Unique Properties of a Renal Sulfotransferase, St1d1, in Dopamine Metabolism

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ABSTRACT

Although catecholamine sulfation is higher in the kidney than in the liver of mice, no detectable amounts of previously reported sulfotransferases (STs) such as St1a, St1b, St1c, and St1e were expressed in mouse kidney cytosols. A new sulfotransferase (St1d1) cDNA was isolated from kidney cDNA library of BALB/c strain by reverse transcription-polymerase chain reaction (RT-PCR) using information from expressed sequence tags (EST) database. The cDNA sequence resembled that of cDNA reported previously (AA238910) (Sakakibara et al., 1998) but differed in two amino acids, 206Q/K and 216Y/F, in the deduced amino acid sequence. The St1d1 expressed had unique substrate specificities for catecholamine derivatives, which preferred their deaminated metabolites rather than their parent amines. St1d1 showed the highest activity toward 3,4-dihydroxyphenylacetic acid (230.2 ± 2.69 nmol/mg/min) among the examined substrate. St1d1 protein was abundant in kidney, followed by liver, lung, and uterus. Furthermore, an addition of anti-St1d1 serum in the cytosolic reaction mixture resulted in complete inhibition of the sulfotransferase activity suggesting a major role of St1d1 on catecholamine sulfations. No human ST1D ortholog was detected at both mRNA and protein levels, although ST1A5 selectively catalyzing parent amine sulfation was detected in human kidney. These results indicate the functional basis of sulfation and the clear species difference on renal catecholamine metabolisms in mice and humans.

Catecholamines, such as dopamine and norepinephrine, are enzymatically biotransformed through several different pathways (Kopin, 1985). Deaminated metabolites by monoamine oxidase (MAO) are further converted to their alcohols or carboxylic acid derivatives. Catecholamines also undergo the methylation to form O-methylated derivatives. Both deaminated and O-methylated metabolites are detected at 10 to 70 nM levels in plasma. Considerable portions of the metabolites are also excreted as the sulfates in urine in experimental animals and humans. Although the liver is considered to be a major site of the conjugation, sulfotransferase activity for dopamine and p-nitrophenol in mice was found to be higher in kidney than in liver in our preliminary experiments, as reported previously (Wong and Yeo, 1982). These data suggest to us a possible role of the kidney for the biotransformation of phenolic chemicals into the readily excretable sulfates peripherally regulating catecholamine levels.

Cytosolic enzymes mediating sulfation of various endobiotics and xenobiotics have been identified and are now known to constitute a gene superfamily, ST (SULT) 1–5 (Nagata and Yamazoe, 2000). Phenol and estrogen sulfotransferases, including the ST1 family, are detected in livers of humans as well as rodents. Four different cDNAs of arylsulfotransferases have been isolated from mouse cDNA libraries of liver: St1a4 (Kong et al., 1993) and St1b3 (Saeki et al., 1998), olfactory (St1c4) (Tamura et al., 1998), and testis (St1e4) (Song et al., 1995), although substrate specificities of these forms were not yet identified except for St1a4 (Honma et al., 2001). Furthermore, limited amounts of information are available on kidney sulfotransferase form(s). Therefore, kidney sulfotransferase has been investigated to assess the possible role for metabolism of catecholamines. In the present study, we have identified a sole expression of St1d1 among arylsulfotransferases in mouse kidney and characterized the unique substrate specificity.

ABBREVIATIONS: MAO, monoamine oxidase; ST, St, or SULT, sulfotransferase; 4’-OH-PhIP, 2-amino-1-methyl-6-(4-hydroxyphenyl)-imidazo[4,5-b]pyridine; DTT, dithiothreitol; PAPS, 3’-phosphoadenosine-5’-phosphosulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RT-PCR, reverse transcription-polymerase chain reaction; DOPAC, 3,4-dihydroxyphenylacetic acid; bp, base pair(s); kbp, kilobase pair; VMA, vanillylmandelic acid.
Materials and Methods

**Materials**

Restriction endonucleases, DNA modifying enzymes, and Takara Ex Taq were purchased from Takara Shuzo (Kyoto, Japan). Enterokinase was obtained from Bizyme Laboratories, Ltd. (Gwent, UK). Thermo Sequenase cycle sequencing kit was obtained from Shimadzu Co. (Kyoto, Japan). 2-Amino-1-methyl-6-(4-hydroxyphenyl)-imidazo[4,5-b]pyridine (4'-OH-PhIP) was kindly donated by Dr. K. Wakabayashi (National Cancer Center Research Institute, Tokyo, Japan). Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Dithiothreitol (DTT), alkaline phosphatase-conjugated goat anti-rabbit IgG, 5-bromo-4-chloro-3-indolylphosphate, nitro blue tetrazolium, and phenolic chemicals used as substrates were purchased from Sigma-Aldrich (St. Louis, MO). 3'-Phosphoadenosine-5'-phosphosulfate ([35S]PAPS) (2000 mCi/mmol) was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). QIAexpress and nickel-nitrilotriacetic acid agarose were obtained from Qiagen (Chatsworth, CA). Bio-Rad protein assay kit and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) molecular weight standards (low range) were purchased from Bio-Rad (Richmond, CA). All other chemicals used were of the highest grade available.

Human kidney cytosols were obtained from BioChain Institute, Inc. (Hayward, CA). Human livers were from the Department of Anatomic Pathology (School of Medicine, Tohoku University, Sendai, Japan). Experiments on human livers were approved by the Ethics Committee in Tohoku University. BALB/c mice (8-week-old) and Japanese White rabbits (female, 3.0 kg) were obtained from Japan SLC (Shizuoka, Japan).

**Methods**

**Preparation of Cytosols from Mouse Kidney and Liver.** Renal and hepatic cytosols were prepared from 8-week-old male and female BALB/c mice. Kidneys and livers were rinsed with phosphate-buffered saline (PBS) and homogenized with a buffer containing 0.075 M potassium phosphate (pH 7.4), 0.075 M KCl, and 1 mM DTT. The homogenates were centrifuged at 9000 g for 20 min, and the supernatants were centrifuged at 105,000 g for 60 min. The resultant supernatants were used as cytosols. The protein concentration was determined by the method of Bradford (1976) with bovine serum albumin (BSA) as the standard.

**Assays of Sulfotransferase Activities.** Sulfotransferase activities were determined by the radioactivities of the metabolites using [35S]PAPS as a sulfate donor after thin-layer chromatography. The incubation mixture (final volume of 10 μl) consisted of 100 mM Tris-HCl buffer (pH 7.4), 0.3 mM MgCl₂, 2 mM DTT, 1 mg/ml bovine serum albumin, 125 μM [35S]PAPS, 10 or 100 μM substrate dissolved in 1% dimethyl sulfoxide, 2.5 μM of cystosolic protein from male mouse kidney or liver, or 50 ng of 6xHis-St1d1 protein. The reaction was initiated by addition of [35S]PAPS incubated at 37°C for 20 min and then terminated by addition of 5 μl of acetonitrile. Aliquots of the reaction mixture (10 μl) were applied to a thin-layer plate (thin-layer chromatography aluminum plate silica gel 60; Merck, Darmstadt, Germany; or chromatogram sheet 13255; Eastman Kodak, Rochester, NY). Metabolites on the chromatogram were developed with a solvent system of n-propanol/28% aqueous ammonia/water (6:3:1) (Fujita et al., 1999). The radioactive spots were analyzed by FLA-3000 image analyzer (Fuji Film, Tokyo, Japan). The apparent kinetic parameters, Kₘ and Vₘₐₓ, were calculated from double-reciprocal Lineweaver-Burk plots using linear regression analyses. The substrate concentrations used are ranging from 5 to 300 μM for 3,4-dihydroxyphenylacetic acid (DOPAC) and 10 μM to 10 mM for dopamine.

**Isolation of St1d1 cDNA.** St1d1 cDNA fragment was obtained by the PCR from mouse kidney or liver cDNA library using oligonucleotides as the primers (St1d1-5': CGGCCATGCGATGCGATGAAGATCTAAGCTTTTATATGCTATCAGTACCCTAGATGTC and St1d1-3': CGCGAGCTCAACTCTCCCTAGATC) containing Sphi/HindIII restriction sites, respectively. A 5'-region from the initiation codon of St1d1 cDNA fragment contained nucleotides encoding seven additional amino acid residues (AlaCysAspAspAspAspAspAla) as enterokinase cleavage site. The PCR mixture consisted of 1 μl of the template cDNA, 3 μl each of dATP, dCTP, dGTP, and dTTP, 1.5 units of Takara Ex Taq, 20 pmol of St1d1-5' and St1d1-3' primers, and the Ex Taq buffer in a final volume of 30 μl. After an initial denaturation at 94°C for 2 min, the amplification was performed for 30 cycles with 1 min at 94°C for denaturation, 30 s at 55°C for annealing, 2 min at 72°C for extension, and a final extension period of 7 min at 72°C.

**Sequencing.** DNA sequence was determined by using fluorescent-labeled primers and DSS2000L DNA sequencer (Shimadzu Co., Kyoto, Japan) according to the dye-terminator method (Shimada et al., 2002). The PCR mixture (8 μl) contained 5 μg of the template DNA, 1.25 pmol of the fluorescent-labeled primer, and 2 μl of the reagent containing ddATP, ddGTP, ddTTP, or ddCTP. After an initial denaturation at 98°C for 5 min, the amplification was performed for 25 min, with 1 min at 98°C for denaturation, 45 sec at 50°C for annealing, 1.5 min at 72°C for extension, and a final extension period of 1 min at 72°C. After addition of 8 μl of the formamide loading dye for termination of the reaction, denaturation was performed at 98°C for 5 min. These samples were applied to the gel and then electrophoresed for 24 h.

**Expression and Purification of St1d1.** The cDNA obtained (St1d1) was ligated into an expression vector, pQE30 (Qiagen, Tokyo, Japan). The constructed plasmid DNA was transformed into Escherichia coli, M15 [pREP4] strain. Recombinant St1d1 (6xHis-St1d1) was expressed and purified from bacterial cytosols by nickel-nitrilotriacetic acid affinity chromatography (Fujita et al., 1997). 6xHis-St1d1 was eluted with the buffer containing 300 mM NaCl, 10% glycerol, 0.025% Tween 20, 50 mM sodium phosphate (pH 6.0), and 200 mM imidazole. St1a4 and St1b3 were also expressed as histidine-tagged proteins.

**Preparation of Antibody.** A Japanese White rabbit (3.0 kg, female) was immunized intradermally with 100 μg of purified 6xHis-St1d1 in Titer Max GOLD (CytRx, Atlanta, GA) and immunized intravenously 4 weeks later with 100 μg of the protein. After 1 week, 40 ml of the blood was obtained and centrifuged. The resultant supernatant was used as the St1d1 antibody. In Western blotting anti-St1d1 antibody serum recognized only one band in kidney and liver cytosols and did not immunocross with other known arylsulfotransferases examined.

**Electrophoresis and Western Blotting.** Cytosolic proteins (10–100 μg/lane) from mouse kidney or liver were separated by SDS-PAGE using a 10.5% gel and electrophoretically transferred to a nitrocellulose membrane. STs on the membrane were reacted with the antibody (1:1000 dilution) and with alkaline phosphatase-conjugated goat anti-rabbit IgG (1:3000 dilution), 5-bromo-4-chloro-3-indolylphosphate, and nitro blue tetrazolium. The stained sheets were scanned with EPSON Calario GT-8700 (Epson, Tokyo, Japan), and their intensities were measured by use of the NIH Image (version 1.59) software. The contents in each sample were determined using the corresponding recombinant proteins cleaved 6× His by enterokinase (Δ6xHis) as the standards.

**Results**

**Sulfotransferase Activities toward Catecholamines in Cytosols from Mouse Kidney and Liver.** Sulfotransferase activities toward catecholamines at 100 μM substrate...
concentration were compared between kidney and livers (Fig. 1). Renal and hepatic cytosols mediated dopamine sulfation at the rate of 745 ± 36.0 and 220 ± 10.4 pmol/mg/min, respectively. Three-fold higher sulfating rates were also observed in kidney than in liver toward norepinephrine and epinephrine.

Western Blot Analyses of Arylsulfotransferase in Mouse Kidney and Liver. To identify which form(s) of sulfotransferase mediates sulfation of catecholamines in mouse kidney, renal cytosols were subjected to Western blotting analyses as shown in Fig. 2. St1a4 and St1b3 were not detected in kidney, although both forms were detected in livers of male and female mice. Anti-rat ST1B1 sera recognized an unknown band at about 33 kDa, which is distinct from authentic St1b3 and St1a4 protein. In addition, neither St1c4 nor St1e5 proteins were detected in kidney and liver (data not shown).

Structure of a New Renal ST cDNA. The cDNA prepared from BALB/c strain of mice was explored to identify the renal sulfotransferase mediating catecholamine sulfation. A possible sulfotransferase cDNA was isolated and the nucleotide and deduced amino acid sequences of a renal ST are shown in Fig. 3. The identified renal ST mRNA contained an open reading frame of 888 bp, which encoded 295 amino acids with a molecular weight of 35,081 Da. The renal sulfotransferase was closely related to sulfotransferase found in EST database (accession no. AA238910), except for 206Q/K (616C/A) and 216Y/F (629A/T) in the deduced amino acid sequences. Only one fragment of mRNA (AA238910) from EST database exactly matched part of the previously reported sequence (Sakakibara et al., 1998). On the contrary, this newly isolated cDNA was exactly matched in three EST fragments (AA245085, 109895, and 109782) and thus judged to correspond to St1d1 in BALB/c mice. These two closely related St1d1 forms are possible to exist in mouse liver and kidney cytosols.

Characterization of General Substrate Specificity of St1d1. To characterize the substrate specificity of the renal St1d1, St1d1 was expressed in E. coli as a fused protein. As shown in Table 1, recombinant St1d1 catalyzed sulfation of dopamine, in addition to p-nitrophenol, triiodothyronine, and estradiol among phenolic chemicals. St1d1 also mediated sulfation of 2-methylbenzyl alcohol, but showed only marginal activities toward other alcohols.
Sulfotransferase Activity of St1d1 for Catecholamine and Their Metabolites. Due to the high activity toward dopamine, substrate specificity of St1d1 was investigated further on catecholamines and their metabolites at 10 μM substrate concentration (Fig. 4). St1d1 had no sulfating activity on 3,4-dihydroxy-L-phenylalanine (L-DOPA), a precursor of catecholamine.

Other catecholamines such as norepinephrine (6.04 ± 1.59 nmol/mg/min) and epinephrine (1.40 ± 0.07 nmol/mg/min) are also sulfated, in spite of the high activity toward dopamine (22.8 ± 3.14 nmol/mg/min) by St1d1. St1d1 had no sulfating activity with 3-methoxytyramine, normethanephrine, and methanephrine, which were formed from the parent amine by catechol-O-methyltransferase. St1d1, however, showed markedly high activities toward DOPAC (230.2 ± 9.21 nmol/mg/min), 3,4-dihydroxydopamine (20.3 ± 2.69 nmol/mg/min), and 3,4-dihydroxyphenylethylene glycol (209.3 ± 13.7 nmol/mg/min). These metabolites are formed from their parent amines by MAO followed by aldehyde oxidase or aldehyde reductase. St1d1 also catalyzed sulfation of homovanillic acid (116.9 ± 7.04 nmol/mg/min) and 4-hydroxy-3-methoxyphenylethylene glycol (72.4 ± 7.00 nmol/mg/min), except for vanillylmandelic acid (VMA).

Kinetic parameters were examined for dopamine and DOPAC sulfations (Table 2). Apparent K_m and V_max values of St1d1 were 713.2 ± 14.2 μM and 1474 ± 9.03 nmol/mg of protein/min, respectively, for dopamine sulfation, whereas 35.02 ± 1.13 μM and 1678 ± 159 nmol/mg of protein/min.

![Diagram of sulfotransferase activities toward catecholamines and their metabolites by recombinant St1d1.](https://aspetjournals.org/jpet/2017/811/fig4.png)
were for DOPAC sulfation. The values of $V_{\text{max}}/K_m$ for dopamine and DOPAC sulfations were 2.07 and 47.9, respectively.

**Distribution and Quantification of Cytosolic St1d1 Protein.** St1d1 protein was detected in kidney and liver at about 33 kDa and comigrated with corresponding to 6xHis-St1d1. The levels are higher in kidney than in liver, followed by lung and uterus (Fig. 5). The protein levels in lung and uterus were calculated about 10% of the level in liver. Cytosolic contents of St1d1 in kidney and livers were determined. Mean contents of renal St1d1 were 5.1 and 3.4 μg/mg cytosolic protein in three different male and female mice, respectively. On the contrary, hepatic St1d1 content was rather higher in female mouse (2.0 μg/mg cytosolic protein) than in males (2.5 μg/mg cytosolic protein). To know whether St1d1 is a major form in liver, we also determined the contents of St1a4 and St1b3. St1a4 content was 2.3 and 4.4 μg/mg cytosolic protein in male and female livers, respectively. St1b3 content was 0.5 and 0.4 μg/mg hepatic cytosol from male and female, respectively.

**Role of St1d1 on Renal Dopamine and DOPAC Sulfations.** To assess the role of St1d1 on renal catecholamine sulfation, anti-St1d1 antisera were added in the incubation mixture containing DOPAC and renal cytosols. To verify the form-specificity of anti-St1d1 antiserum, the antiserum was added to an incubation system containing recombinant St1d1. Each value represents the mean ± S.D. of at least three different determinations. Sulfotransferase activities were assayed using $[^35]$S-PAPS as a sulfate donor. The assays were performed at pH 7.4 in the presence of 10 μM to 10 mM dopamine or 5 to 200 μM and 50 ng of recombinant St1d1.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
<th>$V_{\text{max}}$ (nmol/mg protein/min)</th>
<th>$V_{\text{max}}/K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>713.2 ± 14.2</td>
<td>1474 ± 9.03</td>
<td>2.07 (1)</td>
</tr>
<tr>
<td>DOPAC</td>
<td>35.0 ± 1.1</td>
<td>1678 ± 159</td>
<td>47.9 (23.14)</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study, St1d1 is shown to be a major arylsulfotransferase in mouse kidney, and no other St1 forms, like St1a4 and St1b3, were detected in contrast to their high expression in livers.

These data indicate the primary role of St1d1 for catecholamine sulfation in this tissue. Unique properties of St1d1, which showed higher activities toward the deaminated derivatives of catecholamine, such as DOPAC, 3,4-dihydroxymandelic acid, and 3,4-dihydroxyphenylglycol, rather than parent amines, are characterized by the use of the recombinant protein.

From kinetic parameters of St1d1, the value of $V_{\text{max}}/K_m$ for DOPAC is 20-fold higher than that for dopamine. These data suggest that DOPAC is a selective substrate for St1d1. The substrate specificity of St1d1 was distinct from those of a human catecholamine sulphotransferase, ST1A5 form (Honma et al., 2001). As shown in our previous report, ST1A5 have the higher activity toward parent catecholamines rather than the deaminated derivatives.

Chemicals containing acidic carboxyl groups are rather poor substrates for previously known sulphotransferases included in the ST1 family. St1d1, however, catalyzes sulfation of phenol-carboxylic acids such as DOPAC and homovanillic acid, except for VMA. The latter is consistent with reported data that VMA is excreted mainly in urine as unconjugated in rodents. Presence of methylethyl moieties in catecholamines seems to have complex influences on the St1d1-mediated sulfations. The substrate specificities are unlikely to be determined only by regioselectivity, chemico-physical properties such as acidity and/or hydrophobicity. The sole expression of St1d1 in kidney among ST1 forms was observed in the present study. This phenomenon is in contrast to the expression of multiple families of sulphotransferase in liver. Recently, another sulphotransferase form, which is an ortholog to human SULT1C2 (ST1C2), was detected in kidney (Sugimura et al., 2002). The recombinant protein showed only p-nitrophenol sulfation, and the functional role of mouse SULT1C2 in kidney was not reported.

In rodents, plasma concentration of dopamine was ca. 5 μM (Tsunoda et al., 2001), and renal concentrations of dopamine and DOPAC were reported at 200 and 20 nM, respec-
tively (Shoaf and Elchisak, 1983; Eldrup and Richter, 2000). These values were estimated to be 1/100 of $K_m$ values for recombinant St1d1 in the presence of $125\mu M$ PAPS as shown in Table 2. Any reason for this discrepancy remained obscure.

These are few reports on urinary excretion of catecholamines in rodents. DOPAC sulfate is a major urinary excretion in rats. Renal St1d1, in our preliminary experiment, was stained immunohistochemically at proximal and distal tubule and collecting duct. The presence of organic cation transporter (OCT) is reported in rats and human kidneys (Inui et al., 2000). Combined with the information, it is possible that dopamine and DOPAC are taken into the renal tubule by transporters like OCT1/2 and then sulfated with St1d1 in mice. The sulfate esters are excreted in urine through transporters. These results suggest that renal St1d1 is also involved in excretion of catecholamines to maintain the homeostasis of the whole body.

During the preparation of this study, a recent report showed that the expression of canine SULT1D (ST1D) is expressed in kidney and shown the sulfotransferase activity...
toward dopamine (Tsoi et al., 2001). Rat ST1D2 cDNA is also isolated, although the substrate specificity has not yet been characterized.

Human SULT1D (ST1D) is suggested to be a pseudogene because of the existence of mutated splice donors/acceptors site and in-frame stop codons (Meinl and Glatt, 2001). These data, together with our present result, suggest that ST1D form is not expressed as a functional protein in human kidney. Thus ST1A5, instead of ST1D form, has a primary role in controlling catecholamine sulfation in human kidney.

In the present study, St1d1 (mouse) and ST1A5 (human) are first identified as renal neurotransmitter sulfotransferases and suggest the involvement in the excretion profile of the sulfoconjugated catecholamines in urine.

References


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