J. Biochem. 123, 740-746 (1998)

Molecular Cloning, Expression, and Enzymatic Characterization of Rabbit Hydroxysteroid Sulfotransferase AST-RB2 (ST2A8)[1]

Kouichi Yoshinari,* Kiyoshi Nagata,*,[2] Toshifumi Shiraga,† Kazuhide Iwasaki,† Takehisa Hata,† Makoto Ogino,* Rika Ueda,* Ken-ichi Fujita,* Miki Shimada,* and Yasushi Yamazoe*

**Division of Drug Metabolism and Molecular Toxicology, Faculty of Pharmaceutical Sciences, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai 980-8578; and †Department of Pharmacokinetics and Drug Metabolism, Pharmaceutical and Pharmacokinetic Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532-8514*

Received for publication, November 7, 1997

Cytosolic sulfotransferases, which consist of at least three gene families, play a major role in activation and detoxification of both endogenous and exogenous chemicals. We recently purified a rabbit sulfotransferase, AST-RB2, showing high activities to both hydroxysteroids and amines. To characterize this enzyme, a rabbit cDNA library was screened using anti-AST-RB2 antibodies. The isolated cDNA was judged to encode AST-RB2 (ST2A8) based on the amino acid sequences of peptide fragments obtained from purified AST-RB2. The cDNA showed high similarity to other mammalian hydroxysteroid sulfotransferases (ST2) at the amino acid level (58-68%), but low similarity to aryl sulfotransferases (ST1) (less than 37%). The protein expressed in *Escherichia coli* catalyzed sulfation of typical ST2 substrates. Therefore, ST2A8 was judged to belong to the ST2 family from both its primary structure and substrate specificity. The ST2A8 protein expressed in *E. coli* clearly differed from rat ST2A1 and ST2A2 on its localization (cytosol/insoluble fraction ratio). ST2A8 had no activity to lithocholate, but showed the highest catalysis on dehydroepiandrosterone and testosterone among the four forms (ST2A1, ST2A2, ST2A3, and ST2A8), indicating a clear difference between ST2A forms in substrate specificity to endogenous chemicals.

Key words: bacterial expression, cDNA, hydroxysteroid, rabbit, sulfotransferase.

Sulfation plays a major role in detoxification and activation of both endogenous and exogenous chemicals, such as hormones, neurotransmitters, drugs, and carcinogens (*1-3*). These reactions are mediated by cytosolic sulfotransferases, which transfer sulfate of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to substrates. Based on similarity of their deduced amino acid sequences, cytosolic sulfotransferases are known to constitute a gene superfamily (*4*). These enzymes have been classified into two families, ST1[3] (SULT1) and ST2 (SULT2) in mammals (*4*). The former contains sulfotransferases mainly catalyzing the sulfation of phenolic compounds and is subdivided into four subfamilies, ST1A, ST1B, ST1C, and ST1E. The latter family contains enzymes predominantly catalyzing the sulfation of hydroxysteroids and includes a single subfamily, ST2A.

Recently, we purified two sulfotransferases, AST-RB1 and AST-RB2, from male rabbit livers (Ref. *5* and Shiraga *et al.*, in preparation). These enzymes mediated *N*-sulfation of amino compounds. Purified AST-RB1 catalyzed *N*-sulfation of various types of amines, 4-phenyl-1,2,3,6-tetrahydropyridine (PTHP) (alicyclic amine), desipramine (DMI) (alkyl amine), and aniline (aryl amine), suggesting the existence of a new type of sulfotransferase that selectively mediates sulfamate formation. These results prompted us to isolate the cDNA encoding AST-RB1. The deduced amino acid sequence of the isolated cDNA showed less than 38% identity with those of other mammalian sulfotransferases reported previously (*6*). These data indicate that a third gene family of mammalian sulfotransferases (ST3A1) encodes AST-RB1, which predominantly catalyzes *N*-sulfa-tion of amines (*6*). On the other hand, AST-RB2 catalyzes sulfation of dehydroepiandrosterone (DHEA) as well as DMI, suggesting a clear difference in catalytic properties between AST-RB1 and AST-RB2. Hydroxysteroid sulfotransferases mediate sulfation of various endogenous alcohols, such as pregnenolone, DHEA, and bile acids (*2, 7, 8*). Sulfation is now considered to be essential for the biosynthesis, translocation as well as

[1] The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number AB006053.

[2] To whom correspondence should be addressed. Phone: +81-22-217-6830, Fax: +81-22-217-6826, E-mail: nagataki@mail.cc.tohoku.ac.jp

Abbreviations: PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PTHP, 4-phenyl-1,2,3,6-tetrahydropyridine; DMI, desipramine; DHEA, dehydroepiandrosterone; RT-PCR, reverse transcription-polymerase chain reaction.

[3] No widely recognized nomenclature system of sulfotransferases is yet available. In this article, therefore, we use the system employed in our previous reports (*4, 25*).

excretion of steroids (*2, 7, 8*).

In the present study, we have performed molecular cloning of AST-RB2 (ST2A8) cDNA, which belongs to ST2 family, and characterized its enzymatic properties in comparison with rat and human enzymes of ST2 family by using recombinant proteins expressed in bacteria.

MATERIALS AND METHODS

Materials—A λgt11 cDNA library of a male rabbit liver was obtained from CLONTECH (Palo Alto, CA). Lambda EMBL3 vector digested with *Bam*HI/*Eco*RI and Gigapack II gold packaging extract were purchased from STRATAGENE (La Jolla, CA). DNA labeling kit was purchased from Nippon Gene (Toyama). Restriction endonucleases, bacterial alkaline phosphatase, T4 DNA ligase, and *TaKaRa Ex Taq* polymerase were from Takara Shuzo (Kyoto). Thermo Sequenase fluorescent labeled primer cycle sequencing kit and [α-^{32}P]dCTP (3,000 mCi/mmol) were obtained from Amersham Japan (Tokyo). Dye primers for ABI373A DNA autosequencer were from Perkin Elmer Japan (Urayasu). Ready-To-Go You-Prime First-Strand Beads and Chelating Sepharose Fast Flow were obtained from Pharmacia Japan (Tokyo). The QIAexpressionist was purchased from Qiagen (Chatsworth, CA). All hydroxysteroids and bile acids were obtained from Sigma Chemical (St. Louis, MO). PAPS was obtained as described previously (*9, 10*). ^{35}S-PAPS was purchased from DuPont/NEN research Products (Boston, MA).

Screening of Rabbit cDNA and Genomic Libraries—A λgt11 cDNA library of a male rabbit liver was immunoscreened with anti-AST-RB2 polyclonal antibodies prepared by immunization of guinea pigs with purified AST-RB2, and 10 positive clones were obtained. The phage DNAs were extracted and purified as described previously (*11*). The longest insert cDNA (Rb2-5 clone) was sequenced using dye primers and Thermo Sequenase with a ABI373A DNA sequencer (Perkin Elmer Japan) according to the dideoxy method (*12*) in conjunction with subcloning into pUC18 and pUC19 vectors (*11*). Sequence data were compiled and analyzed by use of the GeneWorks software (IntelliGenetics, CA). Comparison of the deduced amino acid sequence with those of other sulfotransferases suggested that this cDNA fragment lacked about 20-50 bp at around the initiation codon. Further sequencing of other cDNAs did not provide information on the missing portion. Therefore, we isolated a genomic clone including a portion of the lacking 5′-cDNA sequence from a rabbit genomic library using a 137 bp DNA fragment obtained by the digestion of the Rb2-5 clone's cDNA with *Hin*fI as a probe (*Hin*fI recognition site is boxed in Fig. 1). The rabbit genomic library was constructed in a *Bam*HI site of an EMBL3 vector with partially *Sau*3AI-digested genomic DNA of a New Zealand white rabbit. One positive clone (RBG2-4) was isolated from 5×10^5 phage plaques of the library. The insert DNA was purified and digested with *Bam*HI. A DNA fragment hybridized with the probe was sequenced. The genomic DNA included an identical sequence with the cDNA fragment (Rb2-5) overlapping in 113 bases (underlined in Fig. 1), and an ATG codon was found 23 bp upstream from the 5′-terminal of Rb2-5 clone cDNA.

Reverse Transcription-Polymerase Chain Reaction—To further verify whether the sequence of AST-RB2 mRNA is identical with that determined from cDNA and genomic DNA, we performed reverse transcription-polymerase chain reaction (RT-PCR). The first-strand cDNA was synthesized with Ready-To-Go You-Prime First-Strand Beads from total RNAs obtained from male rabbit livers as described previously (*13*), and two oligonucleotides, RB2-Met and PolyT, were used as the primers. The nucleotide sequences of RB2-Met and PolyT with a restriction site of *Pst*I and *Sma*I, respectively, are shown in Table I. PCR reaction mixture (100 μl) contained 2 μl of the first-strand cDNA solution as a template, 20 pmol of each primer, 0.2 mM each of dATP, dCTP, dTTP, and dGTP, 0.5 unit of *TaKaRa Ex Taq* and the *Ex Taq* buffer. After an initial denaturation at 94°C for 3 min, the amplification cycle with 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C was performed for 40 cycles. The PCR product was digested with *Pst*I and *Sma*I, subcloned into pUC18, and sequenced. The nucleotide sequence of the RT-PCR product completely matched that determined in the previous experiments with cDNA and genomic DNA.

Construction of Expression Vectors—A DNA fragment including the open reading frame of AST-RB2 was obtained by PCR as below. Two oligonucleotides, RB2-5′ and RB2-3′, were used as the primers (Table I). RB2-5′ has a *Bam*HI restriction site and a sequence encoding an enterokinase cleavage site at the 5′-upper region of the initiation codon, and RB2-3′ has a *Hin*dIII restriction site at the 3′-terminal. The PCR was carried out as described above with 5 ng of the phage DNA (Rb2-5), and 20 pmol each of RB2-5′ and RB2-3′ as the template and the primers, respectively. The product was digested with *Bam*HI and *Hin*dIII and ligated into a bacterial expression vector, pQE30 (Qiagen).

For construction of ST2A1/ST2A2 and ST2A3 expression vectors, first-strand cDNAs of a female Sprague-Dawley rat and a human liver were synthesized with Ready-To-Go You-Prime First-Strand Beads and used as templates for PCR amplification, respectively. The sequences of the primers used are shown in Table I: 2A-5′ and

TABLE I. **Sequences of the oligonucleotides used.**

Name	Sequence (5′→3′)
RB2-Met	GCCTGCAGATGACCCAGAAGAAT
PolyT	GGCCCGGGTTTTTTTTTTTTTTTT
RB2-5′	GCGGATCCGATGACGATGACAAAATGACCCAGAAGAATATATGGTACGAAGGGATTGCCTTCCCTTTCGTGGG
RB2-3′	GCAAGCTTGTTGTTACCATGGGAACAGCTCTTGGGGG
2A-5′	GCGCATGCGATGACGATGACAAAATGCCAGACTATACTTGGTTTGAAGG
2A1-3′	GCAAGCTTCGATGACCACATTCAG
2A2-3′	GCAAGCTTCAATGACCACATTCAC
2A3-5′	GCGGATCCGATGACGATGACAAAATGTCGGACGATTTCTTATGG
2A3-3′	GCAAGCTTTTGGACGTTATTCCCATGGG

2A1-3′ for ST2A1, 2A-5′ and 2A2-3′ for ST2A2, and 2A3-5′ and 2A3-3′ for ST2A3. Expression vectors were constructed in the same way as for AST-RB2 described above.

The resultant recombinant sulfotransferases have 17 or 18 additional amino acids including a sequence of six histidine residues, and therefore we refer to them as His-2A8, His-2A1, His-2A2, and His-2A3. The additional peptide does not alter the enzymatic properties of sulfotransferases (Yoshinari and Fujita unpublished data and Refs. *14* and *15*).

Preparation of Escherichia coli Cytosols—*Escherichia coli* strain M15 cells were transformed with the construct plasmids and grown in LB medium. At the log phase, expression of recombinant proteins was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside, and the incubation was continued for an additional 3 h. Cells were suspended in 10 mM potassium phosphate buffer (pH 7.4) containing 1 mM DTT, and sonicated (whole cell fractions). Cytosol fractions were prepared by centrifugation at $105,000 \times g$ for 60 min at 4°C, and the pellets were resuspended with the same volume of phosphate-buffered saline containing 0.5% Triton X-100 (insoluble fractions).

The protein concentrations were determined by the method of Lowry *et al.* (*16*) with bovine serum albumin as the standard.

Western Blot Analysis—Whole cell, cytosol and insoluble fractions were subjected to 10% SDS-PAGE. The separated proteins were visualized by Coomassie Brilliant Blue G250 staining or transferred to nitrocellulose sheets for immunostaining with anti-STa (kindly donated by Dr. Watabe). The visualized bands were scanned (Nikon AX-1200) and the intensities were calculated by use of the NIH Image (Version 1.59) software.

Sulfation Assays—Sulfating activities toward hydroxysteroids were calculated from the radioactivities of the metabolites obtained with ^{35}S-PAPS as a sulfate donor after thin layer chromatography (*9*). Assay conditions were determined based on the results on rat and human hydroxysteroid sulfotransferases reported by Ogura *et al.* (*17*) and Forbes *et al.* (*18*), respectively. A typical incubation mixture consisted of 50 mM Tris-HCl buffer (pH 7.4), 1 mM DTT, 5 mM $MgCl_2$, 10 μM substrates, 125 μM ^{35}S-PAPS (0.1-0.2 Ci/mmol), and adequate amounts of cytosolic proteins in a final volume of 10 μl. The reaction was initiated by addition of ^{35}S-PAPS and terminated by addition of 5 μl of ice-cold acetonitrile after incubation at 37°C for 10-40 min. Portions (10 μl) of the reaction mixtures were applied to thin layer plate of silica gel 60 F_{254} (250 μM; Merck, Darmstadt, Germany) and developed with *n*-butanol:acetic acid:water (4:1:2). The radioactive spots were analyzed by use of a BAS1000 image analyzer (FujiFilm, Tokyo).

RESULTS

Structure of AST-RB2 cDNA—Nucleotide and deduced amino acid sequences of AST-RB2 are shown in Fig. 1. A putative initiation codon (bases 1-3), a termination codon (bases 859-861), and poly(A) addition signals (bases 1036-1041 and 1043-1048) are double-underlined. The *Hin*fI restriction site is boxed. Underlined sequences represent bases matched between the cDNA and the genomic DNA. AST-RB2 mRNA contained an open reading frame of 858 bp encoding 286 amino acids with a molecular weight of 33,544.

The amino acids shaded in Fig. 1 were matched with the peptide sequences obtained by digestion of purified AST-RB2 with Lys-C endoproteinase followed by Edman sequencing. The matched portions covered 37% of the whole coding region, and this supports the idea that the cDNA encodes AST-RB2.

Comparison of Amino Acid Sequences—The amino acid sequence deduced from AST-RB2 cDNA was compared with those of rat, human, and rabbit sulfotransferases (Table II). The amino acid sequence of AST-RB2 had 58-68% identity with those of ST2A[3] sulfotransferases [ST2A1 (*19*), ST2A2 (*20*), and ST2A3 (*21*)] and only 35-37% identity with ST1 enzymes [ST1A1 (*22*), ST1B1 (*4*), ST1C1 (*23*), and ST1E2 (*24*)] and ST3A1 (*6*). Therefore, we termed this form ST2A8 according to our nomenclature system previously reported (*4, 25*).

Expression of Recombinant Enzymes—To compare enzymatic properties of rabbit, rat, and human hydroxysteroid sulfotransferases (ST2A), recombinant proteins were expressed in *E. coli*. During preparation of their cytosol fractions, we observed clear differences in localization of individually expressed proteins (Fig. 2A). The subcellular localization of the proteins was determined using anti-STa polyclonal antibodies, which react with all four recombinant sulfotransferases (Fig. 2B). Only 2% of His-2A1 and 4% of His-2A2 expressed were recovered in cytosols. On the other hand, His-2A3 was enriched in the cytosol fraction, amounting to about 31% of the total expressed protein. Recovery of His-2A8 in cytosols was about 11%, which was intermediate between rat His-2A1/2A2 and human His-2A3.

Comparison of Substrate Specificity—Using bacterial cytosolic fractions, sulfating activities of these recombinant sulfotransferases were determined towards hydroxysteroids and bile acids (Table III). Due to the variability of expressed protein amounts in cytosols, results were expressed relative to a standard activity (pregnenolone sulfation). This activity was chosen because pregnenolone is a common precursor for biosynthesis of steroid hormones in rabbits, rats, and humans. His-2A8 sulfated DHEA at the highest rate among the chemicals examined, followed by pregnenolone and testosterone. His-2A8 was also active towards 3β-hydroxy-5-cholenoic acid (3β-hydroxy-5-cholen-24-oic acid), but showed no detectable activity for lithocholic acid (5β-cholan-24-oic acid-3α-ol). His-2A3 showed the highest activity towards DHEA and was also active towards pregnenolone, androsterone, and testosterone. His-2A1 and His-2A2 showed distinct substrate specificities and catalyzed sulfation of corticosterone and cortisol at relatively high rates. In addition, His-2A1 is unique among the four forms in catalyzing sulfation of 3β-hydroxy-5-cholenoic acid and corticosterone at high rates.

DISCUSSION

We have recently purified two sulfotransferases, AST-RB1 and AST-RB2 from male rabbit livers (Ref. *5* and Shiraga *et al.*, in preparation). Both forms are unique in catalyzing amine sulfation. To identify the primary structures and enzymatic properties of these enzymes, we isolated their

cDNAs. In the present study, we have isolated of AST-RB2 cDNA, identified its gene family and characterized the enzymatic properties of the protein expressed in *E. coli.*

As shown in Table I, the deduced amino acid sequence of AST-RB2 share high degrees of similarity with those of other ST2A enzymes, but not with ST1 enzymes, suggesting that AST-RB2 belongs to the ST2A subfamily. Thus, we termed it ST2A8 according to our previous nomenclature system (*4, 25*). In rats and guinea pigs, the presence of multiple mRNAs of the ST2A subfamily has been reported (*26–28*). Thus, plural forms of ST2A may also be contained in rabbits. However, we were not able to isolate a cDNA distinct from ST2A8 cDNA using anti-AST-RB2 antibodies. In addition, screening of a rabbit genomic library (approximately 5×10^5 plaques) yielded only one genomic clone (RBG2-4), which corresponded to ST2A8. These results suggest the major role of ST2A8 (AST-RB2) in steroid and bile acid sulfation in rabbits, although further studies are necessary to confirm this.

To compare the enzymatic properties of ST2A8 and other ST2A enzymes, namely, ST2A1, ST2A2, and ST2A3, substrate specificities towards steroid hormones and bile acids were determined. His-2A8 showed high activities to pregnenolone, DHEA, testosterone, and 3β-hydroxy-5-

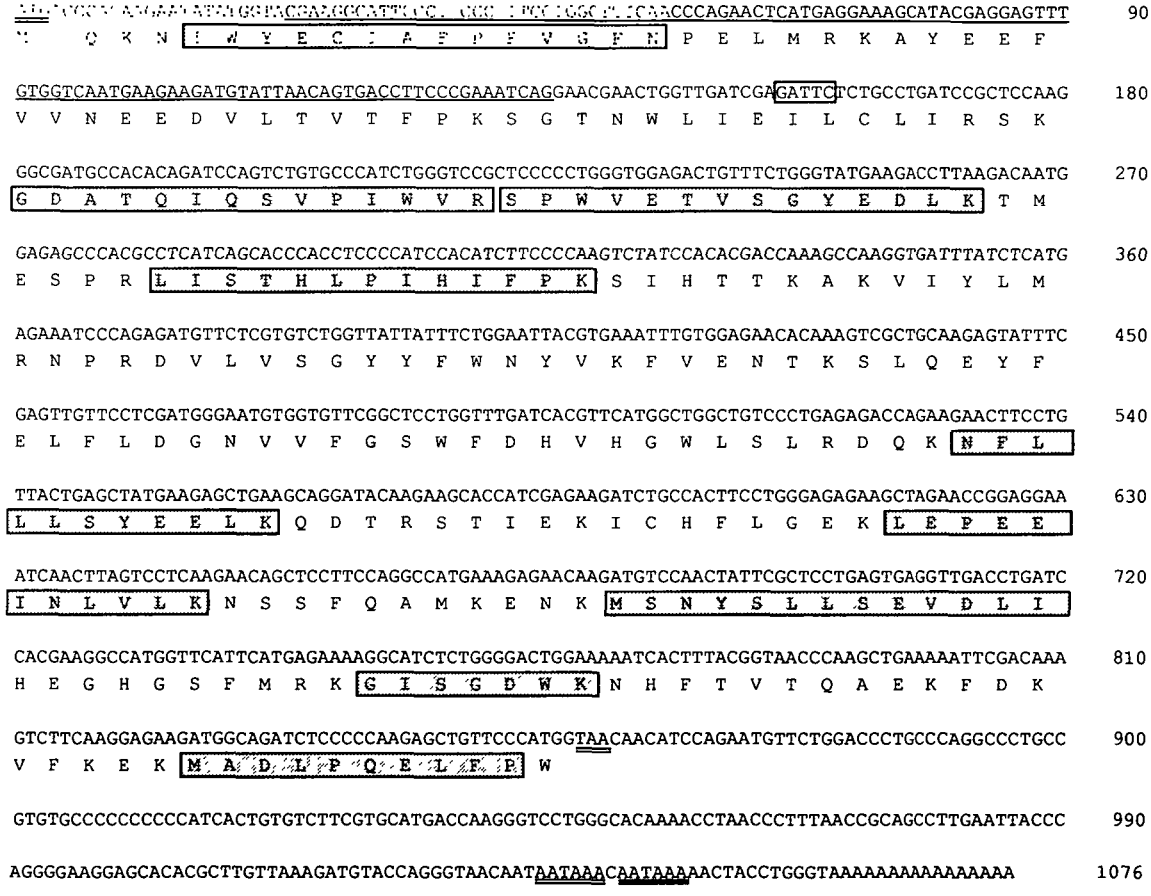

Fig. 1. **Nucleotide and deduced amino acid sequences of AST-RB2 cDNA.** Putative initiation and termination codons, and poly(A) addition signal are double-underlined. Underlined sequences represent bases matched between the cDNA (Rb2-5) and the genomic DNA (RBG2-4). The *Hinf*I restriction site is boxed.

TABLE II. **Similarity of mammalian cytosolic sulfotransferases.** The percent identities of the deduced amino acids were calculated by use of the GeneWorks software (IntelliGenetics). Sulfotransferases are arbitrarily named from the similarity of their primary structures.

	ST1A1[a]	ST1B1[b]	ST1C1[c]	ST1E2[d]	ST2A1[e]	ST2A2[f]	ST2A3[g]	ST3A1[h]
ST2A8 (AST-RB2)	36	37	36	36	**58**	**60**	**68**	35
ST1A1		52	50	50	34	35	36	38
ST1B1			50	45	35	35	39	36
ST1C1				46	36	37	36	36
ST1E2					34	34	35	36
ST2A1						90	63	36
ST2A2							62	36
ST2A3								37
ST3A1								

[a]Ozawa *et al.* (*22*). [b]Yamazoe *et al.* (*4*). [c]Nagata *et al.* (*23*). [d]Demyan *et al.* (*24*). [e]Ogura *et al.* (*19*). [f]Ogura *et al.* (*20*). [g]Otterness *et al.* (*21*). [h]Yoshinari *et al.* (*6*).

cholenoic acid. His-2A8 and His-2A3 showed similar substrate specificities, but differed in the ratio of testosterone sulfation to androsterone sulfation. In addition, all four enzymes mediated sulfation of bile acids (3β-hydroxy-5-cholenoic acid and lithocholic acid) except for His-2A8 on lithocholic acid. These results confirmed that bile acid sulfation is a common property of ST2A subfamily sulfotransferases, as reported previously (*17, 29*). Urinary excretion of sulfated lithocholate has been reported to be low or undetectable in rabbits (*29*). These results agree with the absence of detectable activity of ST2A8 on lithocholic acid sulfation in our present study.

Although showing 90% identities, His-2A1 and His-2A2 displayed clear differences in substrate specificity (Table III). Most of the substitutions of amino acids between ST2A1 and ST2A2 occur in a region just upstream of the conserved site (Fig. 3) which is proposed as a putative PAPS-binding site (*30*). Moreover, similar diversity was observed in the corresponding regions of ST2A8 and ST2A3. The results imply that the region may be involved in substrate recognition of ST2A subfamily enzymes.

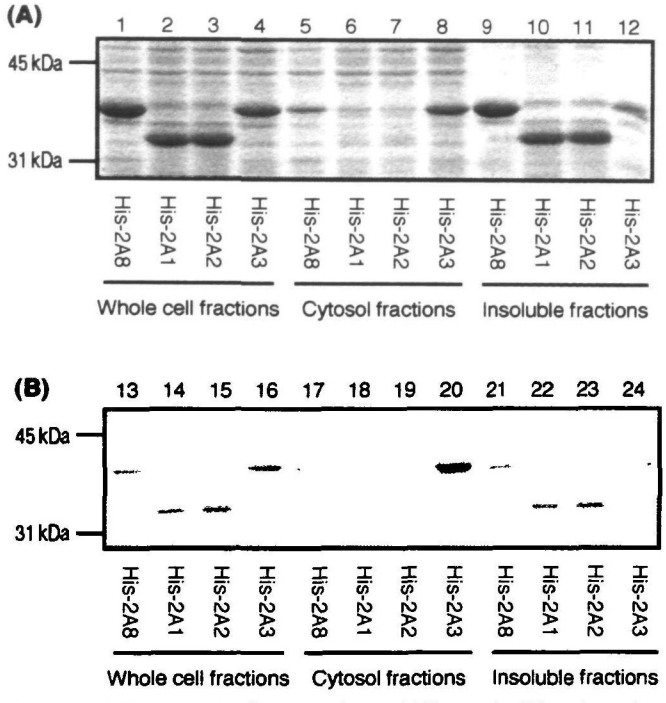

Fig. 2. **Difference in localization of His-2A8, His-2A1, His-2A2, and His-2A3 expressed in *E. coli*.** Proteins of whole cell, cytosolic and insoluble fractions were separated on 10% SDS-PAGE, and the separated proteins were subjected to Coomassie Brilliant Blue G250 staining (A) or transferred to a nitrocellulose membrane for immunostaining with polyclonal antibodies raised against STa (B). Proteins equivalent to 50 μl (lanes 1-12), 2 μl (lanes 13-16, 21-24), or 20 μl of the bacterial cultures (lanes 17-20) were applied. Size markers are indicated on the left.

During preparation of cytosolic fractions of *E. coli*, we observed that most of expressed His-2A1 and His-2A2 proteins were localized in the insoluble fractions, while considerable amounts of His-2A8 and His-2A3 were recovered in cytosols. The exact mechanism behind the difference in cytosolic/insoluble fraction ratios is unclear, but hydropathy may be involved. Thus, hydropathy of ST2A8, ST2A1, ST2A2, and ST2A3 were calculated using the Kite-Doolittle algorithm (*31*) with an 11-amino-acid-window by use of the GeneWorks software, and the mean values were −31.7, −23.0, −26.4, and −40.1 (negative number represents hydrophilicity), respectively. Solubility of expressed sulfotransferases was well correlated with the hydropathy number. In addition, clear differences were observed in their mobilities on SDS-PAGE, although His-2A8, His-2A1, His-2A2, and His-2A3 all had calculated molecular weights of about 35,000. The estimated molecular mass of His-2A1 and His-2A2 from the mobility were both about 34,000 Da, which was consistent with those calculated from their amino acid compositions. The estimated molecular mass of His-2A8 and His-2A3 were, in contrast, about 40,000 Da, which were higher than those calculated from their amino acid compositions. These results also suggest that high hydrophobicity of ST2A1 and ST2A2 may result in the low recovery of the expressed proteins in bacteria cytosols, although further study is necessary to substantiate the exact mechanism behind the different localization.

Originally, AST-RB2 (ST2A8) was purified as a sulfotransferase showing high activities for amine sulfation together with AST-RB1 (ST3A1). In addition, Naritomi *et al.* (*32*) purified an alicyclic amine *N*-sulfotransferase from female rat livers, whose N-terminal amino acid sequence is similar to those of ST2A enzymes. These results suggest that the ST2A family of enzymes as well as ST3A1 catalyze amine sulfation *in vivo*. We are now investigating this possibility.

In conclusion, we have isolated the cDNA encoding AST-RB2 (ST2A8) and proven it to be a member of the

TABLE III. **Relative sulfating activities of His-2A8, His-2A1, His-2A2, and His-2A3.** The assays were carried out with 10 μM substrates and 125 μM ^{35}S-PAPS as a sulfate donor using cytosolic fractions. After reactions, aliquots of the reacted mixtures were subjected to thin layer chromatography. Radioactivities of the sulfates were calculated by use of BAS1000 image analyzer (FujiFilm). All data shown are the mean±SD of three determinations.

Cytosol	Substrate[a]							
	PRGN	DHEA	CORC	CORS	TEST	AND	LTCA	3HCA
His-2A8	100±9[b]	440±24	2±0.1	0.3±0.2	58±3.0	11±0.4	ND[c]	63±5
His-2A1	100±8[b]	43±3	54±4.0	3.9±0.2	3±0.1	5±0.1	2.3±1.1	112±3
His-2A2	100±8[b]	201±11	11±1.0	3.9±0.2	50±0.5	61±2.0	9.4±1.2	61±5
His-2A3	100±10[b]	177±32	4±0.3	0.4±0.1	15±0.5	44±3.0	2.1±1.3	45±2

[a]Substrates are abbreviated as follows: PRGN, pregnenolone; DHEA, dehydroepiandrosterone; CORC, corticosterone; CORS, cortisol; TEST, testosterone; AND, androsterone; LTCA, lithocholic acid; 3HCA, 3β-hydroxy-5-cholenoic acid. [b]Sulfating activities are shown as relative activities (% of those towards pregnenolone) for each enzyme. The activities towards pregnenolone of His-2A8, His-2A1, His-2A2, and His-2A3 were 5,380, 96, 711, and 12,700 pmol/mg of cytosolic protein/min, respectively. [c]ND represents activities below the detection limit [6 pmol/mg of cytosolic protein/min (0.001%)] for His-2A8.

```
ST2A1   MP-DYTWFEGIPFHAFGISKETLQNVCNKFVVKDEDLILLAYPKSGTNWLIEIVCLIQTKGDPKWIQSVTIWDRS   74
ST2A2     -          P    P                E      T                                    74
ST2A3     SD FL      A PTM FRS    RK RDE   IR    V I T          A  L  MHS    A       P  E     75
ST2A8     TQKNI Y    A PFV FNP LMRKAYEE    NE   VLTVTF           L   RS    ATQ     P  V     75

ST2A1   PWIETDVGYDILIKKKGPRLMTSHLPMHLFSKSLFSSKAKVIYLVRNPRDVLVSGYYFWGNSTLAKKPDSLGTYV  149
ST2A2         L   M          I                                 I                KT      149
ST2A3     V SEI   TA SETES    FS     IQ  P  F                  M            F  K MKFI    K WEE F  150
ST2A8     V  VS   ED KTMES    IST    I I P  IHTT               M                NYVKFVENTK  QE F  150

ST2A1   EWFLKGNVLYGSWFEHIRAWLSMQEWDNFLLLYYEDMKKDTMGTIKKICDFLGKKLEPDELDLVLKYSSFQVMKE  224
ST2A2           P                R                                                      224
ST2A3       CQ T        D  HG MP R EK      S  EL Q  GR  E   Q    T   E  N I  N    S     225
ST2A8     L  D    VF    D VHG    LRDQK      S  EL Q  RS  E   H    E   E IN   N    A     225

ST2A1   NDMSNYSLL-MKKSIFTGTG-LMRKGTVGDWKNHFTVSQAEAFDKVFQEKMAGFPPGMFPWE                  284
ST2A2     N     N  -  EL LP FT-    N  T          A                          D           284
ST2A3     K         S-VDYVVDKAQ- L    VS         A   D   L       DL REL               285
ST2A8     K         SEVDL HE H SF     IS         T   K     K     DL QEL   -           286
```

Fig. 3. **Comparison of the deduced amino acid sequences.** The deduced amino acid sequences of ST2A1, ST2A2, ST2A3, and ST2A8 (AST-RB2) were aligned. In the sequences of ST2A2, ST2A3, and ST2A8, only the amino acids different from those of ST2A1 are shown. The box represents the putative PAPS-binding motif GXXGXXK.

ST2A subfamily. ST2A8 expressed in *E. coli* showed high activities towards pregnenolone, DHEA, testosterone, and 3β-hydroxy-5-cholenoic acid, and its substrate specificity was more closely similar to that of His-2A3 rather than His-2A1 and His-2A2.

We thank Prof. T. Watabe (School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo) for generous donating of anti-STa polyclonal antibodies.

REFERENCES

1. De Meio, R.H. (1975) Sulfate activation and transfer in *Metabolic Pathways* (Greenberg, D.M., ed) Vol. 7, 3rd ed., pp. 287-358, Academic Press, New York
2. Jakoby, W.B., Sekura, R.D., Lyon, E.S., Marcus, C.J., and Wang, J. (1980) Sulfotransferases in *Enzymatic Basis of Detoxication* (Jakoby, W.B., ed.) Vol. 2, pp. 199-228, Academic Press, New York
3. Yamazoe, Y. and Kato, R. (1995) Structure and function of sulphotransferases in *Advances in Drug Metabolism in Man* (Pacifici, G.M. and Fracchia, G.N., eds.) pp. 659-678, European Commission, Brussels
4. Yamazoe, Y., Nagata, K., Ozawa, S., and Kato, R. (1994) Structural similarity and diversity of sulfotransferases. *Chem.-Biol. Interact.* **92**, 107-117
5. Shiraga, T., Iwasaki, K., Tozuka, Z., Hata, T., Yamazoe, Y., and Ohno, Y. (1996) Purification and characterization of amine sulfotransferases from male rabbit livers (in Japanese) in *Proceeding of the 116th Meeting of the Pharmaceutical Society of Japan* Vol. 3, p. 53
6. Yoshinari, K., Nagata, K., Ogino, M., Fujita, K., Shiraga, T., Iwasaki, K., Hata, T., and Yamazoe, Y. (1998) Molecular cloning and expression of an amine sulfotransferase cDNA: A new gene family of cytosolic sulfotransferases in mammals. *J. Biochem.* **123**, 479-486
7. Jakoby, W.B., Duffel, M.W., Lyon, E.S., and Ramaswamy, S. (1984) Sulfotransferases active with xenobiotics-comments on mechanism in *Progress in Drug Metabolism* (Bridges, J.W. and Chasseaud, L.F., eds.) Vol. 8, pp. 11-33, Taylor & Francis, London
8. Singer, S.S. (1985) Preparation and characterization of different kinds of sulfotransferase in *Biochemical Pharmacology and Toxicology. Methodological Aspects of Drug-Metabolizing Enzymes* (Zakim, D. and Vessey, D.A., eds.) Vol. 1, pp. 95-159, Wiley, New York
9. Iwasaki, K., Shiraga, T., Noda, K., Tada, K., and Noguchi, H. (1986) *N*-Sulphoconjugation of alicyclic, alkyl- and aryl-amines in vivo and in vitro. *Xenobiotica* **16**, 651-659
10. Iwasaki, K., Shiraga, T., Tada, K., Noda, K., and Noguchi, H. (1986) Age- and sex-related changes in amine sulphoconjugations in Sprague-Dawley strain rats. Comparison with phenol and alcohol sulphoconjugations. *Xenobiotica* **16**, 717-723
11. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
12. Sanger, F., Nicklen, S., and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467
13. Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159
14. Marsolais, F. and Varin, L. (1995) Identification of amino acid residues critical for catalysis and cosubstrate binding in the flavonol 3-sulfotransferase. *J. Biol. Chem.* **270**, 30458-30463
15. Lewis, A.J., Kelly, M.M., Walle, U.K., Eaton, E.A., Falany, C. N., and Walle, T. (1996) Improved bacterial expression of the human P form phenolsulfotransferase. Applications to drug metabolism. *Drug Metab. Dispos.* **24**, 1180-1185
16. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275
17. Ogura, K., Satsukawa, M., Okuda, H., Hiratsuka, A., and Watabe, T. (1994) Major hydroxysteroid sulfotransferase STa in rat liver cytosol may consist of two microheterogeneous subunits. *Chem.-Biol. Interact.* **92**, 129-144
18. Forbes, K.J., Hagen, M., Glatt, H., Hume, R., and Coughtrie, M.W.H. (1995) Human fetal adrenal hydroxysteroid sulphotransferase: cDNA cloning, stable expression in V79 cells and functional characterisation of the expressed enzyme. *Mol. Cell. Endocrinol.* **112**, 53-60
19. Ogura, K., Kajita, J., Narihata, H., Watabe, T., Ozawa, S., Nagata, K., Yamazoe, Y., and Kato, R. (1989) Cloning and sequence analysis of a rat liver cDNA encoding hydroxysteroid sulfotransferase. *Biochem. Biophys. Res. Commun.* **165**, 168-174
20. Ogura, K., Kajita, J., Narihata, H., Watabe, T., Ozawa, S., Nagata, K., Yamazoe, Y., and Kato, R. (1990) cDNA cloning of the hydroxysteroid sulfotransferase STa sharing a strong homology in amino acid sequence with the senescence marker protein SMP-2 in rat livers. *Biochem. Biophys. Res. Commun.* **166**, 1494-

1500
21. Otterness, D.M., Wieben, E.D., Wood, T.C., Watson, W.G., Madden, B.J., McCormick, D.J., and Weinshilboum, R.M. (1992) Human liver dehydroepiandrosterone sulfotransferase: molecular cloning and expression of cDNA. *Mol. Pharmacol.* **41**, 865-872
22. Ozawa, S., Nagata, K., Gong, D.W., Yamazoe, Y., and Kato, R. (1990) Nucleotide sequence of a full-length cDNA (PST-1) for aryl sulfotransferase from rat liver. *Nucleic Acids Res.* **18**, 4001
23. Nagata, K., Ozawa, S., Miyata, M., Shimada, M., Gong, D.W., Yamazoe, Y., and Kato, R. (1993) Isolation and expression of a cDNA encoding a male-specific rat sulfotransferase that catalyzes activation of *N*-hydroxy-2-acetylaminofluorene. *J. Biol. Chem.* **268**, 24720-24725
24. Demyan, W.F., Song, C.S., Kim, D.S., Her, S., Gallwitz, W., Rao, T.R., Slomczynska, M., Chatterjee, B., and Roy, A.K. (1992) Estrogen sulfotransferase of the rat liver: complementary DNA cloning and age- and sex-specific regulation of messenger RNA. *Mol. Endocrinol.* **6**, 589-597
25. Nagata, K., Yoshinari, K., Ozawa, S., and Yamazoe, Y. (1997) Arylamine activating sulfotransferase in livers. *Mutation Res.* **376**, 267-272
26. Watabe, T., Ogura, K., Satsukawa, M., Okuda, H., and Hiratsuka, A. (1994) Molecular cloning and functions of rat liver hydroxysteroid sulfotransferases catalysing covalent binding of carcinogenic polycyclic arylmethanols to DNA. *Chem.-Biol. Interact.* **92**, 87-105
27. Lee, Y.C., Park, C.S., and Strott, C.A. (1994) Molecular cloning of a chiral-specific 3α-hydroxysteroid sulfotransferase. *J. Biol. Chem.* **269**, 15838-15845
28. Luu, N.X., Driscoll, W.J., Martin, B.M., and Strott, C.A. (1995) Molecular cloning and expression of a guinea pig 3-hydroxysteroid sulfotransferase distinct from chiral-specific 3α-hydroxysteroid sulfotransferase. *Biochem. Biophys. Res. Commun.* **217**, 1078-1086
29. Barnes, S., Buchina, E.S., King, R.J., McBurnett, T., and Taylor, K.B. (1989) Bile acid sulfotransferase I from rat liver sulfates bile acids and 3-hydroxy steroids: purification, N-terminal amino acid sequence, and kinetic properties. *J. Lipid Res.* **30**, 529-540
30. Satishchandran, C., Hickman, Y.N., and Markham, G.D. (1992) Characterization of the phosphorylated enzyme intermediate formed in the adenosine 5′-phosphosulfate kinase reaction. *Biochemistry* **31**, 11684-11688
31. Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132
32. Naritomi, Y., Niwa, T., Shiraga, T., Iwasaki, K., and Noda, K. (1994) Isolation and characterization of an alicyclic amine *N*-sulfotransferase from female rat liver. *Biol. Pharm. Bull.* **17**, 1008-1011