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Sulfation of Catecholamines and Serotonin by SULT1A3 Allozymes

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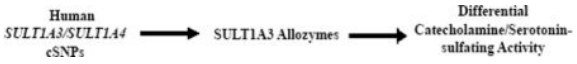
Abstract

Previous studies have demonstrated the involvement of sulfoconjugation in the metabolism of catecholamines and serotonin. The current study aimed to clarify the effects of single nucleotide polymorphisms (SNPs) of human *SULT1A3* and *SULT1A4* genes on the enzymatic characteristics of the sulfation of dopamine, epinephrine, norepinephrine and serotonin by SULT1A3 allozymes. Following a comprehensive search of different *SULT1A3* and *SULT1A4* genotypes, twelve non-synonymous (missense) coding SNPs (cSNPs) of *SULT1A3/SULT1A4* were identified. cDNAs encoding the corresponding SULT1A3 allozymes, packaged in pGEX-2T vector were generated by site-directed mutagenesis. SULT1A3 allozymes were expressed, and purified. Purified SULT1A3 allozymes exhibited differential sulfating activity toward catecholamines and serotonin. Kinetic analyses demonstrated differences in both substrate affinity and catalytic efficiency of the SULT1A3 allozymes. Collectively, these findings provide useful information relevant to the differential metabolism of dopamine, epinephrine, norepinephrine and serotonin through sulfoconjugation in individuals having different *SULT1A3/SULT1A4* genotypes.

Graphical abstract



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Keywords

Single nucleotide polymorphisms; cytosolic sulfotransferase; SULT1A3; sulfation; catecholamines; serotonin

1. Introduction

Catecholamines, including dopamine (DA), epinephrine (EP) and norepinephrine (NE), and serotonin (5-hydroxytryptamine; 5-HT) constitute an important group of monoamine neurotransmitters/hormones that play key roles in the regulation of physiological processes such as mood, body temperature, heart rate, blood pressure, gastrointestinal motility and secretions, as well as the development of various neurological, psychiatric, endocrine, and cardiovascular diseases [1–4]. They are synthesized centrally and peripherally in, for example, the adrenal glands, gastrointestinal tract, kidneys, pancreas, and immune system [5–12]. For 5-HT, its sulfated derivative, 5-HT sulfate, has also been detected in blood and cerebrospinal fluids [13,14]. Studies have shown that more than 98% of DA and approximately 80% of total EP and NE are present in the sulfated form in circulation [15,16]. Thus, sulfation has been proposed to play important roles in the regulation and biotransformation of catecholamines as well as 5-HT [17–19].

In mammals, sulfation as catalyzed by the cytosolic sulfotransferases (SULTs) has been shown to be involved in the metabolism and elimination of xenobiotics as well as the homeostasis of key endogenous compounds such as catecholamines, 5-HT, steroid/thyroid hormones, cholesterol, and bile acids [20–22]. The SULTs mediate the transfer of a sulfonate group from the donor co-substrate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to the hydroxyl or amino group of an acceptor substrate compound [23]. While there are occasionally exceptions, sulfate-conjugated compounds generally become inactive and more hydrophilic and thus can be eliminated more easily from the body [21,22]. Of the 13 human SULTs, SULT1A3 has been identified as the main enzyme responsible for sulfating catecholamines and 5-HT [24,25]. It is noted that SULT1A3 is found only in humans and closely related primates [26]. Interestingly, genomic studies have revealed the duplication of the gene coding for SULT1A3 during the evolutionary process [27,28]. Two genes, designated *SULT1A3* and *SULT1A4*, located on chromosome 16 have been identified and shown to encode identical protein products, collectively called SULT1A3 [28,29]. Importantly, ethnic-specific inherited alterations in catecholamine sulfation in humans have been reported [30]. An intriguing question is whether the genetic polymorphisms of *SULT1A3* and *SULT1A4* may affect the enzymatic activity of the resulting SULT1A3 protein product and thus the homeostasis of catecholamines and 5-HT and possibly pathophysiological conditions associated with these latter compounds.

The current study was based on the hypothesis that nonsynonymous missense single nucleotide polymorphisms (SNPs) of *SULT1A3* and *SULT1A4* genes could lead to SULT1A3 allozymes with differential sulfating activities toward DA, EP, NE and 5-HT. We report in this paper a systematic database search for human *SULT1A3* and *SULT1A4* SNPs. Twelve missense SNPs were identified and the cDNAs and the corresponding SULT1A3

allozymes were generated, expressed, and purified. Purified SULT1A3 allozymes were analyzed for their enzymatic characteristics toward catecholamines and 5-HT.

2. Materials and methods

2.1. Materials

Adenosine 5′-triphosphate (ATP), dithiothreitol (DTT), dimethyl sulfoxide (DMSO), N-2-hydroxylpiperazine-N′-2-ethanesulfonic acid (HEPES), DA, EP and 5-HT were products of Sigma Chemical Company (St. Louis, MO, USA). NE was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Cellulose thin-layer chromatography (TLC) plates were from EMD Millipore Corporation (Burlington, MA, USA). 3′-Phosphoadenosine-5′-phospho[^{35}S]sulfate (PAP[^{35}S]) was prepared using ATP and carrier-free [^{35}S]sulfate based on a previously established protocol [31]. Oligonucleotide primers were synthesized by Eurofins Genomics (Louisville, KY, USA). X-Ray films were products of Research Products International Corporation (Mt Prospect, IL, USA). Prime STAR GXL DNA Polymerase was purchased from Clontech Laboratories, Inc. (Mountain View, CA, USA). PCR kit was from G Biosciences (St. Louis, MO, USA). Protein molecular weight markers were from Bioland Scientific LLC (Paramount, CA, USA). QIAprep Spin Miniprep Kit was a product of QIAGEN (Germantown, MD, USA). Ecolume scintillation cocktail was from MP Biomedical LLC (Irvine, CA, USA). Glutathione Sepharose was purchased from GE Healthcare Life Sciences (Pittsburgh, PA, USA). All other chemicals were of the highest grades commercially available.

2.2. Database search

A comprehensive search was performed for *SULT1A3* and *SULT1A4* SNP clones deposited in two databases located at, respectively, the U.S. National Center for Biotechnology Information (NCBI) and the UniProt Knowledgebase (UniProtKB). Moreover, the NCBI PubMed was searched for previous studies (for example, Thomae et al. 2003 [30] and Hildebrandt et al. 2004 [28]) describing SNPs of these two genes.

2.3. Generation, expression, and purification of SULT1A3 allozymes

Site-directed mutagenesis was performed to generate cDNAs encoding SULT1A3 allozymes packaged in pGEX-2TK vector. Briefly, mutagenic primers (cf. Table 1) corresponding to specific *SULT1A3/SULT1A4* missense cSNPs were designed and synthesized. Wild-type SULT1A3 cDNA packaged in pGEX-2TK prokaryotic vector was used as the template in conjunction with specific mutagenic primers to amplify mutated SULT1A3 cDNAs coding for different SULT1A3 allozymes. The sequences of the “mutated” cDNAs were verified by nucleotide sequencing [32]. The pGEX-2TK vector harboring individual mutated SULT1A3 cDNA was transformed into BL21 *E. coli* cells. After induction of recombinant SULT1A3 expression by IPTG, affinity chromatography using glutathione-Sepharose was performed, followed by thrombin digestion to release purified recombinant SULT1A3 allozyme. The purity of purified recombinant allozymes was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [33,34]. The concentrations of these allozymes were measured based on Bradford protein assay [35].

2.4. Enzymatic assay

To analyze the sulfating activity of SULT1A3 allozymes toward DA, EP, NE and 5-HT, an established assay protocol was employed [36]. Two different concentrations were tested for each of the four substrates. Radioactive PAP[^{35}S] was used as the sulfate donor. The assays were performed at pH 7.4 and allowed to proceed at 37°C for 10 min, followed by TLC separation of the reaction mixtures. Upon completion of TLC, autoradiography was performed using an X-ray film to locate the position of sulfated product. The radioactive sulfated product spot was then cut out and the sulfated product therein eluted for the quantitative measurement of [^{35}S]-radioactivity using a liquid scintillation counter. Specific activity of the SULT1A3 allozymes was calculated based on the [^{35}S]-radioactivity determined. In kinetic experiments, different substrate concentrations, 0, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 75 and 100 μM for DA and EP, 0, 0.001, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 150 and 200 μM for NE, and 1, 10, 50, 100, 125, 250, 500, 750 and 1000 μM for 5-HT.

2.5. Statistical analysis

Data obtained from the kinetic experiments were analyzed based on the non-linear regression of the Michaelis-Menten equation to calculate the kinetic constants. GraphPad Prism 7 software was used in data analysis.

3. Results

3.1. Analysis of human *SULT1A3* and *SULT1A4* single nucleotide polymorphisms

SNPs of *SULT1A3* and *SULT1A4* genes, as identified in above-mentioned database search, were categorized according to the location of variations in each of the two genes. For the *SULT1A3* gene, a total of 38 SNPs, including 2 in the 3′-untranslated region (3′-UTR), 19 in the intron regions, 6 synonymous cSNPs, one nonsense cSNP, and 10 missense cSNPs, were identified. For the *SULT1A4* gene, a total of 39 SNPs, including 1 in the 3′-UTR, 25 in the intron regions, 7 synonymous cSNPs, and 6 missense cSNPs, were identified. Of the 16 missense cSNPs found for both *SULT1A3* and *SULT1A4* genes, 13 distinct amino acid alterations were found. The designated names and their SNP ID number for these 13 SNPs are: SULT1A3-T7P (Reference SNP (rs)776817009/ rs754600221), SULT1A3-S8P (rs767263838), SULT1A3-R9C (rs762151655/ rs752303630), SULT1A3-P10L (rs757573592), SULT1A3-V15M (rs750575779/ rs758881470), SULT1A3-V18F (rs553050853), SULT1A3-P19L (rs747088850), SULT1A3-P101L (rs751527244), SULT1A3-P101H, SULT1A3-R144C and SULT1A3-K234N [28], SULT1A3-N235T (UniProt P0DMM9) and SULT1A3-S290T (UniProt P0DMM9). Figure 1 illustrates the locations of amino acid variations associated with above-mentioned cSNPs, together with previously reported sequences/residues involved in PAPS-binding, substrate-binding, and/or catalysis. Examination of the reported crystal structures of SULT1A3 [37] revealed that these amino acid residues are positioned close to the surface of the molecule (Figure 2). Interestingly, many of them are associated with the three important loops/segments, Asp66-Met77, Ser228-Gly259, and Lys85-Pro90, which have been proposed to be involved in the formation of a gate that governs the substrate selectivity [38] (cf. Figure 2).

3.2. Expression and purification of recombinant human SULT1A3 allozymes

cDNAs corresponding to the twelve chosen SULT1A3 missense genotypes packaged in pGEX-2TK prokaryotic expression vector were individually transformed into BL21 *E. coli* host cells for the expression of SULT1A3 allozymes. To purify the SULT1A3 allozymes, glutathione-Sepharose was used to fractionate the GST-SULT1A3 proteins from the transformed *E. coli* cell homogenates, followed by thrombin digestion to release SULT1A3 allozymes. Purified SULT1A3 allozymes appeared to be highly homogeneous as judged by SDS-PAGE. As shown in Figure 3, the apparent molecular weights of SULT1A3 allozymes were similar to the predicted molecular weight (34,196) of wild-type SULT1A3.

3.3. Enzymatic characterization of the SULT1A3 allozymes

Purified wild-type and SULT1A3 allozymes were characterized with regard to their sulfating activity with catecholamines and 5-HT as substrates. In the initial experiments, the specific activity of wild-type and SULT1A3 allozymes was determined using two different concentrations, one considerably below and the other close to the reported K_m [6.46 ± 0.59, 9.16 ± 1.81, 10.65 ± 1.14 and 71.38 ± 7.99 μM], of each of the four substrates (DA, EP, NE and 5-HT). Results obtained are shown in Figures 4–7.

With DA as the substrate—At low substrate (un-saturating) concentration (0.5 μM), SULT1A3-S8P showed a higher specific activity than the wild-type enzyme, while seven other SULT1A3 allozymes (SULT1A3-T7P to SULT1A3-P101L) displayed comparable specific activities with the wild-type (Figure 4A). In contrast, the specific activities determined for the other five allozymes (SULT1A3-P101H, SULT1A3-R144C, SULT1A3-K234N, SULT1A3-N235T and SULT1A3-S290T) were considerably lower than that of the wild-type enzyme. Among these five latter SULT1A3 allozymes, SULT1A3-N235T exhibited much lower activity (16.4 % of that of the wild-type enzyme) than the other four. At higher substrate concentration (5 μM), eight SULT1A3 allozymes showed comparable or slightly higher specific activities, compared with the wild-type enzyme, whereas four allozymes (SULT1A3-P101H, SULT1A3-R144C, SULT1A3-K234N and SULT1A3-N235T) showed lower DA-sulfating activity than the wild-type enzyme, with SULT1A3-N235T showing the lowest specific activity (Figure 4B).

With EP as the substrate—At low substrate (un-saturating) concentration (1 μM), eight SULT1A3 allozymes displayed higher specific activities than the wild-type enzyme, with SULT1A3-P10L and SULT1A3-P101L showing the highest specific activities (Figure 5A). The other four SULT1A3 allozymes showed lower specific activities than the wild-type, with SULT1A3-N235T displaying the lowest specific activity. At higher substrate concentration (10 μM), all SULT1A3 allozymes showed specific activities that were close to that of the wild-type enzyme except SULT1A3-N235T which displayed a specific activity nearly 10 times lower than the wild-type (Figure 5B).

With NE as the substrate—At low substrate (un-saturating) concentration (1 μM), all SULT1A3 allozymes exhibited lower NE-sulfating activities than the wild-type enzyme (Figure 6A). Among them, SULT1A3-R9C and SULT1A3-N235T showed much lower activities (~8 and 10 times, respectively) than the other ten SULT1A3 allozymes. At higher

substrate concentration (10 μM), all SULT1A3 allozymes displayed similarly lower specific activities than the wild-type enzyme, with SULT1A3-R9C and SULT1A3-N235T again showing much lower specific activities than the rest (Figure 6B).

With 5-HT as the substrate—At low substrate (un-saturating) concentration (10 μM), SULT1A3-S8P displayed a slightly higher activity than the wild-type enzyme, while the other eleven SULT1A3 allozymes all showed lower specific activities than the wild-type enzyme, with SULT1A3-R9C, SULT1A3-R144C and SULT1A3-N235T showing the lowest 5-HT-sulfating activity than the rest (Figure 7A). At higher substrate concentration (100 μM), four SULT1A3 allozymes, SULT1A3-T7P, SULT1A3-S8P, SULT1A3-V15M and SULT1A3-P101H, displayed specific activities comparable to that of the wild-enzyme. The other eight SULT1A3 allozymes showed considerably lower specific activities than the wild-type enzyme, with SULT1A3-R9C, SULT1A3-R144C and SULT1A3-N235T again displaying much lower specific activities than the other five allozymes (Figure 7B).

3.4. Kinetic Analyses

To examine further the impact of the amino acid variations on the catecholamine/5-HT-sulfating activity of the SULT1A3 allozymes, kinetic studies were performed. Assays were carried out using varying concentrations of each of the four substrates (DA, EP, NE and 5-HT) at pH 7.4. Figure 8 shows the concentration-dependent sulfation of DA, EP, NE and 5-HT, respectively, by the wild-type SULT1A3. The arrow signs indicated the concentrations at which substrate inhibition was observed. Data obtained from the kinetic experiments using the wild-type and each of the twelve SULT1A3 allozymes were processed to generate Michaelis-Menten saturation curves and Lineweaver-Burk plots using GraphPad Prism 7 software for the determination of kinetic constants (K_m, V_{max}, and V_{max}/K_m).

With DA as the substrate—As shown in Table 2, the K_m value (6.46 ± 0.59 μM) determined for the wild-type SULT1A3 was lower than those of all SULT1A3 allozymes. Of the twelve SULT1A3 allozymes, SULT1A3-P101H, SULT1A3-R144C and SULT1A3-N235T, displayed K_m values (12.72 ± 3.29, 13.26 ± 2.99 and 12.91 ± 1.29 μM, respectively) that were approximately two times that (6.46 ± 0.59 μM) of the wild-type. In terms of the V_{max}, most SULT1A3 allozymes showed comparable values to that of the wild-type enzyme, except SULT1A3-K234N. The V_{max}/K_m values, reflecting the catalytic efficiency, of all SULT1A3 allozymes were all lower than that of the wild-type enzyme. Notably, three of them, SULT1A3-P101H, SULT1A3-R144C and SULT1A3-N235T, showed calculated values (3.55, 3.38 and 3.33, respectively) that were nearly half of that (6.32) of the wild-type enzyme.

With EP as the substrate—As shown in Table 3, with small fluctuations, the K_m values determined for all SULT1A3 allozymes were comparable to that of the wild-type enzyme, except for SULT1A3-N235T which showed a K_m (200.1 ± 64.51 μM) more than twenty times that of the wild-type. A similar situation was found with the V_{max}, with all SULT1A3 allozymes showing values comparable to that of the wild-type SULT1A3, except for SULT1A3-N235T. Based on these data, the calculated V_{max}/K_m of SULT1A3-N235T (0.13) was near 30 times lower than that of the wild-type (3.85). Of the rest of the SULT1A3

allozymes, only SULT1A3-S290T showed considerable lower value of V_{max}/K_m than the wild-type.

With NE as the substrate—As shown in Table 4, all SULT1A3 allozymes showed higher K_m values than the wild-type enzyme, with SULT1A3-R9C and SULT1A3-N235T displaying much higher K_m values (9.4 and 5.0 times that of the wild-type) than the rest. The V_{max} values determined for all SULT1A3 allozymes were all lower than that of the wild-type except SULT1A3-P101L, whereas SULT1A3-N235T showed the lowest V_{max} value which was only 30% that of the wild-type enzyme. Accordingly, while all SULT1A3 allozymes showed lower V_{max}/K_m than the wild-type, SULT1A3-R9C and SULT1A3-N235T showed much lower values (~14 and 16 times lower, respectively) than the wild-type.

With 5-HT as the substrate—As shown in Table 5, all SULT1A3 allozymes showed higher K_m values than the wild-type (at 71.38 ± 7.99 μM), with SUT1A3-R9C, SULT1A3-P10L, SULT1A3-P101L, SULT1A3-R144C, SULT1A3-N235T and SULT1A3-S290T displaying much higher K_m values (216.6 ± 27.32, 102.40 ± 15.99, 213.9 ± 33.26, 281.0 ± 9.16, 8112.0 ± 866 and 165.6 ± 21.58 μM, respectively) than the rest. With respect to V_{max}, the fluctuations were smaller among SULT1A3 allozymes, except for SULT1A3-N235T which displayed a V_{max} which was three times that of the wild-type. Based on these data, six of the twelve SULT1A3 allozymes (SULT1A3-R9C, SULT1A3-P10L, SULT1A3-P101L, SULT1A3-R144C, SULT1A3-N235T and SULT1A3-S290T) showed notably lower V_{max}/K_m than the wild-type enzyme.

4. Discussion

Previous studies have demonstrated sulfoconjugation as an important pathway in the biotransformation of catecholamines and 5-HT in humans, and SULT1A3 was identified as the major enzyme responsible for the sulfation of these monoamine neurotransmitters/hormones [24,25,39,40]. The current study aimed to systematically evaluate the effects of the genetic polymorphisms on the sulfating activity of SULT1A3 allozymes. Genetic polymorphism of human *SULT1A3* was first reported in a study of a group of 232 individuals in which considerable variations in catecholamine-sulfating activity in the platelet samples prepared from these subjects were found [41]. In a later study using DNA samples from 60 African-American and 60 Caucasian-American subjects, a non-synonymous cSNP of the *SULT1A3* gene was detected [30]. In a similar study, three additional non-synonymous cSNPs of *SULT1A3* were found, and the corresponding SULT1A3 allozymes expressed were shown to exhibit varying DA-sulfating activity [28]. It therefore appears that the genetic polymorphisms may play a critical role in affecting the functional activity of SULT1A3 protein products, and such differences may possibly impact on the metabolism of catecholamine/5-TH through sulfation in individuals with different *SULT1A3* and *SULT1A4* genotypes. In the current study, we performed a systematic database search for human *SULT1A3* and *SULT1A4* SNPs. Thirteen missense cSNPs were identified. Site-directed mutagenesis was used to generate cDNAs for the expression of corresponding SULT1A3 allozymes. Twelve of the thirteen SULT1A3 allozymes were

successfully expressed and purified, with one being present in inclusion body form and could not be purified.

The twelve SULT1A3 allozymes prepared were first analyzed for their catecholamine/5-HT-sulfating activity in comparison with the wild-type enzyme. Activity data shown in Figures 4–7 revealed differential DA-, EP-, NE-, and 5-HT-sulfating activity among all twelve SULT1A3 allozymes. It was noted that the variations in DA-sulfating activities of SULT1A3 allozymes were markedly smaller than the variations in their sulfating activities toward EP, NE, and 5-HT. It should be pointed out that of the twelve SULT1A3 allozymes examined, four, SULT1A3-P101L, SULT1A3-P101H, SULT1A3-R144C and SULT1A3-K234N, had been studied previously [28,30]. While the results reported in the previous studies showed some minor differences in comparison with the results obtained in the current study, the trend of the variations in their DA-sulfating activity appeared the same. It is noted that different DA and PAPS concentrations were used in the assays performed in the previous and current studies. Moreover, purified SULT1A3 allozymes were used in the current study, as compared with transfected COS-1 cell lysates used in the previous studies [28,30].

Kinetic constants shown in Tables 2–5 revealed distinct substrate affinity (as reflected by the K_m) and catalytic activity (as reflected by the V_{max}) of different SULT1A3 allozymes in catalyzing the sulfation of DA, EP, NE, and 5-HT. It was noted that despite their considerable differences in K_m and V_{max} with each of the four substrates, variations in catalytic efficiency (as reflected by V_{max}/K_m) were found to be smaller with DA than with any of the other three compounds as substrate. With DA as substrate, variations in V_{max}/K_m were less than 47.3 % for all twelve SULT1A3 allozymes in comparison with the wild-type enzyme. With EP, NE, or 5-HT as substrate, variations in V_{max}/K_m were found to be as high as 99.96, 99.94, and 99.84 times, compared with the wild-type. For example, SULT1A3-N235T, which showed the lowest specific activities (cf. Figures 4–7) and catalytic efficiencies (Tables 2–5) among all SULT1A3 allozymes, while displaying a catalytic efficiency approximately 52.6 % that of the wild-type enzyme toward DA, exhibited much lower catalytic efficiencies (3.3 %, 6 %, and 2.5 %, respectively) toward EP, NE and 5-HT. Whether the lower degree of variations in DA-sulfating activity of SULT1A3 allozymes indicates the critical importance of the maintenance of the homeostasis of dopamine, in comparison with other monoamine compounds, remains to be clarified. As noted earlier, studies have shown that more than 98% of DA in circulation is present in sulfoconjugated form [15], and sulfoconjugation has been reported as a high capacity (not easily saturated) pathway involved in the biotransformation of DA [42,43]. Moreover, DA, among other monoamines, has been demonstrated to induce its own metabolism by SULT1A3, possibly for protecting neurons from elevated DA levels, and that abnormal SULT1A3 activity may pose as a risk factor for DA-associated neurotoxicity [44]. In relation to this latter point, it has been reported that abnormal levels of catecholamines and 5-HT and/or their conjugates correlated with certain pathological conditions, including neurodegenerative diseases [45–47] attention deficit hyperactivity disorder (ADHD) [48,49], migraine [50,51], atrial fibrillation and blood pressure changes [51], and myocardial infarction [52,53].

Several crystal structures of SULT1A3 have been reported [26,37,54]. Some of the structural elements involved in the functioning of the enzyme include a catalytically important residue

His108, the PAPS interacting regions (residues ^{45}TYPKSGTT^{52}, Arg130, Ser138, and residues ^{257}RKG^{259}), the substrate binding regions (including particularly, residues Asp86 and Glu146) which were proposed to play crucial roles in substrate specificity and sulfating activity [37], the C-terminal dimerization motif (residues Lys265-Glu274) with the sequence KXXXTVXXXE [55], as well as the N-terminal region that contains βA- and βB-sheets which were thought to be structural components important for the SULT folding [56]. Moreover, three loop segments, Asp66-Met77, Ser228-Gly259, and Lys85-Pro90, have been proposed to be involved in the formation of a gate that governs the substrate selectivity (cf. Figure 2) [38]. Of the SULT1A3 allozymes investigated, SULT1A3-T7P, SULT1A3-S8P and SULT1A3-R9C contain amino acid substitutions in the N-terminal region. It was noted that the substitutions of a polar amino acid (Thr, Ser, or Arg) with a non-polar or turn-inducing amino acid (Pro or Cys) in these there SULT1A3 allozymes resulted in lower catalytic efficiencies toward the four substrates tested (DA, EP, NE and 5-HT). From the perspective of the substrate, the differences in the chemical structures of the four monoamine compounds may also contribute to the differential catalytic efficiencies of these SULT1A3 allozymes. In the case of SULT1A3-R9C allozyme, the replacement of Arg, a positively charged amino acid, may possibly abolish H-bonding, salt-bridge, and/or van der Waals interactions [57–59]. Among the four monoamine substrates, NE and 5-HT were more highly affected by the R9C substitution, which might be related to the differences in the positions of their constituent functional groups. The substitution of a non-polar or turn-inducing amino acid (Pro or Val) with a non-polar or an aromatic amino acid (Leu, Met, or Phe) in SULT1A3-P10L, SULT1A3-V15M and SULT1A3-V18F also resulted in lower catalytic efficiencies. It appeared that Leu that replaces Pro in SULT1A3-P10L allozyme might have resulted in conformational changes that rendered the decrease in catalytic efficiency of this allozyme. Specifically, the substituted Val residue in SULT1A3-V15M and SULT1A3-V18F carries a more bulky side chain, which originally may compel the enzyme to adopt a relatively more restricted conformation. The results obtained with these two allozymes appeared to be compatible with the postulation that βA- and βB-sheets present in the N-terminal region represent important structural components of the SULT1A3 molecule [56]. It should be pointed out that the three N-terminal residues (Thr7, Ser8, and Arg9) as well as Val15, Val18, and Arg144 (as discussed below) are located near the proposed gate through which substrates must pass to enter the active site [38]. SULT1A3-P101L and SULT1A3-P101H, both with a turn-inducing proline residue replaced by either a non-polar Leu or a basic His residue, also showed differential sulfating efficiencies toward the four monoamine substrates. A previous study indicated that amino acid residues 84–104, together with residues 145–154, are involved in reshaping the substrate binding pocket, narrowing its cavity volume, and enabling the substrate binding [37]. SULT1A3-R144C showed lower catalytic efficiencies toward the four substrates tested (particularly, DA and 5-HT). Substitution of a basic Arg residue with a non-polar Cys residue is considered non-conservative, which could in part explain the impairment in the catalytic efficiency of this allozyme. Additionally, Arg144 is located within the amino acid residues 143–148 segment, which has been proposed to contribute to substrate-binding and catalysis of both human SULT1A1 and SULT1A3 [60]. Spatially, Arg144 residue is very close to the amino acid segment spanning residues 145–154, which has been proposed to be involved in the substrate binding pocket [37]. SULT1A3-K234N and SULT1A3-N235T carry amino acid

substitutions close to the C-terminal region. Both these two SULT1A3 allozymes showed lower catalytic efficiencies than the wild-type enzyme toward the four substrates tested, with SULT1A3-N235T displaying the lowest specific activity and catalytic efficiency with DA, EP, NE and 5-HT in fact among all 12 SULT1A3 allozymes analyzed. Lys234 and Asn235 residues in the wild-type enzyme are located in the α15 sheet as revealed in the SULT1A3 crystal structure. This region has been proposed to be involved indirect binding of PAPS. These two residues also may contribute to restricting the conformations that allow for substrate binding when the c-substrate (PAPS) is bound [56]. Moreover, Lys234 and Asn235 residues are associated with a segment (Loop 2; Ser228-Gly259) that constitutes the substrate access gate [38] that governs the substrate selectivity. In the case of SULT1A3-N235T, the bulky side chain of the substituting Thr may have more difficulty in fitting into the alpha-helical element as revealed in the SULT1A3 crystal structure. Previous studies have shown that a common SULT1A1 allozyme (SULT1A1-Asn235Thr) displayed a substantially higher K_m toward 4-nitrophenol [61,62]. For SULT1A3-S290T, although the substitution of Ser with Thr does not seem to be dramatic from the chemistry standpoint, it showed differential catalytic efficiencies toward monoamines, with a bigger variation with 5-HT and lower variation with DA. Whether these variations are due to its close proximity (position 290) to the conserved KTVE motif as revealed in the SULT1A3 crystal structure [26,55] remains to be clarified.

To summarize, we have generated, expressed, and purified twelve of the thirteen known human SULT1A3 allozymes. Enzymatic characterization of the purified SULT1A3 allozymes revealed differential substrate binding affinity and catalytic activity toward the four monoamines tested as substrates. These results may have implications in the differential metabolism of monoamine neurotransmitters in individuals with distinct *SULT1A3*/*SULT1A4* genotypes that code for different SULT1A3 allozymes. Moreover, pending further studies, the results obtained may provide clues to the link of particular *SULT1A3/SULT1A4* genotype s to certain neuropathological disorders associated with abnormal levels of the monoamines that are used as substrates by SULT1A3.

Acknowledgments

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Abbreviations

DA	dopamine
EP	epinephrine
NE	norepinephrine
5-HT	serotonin
PAPS	3′-phosphoadenosine-5′-phosphosulfate
SULT	cytosolic sulfotransferase
TLC	thin-layer chromatography

SNP single nucleotide polymorphism

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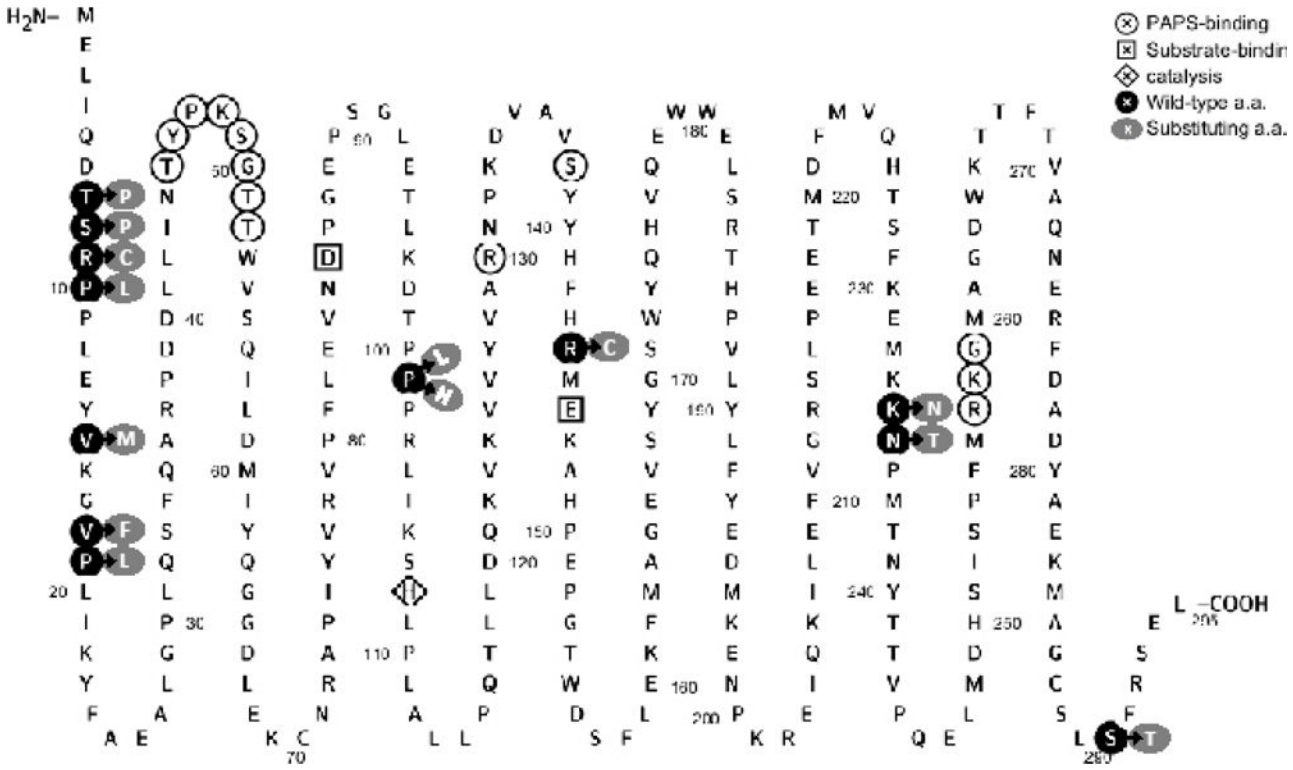

Figure 1.

Amino acid sequence of the human SULT1A3 showing the locations of amino acid residues involved in the *SULT1A3/SULT1A4* cSNPs and sequences/residues reported to be involved in PAPS-binding, substrate-binding, and/or catalysis. Residues circled with white background are involved in PAPS-binding. Residues enclosed in square are involved in substrate-binding. Residue enclosed in diamond is involved in catalysis. Residues circled with black background refer to the locations of amino acid substitutions in the polypeptide chain of the SULT1A3 molecule. Residues circled with gray background refer to the substituting amino acids. The figure was generated using Protter, a web tool for interactive protein feature visualization [63].

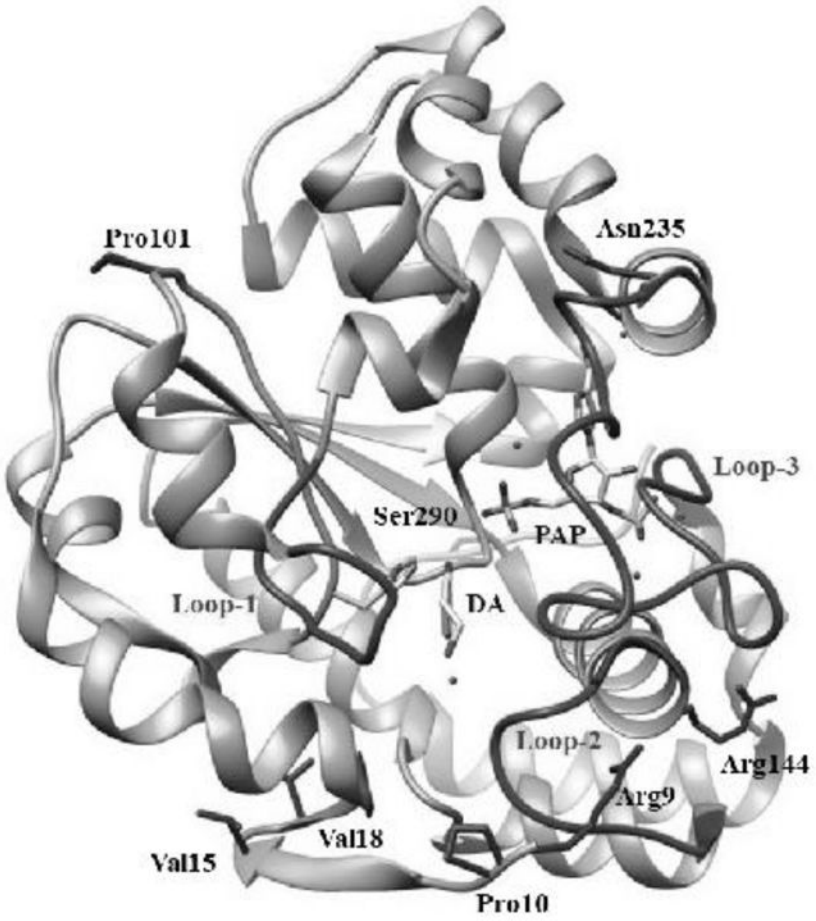

Figure 2.

Ribbon diagram of the structure of human SULT1A3-dopamine-PAP complex showing the locations of amino acid residues involved in the *SULT1A3/SULT1A4* cSNPs. The structure of SULT1A3 (Protein Data Bank code: 2A3R [37]) was edited using USCF Chimera, a molecular modeling software [64]. DA and PAP molecules in the structure are shown by bond structures. Loops 1, 2, and 3 refer to Asp66-Met77, Ser228-Gly259, and Lys85-Pro90 segments previously reported to form a gate for substrate entry. Side chains of the amino acid residues involved in the *SULT1A3/SULT1A4* cSNPs, Arg9, Pro10, Val15, Val18, Pro101, Arg144, Asn235, Ser290, are indicated by bond structures.

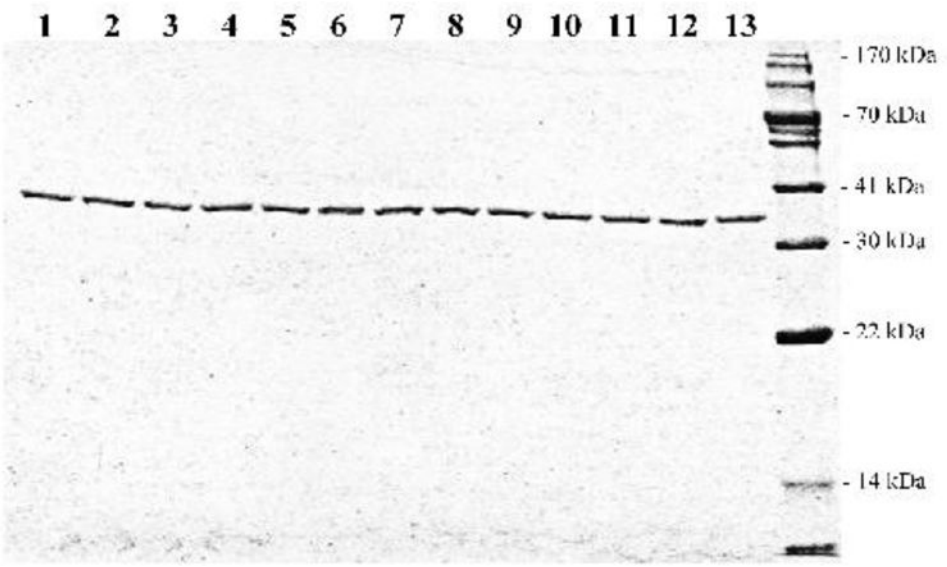

Figure 3.

SDS gel electrophoretic pattern of the purified human SULT1A3 allozymes. SDS-PAGE was performed on a 12% gel, followed by Coomassie Blue staining. Samples analyzed in lanes 1 through 13 correspond to SULT1A3-WT (wild-type), SULT1A3-T7P, SULT1A3-S8P, SULT1A3-R9C, SULT1A3-P10L, SULT1A3-V15M, SULT1A3-V18F, SULT1A3-P101L, SULT1A3-P101H, SULT1A3-R144C, SULT1A3-K234N, SULT1A3-N235T and SULT1A3-S290T. Positions of protein molecular weight markers are indicated on the right.

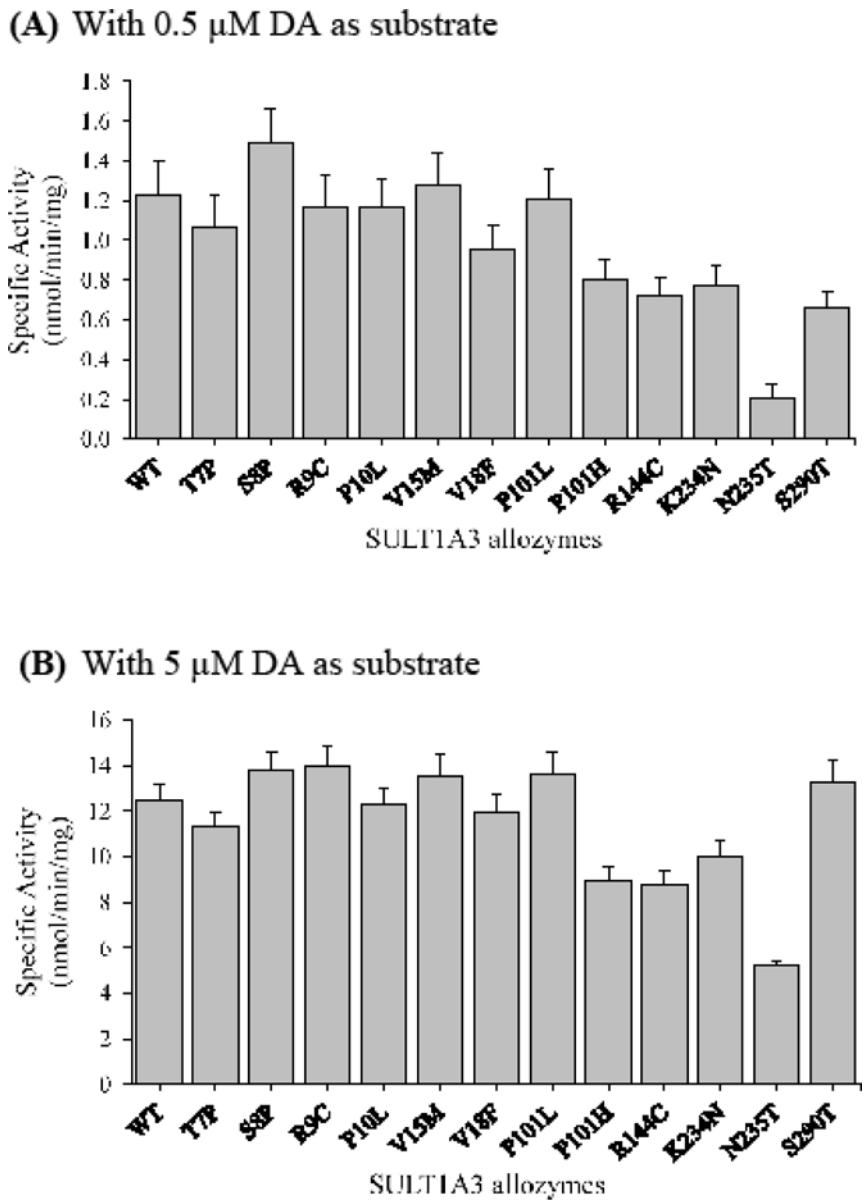

Figure 4.

Specific activities of the sulfation of dopamine (DA) by human SULT1A3 allozymes. Concentrations of dopamine used in the enzymatic assays were 0.5 μM (A) and 5.0 μM (B). Specific activity refers to nmol dopamine sulfated/min/mg of purified allozyme. Data shown represent mean ± standard deviation derived from three determinations. WT refers to wild-type SULT1A3.

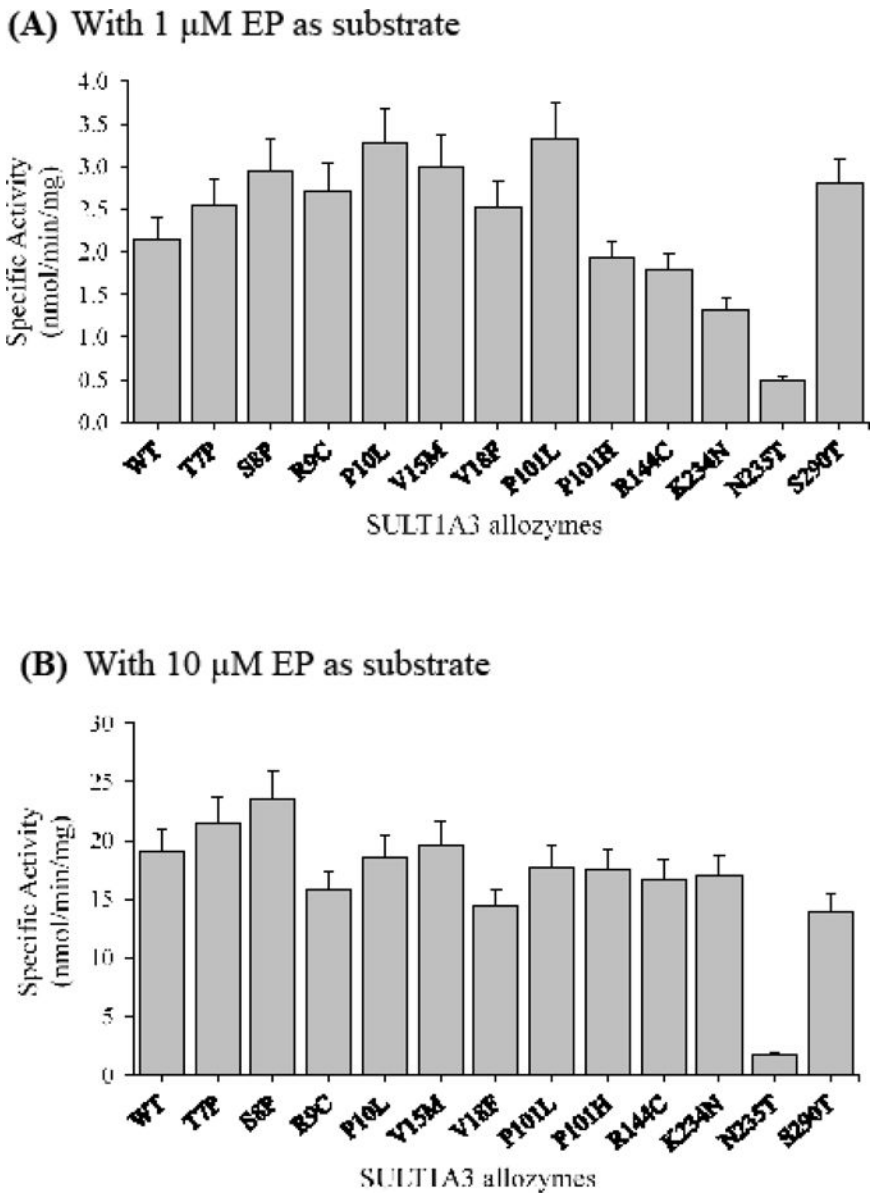

Figure 5.

Specific activities of the sulfation of epinephrine (EP) by human SULT1A3 allozymes. Concentrations of epinephrine used in the enzymatic assays were 1 μM (A) and 10 μM (B). Specific activity refers to nmol epinephrine sulfated/min/mg of purified allozyme. Data shown represent mean ± standard deviation derived from three determinations. WT refers to wild-type SULT1A3.

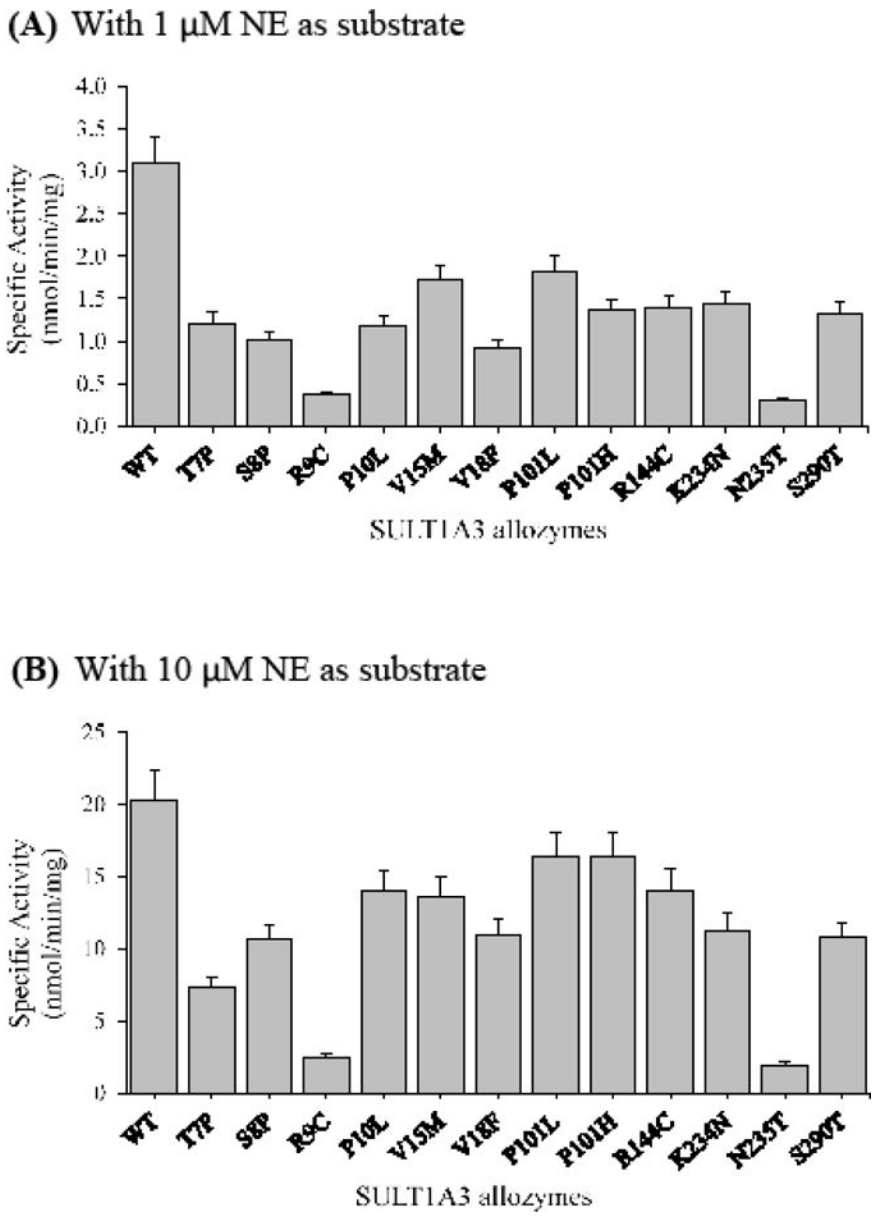

Figure 6.

Specific activities of the sulfation of norepinephrine (NE) by human SULT1A3 allozymes. Concentrations of norepinephrine used in the enzymatic assays were 1 μM (A) and 10 μM (B). Specific activity refers to nmol norepinephrine sulfated/min/mg of purified allozyme. Data shown represent mean ± standard deviation derived from three determinations. WT refers to wild-type SULT1A3.

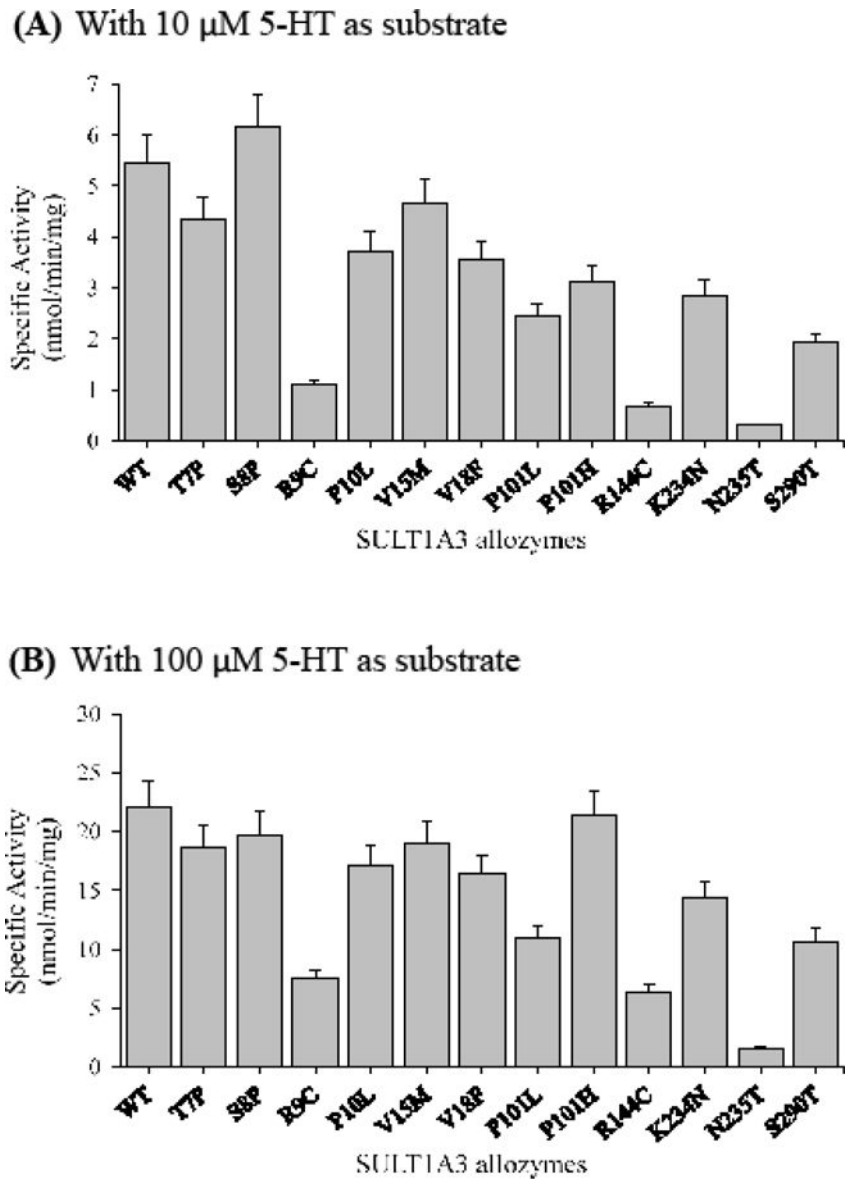

Figure 7.

Specific activities of the sulfation of serotonin (5-HT) by human SULT1A3 allozymes. Concentrations of serotonin used in the enzymatic assays were 10 μM (A) and 100 μM (B). Specific activity refers to nmol serotonin sulfated/min/mg of purified allozyme. Data shown represent mean ± standard deviation derived from three determinations. WT refers to wild-type SULT1A3.

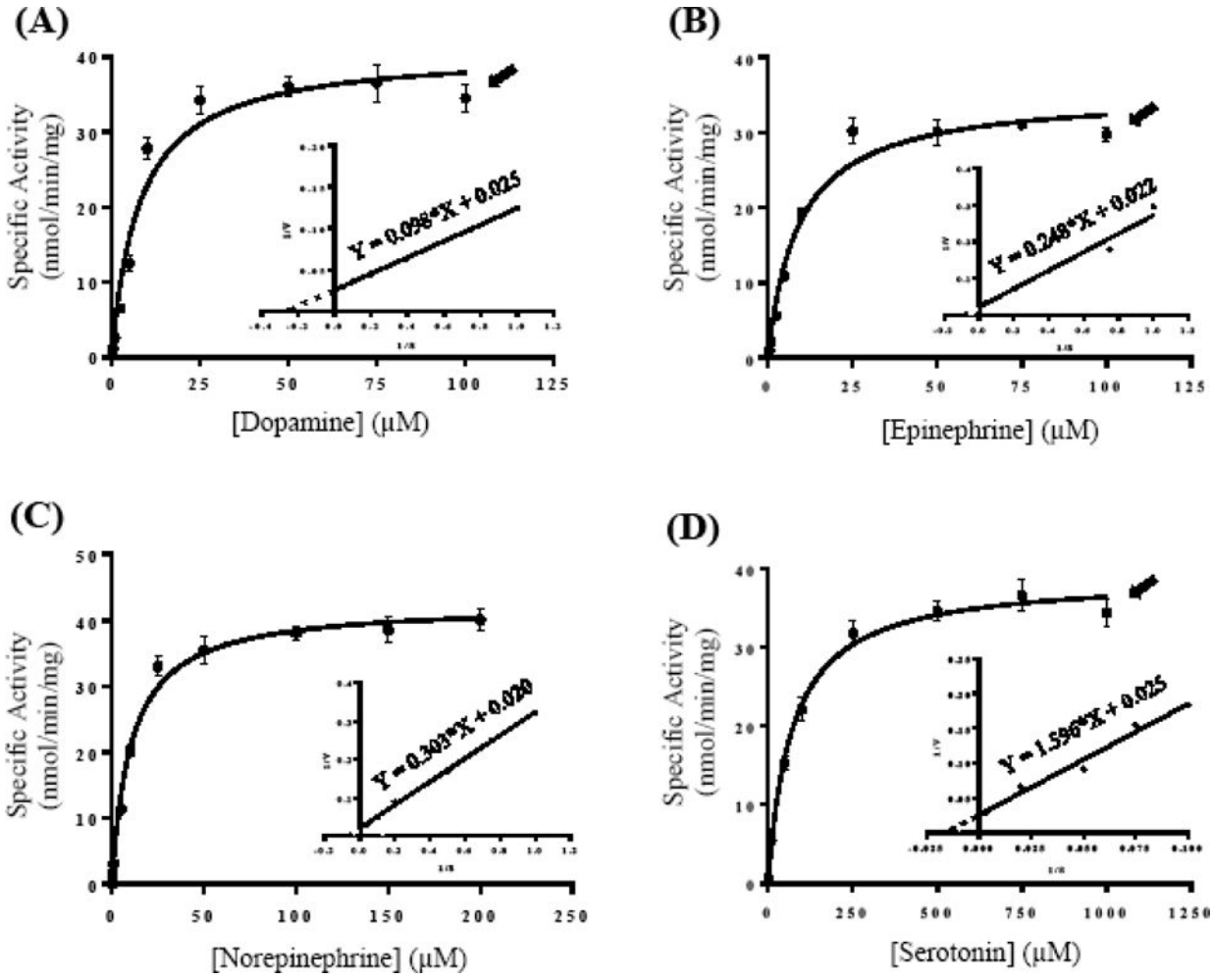

Figure 8.

Kinetic analysis for the sulfation of catecholamines and serotonin by wild-type human SULT1A3. Panels (A), (B), (C) and (D) illustrate the Michaelis–Menten saturation curves for the sulfation of dopamine (DA), epinephrine (EP), norepinephrine (NE), and serotonin (5-HT), respectively. The insets show the Lineweaver-Burk plots generated based on the data shown in each of the four panels. Arrow signs indicate the concentrations at which substrate inhibition started taking place. Data shown represent calculated mean ± standard deviation derived from three experiments.

Table 1

Mutagenic primer sets used in the PCR-amplification of the cDNAs encoding human SULT1A3 allozymes

SULT1A3 Allozymes	MAF[1]	Primers
SULT1A3-T7P	0.0003532[2]	5′- ATGGAGCTGATCCAGGA C[3]CCTCCCGCCCGCCACTGG -3′ 5′- CCAGTGGCGGGCGGGAGGGGTCCTGGATCAGCTCCAT -3′
SULT1A3-S8P	0.0000112[2]	5′- GAGCTGATCCAGGACACC CCCCGCCCGCCACTGGAGT -3′ 5′- ACTCCAGTGGCGGGCGGGGGGTGTCCTGGATCAGCTC -3′
SULT1A3-R9C	0.00006645[2]	5′- CTGATCCAGGACACCTCC TGCCCGCCACTGGAGTACG -3′ 5′- CGTACTCCAGTGGCGGGCAGGAGGTGTCCTGGATCAG -3′
SULT1A3-P10L	0.00000914[2]	5′- TCCAGGACACCTCCCGCC TGCCACTGGAGTACGTGAA -3′ 5′- TTCACGTACTCCAGTGGCAGGCGGGAGGTGTCCTGGA -3′
SULT1A3-V15M	0.00004058[2]	5′- CGCCCGCCACTGGAGTAC ATGAAGGGGGTCCCGCTCA -3′ 5′- TGAGCGGGACCCCCTTCATGTACTCCAGTGGCGGGCG -3′
SULT1A3-V18F	0.0002[2]	5′- CTGGAGTACGTGAAGGGG TTCCCGCTCATCAAGTACT -3′ 5′- AGTACTTGATGAGCGGGAACCCCTTCACGTACTCCAG -3′
SULT1A3-P101L	0.025[4]	5′- CTCTGAAAGACACACCGC TCCCACGGCTCATCAAGTC -3′ 5′- GACTTGATGAGCCGTGGGAGCGGTGTGTCTTTCAGAG -3′
SULT1A3-P101H	0.004[4]	5′- CTCTGAAAGACACACCGC ACCCACGGCTCATCAAGTC -3′ 5′- GACTTGATGAGCCGTGGGTGCGGTGTGTCTTTCAGAG -3′
SULT1A3-R144C	0.025[4]	5′- TCCTACTACCATTTCCAC TGTATGGAAAAGGCGCACC -3′ 5′- GGTGCGCCTTTTCCATACAGTGGAAATGGTAGTAGGA -3′
SULT1A3-K234N	0.042[4]	5′- GTTCAAGGAGATGAAGAA TAACCCTATGACCAACTAC -3′ 5′- GTAGTTGGTCATAGGGTTATTCTTCATCTCCTTGAAC -3′
SULT1A3-N235T	–	5′- TCAAGGAGATGAAGAAGA CCCCTAT GACCAACTACAC -3′ 5′- GTGTAGTTGGTCATAGGGGTCTTCTTCATCTCCTTGA -3′
SULT1A3-S290T	–	5′- TGGCAGGCTGCAGCCTCA CCTTCCGCTCTGAGCTGTG -3′ 5′- CACAGCTCAGAGCGGAAGGTGAGGCTGCAGCCTGCCA -3′

[1]Minor allele frequency.

[2]Allele frequencies as mentioned in NCBI, SNPs.

[3]letters in borders are altered/mutated nucleotides (SNPs).

[4]Allele frequencies as mentioned by previous genetic studies [28,30]

Table 2

Kinetic parameters of the wild-type human SULT1A3 and allozymes with dopamine as a substrate.

SULT1A3 allozymes	Km (μM)	Vmax (nmol/min/mg)	Vmax/Km
1A3-WT[1]	6.46 ± 0.59	40.82 ± 1.14	6.32
1A3-T7P	8.50 ± 2.06	36.76 ± 2.28	4.32
1A3-S8P	8.86 ± 1.70	44.15 ± 2.19	4.98
1A3-R9C	9.06 ± 2.07	45.48 ± 2.69	5.02
1A3-P10L	8.56 ± 2.35	42.82 ± 3.00	5.00
1A3-V15M	8.84 ± 2.11	45.26 ± 2.78	5.12
1A3-V18F	8.81 ± 2.41	41.32 ± 2.91	4.69
1A3-P101L	9.06 ± 1.39	43.18 ± 1.72	4.77
1A3-P101H	12.72 ± 3.29	45.10 ± 3.32	3.55
1A3-R144C	13.26 ± 2.99	44.77 ± 2.90	3.38
1A3-K234N	7.54 ± 2.09	30.74 ± 2.12	4.08
1A3-N235T	12.91 ± 1.29	42.99 ± 1.21	3.33
1A3-S290T	6.56 ± 1.77	35.03 ± 2.29	5.34

[1]Wild-type human SULT1A3.

Table 3

Kinetic parameters of the wild-type human SULT1A3 and allozymes with epinephrine as a substrate.

SULT1A3 allozymes	Km (μM)	Vmax (nmol/min/mg)	Vmax/Km
1A3-WT[1]	9.16 ± 1.81	35.30 ± 1.82	3.85
1A3-T7P	8.90 ± 0.89	33.72 ± 0.87	3.79
1A3-S8P	9.73 ± 0.47	35.60 ± 0.45	3.66
1A3-R9C	9.44 ± 0.40	30.65 ± 1.34	3.25
1A3-P10L	10.86 ± 0.48	38.66 ± 0.46	3.56
1A3-V15M	9.05 ± 1.22	34.38 ± 1.20	3.80
1A3-V18F	9.62 ± 1.08	30.69 ± 0.91	3.19
1A3-P101L	8.83 ± 1.23	34.97 ± 1.26	3.96
1A3-P101H	9.67 ± 1.71	33.95 ± 1.58	3.51
1A3-R144C	11.19 ± 1.69	34.70 ± 1.42	3.10
1A3-K234N	8.58 ± 1.24	29.30 ± 1.08	3.41
1A3-N235T	200.1 ± 64.51	25.85 ± 6.05	0.13
1A3-S290T	11.12 ± 0.45	29.60 ± 1.27	2.66

[1]Wild-type human SULT1A3.

Table 4

Kinetic parameters of the wild-type human SULT1A3 and allozymes with norepinephrine as a substrate.

SULT1A3 allozymes	Km (μM)	Vmax (nmol/min/mg)	Vmax/Km
1A3-WT[1]	10.65 ± 1.14	42.53 ± 0.99	3.99
1A3-T7P	18.78 ± 2.91	27.34 ± 1.07	1.36
1A3-S8P	15.80 ± 3.02	32.62 ± 1.51	2.06
1A3-R9C	99.98 ± 15.44	27.91 ± 3.24	0.28
1A3-P10L	17.10 ± 1.85	37.23 ± 0.99	2.18
1A3-V15M	13.17 ± 2.12	36.13 ± 1.34	2.74
1A3-V18F	12.49 ± 3.53	27.82 ± 1.79	2.23
1A3-P101L	13.41 ± 2.10	42.86 ± 1.55	3.20
1A3-P101H	11.52 ± 2.54	35.92 ± 1.77	3.12
1A3-R144C	13.49 ± 2.71	36.77 ± 1.71	2.73
1A3-K234N	14.32 ± 2.70	32.43 ± 1.44	2.26
1A3-N235T	53.46 ± 4.76	12.88 ± 0.41	0.24
1A3-S290T	10.99 ± 1.62	22.92 ± 0.74	2.09

[1]Wild-type human SULT1A3.

Table 5

Kinetic parameters of the wild-type human SULT1A3 and allozymes with serotonin as a substrate.

SULT1A3 allozymes	Km (μM)	Vmax (nmol/min/mg)	Vmax/Km
1A3-WT[1]	71.38 ± 7.99	38.99 ± 0.98	0.55
1A3-T7P	72.38 ± 6.29	32.22 ± 2.68	0.45
1A3-S8P	72.75 ± 10.97	39.48 ± 1.45	0.54
1A3-R9C	216.60 ± 27.32	26.19 ± 1.15	0.12
1A3-P10L	102.40 ± 15.99	34.01 ± 1.46	0.33
1A3-V15M	83.18 ± 11.32	34.51 ± 1.20	0.41
1A3-V18F	78.08 ± 10.24	29.07 ± 0.95	0.37
1A3-P101L	213.90 ± 33.26	34.49 ± 1.87	0.16
1A3-P101H	76.49 ± 6.82	37.90 ± 2.90	0.50
1A3-R144C	281.00 ± 9.16	24.17 ± 1.30	0.09
1A3-K234N	73.41 ± 7.92	25.38 ± 1.66	0.35
1A3-N235T	8112.0 ± 866	117.50 ± 14.3	0.014
1A3-S290T	165.60 ± 21.58	26.98 ± 1.13	0.16

[1]Wild-type human SULT1A3.