A3 structure. Conversely, SULT1A3 does exist as a dimer in solution (15) but the asymmetric unit in these crystals contains only one monomer. Thus if SULT1A3 did crystallize as a dimer, the monomers must be related by a crystallographic symmetry axis. Of the possible dimers generated by the crystal symmetry in the SULT1A3 structure, only one involves a 2-fold symmetric dimer with a significant interface. In this dimer, a loop involving residues 84–92 from each monomer is inserted into the active site of the other (Fig. 2). Although this is consistent with the observation that residues within this loop are important for the activity and selectivity of the enzyme (14), several features of the interactions across the interface led us to question whether this structure corresponds exactly to the catalytically active dimeric state. First, the inserted loop blocks off the channel that would be the most likely route of acceptor substrate entry. Second, the loop would occlude binding of substrates with a significant substituent para to the nucleophilic hydroxyl group of phenols and/or catechols that are substrates and possibly hinder the interaction between Glu$^{146}$ and substrates containing an appropriately located amine function (such as dopamine), which is inferred from our previous studies (13) and from the molecular modeling/QSAR data reported here. It is also worth noting that the positions of residues in this interface are not as well defined as in the bulk of the structure, the temperature factors for resi-
dyes in this loop are higher than the average temperature factors for the molecule, and the whole loop is displaced relative to mEST. This suggests that the loop may be mobile and have the potential to move on binding of acceptor substrate. We are currently exploring the dimer interface further using site-directed mutagenesis experiments. The lack of structure seen in the loop region may be a feature that allows entry of acceptor substrate to the active site; binding of substrate may induce the loop region may be a feature that allows entry of acceptor substrate to the active site; binding of substrate may induce structure and allow the outer surface of the active site to form. However, no such structural alteration in mEST was observed following binding of β-estradiol (11).

Recognition of PAP is the same as in mEST, although it is interesting to note that parts of the 3′-phosphate binding site are not well ordered whereas the 5′ site is. The 3′ site in mEST comprises the side chains of Arg130 and Ser138, together with the main chain amide nitrogens of residues 258–259 and the guanidinium group of Arg257. The interactions with Arg130 and Ser138 are conserved for SULT1A3, but residues 258 and 259 cannot be seen in the SULT1A3 structure. This may indicate that the 5′ site is a more important means of recognition, a suggestion supported by the observation that, in the unliganded form of the crystal, sulfate occupies the 5′ site. The 5′-phosphate serves to orient the sulfate group for transfer and possibly requires greater constraints on its position. It is possible to build a model for PAPS in its binding pocket from the SULT1A3-PAP structure (Fig. 3), and the implications for sulfonyl transfer and intermediate stabilization are in general agreement with what has been outlined for mEST (11, 12). In contrast, the nature of the substrates for these two enzymes is quite different, and it is important to consider how differentiation between substrates is achieved.

Substrate Specificity and Enzyme Kinetics of Recombinant SULT1A3—To begin probing the active site of SULT1A3 with a view to understanding the molecular basis of SULT substrate specificity, we conducted a detailed analysis of the kinetic properties of the enzyme using a series of catechols and phenols (Table II). To ensure that these determinations were reproducible, we measured the $K_m$ and $V_{max}$ values for 4 of these compounds (phenol, 4-chlorophenol, 4-methylphenol, 4-methoxyphenol) with SULT1A3 and SULT1A1 on three separate occasions with three preparations of enzyme. Standard deviations were all less than 15% of means (not shown).

Our results clearly confirm that, of all the compounds tested, dopamine was the most selective substrate for SULT1A3. Other catecholamines, such as norepinephrine, dobutamine, and isoprenaline were also good substrates for SULT1A3, with $K_m$ values < 5 μM. None of the phenols, with the exception of the esters butyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate, had a $K_m$ value for SULT1A3 of less than 100 μM, and 4-ethyl phenol and 4-aminophenol were not metabolized at all. In contrast, all compounds tested were low $K_m$ substrates for SULT1A1 ($K_m$ between 0.8 μM and 7.6 μM), with the exception of 4-aminophenol ($K_m$ = 34 μM), dopamine ($K_m$ = 109 μM), and tyramine ($K_m$ = 8 μM), and there was little discrimination between the compounds, confirming the perception that this enzyme has very broad substrate specificity (20). The wide tissue distribution and broad substrate specificity of SULT1A1

![Fig. 3. Model of the SULT1A3 active site.](image)

**Table II**

| Compound                  | $K_m$ (μM) | $V_{max}$ (nmol/min/mg) | $V_{max}$/$K_m$ |
|--------------------------|------------|-------------------------|-----------------|
| Phenol                   | 11.0       | 137                     | 12              |
| 4-Chlorophenol           | 16.7       | 133                     | 7.9             |
| Butyl 4-hydroxybenzoate  | 20.0       | 80                      | 4.0             |
| Tyramine                 | 21.0       | 283                     | 13              |
| 4-Methylcatechol         | 21.9       | 415                     | 19              |
| Propyl 4-hydroxybenzoate | 30.0       | 94                      | 3.1             |
| 3,4-Dihydrobenzylglycol  | 33.4       | 48                      | 1.4             |
| 4-Isopropylcatechol      | 33.6       | 793                     | 23              |
| Ethyl 4-hydroxybenzoate  | 100        | 118                     | 1.2             |
| 4-Phenylazophenol        | 124        | 58                      | 0.5             |
| 4-Chlorophenol           | 130        | 241                     | 1.9             |
| 4-Bromophenol            | 147        | 210                     | 1.4             |
| 4-Iodophenol             | 165        | 143                     | 0.9             |
| 4-Cyclopentlyphenol      | 199        | 50                      | 0.3             |
| 4-sec-Butylphenol        | 214        | 23                      | 0.1             |
| Fluorophenol             | 253        | 291                     | 1.2             |
| 3-Methoxyphenol          | 286        | 104                     | 0.4             |
| Methyl 4-hydroxybenzoate | 295        | 143                     | 0.5             |
| Phenolphthalein          | 305        | 30                      | 0.1             |
| Phenol                   | 567        | 207                     | 0.4             |
| 4-Methylphenol           | 746        | 196                     | 0.3             |
| Ethylphenol              | 878        | 147                     | 0.2             |
| 4-Methoxyphenol          | 1080       | 124                     | 0.1             |
| 4-n-Propylphenol         | 1388       | 229                     | 0.2             |
| 4-Isopropylphenol        | 2081       | 105                     | 0.05            |
| 4-Nitrophenol            | 2641       | 311                     | 0.1             |
| 4-Hydroxybenzyleanide    | 4091       | 240                     | 0.06            |
| 4-Butylphenol            | ND         | ND                      | ND              |
| 4-Aminophenol            | ND         | ND                      | ND              |
Table III
Kinetic properties of recombinant SULT1A3 and SULT1A1 toward paired catechols and phenols

|                 | SULT1A3                      | SULT1A1                      |
|-----------------|------------------------------|------------------------------|
|                 | \(K_m\) | V\(_{\text{max}}\) | \(V_{\text{max}}/K_m\)   | \(K_m\) | V\(_{\text{max}}\) | \(V_{\text{max}}/K_m\)   |
| Catechol        | 11     | 117              | 11                         | 3.9     | 40                | 10                         |
| Phenol          | 567    | 207              | 0.4                        | 4.5     | 151               | 34                         |
| 4-Chlorocatechol | 9.2    | 184              | 20                         | 1.4     | 16                | 11                         |
| 4-Chlorophenol  | 130    | 241              | 1.9                        | 6.0     | 487               | 81                         |
| 4-Methylcatechol| 22     | 215              | 10                         | 3.7     | 52                | 14                         |
| 4-Methylphenol  | 746    | 196              | 0.3                        | 7.6     | 572               | 75                         |
| 4-Isopropylcatechol | 34   | 793              | 23                         | 4.4     | 79                | 18                         |
| 4-Isopropylphenol | 2081  | 105              | 0.05                       | 7.5     | 244               | 33                         |
| Dopamine        | 2.2    | 501              | 228                        | 109     | 37                | 0.4                        |
| \(\rho\)-Tyramine | 21    | 282              | 13                         | 8000    | 50                | 0.01                       |

Suggest that this enzyme is probably the major "chemical defense" sulfotransferase (6).

To determine whether the catechol function influenced the substrate specificity of SULT1A3, we compared the kinetic properties of this enzyme and of SULT1A1 toward a series of paired catechols and phenols (Table III). These data show that SULT1A3 has significantly lower \(K_m\) values for the catechol forms than for the corresponding phenols, although in general there was little difference in \(V_{\text{max}}\) (4-isopropylcatechol/4-isopropylphenol and dopamine/tyramine being the exceptions, where the \(V_{\text{max}}\) for the phenol form was substantially lower). Conversely, SULT1A1 showed much less discrimination in terms of \(K_m\) between catechols and phenols (with the exception of dopamine/tyramine), but the \(V_{\text{max}}\) values were consistently higher for the phenol form. SULT1A1 demonstrated a lower \(K_m\) value for every phenol and catechol tested, with the exception of dopamine and tyramine.

**QSAR Analysis of SULT1A3**—QSAR analysis was used to help identify features that determine the suitability of molecules as substrates for SULT1A3. We calculated a range of physicochemical descriptors for a series of thirty-nine 4-substituted phenols and catechols that are substrates for SULT1A3, which were then compared with the experimentally determined \(K_m\) value for each compound measured with SULT1A3. Two structure-activity relationships were derived that best described the observed \(K_m\) data, and these are given in Equations 1 and 2. Plots of predicted against experimental log(1/\(K_m\)) values are shown in Fig. 4.

The first analysis (Equation 1; Fig. 4A) indicates that hydrogen bond donor and acceptor groups are important for binding of substrate to the enzyme; an increased number of hydrogen bonding groups leads to smaller \(K_m\) values. Thus the extra hydrogen bond donor groups on compounds such as tyramine, dopamine, and the catechols probably contribute to their relatively strong binding to SULT1A3. Another important parameter was cLogD, where the more hydrophobic the molecule the lower the \(K_m\) value is predicted to be. The active site of the enzyme contains 3 conserved phenylalanine residues, which will likely create a hydrophobic environment. It is also clear from this model that \(K_m\) is dependent upon the size of the para substituent, in particular the width (or Y values), with narrower molecules being favored. An example of this is the contrast between 4-isopropylphenol and 4-\(\rho\)-propylphenol, where the \(K_m\) value for the former is nearly double that for the latter (Table II). Also, 4-\(\rho\)-butylphenol is not metabolized at all by SULT1A3, whereas the \(n\)- and sec-isomers are both substrates. The second QSAR model (Equation 2, Fig. 4B) also identified cLogD as an important parameter, and again molecules with low molecular volume (\(V_m\)) are predicted to have higher affinity.

![Fig. 4. QSAR analysis of SULT1A3](image-url)

**Table III**

|                      | SULT1A3 | SULT1A1 |
|----------------------|---------|---------|
| \(K_m\) nmol/min/mg  |         |         |
| Catechol             | 11      | 117     |
| Phenol               | 567     | 207     |
| 4-Chlorocatechol     | 9.2     | 184     |
| 4-Chlorophenol       | 130     | 241     |
| 4-Methylcatechol     | 22      | 215     |
| 4-Methylphenol       | 746     | 196     |
| 4-Isopropylcatechol  | 34      | 793     |
| 4-Isopropylphenol    | 2081    | 105     |
| Dopamine             | 2.2     | 501     |
| \(\rho\)-Tyramine    | 21      | 282     |

\(R^2 = 0.86; V^2 = 0.81; n = 39; S.E. = 0.14\).
for SULT1A3. This model also supports the evidence from molecular modeling and site-directed mutagenesis experiments (see below) indicating that the enzyme favors catechols over phenols and that an amine nitrogen at the β-carbon of the 4-substituent is a very important indicator of SULT1A3 selectivity, presumably through its interaction with Glu146. We conclude that this interaction between the amine nitrogen and Glu146 is the major factor determining the low Km values obtained for SULT1A3 with the catecholamines dopamine, norepinephrine, isoprenaline, and dobutamine (Table II) and explains why dopamine, for example, is a highly specific substrate for SULT1A3 compared with other catechols without the nitrogen in this position.

The Km data obtained for SULT1A1 were not subjected to QSAR analysis as, with the exception of dopamine and tyramine, there was very little variation in affinity of the enzyme within the group of substrates studied.

Molecular Modeling of SULT1A3—Although crystals of SULT1A3 could not be obtained with substrate bound (possibly due to substrate entry “channel” occlusion by the crystalline dimer interface), it is possible to model the position of the substrate dopamine based on the location of estradiol in the mEST structure (Fig. 3). The substrate binding site in SULT1A3 appears to be formed by Phe24, Phe81, and Phe142. These 3 residues are absolutely conserved across human SULTs 1A1, 1A2, and 1A3 (Phe142 is conserved in all SULTs); however in other sulfotransferases, where there is not a strong preference for phenolic substrates, variation is observed at positions corresponding to residues 24 and 81. For example, all human and rat steroid (alcohol) sulfotransferases of the SULT2A family have a Trp at the position equivalent to Phe24 in SULT1A3. In mEST position 24 is occupied by a Tyr, while the other two are conserved, and Tyr240 and Met248 also contribute to the substrate binding site. Although Tyr240 and Met248 are not visible in the SULT1A3 structure reported here, they are highly conserved in the SULT family, and it is assumed that they will perform a similar function in SULT1A3.

The phenolic hydroxyl group of SULT1A3 substrates can be placed in a location similar to that of estradiol in mEST, adjacent to His308 the proposed catalytic base, and in an appropriate position for apical nucleophilic attack on the sulfur in PAPS (11) (Fig. 3). The ϵ-nitrogen of His308 is hydrogen-bonded to the backbone carbonyl of Thr45 (also highly conserved), orienting the unprotonated ϵ-nitrogen toward the substrate. Interestingly, in the case of catechol substrates, the two hydroxyls could be positioned within hydrogen bonding distance of the unprotonated ring nitrogen of His308, but this arrangement is unlikely to occur during catalysis as it would inhibit removal of a proton from either. Instead the ring of the catechol can be flipped to direct the passive hydroxyl away from His308 and toward the predicted location of the hydroxyl of Tyr240. This is consistent with our observation that, for otherwise equivalent pairs of phenols and catechols, SULT1A3 shows a significantly lower Km for the catechol form compared with the phenol, although there are much smaller effects on Vmax (Table III). It is also clear that, with a minor shift in the orientation of the substrate, the hydroxyl at either the 3- or 4-position of catechols such as dopamine could be placed in a suitable orientation for sulfation. Physiologically, dopamine 3-O-sulfate predominates over the 4-O-sulfate in humans (21, 22), although other mechanisms such as specificity of transport proteins and/or of sulfate hydrolysis by arylsulfatase(s) (23) may influence circulating levels. It has recently been demonstrated that in the case of l-Dopa, SULT1A3 has absolute selectivity for sulfation at the 3-O-position (24).

Effect of the Glu146 → Ala Mutation on SULT1A3 Substrate Specificity—The broad substrate specificity of SULT1A1, where large variations in the nature of the para substituent are accompanied by virtually no change in the Km, is in marked contrast to the high degree of selectivity exhibited by SULT1A3. The only residue that differs between them, which would contact phenols with small substituents is at position 146. Glu146 in SULT1A3 thus appears to have a key role in substrate selection-rejecting groups with a bulky or noncationic substituent meta or para to the hydroxyl group destined for sulfation. To investigate further the function of Glu146 in controlling the substrate specificity of SULT1A3, we first tested 6 members of the series of 4-substituted phenols (including some of the “worst” substrates for SULT1A3) with the E146A mutant of SULT1A3 (13) (Table IV). Mutating Glu146 in SULT1A3 to Ala146 (as it is in SULT1A1) dramatically reduced the Km values for 4-isopropylphenol, 4-t-butylphenol, 4-methoxyphenol, and 4-hydroxybenzylcyanide, strongly suggesting that Glu146 plays a critical role in limiting substrate access and/or binding to SULT1A3. It is rather surprising, however, that the A146E mutant of SULT1A1 showed no activity toward dopamine (14).

Steric considerations suggest that when substrates such as dopamine or tyramine are docked into the active site of SULT1A3, the α-β bond of the ethylamine side chain would lie perpendicular to the aromatic ring. Directing the side chain toward the side facing Phe24 means the amine would be adjacent to Glu146, the proposed regulator of substrate access/bind-}
away from Glu146, and the carbonyl oxygen can hydrogen bond with His149. Thus propyl 4-hydroxybenzoate and butyl 4-hydroxybenzoate had much lower $K_m$ values with the wild-type SULT1A3 than the other compounds tested and demonstrated a much less marked change in $K_m$ (only about a 2-fold reduction) when Glu146 was replaced by Ala. His149 is conserved in SULT1A1 but is replaced by Tyr in mEST and SULT1A2.

Glu146 also appears to have a role in determining the lower $K_m$ values exhibited by SULT1A3 with catechols compared with phenols. This is apparent from the fact that, whereas the reduced $K_m$ for catechols could be explained simply by hydrogen-bonding with Tyr240, substantial differences between catechols and phenols were not observed for SULT1A1, where Tyr240 is conserved. Thus we measured sulfotransferase kinetic parameters for the E146A mutant of SULT1A3 toward 5 pairs of phenols and catechols and compared these with data obtained from wild-type SULTs 1A3 and 1A1 (Table V).

The single amino acid change in SULT1A3 E146A had a dramatic effect, abolishing the preference for catechols through a reduction in $K_m$ for the phenol form. $K_m$ values for catechols were not altered substantially and neither were $V_{\text{max}}$ values for either the phenol or catechol form (with the exception of 4-isopropylcatechol where both $K_m$ and $V_{\text{max}}$ were reduced for the mutant enzyme). As could have been predicted, the only exception to this trend was the dopamine/tyrine pair, where $K_m$ increased and $V_{\text{max}}$ reduced, consistent with the importance of the interaction between the amino group and Glu146. The means by which Glu146 selects against phenols relative to catechols are not clear. It may be that Glu146 forces substrates into an orientation where hydrogen-bonding to Tyr240 is required; alternatively Glu146 in SULT1A3 could be more restrictive than Ala146 in SULT1A1 in allowing sulfation at a hydroxyl para to the substituent.

### Table V

| Compound          | SULT1A3 E146A | SULT1A3wt | SULT1A1 |
|-------------------|---------------|-----------|---------|
|                   | $K_m$ [mM]    | $V_{\text{max}}$ [nmol/min/mg] | $V_{\text{max}}/K_m$ [nmol/min/mg] | $K_m$ [mM]    | $V_{\text{max}}$ [nmol/min/mg] | $V_{\text{max}}/K_m$ [nmol/min/mg] | $K_m$ [mM]    | $V_{\text{max}}$ [nmol/min/mg] | $V_{\text{max}}/K_m$ [nmol/min/mg] |
| Catechol          | 16            | 117       | 10.6    | 3.9       | 40          | 10.3          | 3.9       | 40          | 10.3                      |
| Phenol            | 64            | 207       | 0.4     | 4.5       | 151         | 33.6          | 4.5       | 151         | 33.6                      |
| 4-Chlorocatechol   | 5             | 184       | 20.0    | 1.4       | 487         | 81.2          | 1.4       | 487         | 81.2                      |
| 4-Chlorophenol     | 21            | 241       | 1.9     | 3.7       | 52          | 14.1          | 3.7       | 52          | 14.1                      |
| 4-Methylcatechol   | 16            | 215       | 9.8     | 3.7       | 52          | 14.1          | 3.7       | 52          | 14.1                      |
| 4-Methylphenol     | 40            | 746       | 0.3     | 7.6       | 572         | 75.3          | 7.6       | 572         | 75.3                      |
| 4-Isopropylcatechol| 9             | 793       | 23.6    | 4.4       | 79          | 18.0          | 4.4       | 79          | 18.0                      |
| 4-Isopropylphenol  | 65            | 229       | 0.2     | 6.8       | 242         | 35.6          | 6.8       | 242         | 35.6                      |
| Dopamine          | 23            | 501       | 227.7   | 109       | 37          | 0.3           | 109       | 37          | 0.3                       |
| Tyramine          | 727           | 282       | 13.4    | 8000      | 50          | 0.01          | 8000      | 50          | 0.01                      |

### CONCLUSION

The crystal structure of human dopamine sulfotransferase strongly suggests that cystolic sulfotransferases share a common reaction mechanism and that key conserved amino acids participate in PAPS and substrate binding and sulfuryl transfer. SULT1A3 displays significant selectivity for catechols, and the importance of Glu146 in SULT1A3 substrate specificity appears more fundamental than previously indicated. It seems to be involved not only in “attracting” natural substrates such as dopamine but also in “repelling” many phenolic xenobiotics with hydrophobic or bulky substituents. It also appears to be involved in the selectivity of SULT1A3 for catechols over phenols. This most likely reflects the different functions of the SULT1A3 and SULT1A1 isoforms in humans, where SULT1A3 has evolved to produce endogenous catecholamine sulfates (and also probably to detoxify certain xenobiotic catechols) and SULT1A1 plays a general chemical defense role, with the ability to sulfate many different chemicals with high affinity. The high degree of selectivity displayed by SULT1A3 probably reflects a necessity to prevent (competing substrate) inhibition of catecholamine sulfate production by other xenobiotics. The substitution of a single acidic amino acid at position 146 seems to be a highly efficient mechanism for producing an enzyme with this function.

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### REFERENCES

1. Coughtrie, M. W. H., Sharp, S., Maxwell, K., and Innes, N. P. (1998) Chem.- Biol. Interact. 109, 3–21.
2. Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C., and Raftogianis, R. B. (1997) FASEB J. 11, 3–14.
3. Klaassen, C. D., and Boles, J. W. (1997) FASEB J. 11, 404–418.
4. Duffell, M. W. (1997) in Comprehensive Toxicology (Guengerich, F. P., ed) pp. 363–385, Elsevier Science Ltd., Oxford.
5. Glatt, H. R. (1997) FASEB J. 11, 314–321.
6. Coughtrie, M. W. H. (1996) Hum. Exp. Toxicol. 15, 547–555.
7. Rupper, G. L., Sharp, S., Jones, A. L., Glatt, H., Mills, J. A., and Coughtrie, M. W. H. (1986) Xenobiotica 16, 1113–1119.
8. Dousa, M. K., and Tyce, G. M. (1988) Proc. Soc. Exp. Biol. Med. 188, 427–434.
9. Zhang, H. P., Varma-lova, O., Vargass, F. M., Falany, C. N., and Lehy, T. S. (1998) J. Biol. Chem. 273, 10888–10892.
10. Kakuta, Y., Pedersen, L. G., Carter, C. W., Negishi, M., and Pedersen, L. C. (1997) Nature Struct. Biol. 4, 904–908.
11. Kakuta, Y., Petrozhenko, E. V., Pedersen, L. C., and Negishi, M. (1998) J. Biol. Chem. 273, 27225–27230.
12. Dajani, R., Hoo, A. M., and Coughtrie, M. W. H. (1998) Mol. Pharmacol. 54, 942–948.
13. Brix, R. A., Duggleby, R. G., Gaedigk, A., and McManus, M. E. (1999) Biochem. J. 337, 337–343.
14. Dajani, R., Sharp, S., Graham, S., Bethell, S. S., Cooke, R. M., Jamieson, D. J., and Coughtrie, M. W. H. (1999) Protein Expression Purif. 16, 11–18.
15. Bronaugh, R. L., Hattox, S. E., Hoehn, M. M., Murphy, R. C., and Rutledge, C. O. (1995) J. Pharmacol. Exp. Ther. 195, 441–452.
16. Scott, M. C., and Elhissak, M. A. (1987) J. Chromatogr. 413, 17–23.
17. Strobel, G., Werle, E., and Weicker, H. (1986) Biochem. J. 240, 343–351.
18. Suiko, M., Sakakibara, Y., Awan-Khan, R., Sakaida, H., Yoshikawa, H., Ranaizinghe, J. G. S., and Liu, M.-C. (1998) J. Biochem. (Tokyo) 124, 707–711.
19. Hochhaus, G., and Mullmann, H. (1992) Int. J. Clin. Pharmacol. Ther. Toxicol. 30, 342–362.
20. Eaton, E. A., Walle, U. K., Wilson, H. M., Aberg, G., and Walle, T. (1996) Br. J. Clin. Pharmacol. 41, 201–206.