Structure-Function Relationships in the Stereospecific and Manganese-dependent 3,4-Dihydroxyphenylalanine/ Tyrosine-sulfating Activity of Human Monoamine-form Phenol Sulfo transferase, SULT1A3*

Received for publication, April 1, 2002, and in revised form, November 5, 2002
Published, JBC Papers in Press, November 6, 2002, DOI 10.1074/jbc.M203108200

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The human monoamine-form phenol sulfotransferase (PST), SULT1A3, has a unique 3,4-dihydroxyphenylalanine (Dopa)/tyrosine-sulfating activity that is stereospecific for their L-form enantiomers and can be stimulated dramatically by Mn²⁺. This activity is not present in the simple phenol-form PST, SULT1A1, which is otherwise >93% identical to SULT1A3 in amino acid sequence. The majority of the differences between these two proteins reside in two variable regions of their sequences. Through the characterization of chimeric PSTs where these two regions were exchanged between them, it was demonstrated that variable Region II of SULT1A3 is required for the stereospecificity of its Dopa/tyrosine-sulfating activity, whereas variable Region I of SULT1A3 is required for the stimulation by Mn²⁺ of this activity. Further studies using point-mutated SULT1A3s mutated at amino acid residues in these two regions and deletional mutants missing residues 84–86 and 84–90 implicated residue Glu-146 (in variable Region II of SULT1A3), as well as the presence of residues 84–90 of variable Region I, in the stereospecificity in the absence of Mn²⁺. Residue Asp-86 (in variable Region I of SULT1A3), on the other hand, is critical in the Mn²⁺ stimulation of the Dopa/tyrosine-sulfating activity of SULT1A3. A model is proposed, with reference to the reported x-ray crystal structure of SULT1A3, to explain how the normal role of SULT1A3 in dopamine regulation may be subverted in the presence of Mn²⁺. These studies could be relevant in understanding the stereoselective action of SULT1A3 on chiral drugs.

The sulfotransferases (STs),¹ which are ubiquitous in both plants and animals, catalyze the sulfation of hydroxyl or amino groups on a variety of target acceptor molecules (1, 2). These enzymes all use adenosine 3’-phosphate, 5’-phosphosulfate (PAPS) as the sulfonyl group donor (3) and share sequences responsible for PAPS binding (4). Although the membrane-bound STs use proteins, glycolipids, and other macromolecules as acceptor substrates, the cytosolic STs sulfate smaller molecules and are part of the Phase II detoxification pathway for the biotransformation/excretion of drugs and xenobiotics (1, 2). Increasingly, the cytosolic STs have also been shown to be important in regulating the levels and/or activities of endogenous compounds such as thyroid and steroid hormones, catecholamines, and bile acids (5, 6).

Based on their sequences, the cytosolic STs have been classified into several gene families (4). Two human cytosolic STs, the monoamine-form and the simple phenol-form phenol sulfotransferases, named SULT1A3 and SULT1A1, respectively (4), show an extensive (>93%) identity in their amino acid sequences (cf. Fig. 1A) and yet vary widely in their substrate specificity and other properties (7–9). They have thus served as an ideal model system to study structure/function relationships in these proteins. Examination of their aligned sequences revealed that most of the differences between SULT1A3 and SULT1A1 occur in two variable regions, designated Region I (encompassing amino acid residues 84–89) and Region II (including residues 143–148) (cf. Fig. 1A). Based on the hypothesis that the differences in these two variable regions may account for the distinct properties of SULT1A3 and SULT1A1, we had prepared chimeric proteins (7), where these two regions were reciprocally exchanged (cf. Fig. 1B). Characterization of these chimeras indicated that both Regions I and II were indeed critical for the specificity of SULT1A3 for dopamine and of SULT1A1 for p-nitrophenol (7). To extend the study further, we and others (8, 10, 11) had, by site-directed mutagenesis, exchanged amino acid residues in these two regions between SULT1A3 and SULT1A1. Results from these studies implicated residue 146 in Region II and residues 86 and 89 in Region I, as important in determining the specificity of the enzymes for their respective substrates.

SULT1A3 is present in brain where it is believed to sulfate monoamine neurotransmitters (particularly dopamine) with high activity and thus to regulate their levels (5). It also serves a detoxifying function in the intestine, where it may detoxify potentially lethal dietary monoamines (12). Besides the activity toward its physiological substrate, dopamine, for which it has a \( K_m \) of 2 \( \mu M \), SULT1A3 has been shown recently to display a unique Dopa/tyrosine-sulfating activity at higher (millimolar and sub-millimolar) concentrations of these substrates, which can be dramatically stimulated by Mn²⁺ (13).

In this study, chimeras and site-directed mutants were used to explore the structural basis for the stereoselectivity and

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¹ This work was supported in part by a grant from the American Heart Association (Texas Affiliate) (to M. C. L.), a UTHCT President’s Council Research Membership Seed Grant (to M. C. L.), and an award from the Naito Foundation (to M. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This advertisement must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: ST, sulfotransferase; PAPS, adenosine 3’-phosphate, 5’-phosphosulfate; Dopa, 3,4-dihydroxyphenylalanine; TAPS, 3-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino-1-propane-sulfonic acid; TLC, thin-layer chromatography.

This paper is available on line at http://www.jbc.org

Printed in U.S.A.

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Mn$^{2+}$ stimulation of the Dopa/tyrosine-sulfating activity of SULT1A3. SULT1A3 is present as a homodimer in its native state (14). Kinetic studies of the Dopa/tyrosine-sulfating activity of SULT1A3 (15) suggested that, in the case of D-tyrosine, where a 4-hydroxyphenyl group is to be sulfated, the tyrosine-Mn$^{2+}$ and tyrosine-Mn$^{2+}$-tyrosine complexes may be the real and obligatory substrates. In the case of Dopa, where we had shown that sulfation takes place exclusively at the 3-hydroxyphenyl group (16), the substrate-Mn$^{2+}$ complexes appeared to be better substrates than D-Dopa. Thus the kinetic data implied that Mn$^{2+}$ while interacting with the carboxyl group of the Dopa/tyrosine substrate to form the complex, also coordinates with amino acid residues on the enzyme. Previous x-ray crystallography study on SULT1A3 (17) has suggested that residues 84–92 corresponding to the variable Region 1 (cf. Fig. 1A) form a “mobile” loop. Based on the amino acid residue in SULT1A3 identified as interacting with Mn$^{2+}$ in the present study, a model has been proposed, in reference to the above-mentioned mobile loop, to explain how Mn$^{2+}$ exerts its stimulatory effect on the sulfation of tyrosine and Dopa by the enzyme. This study also identified the amino acid residues and regions responsible for the unusual stereospecificity of SULT1A3 for the D-enantiomers of Dopa and tyrosine. The model developed may prove useful in understanding the stereoselective action of SULT1A3 on chiral drugs (18–21) and the possible effects of metal ions on this activity.

**EXPERIMENTAL PROCEDURES**

**Materials**—L-Tyrosine, D-tyrosine, L-Dopa, D-Dopa, ATP, 3-[12-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino-1-propanesulfonic acid (TAPS); SDS, di ethiothreitol, EDTA (tetrasodium salt), isopro pyl β-thiogalactopyranoside, thrombin, and PAPS were from Stratagene. QuikChange site-directed mutagenesis kit and XL1-Blue Epicurian coli competent cells were purchased from Stratagene. Oligonucleotide primers were synthesized by MWG Biotech. pGEX-2TK glutathione S-transferase gene fusion vector, E. coli BL21 host cells, and glutathione-Sepharose were products of Amersham Biosciences. Carrier-free sodium $[^{35}S]$sulfate and E. coli liquid scintillation fluid were from ICN Biomedicals. Recombinant human bifunctional ATP sulfurylase/adenosine $5'$-phosphosulfate kinase, and its purity was determined as described previously (25). The [35S]PAPS synthesized was then adjusted to the required concentration and specific activity by the addition of cold PAPS. SDS-polyacrylamide gel electrophoresis was performed on 12% polyacrylamide gels using the method of Laemmli (26). Protein determination was based on the method of Bradford with bovine serum albumin as standard (27).

**RESULTS AND DISCUSSION**

Despite recent efforts from several laboratories (7–11), there is still relatively scant information concerning the structure/function relationships of cytosolic ST enzymes. As noted earlier, SULT1A3 and SULT1A1, which are $>$93% identical in amino acid sequence and yet display distinct substrate specificity and other properties, provide an excellent model for studies in this regard. In contrast to SULT1A1, SULT1A3 exhibits two unique activities. The sulfating activity toward dopamine, which presumably helps to regulate the levels of this endogenous compound, has a pH optimum of ~7.0 and a $K_m$ of 2 μM (in the physiological range) (14, 28). Mn$^{2+}$ stimulated this activity to a comparatively smaller extent (~2- to 3-fold) (13) but appeared to increase the $K_m$ for dopamine slightly (15). The Dopa/tyrosine-sulfating activity of SULT1A3, on the other hand, has the hallmarks of a detoxifying activity, with a pH optimum between 8 and 9 (29) and a $K_m$ (if we consider the substrate rather than substrate-Mn$^{2+}$ complex) in the millimolar to sub-millimolar range (7, 13). In contrast to the dopamine-sulfating activity, the Dopa/tyrosine-sulfating activity can be stimulated much more dramatically by Mn$^{2+}$ and, intriguingly, displays stereospecificity for the D-form Dopa/tyrosine enantiomers (13). As discussed before, our recent kinetic studies (15) suggested that Dopa and tyrosine form complexes with Mn$^{2+}$ (the pH dependence of the reaction forming the complex may explain the different pH optimum for the Dopa/tyrosine-sulfating activity compared with that for the dopamine sulfation, which does not involve such a complex) that serve either as the obligatory (in the case of tyrosine) or as a better (in the case of Dopa) substrate. The log K for the formation of the tyrosine-Mn$^{2+}$ complex is 1.5, whereas that for the tyrosine-Mn$^{2+}$-tyrosine complex is 5.0 (30, 31). Mn$^{2+}$ coordinates not only with...
Dopa/Tyrosine Sulfation by Human SULT1A3

Fig. 1. A, amino acid sequence comparison of SULT1A3 and SULT1A1. Identical amino acid residues are boxed. B, schematic representation of the molecular architecture of wild-type SULT1A3 (labeled MMMM), SULT1A1 (labeled PPPP), and chimeras.

The carboxyl group of the substrates (to form the complexes) but also with amino acid residues on the enzyme (on formation of the enzyme-substrate complex). Our previous studies using chimeric and point-mutated SULT1A3/SULT1A1s had revealed that two variable regions (cf. Fig. 1A), and in particular residues 86, 89, and 146 therein, are important in determining the distinct substrate specificity of these two otherwise highly homologous enzymes. The current study aimed to elucidate further the structural determinants for the unique stereoselectivity and Mn$^{2+}$ dependence of the Dopa/tyrosine-sulfating activity of SULT1A3.

Differential Roles of Variable Regions I and II of SULT1A3 in the Stereospecific Dopa/Tyrosine Sulfation and Its Stimulation by Mn$^{2+}$—This was studied by testing wild-type SULT1A3 and SULT1A1 and chimeric SULT1A3/SULT1A1s (cf. Fig. 1B) for their sulfation activities toward the Dopa and tyrosine enantiomers, in the presence or absence of Mn$^{2+}$. Our previous kinetic studies (15) had confirmed that Mn$^{2+}$ could stimulate dramatically the sulfation activity of wild-type SULT1A3 toward the tyrosine enantiomers. Mn$^{2+}$, when added at a 5 mM concentration to the assay mixture, stimulated the sulfation activity of SULT1A3 toward D-tyrosine by two orders of magnitude in comparison with the basal activity determined without Mn$^{2+}$, whereas the activity toward L-tyrosine was stimulated about ten times (15). The same assay conditions were used to evaluate the stimulatory effect of Mn$^{2+}$ on the sulfation activities of wild-type SULT1A3 and SULT1A1 and their various chimeras. The activity data obtained (Table I) confirmed that the Mn$^{2+}$-stimulated activity with D-tyrosine as substrate for wild-type SULT1A3 was about 135 times the basal activity. When L-tyrosine was used as substrate, the stimulation was 14-fold. Therefore, as in our previous findings, wild-type SULT1A3 (designated MMMM for comparison with the chimeras; cf. Fig. 1B) showed a distinct preference for the D-form enantiomer of tyrosine relative to the L-enantiomer. Wild-type SULT1A1 (PPPP), on the other hand, showed no sulfation activity toward either tyrosine enantiomer, with or without Mn$^{2+}$. The data in Table I were analyzed to pinpoint which of the four regions (the two flanking regions and the middle variable Regions I and II) in the SULT1A3 molecule are required, respectively, for the basal tyrosine-sulfating activity, the stereoselectivity, and the Mn$^{2+}$ stimulation. Interestingly, the PMMP chimera displayed essentially the same level of basal activity, extent of stimulation by Mn$^{2+}$, and stereoselectivity as wild-type SULT1A3 (MMMM), whereas wild-type SULT1A1 (PPPP) showed none of these effects. These results indicated that the variable Regions I and II of the SULT1A3 can fully account for the tyrosine-sulfating activity and Mn$^{2+}$ stimulation effect. Data on the chimeras with the variable Region II derived from SULT1A1 (MPPM, MMPM, and PMPP) showed that they were all incapable of catalyzing the sulfation of tyrosine enantiomers, indicating an absolute requirement for the variable Region II of the SULT1A3 molecule for the basal tyrosine-sulfating activity. In contrast, the chimeras MPPM and PPMP showed a small basal activity with D-tyrosine as substrate but without any Mn$^{2+}$ stimulation effect. With L-tyrosine as substrate, however, no activity was detected with either of these two chimeras. These latter results suggested indirectly that the variable Region I of the SULT1A3 molecule, although not absolutely required for, may contribute substantially to its D-tyrosine sulfation activity. Moreover, the data indicated that this variable Region I is essential for the Mn$^{2+}$ stimulation effect with D-tyrosine as substrate.

A limitation in the use of tyrosine as substrate is that the basal activities detected for the wild-type and chimeric SULTs were all quite low. Although the above-mentioned data indicated that the SULT1A3 Region I is required for the Mn$^{2+}$ stimulation effect, it was difficult to determine whether it is truly required for the stereoselectivity of SULT1A3 for the D-form tyrosine enantiomer. To better address this issue, Dopa enantiomers were used as substrates. Our previous kinetic studies (15) had demonstrated that wild-type SULT1A3 displayed significantly higher basal sulfation activities toward Dopa. Although much less dramatic compared with the sulfation of tyrosine, a 2- to 3-fold stimulation (at the Dopa concentrations used) of the Dopa-sulfating activity was observed in the presence of Mn$^{2+}$. Therefore, using Dopa as substrate may prove to be more useful in investigating the structural requirements for the stereoselectivity and Mn$^{2+}$ stimulation effect. The sulfation activities of wild-type SULT1A3 and SULT1A1 and the chimeras toward D- or L-Dopa, in the presence or absence of 2.5 mM Mn$^{2+}$, were determined. The results obtained are compiled in Table II. Wild-type SULT1A3 showed a distinct preference for d-Dopa. The Mn$^{2+}$-stimulated activity of wild-type SULT1A3 with D-Dopa as substrate was 1.8 times the basal activity, whereas with L-Dopa it was almost 2-fold. The data obtained with the chimeras basically reinforce the conclusions reached using tyrosine as substrate. Moreover, the chimeras with the variable Region I derived from SULT1A1 and Region II from SULT1A3 (i.e., PPMP and MMPM), while exhibiting substantial basal sulfation activities toward both D- and L-Dopa, showed a preference for the D-enantiomer, especially with the PPMP chimera where the activities were higher. There appeared to be no significant Mn$^{2+}$ stimulation effects with these chimeras. Because the absence of SULT1A3 variable Region I abolished the Mn$^{2+}$ stimulation effect it follows that this Region I is required for the Mn$^{2+}$ stimulation effect. The stereoselectivity for the D-enantiomer, however, is present regardless of whether Region I is from the SULT1A3 or SULT1A1. The variable Region II of SULT1A3 may be required for its stereoselectivity for the D-enantiomers. Moreover, the variable Region II of SULT1A3 is absolutely required for the basal Dopa/tyrosine-sulfating activity, whereas this variable
Table I
Specific activities of the wild-type and chimeric SULT1A3/SULT1A1s with D- or L-tyrosine in the presence or absence of Mn

The values are the calculated mean ± S.D. from three determinations. The concentration of D- or L-tyrosine used was 5 mM, and the concentration of Mn

| Substrate used | MMMM | PMMP | MMMP | PPMP | PPPP | MMMP | PMMP | PPPP |
|----------------|------|------|------|------|------|------|------|------|
| D-p-Tyrosine + Mn

| 602 ± 63 | 619 ± 73 | ND | ND | ND | ND | ND | ND |
|----------|----------|----|----|----|----|----|----|
| D-p-Tyrosine | 4.5 ± 0.5 | 6.2 ± 0.6 | 0.6 ± 0.1 | 1.1 ± 0.1 | ND | ND | ND | ND |
| L-p-Tyrosine + Mn

| 14.5 ± 0.7 | 15.7 ± 0.4 | ND | ND | ND | ND | ND | ND |
| L-p-Tyrosine | 1.0 ± 0.1 | 1.2 ± 0.1 | ND | ND | ND | ND | ND | ND |

Table II
Specific activities of the wild-type and chimeric SULT1A3/SULT1A1s with D- or L-Dopa in the presence or absence of Mn

The values shown represents means ± S.D. derived from three determinations. The concentration of D- or L-Dopa used was 1 mM, and the concentration of Mn

| Substrate used | MMMM | PMMP | MMMP | PPMP | PPPP | MMMP | PMMP | PPPP |
|----------------|------|------|------|------|------|------|------|------|
| D-Dopa + Mn

| 1545 ± 3 | 1649 ± 66 | 8.0 ± 0.4 | 52.7 ± 4.4 | ND | ND | ND | ND |
| D-Dopa | 845 ± 43 | 871 ± 46 | 7.6 ± 3.8 | 42.8 ± 4.9 | ND | ND | ND | ND |
| L-Dopa + Mn

| 49.9 ± 5.4 | 57.4 ± 4.8 | 1.7 ± 0.3 | 6.8 ± 1.1 | ND | ND | ND | ND |
| L-Dopa | 25.9 ± 1.3 | 25.0 ± 0.4 | 2.5 ± 0.3 | 7.8 ± 0.1 | ND | ND | ND | ND |

Region I, although not absolutely required, substantially enhances this sulfation activity.

Identification of Specific Amino Acid Residues Required for the Mn

Stimulation of the Sulfation of Tyrosine and Dopa Enantiomers—To investigate further the structural determinants for the Mn

stimulation of the sulfation of tyrosine and Dopa enantiomers, point-mutated SULT1A3s, targeted at specific amino acid residues within the variable Regions I and II, were tested. By employing the site-directed mutagenesis technique, we obtained expressed, and purified nine point-mutated SULT1A3s (five single mutants, three double mutants, and a triple mutant) targeted at amino acid residues in the two variable regions (8). One other single mutant involved a conserved residue Lys-48 that most likely participates in catalysis (8). The sulfation activities of these point-mutated SULT1A3s toward D- or L-tyrosine, in the presence or absence of Mn

Fig. 2 shows a bar graph plotted based on the results obtained in experiments in which the Mn

stimulated D-tyrosine-sulfating activities of wild-type and mutant SULT1A3s were compared with their basal activities. In the absence of Mn

Though the extent of stimulation by Mn

was considerably lower in all cases. One exception, however, is that the E146A mutant exhibited a considerable stimulation by Mn

of its sulfation activity toward L-tyrosine, albeit at lower activity levels. This was in fact also the case in its sulfation activity toward D-tyrosine, which showed a four times stimulation by Mn

(this Mn

stimulation effect in Fig. 2, however, is overshadowed by the much more dramatic Mn

stimulation effects observed with wild-type SULT1A3 and N85K, E89I, H143Y, and N85K/E89I mutant clones). These latter results therefore indicate that the Glu-146 residue is not pivotal for the Mn

stimulation of the sulfation of the tyrosine enantiomers by SULT1A3.

To summarize, the data in Figs. 2 and 3 appear to rule out the requirement for residues Asn-85, Glu-89, Glu-146, and His-143 in the Mn

stimulation of the tyrosine-sulfating activity of SULT1A3. The role of residue Asp-86 is not as clear. Whether the lowered sulfation activities were the result of a drastic loss of the basal tyrosine-sulfating activity (as appeared to be the case with the E146A mutant) or a loss of the Mn

stimulation effect or both was not clear. To resolve this
issue, we carried out assays using the same set of wild-type and chimeric SULT1A3/SULT1A1s (cf. Fig. 1) with D- and L-Dopa as substrates. As revealed in Table II, wild-type SULT1A3 showed a much higher basal activity toward Dopa, as well as a clearly detectable stimulation by Mn$^{2+}$/H11001 (though not as dramatic as in the case of tyrosine). Therefore, using D- and L-Dopa as substrates, it may be possible to uncouple the Dopa/tyrosine-sulfating activity from its stimulation by Mn$^{2+}$.

Fig. 4 shows a bar graph plotted based on the results obtained in experiments in which the Mn$^{2+}$/H11001-stimulated D-Dopa-sulfating activities of wild-type and mutant SULT1A3s were compared with their basal activities. In contrast to the case with tyrosine as substrate, considerably higher basal activities for wild-type and mutant SULT1A3s were observed even in the absence of Mn$^{2+}$. It is evident from the figure that the Mn$^{2+}$-stimulated D-Dopa-sulfating activity associated with the wild-type enzyme was essentially retained in the N85K, E89I, and H143Y mutants, as well as in the N85K/E89I double mutant. It is also clear that for the D86A mutant and the double (N85K/D86A and D86A/E89I) and triple (D86A/E89I/E146A) mutants involving D86A mutation, there was no longer noticeable Mn$^{2+}$ stimulation of the basal activities toward D-Dopa. Besides reinforcing our earlier conclusion with regard to residues Asn-85, Glu-89, His-143, and Glu-146 not being required for the Mn$^{2+}$ stimulation effect, these results unequivocally confirm the primary requirement for residue Asp-86 in the Mn$^{2+}$ stimulation of the Dopa/tyrosine-sulfating activity of SULT1A3. Because residue Asp-86 is present within the variable Region I, the above-mentioned results are in line with the earlier conclusion that the presence of the variable Region I from SULT1A3 is required for the Mn$^{2+}$ stimulation of the Dopa/tyrosine-sulfating activity (cf. above-mentioned studies using the chimeras).

As a negatively charged residue, Asp-86 is inherently capable of mediating the binding of Mn$^{2+}$ to SULT1A3. Identification of Specific Amino Acid Residues Required for the Stereospecificity of the Sulfation of Tyrosine and Dopa Enantiomers—Fig. 5 shows a bar graph plotted based on the data in which the D-tyrosine-sulfating activities of wild-type and point-mutated SULT1A3s toward 5 mM L-tyrosine, whereas the black bars represent the activity toward the D-enantiomer (both in the presence of 5 mM Mn$^{2+}$). The activities are expressed in nmol of sulfated product produced/min/mg enzyme.
toward 1 mM L-Dopa, whereas the wild-type and point-mutated SULT1A3s represent the sulfation activity of the wild-type and point-mutated SULT1A3s toward 1 mM L-Dopa, whereas the black bars represent the activity toward the p-enantiomer (both in the presence of 2.5 mM Mn$^{2+}$). The activities are expressed in nmol of sulfated product produced/min/mg enzyme.

The above-mentioned studies have pinpointed the residue Glu-146 of SULT1A3 as being primarily responsible for the stereospecificity of SULT1A3 toward DOPA or TYROSINE. This is in accordance with our earlier conclusion from the studies using chimeras that the variable Region II is the one responsible for the stereospecificity for the D-enantiomers of tyrosine or L-Dopa. It is therefore clear that residue Glu-146 is the one that primarily directs the stereospecificity of SULT1A3 for the L-enantiomer of Dopa or tyrosine. This is in accordance with our earlier conclusion from the studies using chimeras that the SULT1A3 variable Region II is the one responsible for the stereospecificity of the Dopa/tyrosine-sulfating activity of SULT1A3.

Sulfation Activities of the Residues 84–86 and 84–90 Deletional SULT1A3 Mutants toward Dopa and Tyrosine: Stereospecificity and Stimulatory Effect of Mn$^{2+}$ — The above-mentioned studies have pinpointed the residue Glu-146 of SULT1A3 as being primarily responsible for the stereospecificity for the L-enantiomers of Dopa and Tyrosine. To further clarify these points, the activities of two deletional SULT1A3 mutants (lacking, respectively, residues 84–86 and residues 84–90) toward tyrosine (5 mM final concentration) and Dopa (1 mM final concentration) enantiomers were measured in the absence of Mn$^{2+}$. The residues 84–86 deletional mutant exhibited no detectable activities toward any of these substrates (or with dopamine). The residues 84–90 deletional mutant displayed detectable activity though this was much lower, as compared with that of the wild-type SULT1A3. Surprisingly, this deletional mutant seemed to have completely lost the stereospecificity for the L-enantiomers of Tyrosine or Dopa as is clear from the data compiled in Table III. This seems to suggest a role for the “mobile loop” (encompassing residues 84–92; see Ref. 17 and below) per se as a steric selector, in the absence of Mn$^{2+}$, for the L-enantiomers of these substrates.

Proposed Model Explaining the Stimulatory Effect of Mn$^{2+}$ and the Stereoselectivity for L-Enantiomers of Dopa/Tyrosine—SULT1A3 is known to be present as a homodimer in its native state (14). Previous x-ray crystallography study (17) had shown that, in the crystal structure, residues 84–92 corresponding to the variable Region I (cf. Fig. 1A) from one monomer form a mobile loop that intercalates into the active site of the other monomer and may block the proper positioning of certain acceptor substrates. In addition to this mobile loop, the active site is also guarded by key residues (particularly Glu-146) (10, 11) from the variable Region II of the same monomer. This model had been used to explain our previous kinetic studies (15) by suggesting that the mobile loop might not hinder the positioning of the physiological substrate, dopamine, whose positively charged amino group may be stabilized by interaction with the negatively charged Glu-146 residue (8, 10, 11). It was proposed that the variable Region I loop may, however, act to prevent the
positioning of xenobiotic substrates (represented by D-Dopa or D-tyrosine) that carry, for example, an extra carboxyl group, as compared with dopamine. In this model, Mn$^{2+}$ exerts its stimulatory effect by complexing with the negatively charged carboxyl group of Dopa or tyrosine and forming a bridge with a residue in the loop. This would peg back the loop, thus allowing the proper positioning of these substrates.

The essential features of this model could also be used to explain the results obtained in the current study. However, one important modification has to be made. The previously reported x-ray structure of SULT1A3 (17, 32) demonstrated that the interaction between the subunits of the homodimer, in the crystal structure, takes place at the surface near the substrate binding sites of the subunits, which is consistent with the Region I loop from one monomer intercalating into the substrate binding site of the other monomer. This idea seemed to be supported by buried surface area calculations and data on the x-ray structure of other SULT enzymes (33). However, more recent work using cross-linking and limited proteolysis, in conjunction with mass spectrometry (34), suggested that the physiological dimer interface in solution (as opposed to the crystal dimer interface) may be near the C-terminal region of the subunit, away from the surface of the substrate binding site. Site-directed mutagenesis, along with gel filtration data, pinpointed a stretch of 10 amino acid residues near the C-site.

Recent studies on the extent of inhibition of the activity of SULT1A3 and its mutants by 2,6-dichloro-4-nitrophenol (DCNP).

Our model suggests that SULT1A3, under normal circumstances, acts only on its physiological substrate dopamine, thereby regulating its levels. This makes sense from the viewpoint of cellular economy, because sulfation is an energetically expensive process that uses PAPS, the synthesis of one molecule of which requires the expenditure of three high energy phosphate bonds of ATP (3). Under conditions of oxidative stress (which may result in the release of Mn$^{2+}$ from mitochondria into the cytosol), however, the constraining loop in the active site may be pinned back by a substrate-Mn$^{2+}$ complex, allowing the proper positioning, and therefore the sulfation, of non-physiological xenobiotic molecules present at higher levels (15). It is possible that other candidate molecules serving as substrates in this way may form complexes with Mn$^{2+}$ with much higher log K values.

The physiological significance, if any, of the Mn$^{2+}$ stimulation of the Dopa/tyrosine-sulfating activity of SULT1A3, and the specificity for the d-enantiomers of these substrates, which has also been demonstrated in cell culture and in cell-free extracts (13, 29), is somewhat speculative and has been considered in a previous report (15). From a more practical perspective, however, the stimulation of the detoxifying activity of SULT1A3 by Mn$^{2+}$ and its stereoselective action may have implications for the detoxifying activity of the SULTs toward chiral drugs (18–21). We are currently investigating the stereoselective action of SULT1A3 and its mutants on some chiral dopamine analogs widely used as drugs. Besides throwing further light on the structure-function relationships we have considered here, the principles emerging may enable some engineering of the protein with regard to its stereospecific action.

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