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Sulfating-Activity and Stability of cDNA-Expressed Allozymes of Human Phenol Sulfotransferase, ST1A3*1 ([213]Arg) and ST1A3*2 ([213]His), Both of Which Exist in Japanese as Well as Caucasians[1]

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We recently found single amino acid substitutions ([213]Arg/His and [223]Met/Val) in polymorphic human phenol-sulfating phenol sulfotransferase (SULT: cDNAs encoding ST1A3, P PST or HAST1/2) among Caucasians and African-Americans. In a Japanese population ($n=143$), allele frequencies of [213]Arg and [213]His were 83.2 and 16.8%, respectively, but the [223]Val allele was not found. [213]His homozygosity was reportedly associated with both very low (>7-fold) sulfating activities of *p*-nitrophenol (at 4 μM) and low thermostability in platelets. Sulfating-activity determinations using recombinant [213]Arg- and [213]His-forms (ST1A3*1 and ST1A3*2, respectively) did not, however, reveal appreciable deficiency in [^{35}S]3′-phosphoadenosine 5′-phosphosulfate (PAPS)-dependent sulfation of *p*-nitrophenol (4 μM) by ST1A3*2 (7.5 *vs.* 10.2 nmol/min/nmol SULT for ST1A3). Kinetic parameters for *p*-nitrophenol for *p*-nitrophenol sulfation supported the slight decrease in sulfating activities at 4 μM (K_m, 0.82 *vs.* 1.75 μM; V_{max}, 13.2 *vs.* 13.1 nmol/min/nmol SULT, respectively, for ST1A3*1 and *2). *p*-Nitrophenyl sulfate-dependent 2-naphthol sulfation by ST1A3*2 was 69% of that by ST1A3*1 ($p<0.05$). However, ST1A3*2 was remarkably unstable at 45 and 37°C as compared to ST1A3*1. The lower *p*-nitrophenol sulfating activity of ST1A3*2 may explain the lower platelet *p*-nitrophenol sulfation in ST1A3*2 homozygotes. Protein instability and ST1A3 gene regulation may be both involved in the polymorphism of *p*-nitrophenol sulfation in human tissues.

Key words: genetic polymorphism, molecular cloning, phenol sulfotransferase, recombinant enzyme, thermostability.

Human cytosolic thermostable phenol sulfotransferase (TS-PSULT), using 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as a sulfate donor, is known to catalyze both the detoxification of endobiotics and xenobiotics and the metabolic activation of therapeutic drugs (*e.g.*, minoxidil) and *N*-hydroxy derivatives of carcinogenic aromatic amines (*1*).

Molecular cloning studies revealed that a protein encoded by P PST (*2*), HAST1 and 2 (*3, 4*), and ST1A3 (*5, 6*) cDNAs corresponded to the major hepatic form of TS-PSULT. The existence of allelic variants was subsequently shown for the TS-PSULT gene encoding four allozymes, ST1A3*1[3] ([37]Arg, [213]Arg, and [223]Met), ST1A3*2 ([37]Arg, [213]His, and [223]Met), ST1A3*3 ([37]Arg, [213]Arg, and [223]Val), and ST1A3*4 ([37]Gln, [213]Arg, and [223]Met) (*5, 7*). The designation of the allozymes, *1, *2, *3, and *4, was according to the report by Raftogianis *et al.* (*7*). The most frequently observed allozyme was ST1A3*1, and the next most frequent was ST1A3*2 (allele frequencies: 0.67 *vs.* 0.31, respectively), whereas the frequency of the *3 and *4 alleles was reportedly 0.01 or less (*7*).

Remarkable individual differences and multimodal dis-

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[3] We used a SULT nomenclature system for human TS-PSULT according to the report by Yamazoe *et al.* (*5*) in the present paper, as a mutual nomenclature system has been unavailable thus far.

Abbreviations: pNPS, *p*-nitrophenyl sulfate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PhIP, 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine; SULT, sulfotransferase; TS-PSULT, thermostable phenol sulfotransferase.

tribution have been observed in TS-PSULT activities due to genetic polymorphism in human liver (*8, 9*) and platelets (*7, 9-11*). Heat stability of TS-PSULT was also documented as characteristic of sulfation pharmacogenetics in human platelets (*7, 9-11*). Platelet TS-PSULTs showed the following phenotypes (*7*): (i) high activity and high thermostability, (ii) low activity and high thermostability, and (iii) low activity and low thermostability. Among the four allozymes, ST1A3*2 (^{213}His-type) was reported to be associated with both very low TS-PSULT activity and low thermal stability in human platelets (*7*).

We also detected variant hepatic mRNAs encoding ST1A3 (^{213}Arg/His and ^{223}Met/Val) (*12*). Genotyping methods developed by us revealed that the frequency of the allele encoding ^{213}His was *ca.* 0.32, while that of ^{223}Val allele was only 0.03. The frequencies of these different alleles were largely consistent with those reported by Raftogianis *et al.* (*7, 12*). In this study, we investigated the frequencies of these different alleles in a Japanese population ($n=143$) and found both Arg and His alleles for codon 213, but no individuals with the ^{223}Val allele. Thus, the ^{213}Arg/His polymorphism proved to be present in both Caucasians and Asians.

The above results led us to compare the sulfating capacities of the ST1A3*1 and *2 allozymes and to examine whether the observed low thermostability and the low TS-PSULT activities in platelets are properties of the ^{213}His-type enzyme itself. We compared catalytic properties of recombinant ST1A3*1 and *2 allozymes prepared by using cDNA-expression systems in *Escherichia coli*, and quantified the allozymes by Western blotting. This enabled us to compare directly the sulfating capacities of the two allozymes on a molecular basis. Thus, the present results provided clues to the molecular mechanisms involved in sulfation polymorphism in human tissues.

EXPERIMENTAL PROCEDURES

Genotyping of Japanese Individuals for ^{213}Arg/His and ^{223}Met/Val Alleles of Human ST1A3 Genes—Genotyping for ^{213}Arg/His and ^{223}Met/Val of the ST1A3 gene was performed as previously described (*12*). The Japanese individuals examined for ^{213}Arg/His and ^{223}Met/Val comprised 143 subjects who had visited medical clinics in Kitakyushu City between September 1993 and April 1995 for regular medical health checks (including blood and urine tests). They comprised 79 males and 64 females (mean age 61.0 years) who showed no evidence of malignancy or inflammatory pathology. All participants gave informed consent after receiving an explanation of the nature of the study.

Genomic DNAs, isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction, were used for the genotyping.

Isolation of a cDNA Encoding Full-Length ^{213}Arg- and ^{213}His-Type Human ST1A3—cDNAs were prepared from poly(A)$^+$ RNA (1 μg) extracted as previously described (*12*) from the liver of an individual possessing both ^{213}Arg and ^{213}His alleles of the ST1A3 gene. cDNA fragments coding for an entire coding region were made by PCR amplification procedures described previously (*12*) using forward (5′-GGAATTCAGGAACATGGAGCTGATCCA-3′) and reverse (5′-ACGAATTCTCGAACTCCTGGGCTC-AAA-3′) primers. The amplified cDNAs were ligated with pUC19 plasmids for clones of cDNAs encoding both Arg and His at codon 213. Plasmids with a ST1A3 insert were screened by PCR-RFLP by treatment with *Hae*II of the PCR product (*ca.* 230 bp) amplified with a forward (5′-GA-GTGGTGGGAGCTGAGCCG-3′) and a reverse (5′-GGAG-ATGCTGTGGTCCATGA-3′) primer and a candidate plasmid as a template. PCR products with ^{213}Arg can be digested with *Hae*II, but those with ^{213}His can not. Thus, we obtained full-length cDNAs encoding ^{213}Arg and ^{213}His-type ST1A3 allozymes.

Construction of E. coli Expression Vectors Carrying Human ST1A3—We attempted to express ^{213}Arg- and ^{213}His-type ST1A3 in *E. coli*, so that the translation should be initiated with the native ATG present in the ST1A3 cDNAs. We altered the nucleotide located two bases upstream of the translation initiation codon of the cDNAs from A to C by PCR amplification using a forward (5′-ACC-ATGGAGCTGATCCAGGACA-3′) and a reverse primer (5′-GGAATTCAAGCTTCGAACTCCTGGGCTCAAA-3′), in order to ligate the cDNAs with an *E. coli* expression vector, pTrcHisB (Invitrogen, Carlsbad, CA) as a *Nco*I-*Eco*RI fragment. The PCR reaction mixture (100 μl) consisted of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 2.5 U of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA), and the primers mentioned above. The mixture was heated at 95°C for 1 min, then amplification was carried out with 20 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 45 s, and primer extension at 72°C for 2 min. After the 20 cycles, the mixtures were kept at 72°C for 7 min. The resultant PCR products and pTrcHisB were each treated with *Nco*I and *Eco*RI and ligated using a DNA ligation kit (Takara-Shuzo, Kyoto). We also modified the terminal sequences of ^{213}Arg-type ST1A3 cDNA by PCR amplification with a forward (5′-GGAATTCAGGAACAT-GGAGCTGATCCA-3′) and a reverse (5′-GGAATTCAAG-CTTCGAACTCCTGGGCTCAAA-3′) primer in order to place *Eco*RI and *Hin*dIII sites at the 5′- and 3′-termini, respectively. The *Eco*RI-*Hin*dIII fragment of wild-type cDNA was ligated with pTrcHisB vector treated with *Eco*RI and *Hin*dIII to produce ^{213}Arg-type ST1A3 enzyme with an additional 47 amino acids containing a stretch of six histidine residues on the N-terminus side (His-tagged ST1A3). This enabled us to purify the His-tagged ST1A3 to electrophoretical homogeneity and to determine contents of the ^{213}Arg- and ^{213}His-type ST1A3. Nucleotide sequences of the cDNA inserts used in the expression systems were determined using an automatic sequencer (Perkin-Elmer ABI 377). The only difference in the encoded amino acid sequences was Arg/His at codon 213. Their deduced amino acids at codons 37 and 223 were Arg and Met, respectively. Thus, the cDNAs for ^{213}Arg- and ^{213}His-type enzymes were verified to encode ST1A3*1 and ST1A3*2, respectively.

*cDNA-Mediated Expression and Purification of ST1A3*1 and *2*—Cultures (150 ml) of plasmid-carrying bacteria in the logarithmic growth phase were treated by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside to induce protein expression and further cultured for 4 h at 37°C. The bacteria producing ST1A3*1 and *2 were lysed by sonication on ice in 10 mM triethanolamine (pH 7.4), 1 mM DTT, and 0.25 M sucrose. After removal of bacterial debris by centrifugation at 3,000×g for 30 min, the expressed ST1A3 allozymes were partially purified on col-

umns of DEAE-Sepharose CL-6B (7 cm × 1.5 cm, Pharmacia Biotech, Uppsala, Sweden). After application of the samples, the columns were washed extensively with 10 mM potassium phosphate buffer (pH 7.5) containing 1 mM DTT and 0.25 M sucrose (Buffer A). The proteins were eluted by stepwise increase of NaCl concentration (100, 150, 200, and 250 mM) in Buffer A. ST1A3*1 and *2 were both eluted at 150-200 mM NaCl. The active fractions were ascertained using the colorimetric 2-naphthol sulfation assay (*p*-nitrophenyl sulfate as a sulfate donor). Protein concentrations of the partially purified ST1A3*1 and *2 were determined to be 0.046 and 0.225 mg/ml, respectively, using a Bio-rad Protein Assay kit (Bradford method).

His-tagged ST1A3*1 was isolated from bacterial culture (150 ml) using Invitrogen's ProBond™ resin under the native conditions recommended by the manufacturer. Briefly, the bacteria were lysed in 10 ml of Native Binding Buffer [20 mM sodium phosphate buffer (pH 7.8) containing 500 mM NaCl], then treated with 100 μg/ml egg white lysozyme and 5 μg/ml RNase. The His-tagged proteins were batch-bound to the resin pre-equilibrated with the Native Binding Buffer. The protein-bound resin was washed three times with 4-ml portions of the Binding Buffer, then three times with 4-ml portions of Native Wash Buffer [20 mM sodium phosphate (pH 7.8) containing 500 mM NaCl]. After separation of the resin from the supernatant, the His-tagged ST1A3*1 was eluted and collected in 1-ml fractions by applying consecutively 5 ml of each of the four Imidazole Elution Buffers with increasing imidazole concentration [50 mM (fractions 1-4), 200 mM (fractions 5-9), 350 mM (fractions 10-14) and 500 mM (fractions 15-24)]. The His-tagged proteins were eluted at the imidazole concentration of 350-500 mM. The proteins in the fractions were analyzed by SDS-PAGE and Coomassie blue staining after being concentrated fivefold by ultrafiltration (Fig. 1). The His-tagged ST1A3*1 in fraction 17, which was electrophoretically homogeneous, was used as a standard for determination of partially purified ST1A3*1 and *2 by immunoblotting. Protein concentration in the column fractions was measured by the fluorescein method (*13*) using BSA as a standard. Concentration of the purified and concentrated His-tagged ST1A3*1 (fraction 17) was 41.0 μg/ml.

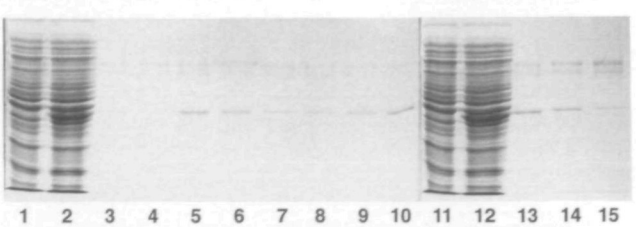

Fig. 1. **SDS-PAGE of column fractions of Invitrogen's ProBond™ resin for purification of bacterially expressed His-tagged ST1A3*1.** His-tagged ST1A3*1 was purified using an Invitrogen's ProBond™ resin column as described in "EXPERIMENTAL PROCEDURES." After being concentrated by *ca.* 5-fold and denatured, 12.5 μl of each fraction was applied on two SDS-PAGE gels (lanes 1-10 and lanes 11-15) together with crude 3,000 × *g*-supernatant of lysate of bacteria transfected with (lanes 2 and 12) or without (lanes 1 and 11) ST1A3*1 cDNA. Lanes 3-10, fractions 10-17; lanes 13-15, fractions 18-20.

Western Blotting—Partially purified cDNA-expressed enzymes, electrophoretically homogeneous His-tagged ST1A3*1, and human liver cytosols were separated on SDS-PAGE and transferred to nitrocellulose membranes. The cytosols were prepared as previously described (*12*) from human livers obtained as excess surgical samples from the J.L. McClellan Memorial Veterans' Administration Medical Center or from the USA Cooperative Human Tissue Network. The membranes were probed with rabbit antibodies (Alpha Diagnostic Intl., San Antonio, TX) raised against a peptide antigen (KVHPEPGTWDSFC) which is specific to ST1A3, and which differs in three amino acids from the corresponding ST1A2 (*5, 12*) sequence (KVYPHPGTWESFC). Immunoreactive proteins were visualized according to ECL™ Western blotting protocols (Amersham International plc, Buckinghamshire, England). The intensities of immunodetectable proteins were analyzed with the aid of ZERO-Dscan™ software (Scanalytics, Billerica, MA) in a Chemiluminescence Detection System, DIANAII (Raytest, Straubenhardt, Germany).

Sulfotransferase Assays—Sulfations of *p*-nitrophenol (4 μM, *14*), 2-naphthol (100 μM), bisphenol A (100 μM), acetaminophen (1 mM, *15*), (+)- and (−)-isoproterenol (500 μM, *15*), and minoxidil (1 mM, *15*) were measured in the presence of 20 μM [^{35}S]PAPS (diluted to 50 mCi/mmol of 2 Ci/mmol, Dupont-New England Nuclear, Wilmington, DE) according to a barium precipitation method (*16*). All reactions were performed for 10 min in a total volume of 100 μl. The reaction mixture for bisphenol A sulfation consisted of 100 μl of 50 mM Tris-HCl (pH 7.4) containing 5 mM $MgCl_2$, 1 mM DTT, 100 μM bisphenol A, and 20 μM [^{35}S]PAPS (50 mCi/mmol), and 40 μl of enzyme solution containing either ST1A3*1 or *2 (1.8 and 9 μg proteins of ST1A3*1- and ST1A3*2-enzyme solution, respectively). The reaction was initiated by the addition of [^{35}S]PAPS and terminated after 10 min by the addition of 20 μl of 0.1 M barium hydroxide. Subsequent barium precipitation was done according to the method by Foldes and Meek (*16*). *p*-Nitrophenyl sulfate (5 mM)-dependent sulfation of 2-naphthol (100 μM) was determined colorimetrically as described previously (*17*). For most substrates, 40 μl of the ST1A3*1 solution was used; but for [^{35}S]PAPS-dependent *p*-nitrophenol and *p*-nitrophenyl sulfate-dependent 2-naphthol sulfations, 10 μl of the ST1A3*1 solution was used to optimize the reaction conditions. For the ST1A3*2 solution, 40 μl was the optimal volume for all the substrates examined. In order to compare sulfating capacities of ST1A3*1 and *2 toward various substrates, allozyme contents in the enzyme solutions used for enzymatic activity measurements were determined on the basis of Western blotting. Sulfating-activities were calculated based on the enzymatic activities and the amounts of the ST1A3*1 and *2 allozymes, and expressed as nmol substrate sulfated/min/nmol SULT. All the assays were done in triplicate experiments, and the results were expressed as mean ± SD. Mean apparent K_ms for PAPS and *p*-nitrophenol for *p*-nitrophenol sulfation catalyzed by the allozymes were determined in the presence of various concentrations (0.625-20 μM) of PAPS (in the presence of 4 μM *p*-nitrophenol) or various concentrations (0.5-4 μM) of *p*-nitrophenol (in the presence of 20 μM [^{35}S]PAPS), respectively, from Lineweaver-Burk plots for three separate experiments.

RESULTS AND DISCUSSION

Table I shows the frequencies of ^{213}Arg/His and ^{223}Met/Val alleles of the ST1A3 gene found in a Japanese population. The ^{213}His allele was found as an infrequent allele in the Japanese population, with slightly lower frequency than those found in Caucasian and Nigerian populations (*7, 12, 18*). The ^{223}Val allele was not found in the Japanese studied, while frequency of this allele in American Caucasians was 0.01-0.03 (*7, 12*). These results imply that difference in the catalytic properties of ^{213}Arg- and ^{213}His-type enzymes should further be evaluated for various substrates, since ethnic variability of drug metabolizing capacities is, in some cases, known to cause serious problems in the developmental stage of therapeutic drugs (*19*). We, therefore, compared sulfating capacities of the ^{213}Arg- and ^{213}His-type enzymes (ST1A3*1 and ST1A3*2, respectively) prepared using cDNA-mediated expression systems in *E. coli*.

TABLE I. **Number of Japanese individuals with ^{213}Arg/His and ^{223}Met/Val alleles of human ST1A3 gene.** Numbers in parentheses represent percentage of total. Genotyping for codon 213 and 223 was done by the method of polymerase chain reaction-restriction fragment length polymorphism described by Ozawa *et al.* (*12*).

		Japanese (n=143)
^{213}Arg/His	aa[a]	99 (69.2)
	ab	40 (28.0)
	bb	4 (2.8)
^{223}Met/Val	aa	143 (100.0)
	ab	0 (0.0)
	bb	0 (0.0)

[a]Frequent and infrequent alleles on codons 213 and 223 denoted as a and b, respectively. For codons 213 and 223, Arg and Met were the frequent alleles, respectively.

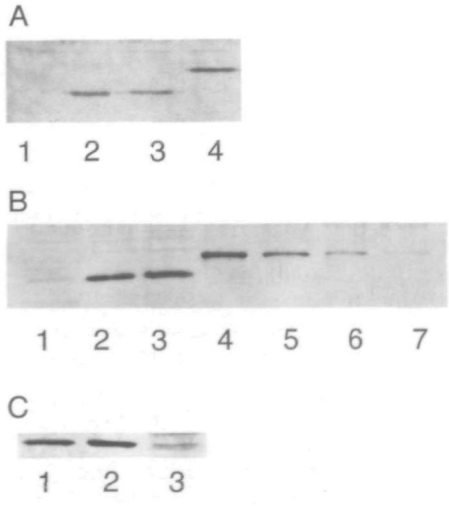

Fig. 2. **Western blot analyses of crude supernatant of *Escherichia coli* expressing ST1A3*1, ST1A3*2, and His-tagged ST1A3*1 (A), and partially purified ST1A3*1 and *2 together with electrophoretically homogeneous His-tagged ST1A3*1 (B), or together with human liver cytosol (124 μg) (C).** A: Crude 3,000×g-supernatant of lysate of null (lane 1) bacteria and those transfected with cDNAs encoding ST1A3*1 and *2 and His-tagged ST1A3*1 (lanes 2, 3, and 4, respectively). In each lane, 1.6 μg of protein was loaded. B: Partially purified ST1A3*1 and *2 (lanes 2 and 3, 0.14 and 2.8 μg of protein, respectively) and electrophoretically homogeneous His-tagged ST1A3*1 (lanes 4-7; 1.63, 0.82, 0.41, 0.20 pmol sulfotransferase, respectively) to determine contents of the ST1A3 allozymes in partially purified enzyme solutions together with cytosolic proteins (16 μg) from a human liver (lane 1). Mobilities of the major immunoreactive protein in human liver cytosol and the partially purified ST1A3*1 and *2 were identical under the experimental conditions. C: Partially purified ST1A3*1 and *2 (lanes 1 and 2, 0.14 and 2.8 μg of protein, respectively) and 124 μg of cytosolic proteins prepared from Pooled Human Liver S9 (Gentest, Woburn, MA) by 105,000×g centrifugation (lane 3). A major immunoreactive protein in human liver cytosol and ST1A3*1 and *2 migrated at the same position under the same experimental conditions as those for B.

To confirm expression of ST1A3*1 and *2 in the *E. coli* expression systems used in this study, we performed immunoblot analysis of crude supernatant of 3,000×g centrifugation using human ST1A3 antibodies whose antigen peptide sequence was the most specific to human ST1A3. Bacteria transfected with expression vector pTrcHisB without SULT cDNA insert failed to produce any immunoreactive protein (Fig. 2A, lane 1). On the other hand, ST1A3*1 (Fig. 2A, lane 2), ST1A3*2 (Fig. 2A, lane 3), and His-tagged ST1A3*1 (Fig. 2A, lane 4) were efficiently expressed. These results verified the expression of ST1A3*1 and *2 in the currently used prokaryotic expression systems. TS-PSULT activities of these bacterially expressed proteins were measured toward 2-naphthol using p-nitrophenyl sulfate as a sulfate donor, which has been shown to be specifically catalyzed by TS-PSULT (*17*). ST1A3*1 and *2 catalyzed 2-naphthol sulfation 5 to 16

TABLE II. **p-Nitrophenyl sulfate-dependent sulfation of 2-naphthol by crude supernatant (3,000×g) of *Escherichia coli* expressing ST1A3*1 and *2 and His-tagged ST1A3*1.** To examine whether ST1A3*1 and *2 and fused ST1A3*1 expressed in *E. coli* were functionally active, crude lysates of cDNA-transfected bacteria were assayed for p-nitrophenyl sulfate-dependent 2-naphthol sulfation (*17*).

	(nmol/min/mg protein)
ST1A3*1	330.8
ST1A3*2	96.5
His-tagged ST1A3*1	260.4
pTrcHisB without SULT cDNA	<3
Human liver (n=12)	20.3 (Range 5-29)

TABLE III. **Sulfating activities toward substrates with phenolic and other structures by partially purified ST1A3*1 and *2 expressed in *Escherichia coli*.** Sulfating activities to each substrate were measured as described in "EXPERIMENTAL PROCEDURES." In order to compare sulfating capacities of ST1A3*1 and *2, sulfating activities were expressed as nmol/min/nmol ST1A3 after the determination of the amount of the allozymes used by Western blot analyses.

	ST1A3*1	ST1A3*2
p-Nitrophenol	10.2±1.2	7.5±2.0
2-Naphthol/PAPS[a]	3.8±1.9	2.5±1.4
2-Naphthol/pNPS[b]	350.6±62.4	241.2±25.7[c]
Bisphenol A	1.6±0.5	1.5±0.1
Acetaminophen	6.2±0.6	6.9±0.1
(+)-Isoproterenol	8.1±0.1	9.4±0.3[d]
(−)-Isoproterenol	4.0±0.4	4.1±0.3
Minoxidil	5.8±0.3	8.5±0.2[d]

[a][^{35}S]PAPS was used as a sulfate donor. [b]p-Nitrophenyl sulfate (pNPS) was used as a sulfate donor and the activity was measured colorimetrically (*17*). [c]$p<0.05$. [d]$p<0.001$.

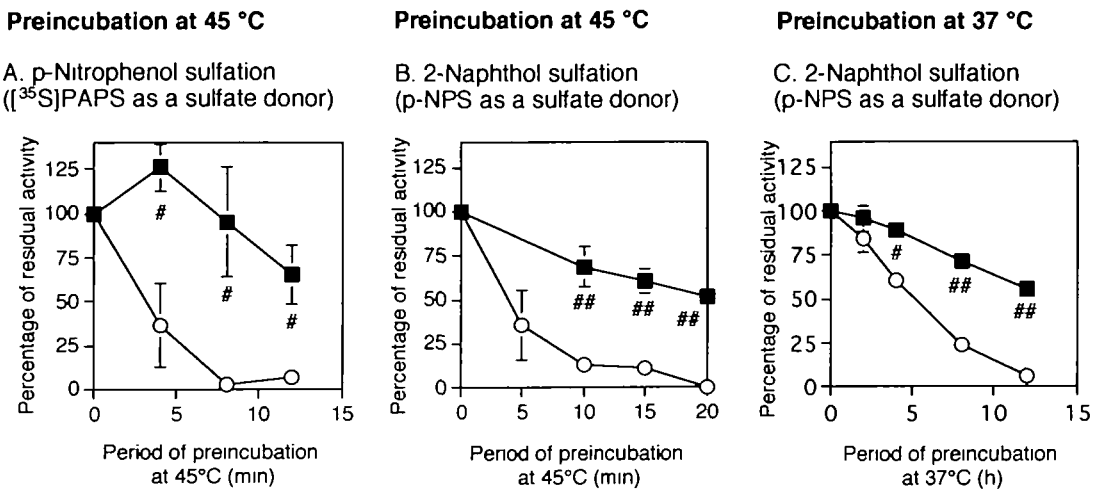

Fig. 3. **Stability of bacterially expressed ST1A3*1 and *2 after preincubation at 45°C (A and B) or 37°C (C) for various periods using [^{35}S]3′-phosphoadenosine 5′-phosphosulfate (PAPS)-dependent *p*-nitrophenol sulfation (A) and *p*-nitrophenyl sulfate-dependent 2-naphthol sulfation (B and C) as marker activities.** Closed and open symbols represent data for ST1A3*1 and *2, respectively. # and ## indicate statistically significant difference ($p<0.01$ and $p<0.002$, respectively) between data for ST1A3*1 and *2 at the corresponding time. A: Partially purified ST1A3*1 and *2 were preincubated at 45°C for various time periods before determination of [^{35}S]PAPS-dependent *p*-nitrophenol sulfation. The data are expressed as percentage of residual enzymatic activity. The activity (mean ± SD) at 0 time was 9.4 ± 1.0 and 7.3 ± 0.7 (nmol/min/nmol SULT) for ST1A3*1 and *2, respectively, in triplicate determinations. B: Partially purified ST1A3*1 and *2 were preincubated as in A before determination of *p*-nitrophenyl sulfate-dependent 2-naphthol sulfation. The data are expressed as percentage of residual enzymatic activity. The activity (mean ± SD) at 0 time was 350.2 ± 62.4 and 226.2 ± 15.0 (nmol/min/nmol SULT) for ST1A3*1 and *2, respectively, in triplicate determinations. C: Partially purified ST1A3*1 and *2 were preincubated at 37°C before determination of *p*-nitrophenyl sulfate-dependent 2-naphthol sulfation. The data are expressed as percentage of residual enzymatic activity. The activity (mean ± SD) at 0 time was 359.5 ± 22.8 and 301.3 ± 0.7 (nmol/min/nmol SULT) for ST1A3*1 and *2, respectively, in triplicate determinations.

TABLE IV. **Kinetic parameters for *p*-nitrophenol sulfation by partially purified ST1A3*1 and *2 expressed in *Escherichia coli*.** [^{35}S]PAPS-dependent *p*-nitrophenol sulfations were measured in order to determine kinetic parameters for the reaction with various concentrations of *p*-nitrophenol as described in "EXPERIMENTAL PROCEDURES." In order to compare sulfating capacities of ST1A3*1 and *2, V_{max} values were expressed as nmol/min/nmol ST1A3 after the determination of the amount of the allozymes used by Western blot analyses.

	K_m (μM)	V_{max} (nmol/min/nmol SULT)	V_{max}/K_m
ST1A3*1	0.82	13.2	16.9
ST1A3*2	1.75	13.1	7.5

times more efficiently than human liver cytosol (Table II). Thus, the bacterial expression systems used yielded functionally active cDNA-expressed ST1A3*1 and *2.

To compare substrate specificities of ST1A3*1 and *2, we measured enzyme activities toward various substrates, after partial purification of the allozymes by DEAE-Sepharose CL-6B according to Lewis *et al.* (*15*). The fractions active in the colorimetric assay (2-naphthol sulfation) were used as the enzyme source. Amounts of immunodetectable ST1A3*1 and *2 were determined by comparison with that of His-tagged ST1A3*1 which had been purified to electrophoretical homogeneity as described in "EXPERIMENTAL PROCEDURES." Figure 2B shows representative immunoblots of three separate protein transfers. Both ST1A3*1 and *2 proteins (lanes 2 and 3, respectively) migrated at the same position as the major immunoreactive protein in human liver cytosol (16 μg, lane 1), even after purification by anion exchange chromatography. The identical mobility on Western blots was reproduced in another experiment using larger amount of human liver cytosol (124 μg, Fig. 2C, lane 3). ST1A3*2 was eluted from the DEAE-Sepharose column as a broad peak, and its specific activity was not raised during the chromatography. This may indicate a difference in protein conformation in aqueous solution because of the ^{213}Arg/His polymorphism. Since considerable difference was anticipated in the specific contents of the ST1A3 allozymes after the purification, 0.14 and 2.81 μg of proteins of ST1A3*1 and *2 enzyme solutions used for enzymatic activity measurements were applied on SDS-PAGE gels to yield comparable intensities of immunoreactive proteins. Thus, mean contents of ST1A3*1 and *2 were 6.74 and 0.52 nmol SULT/mg protein, respectively, in the solutions used for enzyme activity measurements. Concentrations of the allozymes were calculated to be 0.31 and 0.12 pmol SULT/μl for ST1A3*1 and *2, respectively. Then, sulfating-activities of ST1A3*1 and *2 were determined toward various substrates. The results are listed in Table III as mentioned in "EXPERIMENTAL PROCEDURES." The difference in the catalytic activities of ST1A3*1 and *2 on the basis of nmol ST1A3 allozyme was statistically significant in three cases: ST1A3*2 sulfated 2-naphthol (*p*-nitrophenyl sulfate-dependently) at significantly lower rate ($p<0.05$) and (+)-isoproterenol and minoxidil at significantly higher rate ($p<0.001$).

It is important to determine whether *in vivo* functions of these allozymes result in any difference in pharmacokinetics among ST1A3*1 and *2 homozygotes and heterozygotes. Loss of enzyme activities was examined by preincubation of the partially purified ST1A3*1 and *2 at 45°C for various periods. After the treatment, residual [^{35}S]-

PAPS-dependent and *p*-nitrophenyl sulfate-dependent sulfating activities were measured using *p*-nitrophenol and 2-naphthol as substrates, respectively. As shown in Fig. 3, A and B, ST1A3*2 was found to be significantly more thermolabile than ST1A3*1 using the bacterially expressed ST1A3*1 and *2 as enzyme sources. Platelet *p*-nitrophenol sulfating activities derived from ST1A3*2 homozygotes were reportedly more unstable than those from ST1A3*1 homozygotes and ST1A3*1/*2 heterozygotes (*7*). Our results on thermostability were consistent with the platelet results. We also investigated the stability and half-lives of ST1A3*1 and ST1A3*2 after preincubation at 37°C (Fig. 3C). ST1A3*2 was clearly more unstable than ST1A3*1, though a longer period of incubation was required to bring about the loss of enzymatic activities. Half-lives of ST1A3*1 and *2 were 13.7 h and 5.8 h, respectively, suggesting that ST1A3*2 allozyme may have shorter biological half-life in human tissues than ST1A3*1. *p*-Nitrophenol sulfations were catalyzed by ST1A3*1 and *2 with mean apparent K_m values for PAPS of 13.5 and 20.1 μM, respectively, in three separate experiments. K_m values for *p*-nitrophenol were 0.82 and 1.75 μM for the same reaction catalyzed by ST1A3*1 and *2, respectively (Table IV). V_{max} values for *p*-nitrophenol sulfations by ST1A3*1 and *2 were almost identical (13.2 and 13.1 nmol/min/nmol SULT, respectively, Table IV). Four micromolar *p*-nitrophenol, which is likely to be a saturating concentration for both allozymes, is generally employed for human TS-PSULT activity measurement (*7, 9-12, 14*). The current kinetic analyses for *p*-nitrophenol sulfation thus supported the lack of remarkable deficiency of ST1A3*2 in sulfating the substrate at 4 μM. Except for our previous study (*12*), where 20 μM [^{35}S]PAPS was used, the concentration of 0.4 μM PAPS, much lower than K_m, was employed (*7, 9-11, 14*). We also used PAPS concentrations as low as 0.6 μM. The difference in sulfating-activities of *p*-nitrophenol (4 μM) between ST1A3*1 and *2 was less than twofold (0.80 ± 0.08 and 0.52 ± 0.09 nmol/min/nmol SULT ($p<0.05$), respectively, from three separate experiments).

Thus the present results did not show a marked deficiency of ST1A3*2 relative to ST1A3*1 in the sulfating capacities toward the concentrations of the substrates examined under the present experimental conditions. On the other hand, ST1A3*2 was clearly more thermolabile than ST1A3*1 prepared using our cDNA-expression systems, in accordance with the results of Weinshilboum's research group (*7, 9-11, 14*). It was, therefore, suggested that the very low (>7-fold) TS-PSULT activity trait in the platelet of ST1A3*2 homozygotes was unlikely to be fully explained by the observed difference in *p*-nitrophenol-sulfating activities catalyzed by the cDNA-expressed ST1A3*1 and *2 used in our present study. We and others showed that sulfations of *p*-nitrophenol and *N*-hydroxy-PhIP were highly correlated with the contents of TS-PSULT proteins in human livers (*20*) and platelets (*21*). ST1A3*2 had a shorter half-life than ST1A3*1 in the incubation at 37°C (Fig. 3C). It is possible, therefore, that steady-state levels of ST1A3*2 in platelets from ST1A3*2 homozygotes were low due to its instability in the tissue and thus resulted in the low activity trait in those individuals. Our present results provided useful information on ethnic difference in variant alleles encoding ^{213}Arg- and ^{213}His-type ST1A3 and also gave an important clue for elucidation of the molecular mechanisms of the human sulfation polymorphism.

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