ENZYMATIC CHARACTERIZATION AND INTERSPECIES DIFFERENCE OF PHENOL SULFOTRANSFERASES, ST1A FORMS

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ABSTRACT:

Cytosolic sulfotransferases, which mediate activation and detoxification of both endogenous and exogenous compounds, consist of at least five different gene families (ST1 to ST5) in mammals. Several cDNAs corresponding to ST1A forms have been reported, but their functional properties are not well characterized. In addition, only a single form of ST1A sulfotransferase has been reported in each experimental animal species despite the expressions of plural forms in humans. Therefore, enzymatic properties of human ST1A3, ST1A5, rat ST1A1, mouse ST1A4, and newly isolated rabbit ST1A8 have been characterized and compared by use of their recombinant proteins to clarify the functional difference between human and experimental animal ST1A forms. From the results using more than 25 phenolic chemicals, all the experimental animal ST1A forms showed substrate specificities similar to human ST1A3 rather than ST1A5. They showed high affinities toward p-nitrophenol and 6-hydroxymelatonin as found in human ST1A3. These forms also showed high activities toward umbelliferone and naringenin, but very low activities toward catecholamines, representative substrates of human ST1A5. Hepatic contents of experimental animal ST1A forms varied (66–250 pmol/mg of cytosolic protein) but showed the same order as observed with human ST1A3 (120 pmol/mg). Hepatic content of human ST1A5 was about 19-fold less than that of ST1A3. Therefore, ST1A forms identified in experimental animal species correspond to human ST1A3 functionally. For chemicals such as troglitazone and 2-amino-4’-hydroxy-1-methyl-6-phenylimidazo[4,5-b]pyridine, clear species differences were detected among the ST1A forms examined.

Sulfation plays key roles in detoxification and activation of various endogenous and exogenous compounds such as hormones, neurotransmitters, drugs, and carcinogens (De Meio, 1975; Jakoby et al., 1980; Yamazoe and Kato, 1995). These reactions are catalyzed by cytosolic sulfotransferases (STs) in mammals, which transfer SO₃⁻ (sulfate moiety) from 3’-phosphoadenosine-5’-phosphosulfate (PAPS) to substrates.

Species differences are often observed on sulfations of chemicals among human and experimental animal species. For example, a model substrate, 7-hydroxycoumarin (umbelliferone), was preferentially conjugated with sulfate in precision-cut liver slices from rats and mice. The rates of glucuronidation and sulfation were, however, similar in guinea pig, monkey, and human (Steenisma et al., 1994). Mechanisms yielding species differences remained unclear and thus hamper the prediction of the metabolic property of chemicals in humans from experimental animal data.

STs are known to constitute a gene superfamily. This superfamily contains at least five different classes, ST1, ST2, ST3, ST4, and ST5 families in mammals, which are based on their similarities of deduced amino acid sequences (Yamazoe et al., 1994; Weinshilboum et al., 1997; Nagata and Yamazoe, 2000). ST1 family is further subdivided into five subfamilies: ST1A, ST1B, ST1C, ST1D, and ST1E.

ST1A4 forms mainly mediate sulfations of phenols. From human-derived cDNA libraries, three distinct cDNAs of ST1A forms, ST1A2 (also called STP2 or SULT1A2), ST1A3 (STP1, TS-PST, or SULT1A1), and ST1A5 (STM, TL-PST, or SULT1A3), have been isolated and characterized (Zhu et al., 1993; Ozawa et al., 1994; Dooley and Huang, 1996). ST1A2 and ST1A3 catalyze the sulfations of simple phenolic chemicals such as p-nitrophenol (p-NP). ST1A3 shows higher catalytic activity and affinity for p-NP than does ST1A2. In contrast, ST1A5 shows a trivial activity for p-NP, but has high affinity for dopamine (Veronese et al., 1994; Lewis et al., 1996; Fujita...
Materials. Restriction endonucleases, DNA modifying enzymes, and Takara Taq were purchased from Takara Shuzo (Kyoto, Japan). A Agt11 cDNA library of a male rabbit liver was obtained from CLONTECH (Palo Alto, CA). Enterokinase was obtained from Biozyme Laboratories, Ltd. (Gwent, UK). 2-Amino-4-hydroxypyrene (3-OH-PhIP) was kindly donated by Dr. K. Wakabayashi (National Cancer Institute, Bethesda, MD). Sakuranetin and 5,7-dihydroxyflavanone were provided by Meiji Seika Kaisha, Ltd. (Kanagawa, Japan). Derivatives of other flavonoids were generously provided by Dr. I. Nakamura, Osaka City University, Osaka, Japan. 5-Bruridine was obtained from Sigma Chemical Co. (St. Louis, MO). [3H]SIPAP (2000 mCi/mmol) was from New England Nuclear (Boston, MA). QIAexpress and Niti-nitrilotriacetic acid agarose were the product of Qiagen (Chatsworth, CA). Bio-Rad protein assay kit and SDS-polyacrylamide gel electrophoresis (PAGE) molecular weight standards (low range) were from Bio-Rad (Richmond, CA). All other chemicals used were of the highest grade available. Sprague-Dawley rats (8 weeks old), BALB/c mice (8 weeks old), and male New Zealand White rabbits (8 weeks old) were obtained from Japan SLC (Shizuoka, Japan). Human liver samples provided by SRI International Toxicology Laboratory (Menlo Park, CA) and Department of Anatomic Pathology (School of Medicine, Tohoku University, Sendai) were used. Experiments with human livers were approved by the Tohoku University Ethical Committee. Animal experiments were done under the instruction of Tohoku University Animal Care and Use Committee.

Methods. Isolation of rabbit STIA form (STIA8) cDNA. A Agt11 cDNA library of a male rabbit liver was immunoscreened with anti-

\[ \text{His-STIA5} \]

rabbit STIA polyclonal antibody by the method described previously (Yoshinari et al., 1998). After the third screening, positive clones were isolated and purified. Then, DNA sequences of each clone were determined separately using dye primers and Thermo Sequenase with ABI373A DNA sequence (Perkin Elmer Japan, Tokyo, Japan) according to the dideoxy method in conjunction with M13 phage cloning as described (Sambrook et al., 1989). The DNA sequences of the positive clones coincided with each other. Two oligonucleotides (rab.STIA-5': GCCGATTCGATGAGATGACAAAATGGAGACTCATCCAGGACACCTCCGC, and rab.STIA-3': GCCATGCCCCACCCACCATCAGCTGTGAACCGGAAGG) were designated to construct the expression vector. Oligonucleotides have BamHI and SphI restriction sites, respectively. The designated (rabbit STIA8) cDNA fragment was obtained by PCR. The PCR reaction mixture (50 μl) contained 5 ng of the template cDNA; 10 pmol of each 5′ and 3′ primers; 0.2 mM each of dATP, dCTP, dTTP, and dGTP; 0.5 units of TAKARA Ex Taq. and the Ex Taq buffer. After an initial denaturation at 94°C for 3 min, the amplification was performed for 25 cycles, with 1 min at 94°C for denaturation, 30 s at 55°C for annealing, 1 min at 72°C for extension, and a final extension period of 2 min at 72°C.

Construction of expression vectors, expression and purification of recombinant STIA proteins. Designated STIA cDNA fragments contained nucleotides encoding seven additional amino acid residues (GlySerAspAspAspAspAspLys), which was included in a sequence recognition sites of enterokinase next to the N-terminal methionine of the native form. Designated human STIA3, STIA5, and rat STIA1 cDNA fragments were obtained by PCR as described (Fujita et al., 1999a,b). The mouse St1a4 fragment was also obtained by PCR from a mouse male liver cDNA library using oligonucleotides as the primers (mST1A-5': GCGGATCCGATGAGATGACAAAATGGA, and mST1A-3': GCCGCGAATTCGATGAGATGACAAAATGGA). They have BamHI and SalI restriction sites, respectively. The designated STIA cDNAs were ligated into a prokaryotic expression vector, pQE30 (Qiagen). The constructed plasmid DNAs were transformed into Escherichia coli, M15 [pREP4] strain. Recombinant proteins, termed His-STIA1s, were expressed and purified from bacterial cytosols by Nitrilotriacetic acid affinity chromatography. The fusion portion of His-STIA1s was removed to yield His-STIA proteins for standards of immunoblot analyses by use of enterokinase as described (Fujita et al., 1997). The protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Antibody preparation and immunoblot analysis. Japanese White rabbits (2.5 kg, female) were immunized intradermally with 20 to 50 μg of each purified His-STIA protein in complete Freund's adjuvant, and immunity was boosted intravenously with 20 to 50 μg of the protein 3 weeks later. One week after the boost, antisera were obtained and kept at ~80°C until use. Cytosolic proteins (5–50 μg/lane) were separated by 10% sodium dodecyl sulfate-PAGE and then transferred to a nitrocellulose sheet (Whatman, 1979). The sheet was immunostained with the polyclonal antibody (1:3000 dilution) raised against each purified His-STIA1 protein, alkalinephosphatase-conjugated goat anti-rabbit IgG, 5-bromo-4-chloro-3-indolylphosphate, and nitro blue tetrazolium as described (Blake et al., 1984). In the case to determine the contents of STIA3 and STIA8, the polyclonal antibodies raised against the purified His-STIA5 protein were used. The stained sheets were scanned with Nikon AX-1200 and their intensities were measured by use of the NIH image (version 1.59) software (Bethesda, MD). The contents of each STIA form in liver cytosols were determined using corresponding His-STIA proteins as the standards. The antibodies did not cross-react with other subfamilies of ST1 forms (ST1B, ST1C, ST1D, and ST1E) and ST2A forms. Molecular weights of 34,157 for His-STIA1; 34,171 for His-STIA5; 33,802 for His-STIA8, and 33,867 for His-STIA1A; 34,677 for His-ST1a4; and 33,802 for His-ST1A8.
which were derived from their deduced amino acid sequences, were used for the
determination of contents of each ST1A form.

**Assay of sulfation.** Sulfating activities were determined by the radioactivity
tests of the metabolites obtained with [35S]PAPS as a sulfite donor after thin
layer chromatography (Yoshinari et al., 1998). A typical incubation mixture consisted of
50 mM Tris-HCl buffer (pH 7.4), 1 mM dithiothreitol, 20 mM MgCl2, 10 μM substrate, 125 μM [35S]PAPS (0.1–0.2 Ci/mmol), and 50 ng of
His-ST1A protein in a final volume of 10 μl. The reaction was initiated by
addition of [35S]PAPS and terminated by addition of 5 μl of chilled acetonitrile
after incubation at 37°C for 20 min. A portion (10 μl) of the reaction mixtures
was applied to a thin-layer plate (chromatogram sheet 13255; Kodak, Roch-
ester, NY; or thin layer chromatography aluminum plate silica gel 60; Merck,
Darmstadt, Germany). Metabolites on the chromatogram were developed with
a solvent system of n-propanol/amoniam/water (6:3:1). The radioactive spots were
analyzed by a BAS1000 image analyzer (FujiFilm, Tokyo, Japan). All the
substrates dissolved in dimethyl sulfoxide (DMSO) were added to the
final DMSO concentration 0.01 to 0.04%. In the case of inhibitory assays, the
final 0.08% DMSO concentration was used. Sulfating activities showed less
than 3% difference between experiments with 0.04 and 0.08% DMSO toward
salicylic acid, 6-hydroxymelatonin, harmol, naringenin, and dopamine. Mo-
olecular weights of 36,160 for His-ST1A5; 36,174 for His-ST1A3; 35,870 for
His-ST1A1; 36,680 for His-St1a4; and 35,805 for His-ST1A8, which were
derived from their deduced amino acid sequences, were used for the determi-
nation of each sulfating activity. ST1 families’ Km values for PAPS are about
0.5 to 1.0 μM (Fujita et al., 1999b), and there are species differences in PAPS
tissue concentrations (Klaassen and Boles, 1997). In the present study, we used
a high PAPS concentration (125 μM) and relatively short-periods of incubation
to minimize the deviation of the reaction. Deviations start to occur over 20
μM p-NP and troglitazone under our assay conditions.

The apparent kinetic parameters were from the assays examined with several concentrations of p-NP (0.5–10 μM for ST1A3, ST1A1, St1a4, and
ST1A8; 50–1000 μM for ST1A5), dopamine (34–200 μM for ST1A3, ST1A1, and St1a4; 34–1000 μM for ST1A8; and 1–25 μM for ST1A5) and
6-hydroxymelatonin (0.5–50 μM for ST1A3, ST1A1, and St1a8; 0.5–100
μM for St1a4; and 2.5–100 μM for ST1A5). Each reaction showed the lineearity
within the concentration examined.

**Results**

**Comparison of Deduced Amino Acid Sequences.** The deduced
amino acid sequences of ST1A forms examined in the present study (human
ST1A3, ST1A5, rat ST1A1, mouse St1a4, and rabbit ST1A8) and the percentage of identities are shown in Fig. 2 and Table 1,
respectively. These ST1A forms share more than 96% identity with each other.
The highest homology is observed between ST1A3 and ST1A5 (93%). All the experimental animal ST1A forms show slightly
higher identities with human ST1A3 than ST1A5. Especially rabbit
ST1A8, newly isolated from a male rabbit liver, is more closely related (more than 81% identities) to the human ST1A forms than equivalents in rat and mouse (Table 1). As shown in Fig. 2, arrows indi-
cating 121Gln, 185Thr, and 267Thr of ST1A3 and corresponding residues to ST1A3
forms. Human ST1A5 catalyzed sulfation of serotonin and 5-hy-
droxytryptophol (5-HTP), 6-hydroxymelatonin (0.36 nmol/mmol/min), but their ratios to the ST1A3 activity
were 1205 and 13%, respectively. ST1A3 and experimental
animal ST1A forms showed high activities toward 5-hydroxyindole, harmol, and 6-HM. Sulfating activities toward HIAA were not de-
mined in all the ST1A forms (less than 10 pmol/mg/min).

**Contents of ST1A Proteins in Liver Cytosols.** To clarify the enzymatic properties of ST1A forms, recombinant ST1A proteins,
termed His-ST1As, were expressed in *E. coli* and purified by nickel-affinity chromatography. Contents of ST1A forms in liver cytosols were determined by immunoblot analyses using each anti-ΔHis-ST1A
antibody and corresponding ΔHis-ST1A protein as the standard (Ta-
ble 2). The antibodies did not cross-react with other ST1 subfamilies (ST1B, ST1C, ST1D, and ST1E forms) and ST2A forms. ST1A3 and
ST1A5 are immunostained with anti-ΔHis-ST1A antibodies, but
distinguished by their different mobilities on SDS-PAGE.

In human liver cytosols (n = 21), the average content of ST1A3 was about 19 times higher (120 ± 38 pmol/mg cytosolic protein) than
that of ST1A5 (6.4 ± 2.6 pmol/mg). There are noticeable individual
differences (90–248 pmol/mg) in ST1A3 contents. The average content
of ST1A1 was about 1.5 times higher in adult male rats (270 ± 5.9 pmol/mg) than in the females (190 ± 15 pmol/mg). On the other
hand, the average content of St1a4 was about twice as high in adult
female mice (130 ± 5.8 pmol/mg) as in the males (66 ± 2.9 pmol/
mg). The average content of ST1A8 in adult male rabbit was 250 ±
10 pmol/mg.

**Sulfating Activities (Comparison of Substrate Specificities).** The
His-ST1A proteins have 17 additional amino acid residues (MetArg-
GlySerHisHisHisHisGlySerAspAspLysAspLysAspLys), including a histidine
tag and a sequence for the recognition site of enterokinase
next to the N-terminal methionine of the native form. The additional
peptide fused to the N terminus of STs has been shown to have
minimal influence on kinetic parameters (Marsolais and Varin et al.,
1995; Fujita et al., 1999a), although a slight difference was observed
on isoprotoren sulfonylation (Lewis et al., 1996). In our experiments
using His-ST1A3 and ΔHis-ST1A3, both proteins showed consistent
results on p-NP sulfation (Km = 3.00 and 2.62 μM, Vmax = 3.98 and
3.47 nmol/nmol/min, Vmax/Km = 1.32 and 1.33, respectively). Thus,
His-ST1A was used for our present experiments as shown below.

Toward catecholamines, its derivatives, and tyramine, human
ST1A5 showed higher activities (2.93–4.34 nmol/mmol/min) than did
the other ST1A forms except for 4-hydroxy-3-methoxyphenylglycol
and 3,4-dihydroxyphenylacetic acid (Table 3). Especially, ST1A5
activities toward tyramine and norepinephrine were about 62 and 80
times higher than those of ST1A3. Human ST1A3 and experimental
animal ST1A forms mediated these reactions but at very low levels
(0.03–0.59 nmol/mmol/min).

As endogenous indoles, serotonin, 5-hydroxytryptophol, 5-hy-
droxyindoleacetic acid (HIAA), and 6-hydroxymelatonin (6-HM)
were also examined together with model substrates 5-hydroxyindole
and harmol (Table 4). Experimental animal ST1A forms showed activities comparable with human ST1A3 toward these chemicals
except that rat ST1A1 catalyzed 5-hydroxytryptophol sulfation at
twice the rate as did ST1A3. Rabbit ST1A8 had a lower activity
related to human ST1A3 toward these chemicals activities comparable with human ST1A3 toward these chemicals
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twice the rate as did ST1A3. Rabbit ST1A8 had a lower activity
related to human ST1A3 toward these chemicals activities comparable with human ST1A3 toward these chemicals
similar extents of activities toward sakuranetin and DHF (4.22 and 4.20 nmol/nmol/min, respectively), whereas experimental animal ST1A forms and human ST1A5 showed decreased activities for sakuranetin than for DHF.

To compare details on substrate specificities, apparent kinetic parameters for p-NP, dopamine, and 6-HM were determined with 125 μM PAPS at pH 7.4 (Table 6). ST1A3 and ST1A5 showed the lowest \( K_m \) values for p-NP (3.0 μM) and for dopamine (14 μM), respectively. For p-NP, all experimental animal ST1A forms also showed low \( K_m \) (3.2–3.8 μM) and similar \( V_{\text{max}} \) values (3.94–4.66 nmol/nmol/min) with human

**TABLE 1**  

**Similarity of ST1A forms**

<table>
<thead>
<tr>
<th></th>
<th>ST1A5* (Human)</th>
<th>ST1A3* (Human)</th>
<th>ST1A1* (Rat)</th>
<th>ST1A4* (Mouse)</th>
<th>ST1A8* (Rabbit)</th>
<th>ST1A6 (Bovine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST1A5* (human)</td>
<td>93</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1A3* (rat)</td>
<td>76</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1A1* (mouse)</td>
<td>70</td>
<td>72</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1A4* (mouse)</td>
<td>84</td>
<td>85</td>
<td>75</td>
<td>69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1A8* (rabbit)</td>
<td>79</td>
<td>83</td>
<td>77</td>
<td>70</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>ST1A6 (bovine)</td>
<td>79</td>
<td>82</td>
<td>72</td>
<td>69</td>
<td>74</td>
<td>79</td>
</tr>
</tbody>
</table>

* The ST1A forms characterized in the present study.

Fig. 2. Alignment of deduced amino acid sequences among the ST1A forms.

Deduced amino acid sequences of ST1A5, ST1A3, ST1A1, ST1A4, and ST1A8 were aligned. Boxes indicate conserved regions among these ST1A forms. Arrows represent position 121, 185, and 267 of human ST1A3 and corresponding positions of the other forms, which are the ST1A-specific residues. Sites (site A: L\( ^5 \)A/S\( ^6 \)E/S\( ^7 \)L/L/DQK\( ^{K^2} \)\( ^{Y^3} \); site B: L\( ^5 \)^E/S\( ^6 \)HPV; and site C: SLPEET) are highly conserved within ST1A forms.
TABLE 2
Contents of STIA forms
Contents of STIA forms in liver cytosols of individual species were determined by immunoblot analyses as described under Experimental Procedures. Each value represents mean ± S.D.

<table>
<thead>
<tr>
<th>Name</th>
<th>ST1A3 (human)</th>
<th>ST1A5 (human)</th>
<th>ST1A1 (rat)</th>
<th>ST1A4 (mouse)</th>
<th>ST1A8 (rabbit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/ng cytosolic protein [μg/mg cytosolic protein]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1A3 (human)</td>
<td>120 ± 38.0 (n = 21) [4.1 ± 1.3]</td>
<td>6.4 ± 2.6 (n = 21) [0.22 ± 0.09]</td>
<td>270 ± 5.9 (male, n = 3) [9.1 ± 0.2]</td>
<td>66 ± 2.9 (male, n = 3) [2.3 ± 0.1]</td>
<td>250 ± 10.0 (male, n = 3) [8.3 ± 0.4]</td>
</tr>
</tbody>
</table>

TABLE 3
Sulfating activities toward catecholamines and their derivatives
Assays were performed with 10 μM substrates at pH 7.4. After 20-min incubations, reaction mixtures were separated on thin layer plates. The radioactive spots were quantified by BAS1000. Each value represents mean ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Substrate (10 μM)</th>
<th>Sulfating Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST1A5 (Human)</td>
</tr>
<tr>
<td>Tyramine</td>
<td>4.34 ± 0.02 [6199]</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>3.98 ± 0.16 [7955]</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>3.33 ± 0.07 [2559]</td>
</tr>
<tr>
<td>Dopamine</td>
<td>3.07 ± 0.18 [1025]</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>2.93 ± 0.05 [3254]</td>
</tr>
<tr>
<td>HMPG</td>
<td>0.69 ± 0.03 [95]</td>
</tr>
<tr>
<td>DOAPC</td>
<td>0.06 ± 0.00 [23]</td>
</tr>
</tbody>
</table>

HMPG, 4-hydroxy-3-methoxyphenylglycol; DOAPC, 3,4-dihydroxyphenylacetic acid.

TABLE 4
Sulfating activities toward serotonin and its derivatives
Assays were performed as described in Table 3. Each value represents mean ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Substrate (10 μM)</th>
<th>Sulfating Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST1A5 (Human)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>0.36 ± 0.06 [1205]</td>
</tr>
<tr>
<td>5-Hydroxytryptophol</td>
<td>0.36 ± 0.04 [13]</td>
</tr>
<tr>
<td>5-Hydroxyindole</td>
<td>0.59 ± 0.03 [6]</td>
</tr>
<tr>
<td>6-HM</td>
<td>1.84 ± 0.07 [39]</td>
</tr>
<tr>
<td>Harmol</td>
<td>3.93 ± 0.13 [78]</td>
</tr>
<tr>
<td>HIAA</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not detected (less than 10 pmol/mg/min).

TABLE 5
Sulfating activities toward p-NP and other small phenolic chemicals
Assays were performed as described in Table 3. Each value represents mean ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Substrate (10 μM)</th>
<th>Sulfating Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST1A5 (Human)</td>
</tr>
<tr>
<td>p-NP</td>
<td>0.09 ± 0.00 [2]</td>
</tr>
<tr>
<td>HMC</td>
<td>0.17 ± 0.01 [2]</td>
</tr>
<tr>
<td>Umbelliflorone</td>
<td>0.22 ± 0.00 [3]</td>
</tr>
<tr>
<td>Salicylamide</td>
<td>0.34 ± 0.01 [9]</td>
</tr>
<tr>
<td>Naringenin</td>
<td>3.62 ± 0.15 [50]</td>
</tr>
<tr>
<td>DHF</td>
<td>2.52 ± 0.08 [60]</td>
</tr>
<tr>
<td>Sakuranetin</td>
<td>0.04 ± 0.01 [11]</td>
</tr>
</tbody>
</table>

ST1A3. For dopamine, these forms showed about 8 times higher $K_m$ values than that of ST1A5. These values were comparatively similar to that of ST1A3 except that rabbit ST1A8 showed a very high $K_m$ value (more than 500 μM, not exactly determined). Moreover, all the ST1A forms showed low $V_{max}$ values (18–65 μM) and high $V_{max}$ values (11.9–18.3 nmol/nmol/min) for 6-HM.

Dissimilarities were observed in substrate specificities between human ST1A3 and experimental animal ST1A forms toward some chemicals, such as troglitazone (2.53 versus 0.28–0.81 nmol/nmol/min, respectively) and 4′-OH-PhIP (9.41 versus 0.47–1.36 nmol/nmol/min, respectively) (Table 7). ST1A forms from experimental animals showed lower activities toward estradiol (0.14–0.50 nmol/
The present study using recombinant ST1A sulfotransferases has shown that all the experimental animal ST1A forms (rat ST1A1, mouse ST1a4, and rabbit ST1A8) have substrate specificities similar to those of human ST1A3 compared with ST1A5. These forms exhibited substrate preferences for simple phenolic chemicals rather than for catecholamines (Tables 3 and 5). As shown in Table 4, experimental animal ST1A forms also showed substrate specificities comparable with human ST1A3 toward endogenous indoles. The activities catalyzed by human ST1A3 and experimental animal ST1A forms were decreased drastically in the presence of 10 μM mefenamic acid, but sulfating activities catalyzed by human ST1A5 were not inhibited by 100 μM mefenamic acid (Table 8). Hepatic contents were similar among human ST1A3 and experimental animal ST1A forms, whereas the content of human ST1A5 was about 19 times less than that of ST1A3 (Table 2).

To examine in detail the substrate specificities, apparent kinetic parameters for p-NP, dopamine, and 6-HM were determined and compared among these ST1A forms (Table 6). The $K_m$ value of human ST1A3 for p-NP was lower than that of human ST1A5 and the $K_m$ values for dopamine were opposite between the two forms. Their values were largely consistent with those previously reported (Veronese et al., 1994; Lewis et al., 1996; Fujita et al., 1999b). The ST1A forms from experimental animals also showed low $K_m$ values for p-NP, and they showed high $K_m$ values toward dopamine. Thus, these data also support the functional similarity of experimental animal ST1A forms to human ST1A3.

Although the relationship between the structure and function remains obscure, the residue 146 is proposed to be crucial in determining the substrate specificity of both human ST1A3 and ST1A5 (Dajani et al., 1998). Corresponding positions of all experimental animal ST1A forms are alanine as well as human ST1A3, whereas that of human ST1A5 is glutamic acid (Fig. 2). A new ST1A form, ST1A8, has been isolated from a male rabbit liver library and characterized in the present study. At amino acid sequence level, ST1A8 is more similar to the present study. At amino acid sequence level, ST1A8 is more similar to experimental animal species. Its enzymatic properties were similar, but not identical with human ST1A3, rat ST1A1, and mouse St1a4. For instance, $K_m$ value of ST1A8 for dopamine was much higher than that of ST1A3 (Table 2).
of human ST1A3, although $K_m$ values for $p$-NP were nearly the same between ST1A8 and ST1A3. In addition, the sulfating activities of ST1A3, ST1A1, and St1a4 were inhibited about 90 to 95% in the presence of 10 μM mefenamic acid, whereas those of ST1A8 were 30 to 50% inhibited (Table 8). The present study also shows first the enzymatic properties of mouse St1a4. This form shared enzymatic properties similar with rat ST1A1, but the gender difference pattern of hepatic contents was opposite from ST1A1.

As shown in Table 7, experimental animal ST1A forms showed clear differences on enzymatic properties from human ST1A3 toward chemicals such as troglitazone and 4'-OH-PhIP. These results suggest that human ST1A3 has broader substrate specificities than experimental animal ST1A forms. Human ST1A3 showed higher activities toward estradiol and troglitazone than experimental animal ST1A forms. These data suggest that ST1A3 is able to catalyze the sulfation of the phenolic chemicals with large molecular sizes.

On the other hand, human ST1A5 exhibited substrate preferences for biogenic amines such as dopamine, noradrenaline, and norepinephrine. But toward 4-hydroxy-3-methoxyphenylglycol, a metabolite of normetanephrine by monoamine oxidase, ST1A3, ST1A1, and St1a4 also showed similar activities. Moreover, the sulfating activity of ST1A5 toward 3,4-dihydroxyphenylacetic acid was lower than for the other ST1A forms. The amine residue of catecholamine is thus likely to affect the substrate specificities of ST1A5 strongly. In addition to catecholamines, ST1A5 catalyzed sulfations of harmol, 6-hydroxymelatonin, non-nitrogen-containing naringenin, and its derivative 5,7-dihydroxyflavonone effectively.

6-Sulfoxy melatonin is known to be a major urinary metabolite of melatonin (Arendt et al., 1985). All the ST1A forms showed low $K_m$ and high $V_{\text{max}}$ values for 6-hydroxymelatonin, which suggests that these ST1A forms may contribute to the excretion of melatonin.

Toward HIAA, sulfating activities catalyzed by all ST1A forms were not detected. We also examined for 5-hydroxy-tryptophan, L-dopa, and salicylic acid, but sulfating activities toward these chemicals were not detected or very limited (data not shown). Thus, these data suggest phenolic chemicals having carboxyl group are not preferred substrates for ST1A forms.

Human ST1A3 and ST1A5 have been studied in the present study, although another form, ST1A2, is known to express in human liver (Ozawa et al., 1995). ST1A2 shows similar enzymatic properties with ST1A3 and is a minor form compared with ST1A3. Human ST1A3 mRNA was found to be the major transcript form in the liver, representing 43 to 89% of the three related ST1A mRNAs (Ozawa et al., 1998).

The dog ST1A form (arbitrarily termed ST1A7) is reported to sulfate both simple phenols such as $p$-NP and catecholamines such as dopamine (Oddy et al., 1997). It shows, however, low specificities toward tyramine and serotonin. Thus, the dog ST1A7 seems to be an ortholog of human ST1A3 rather than ST1A5. In our preliminary examination, genomic Southern blot analysis using full-length St1a4 cDNA probe suggests a single gene copy of ST1A form in mice (data not shown). Southern blot analysis of rat genomic DNA also indicates the presence of a single gene (ST1A1 gene) copy per haploid genome (Khan et al., 1993). The present simultaneous comparison on substrate specificities and hepatic contents indicates little functional similarity between human ST1A5 and experimental animal ST1A forms. Distribution of human ST1A5 is mainly in extrahepatic tissues, such as brain, platelet, and small intestine (Young et al., 1984; Van Loon and Weinschilbourn, 1984; Aksoy and Weinschilbourn, 1995). Human plasma contains the highest concentration of catecholamines compared with those observed in most experimental animal species (Dousa and Tyce, 1988). These phenomena may imply the recent evolution of ST1A5 in primates for response to high demands of the metabolism.

In conclusion, experimental animal (rat, mouse, and rabbit) ST1A forms showed substrate specificities similar to human ST1A3 rather than ST1A5 for several phenolic chemistries and thus they are likely to be ST1A3 orthologs functionally. These forms, however, showed enzymatic properties distinctive from human ST1A3 on the sulfations of some chemicals such as troglitazone and 4'-OH-PhIP.

### References


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