J. Biochem. 123, 479-486 (1998)

Molecular Cloning and Expression of an Amine Sulfotransferase cDNA: A New Gene Family of Cytosolic Sulfotransferases in Mammals[1]

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Received for publication, October 16, 1997

A cDNA of amine sulfotransferase-RB1 (AST-RB1), which efficiently catalyzes 4-phenyl-1,2,3,6-tetrahydropyridine (PTHP) sulfation, has been isolated by immunoscreening of a rabbit liver cDNA library. The cDNA consisted of 1,117 base pairs and encoded a protein of 301 amino acids with a molecular weight of 35,876. The deduced amino acid sequence matched at six positions those of peptide fragments obtained from purified AST-RB1 protein. The sequence had less than 38% identity at the amino acid level with cytosolic sulfotransferases in mammals, although high degrees of similarity were observed with regions conserved throughout mammalian sulfotransferases. These results indicate that AST-RB1, arbitrarily named sulfotransferase 3A1 (ST3A1), constitutes a new and third gene family of cytosolic sulfotransferases in mammals. ST3A1 expressed in *Escherichia coli* as a fused protein catalyzed sulfation of amines such as PTHP, aniline, 4-chloroaniline, 2-naphthylamine, and desipramine, but barely *O*-sulfation of typical aryl and hydroxysteroid sulfotransferase substrates. These data unequivocally demonstrate the existence of a cytosolic sulfotransferase showing a high selectivity for amine substrates, and indicate that multiple forms of sulfotransferase mediate sulfation of xenobiotics in mammalian livers.

Key words: amino compound, bacterial expression, cDNA, rabbit, sulfotransferase.

Sulfation[3] is a major pathway for detoxification and activation of both endogenous and exogenous compounds such as hormones, bile acids, neurotransmitters, drugs and carcinogens (*1, 2*). This reaction is mediated by cytosolic sulfotransferase(s) to form not only *O*-sulfonates of phenolic or alcoholic compounds but also sulfamates (*N*-sulfates) of amines in the presence of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as a sulfate donor.

Since the first report of Boyland *et al.* (*3*), several amino compounds have been found to be excreted as sulfamates in the urine of mammals (*1, 3-6*). Prosser and Roy (*5*) studied the enzymatic properties of *N*-sulfation using rat and guinea pig livers and demonstrated higher activity in rabbits and guinea pigs than in rats (*7*). Shiraga *et al.* (*8*) have recently reported species- and sex-differences in hepatic *N*-sulfation of various amines (alkyl, alicyclic, and aryl amines). Hepatic sulfation of 4-phenyl-1,2,3,6-tetrahydropyridine (PTHP) was 3.6 times higher in male rabbits than in the females.

From livers of guinea pigs (*9*) and rats (*10*), two sulfotransferases mediating amine sulfation were isolated. The purified preparations of sulfotransferase from guinea pigs were reported to catalyze sulfation of 2-naphthol and dehydroepiandrosterone (DHEA) as well as 2-naphthylamine (*9*). Naritomi *et al.* (*10*) reported that an amine sulfotransferase isolated from livers of female rats had an N-terminal amino acid sequence similar to those of hydroxysteroid sulfotransferases.

Based on similarity of deduced amino acid sequences, sulfotransferases are now known to constitute a gene superfamily (*11*). This superfamily has been classified into two families, ST1 (SULT1) and ST2 (SULT2) in mammals. The former mainly catalyzes sulfation of phenolic residues and includes four subfamilies, ST1A, ST1B, ST1C, and ST1E. The latter, predominantly catalyzing sulfation of alcoholic residues, includes a single subfamily, ST2A. These sulfotransferases are reported mainly to

[1] The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number D86219. A mutual nomenclature system of sulfotransferases is not yet available. In this article, we used the system shown in our previous reports (*11, 19*).

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[3] Sulfotransferase mediates transfer of SO_3^- from PAPS to phenols, alcohols and amines. In this study, "sulfation" is used to indicate these reactions collectively.

Abbreviations: PTHP, 4-phenyl-1,2,3,6-tetrahydropyridine; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; DHEA, dehydroepiandrosterone; ST, sulfotransferase; AST-RB1, amine sulfotransferase-RB1.

catalyze *O*-sulfation, but not *N*-sulfation, except for a few forms. Using the heterologous expressed enzymes, Ozawa *et al.* (*12*) reported that a rat arylsulfotransferase, ST1A1, catalyzed *N*-sulfation of carcinogenic heterocyclic amines. Hence, the existence of an amine-specific sulfotransferase remains uncertain.

Shiraga *et al.* have recently isolated an amine sulfotransferase termed AST-RB1, which showed high activities for *N*-sulfation of amines rather than *O*-sulfation of typical substrates, 2-naphthol and DHEA (Shiraga, in preparation).

In the present study, we have isolated a new sulfotransferase cDNA from a male rabbit liver library using polyclonal antibodies raised against purified AST-RB1. The cDNA was compared at both nucleotide and deduced amino acid levels with ST1 and ST2 cDNAs reported previously, and expressed in *Escherichia coli* to determine the enzymatic properties. The results obtained indicate that the newly isolated cDNA encodes an amine sulfotransferase which belongs to a previously uncharacterized sulfotransferase family.

MATERIALS AND METHODS

Materials—A λgt11 cDNA library of a male rabbit liver was obtained from CLONTECH (Palo Alto, CA). Restriction endonucleases, bacterial alkaline phosphatase, and DNA labeling kit were purchased from Nippon Gene (Toyama). T4 DNA ligase and *TaKaRa Ex Taq* 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 sequencer were from Perkin Elmer Japan (Urayasu). The QIAexpressionist was purchased from Qiagen (Chatsworth, CA). Aniline, desipramine, 2-naphthol, PTHP, and their respective sulfates were obtained as described previously (*13, 14*). Dopamine, DHEA and testosterone were obtained from Sigma Chemical (St. Louis, MO). 4-Chloroaniline was purchased from Wako Pure Chemicals (Osaka). 4-Hydroxybiphenyl was obtained from Tokyo Kasei Industry (Tokyo). ^{14}C-DHEA and ^{35}S-PAPS were purchased from DuPont/NEN Research Products (Boston, MA). PAPS was obtained as described previously (*13, 14*).

Screening of a Rabbit cDNA Library—A λgt11 cDNA library of a male rabbit liver was immunoscreened with anti-AST-RB1 polyclonal antibodies prepared by immunization of guinea pigs with the purified protein, and four positive clones (Rb1-1, Rb1-5, Rb1-6, and Rb1-10) were isolated. The phage DNAs were extracted and purified as described previously (*15*). The cDNA contained in the longest clone (Rb1-5) was cleaved into two cDNA fragments (1 kbp and 150 bp) by digestion with *Eco*RI during the preparation of insert cDNA fragments, and both strands of each fragment were sequenced separately using dye primers and Thermo Sequenase with ABI373A DNA sequencer (Perkin Elmer Japan) according to the dideoxy method in conjunction with M13 phage cloning (*15*). Sequence data were compiled and analyzed by use of the GeneWorks software (IntelliGenetics, CA), which was also used for aligning of nucleotide and amino acid sequences. Purified AST-RB1 was digested with an endoproteinase Lys-C and the polypeptide fragments were subjected to Edman amino acid analyses, and the arrangement of the two cDNA fragments was confirmed by reference to partial peptide sequences obtained from purified AST-RB1. The larger cDNA was followed by the smaller one in the 5′ to 3′ direction.

Expression of Amine Sulfotransferase in E. coli—A DNA fragment encoding the open reading frame of AST-RB1 was obtained by PCR as described below. Two oligonucleotides, ASTRB1-5′ and ASTRB1-3′, were synthesized as primers. The nucleotide sequence of ASTRB1-5′ with a *Bam*HI restriction site and a sequence encoding an enterokinase cleavage site at the 5′-upper region of the initiation codon was 5′-GCGGATCCGATGACGATGACAAAATGGACAACTCACGTAAATATTTATTG-3′ (the initiation codon is underlined). The nucleotide sequence of ASTRB1-3′ with a *Pst*I restriction site at the 3′-lower region of the termination codon was 5′-GCCTGCAGGTGACTATTTTGCACTGTG-3′ (underlined sequence is complementary to the termination signal, TAG). The PCR reaction mixture (100 μl) contained 5 ng of the template phage DNA (Rb1-5), 20 pmol each of 5′- and 3′-primers, 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 was started for 25 cycles, with 1 min at 94°C for denaturation, 1 min at 55°C for annealing, 3 min at 72°C for extension, and a final extension period of 7 min at 72°C. The PCR product was digested with *Bam*HI and *Pst*I and ligated into a bacterial expression vector, pQE30 (Qiagen). *E. coli* strain M15 was transformed with the construct plasmid (pRB1). Thirty milliliters of LB medium were inoculated with 500 μl of an overnight culture of M15 harboring pRB1. After incubation at 37°C for 3 h, the inducer, isopropyl-β-D-thiogalactopyranoside, was added at a final concentration of 1 mM. The incubation was continued for an additional 3 h. Cells were collected by centrifugation and resuspended in 600 μl of 0.1 M potassium phosphate (pH 7.4) containing 1 mM dithiothreitol, and lysed by sonication. Cytosol fractions were prepared from the lysates by centrifugation at 105,000×*g* for 60 min at 4°C and used as enzyme sources for assays of sulfotransferase activity. Cytosols of male rabbit livers were prepared as described (*8*). The protein concentrations were determined by the method of Bradford (*16*) with bovine serum albumin as the standard.

Western Blot Analysis—Cytosolic proteins (0.5 μg from M15 cells and 10 μg from rabbit livers) and purified AST-RB1 (0.05 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrically transferred to a nitrocellulose sheet (*17*). The membrane was immunostained with polyclonal antibodies raised against purified AST-RB1, peroxidase-conjugated rabbit anti-guinea pig IgG, diaminobenzidine and hydrogen peroxide.

Sulfation Assays—Sulfating activities of PTHP, aniline, desipramine, and 2-naphthol were measured using high performance liquid chromatography as described previously (*13, 14*). Sulfating activities of 4-chloroaniline (1 mM), 2-naphthylamine (1 mM), 4-hydroxybiphenyl (200 μM), dopamine (1 mM), and testosterone (200 μM) were calculated from the radioactivity of the metabolites obtained with ^{35}S-PAPS as a sulfate donor after thin layer chromatography. A typical incubation mixture consisted of 0.1 M potassium phosphate (pH 7.4) (for 4-chloroaniline, 2-

naphthylamine, and 4-hydroxybiphenyl) or 50 mM Tris-HCl (pH 7.4) containing 20 mM $MgCl_2$ (for testosterone), and 1 mM dithiothreitol, various concentrations of substrates, 50 μM ^{35}S-PAPS (0.2 Ci/mmol), and adequate amounts of cytosols in a final volume of 10 μl. The reaction was initiated by the addition of ^{35}S-PAPS and terminated by the addition of 5 μl of ice-cold acetonitrile after incubation at 37°C for 15-45 min. Portions (10 μl) of the reaction mixture were applied to a cellulose thin layer plate (13255, Eastman Kodak, CT) and developed with *n*-propanol: ammonia:water (6:3:1). The radioactive spots were scraped from the plates and quantified by liquid scintillation counting. The rates of the sulfation were calculated by subtraction of the corresponding controls (minus substrates). Sulfation of DHEA was assayed as follows: the reaction mixture (0.5 ml) contained 50 μM ^{14}C-DHEA, 0.20 mM PAPS, 5 mM $MgCl_2$, 3 mM 2-mercaptoethanol, 0.1 M potassium phosphate buffer (pH 6.8), and adequate amounts of cytosols. After incubation at 37°C for 10 min, unchanged DHEA was extracted from the reaction mixture twice with 4 ml of *n*-hexane, and the DHEA sulfate in the aqueous solution was determined by the radioactivity with a liquid scintillation counter. The apparent K_m values for PTHP sulfation by cytosols from *E. coli* and from male rabbit livers were determined by linear regression analysis of double-reciprocal plots.

Northern Blot Analysis—Total RNAs were isolated from male rabbit livers (2.5-3.5 kg weight, New Zealand white) as described (*18*). Portions (20 μg) of the total RNAs were subjected to electrophoresis on 1.2% formaldehyde-agarose gel and transferred to nylon membrane (Nytran-plus, Schleicher & Schuell, Dassel, Germany). The membrane was hybridized overnight at 65°C with the ^{32}P-labeled AST-RB1 cDNA prepared by the random primer labeling method, then washed twice at 65°C for 15 min with 20 mM sodium phosphate buffer (pH 7.2) containing 1 mM ethylenediamine-*N,N,N′,N′*-tetraacetic acid and 1% sodium dodecyl sulfate (*15*). Autoradiography was performed at −80°C for 48 h.

RESULTS

Isolation and Sequencing of AST-RB1 cDNA—Four positive clones were isolated from a λgt11 cDNA library of a male rabbit liver by immunoscreening with polyclonal antibodies raised against purified AST-RB1. Of these, the longest cDNA clone (Rb1-5) was digested into two DNA fragments (1 kbp and 150 bp) with *Eco*RI. These fragments were sequenced separately, and the deduced amino acid sequences were compared with amino acid sequences of

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                                                                    A      1
TTTTGCTTCCTCGGCTGATCTACAACAGTGAGTAGGAAAAGGAGAGACGGAGGGTCTCTTTAAAATTTCTGCCTTTTTTCTGCATAACAGGTGAAAAGTT    101

ATGGACAACTCACGTAAATATTTATTGAACTTCAAAGGCTGTAATTTTGAACGTACACTTGTTGATATGAAAATACTGGAAAAGTTAGATGACTTTGAAA    201
M  D  N  S  R  K  Y  L  L  N  F  K  G  C  N  F  E  R  T  L  V  D  M  K  I  L  E  K  L  D  D  F  E  I     34

TCAGAGATGACGATGTCTTCGTAATCACATATCCCAAATCTGGTACTGTCTGGACTCAGCAGATACTAAGTTTGATTTATTTTGAGGGCCACCGAAACAG    301
   R  D  D  D  V  F  V  I  T  Y  P  K  S  G  T  V  W  T  Q  Q  I  L  S  L  I  Y  F  E  G  H  R  N  R     67

AACTGAAAAGTGGGACACGCTTGATAGAGTGCCCTTCTTGGAATACAACATTCGCAAAGTGGACATTGAGAACAGACCATCCCCTCGCCTCTTTGCTTCC    401
   T  E  K  W  D  T  L  D  R  V  P  F  L  E  Y  N  I  R  K  V  D  I  E  N  R  P  S  P  R  L  F  A  S     100

CACCTTCCATATTATTTAGCACCCAAAAGTCTCAAGAACAACAAAGCTAAAATTATTTATGTCTACAGAAATCCTAAGGATGTTTTAATTTCATTTTTCC    501
H  L  P  Y  Y  L  A  P  K  S  L  K  N  N  K  A  K  I  I  Y  V  Y  R  N  P  K  D  V  L  I  S  F  F  H     134

ATTTTTCAAATATGGTGGTTAAATTAGAAGCTTCAAATACCTTAGAAAATTTCATGGAAAAATTTCTAGATGGAAAAGTGGTGGGAAGCATATGGTTTGA    601
   F  S  N  M  V  V  K  L  E  A  S  N  T  L  E  N  F  M  E  K  F  L  D  G  K  V  V  G  S  I  W  F  D     167

TCACATCAGAGGCTGGTATGAACACAAAAATGACTTCAATATTCTGTTCATGATGTATGAAGATATGAAGAAGGATCTCAGAAGTTCAATACTGAAAATC    701
   H  I  R  G  W  Y  E  H  K  N  D  F  N  I  L  F  M  M  Y  E  D  M  K  K  D  L  R  S  S  I  L  K  I     200

AGCAGTTTTCTTGAGAAGGACCTGAGTGAAGAGGAGGTGGATGCCATTGTGAGGCAGGCTACATTTGAAAACATGAAGTTTATTCCACAAGCAAATTATA    801
   S  S  F  L  E  K  D  L  S  E  E  E  V  D  A  I  V  R  Q  A  T  F  E  N  M  K  F  I  P  Q  A  N  Y  N     234

ATAATATTCTAAGCAATGAAATTGGCAGACGACATAATGAGGGAGCTTTCTTGCGCAAAGGTGCTGTTGGAGACTGGAAACACCACATGACTGTGGAGCA    901
   N  I  L  S  N  E  I  G  R  R  H  N  E  G  A  F  L  R  K  G  A  V  G  D  W  K  H  H  M  T  V  E  Q     267

GAGTGAAAGATTTGACAGGATATTCCAGGAGGAGATGAAAGATTTTCCCTTGAAGTTCATCTGGGATCTAAATGATGAGGCGAATTCTAATCACAGTGCA   1001
   S  E  R  F  D  R  I  F  Q  E  E  M  K  D  F  P  L  K  F  I  W  D  L  N  D  E  A  N  S  N  H  S  A     300

AAATAGTCACATAAAATCTTATCCAAAGGTCATATTTTAATATAAGATATTAACTTGTGACTATAATTCAATTGTTAATTATACAACATTATTAATAAAC   1101
K                                                                                                          301

AAAATAATTAACACCG                                                                                          1117
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Fig. 1. **Nucleotide and deduced amino acid sequences of AST-RB1 cDNA.** The initiation and termination codons are underlined, and the poly(A) addition signal is double-underlined. Amino acid sequences identical to polypeptide fragments from purified AST-RB1 are boxed. The shaded box indicates the *Eco*RI restriction site.

peptide fragments obtained from purified AST-RB1 after digestion with endoproteinase Lys-C. An overlapping region, FIWDLNDEANSNHSAK, was found in the C-terminal amino acid sequence deduced from the 1 kbp cDNA and in the N-terminal from the other.

Nucleotide and deduced amino acid sequences of the cDNA are shown in Fig. 1. The putative initiation (at bases 102-104) and termination (at bases 1005-1007) codons are underlined, and the poly(A) addition signal (at bases 1095-1100) is double-underlined. The shaded-box indicates the *Eco*RI restriction site. Rb1-5 clone was thus found to contain an entire open reading frame. The amino acid sequences of the six peptide fragments shown in boxes completely matched the deduced amino acid sequence of the cDNA except for undefined residues. These results suggest that the cDNA included in Rb1-5 clone encodes AST-RB1. The isolated cDNA consisted of 1,117 bp, including an open reading frame of 903 bp encoding 301 amino acids with a molecular weight of 35,876, and 5′- and 3′-untranslated regions of 101 and 113 bp, respectively.

Comparison of Deduced Amino Acid Sequences—The amino acid sequence deduced from AST-RB1 cDNA was compared with those of seven rat sulfotransferases [ST1A1 (*20*), ST1B1 (*11*), ST1C1 (*21*), ST1E2 (*22*), ST2A1 (*23*), ST2A2 (*24*), and ST2A5 (*25*)] reported previously (Table I). ST1 gene family consists of four

TABLE I. **Similarity of AST-RB1 and rat sulfotransferases.** The percent identities were calculated by the use of the GeneWorks software (IntelliGenetics, CA). Rat sulfotransferases are arbitrarily named from the similarity of their primary structures (*11, 19*).

	AST-RB1	ST1A1[a]	ST1B1[b]	ST1C1[c]	ST1E2[d]	ST2A1[e]	ST2A2[f]	ST2A5[g]
ST1A1	38							
ST1B1	36	52						
ST1C1	36	50	50					
ST1E2	36	50	45	46				
ST2A1	36	34	35	36	34			
ST2A2	36	35	35	37	34	90		
ST2A5	37	37	36	38	33	83	87	

[a]Ozawa *et al.* (*20*). [b]Yamazoe *et al.* (*11*). [c]Nagata *et al.* (*21*). [d]Demyan *et al.* (*22*). [e]Ogura *et al.* (*23*). [f]Ogura *et al.* (*24*). [g]Watabe *et al.* (*25*).

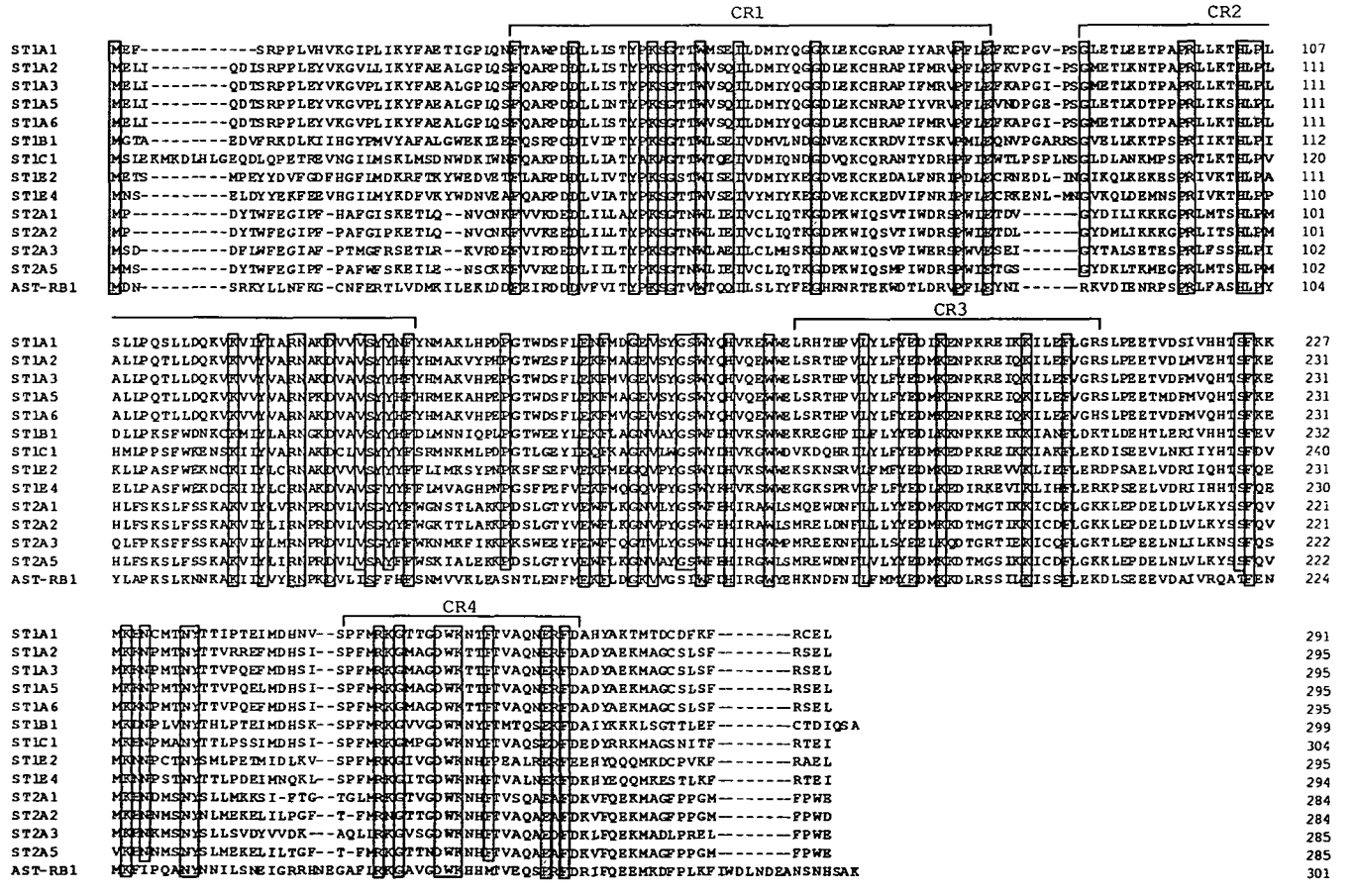

Fig. 2. **Alignment of amino acid sequence deduced from AST-RB1, rat, and human sulfotransferase cDNAs.** Amino acids conserved in all sulfotransferases are shaded in boxes and those conserved in both ST1 and ST2 families but not in AST-RB1 are boxed. Four regions well-conserved among all three sulfotransferase gene families, CR1-4, were found with the help of the PROTOMAT system. Rat and human sulfotransferases are arbitrarily named from the identities of amino acid sequences as shown in footnote 1.

subfamilies, in which enzymes share more than 45% identity with each other at the deduced amino acid level. Similarly, enzymes of the ST2 family (ST2A subfamily) show more than 62% identity within the family (subfamily). The sequence from the newly isolated cDNA showed less than 38% identity with both families of sulfotransferases.

The amino acid sequence of AST-RB1 was aligned with seven rat sulfotransferases and six human forms [ST1A2 (*26*), ST1A3 (*26*), ST1A5 (*27*), ST1A6 (*27*), ST1E4 (*28*), and ST2A3 (*29*)] as shown in Fig. 2 by use of the GeneWorks software (IntelliGenetics). There are 56 amino acid residues conserved among these 13 human and rat sulfotransferases and most of them (47/56) were also conserved in AST-RB1. We found four well-conserved regions among the families (CR1-4) in the alignment with the help of the PROTOMAT system (*30*).

Immunoblot Analysis of E. coli-Expressed Protein—AST-RB1 cDNA was expressed in *E. coli* strain M15 cells using the bacterial expression vector pQE30. The sulfotransferase expressed as a fused protein (recombinant AST-RB1, rAST-RB1) was used for determining substrate specificity of rAST-RB1. Cytosols prepared from rabbit livers and from M15 cells transfected with AST-RB1 cDNA, and purified AST-RB1 were subjected to Western blot analysis using polyclonal antibodies raised against purified AST-RB1 protein (Fig. 3). In cytosols of male rabbit livers (lane 1), a band was detected at an identical mobility (34 kDa) with purified AST-RB1 (lane 2), although this size was slightly smaller than that estimated from the mass of the deduced amino acids. In cytosols of M15 cells expressing rAST-RB1, a fused protein containing an additional peptide (1,999 Da) was detected, which was 2 kDa larger than that of the native AST-RB1 (lane 3). The antibodies did not react with cytosol from M15 cells containing control pQE30 (lane 4).

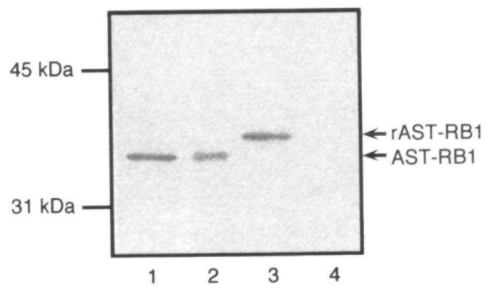

Fig. 3. **Western blot analysis of cytosolic proteins from rabbit livers and *E. coli* cells, and purified AST-RB1.** Proteins were separated on 10% sodium dodecyl sulfate-polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane. The membrane was immunostained with antibodies raised against purified AST-RB1. Lane 1, cytosols from male rabbit livers (10 μg); lane 2, purified AST-RB1 (0.05 μg); lane 3, cytosols of M15 cells expressing rAST-RB1 (0.5 μg); lane 4, cytosols of M15 cells containing control pQE30 (0.5 μg). Size markers are indicated as bars on the left.

Sulfating Activities—To determine catalytic properties of AST-RB1 expressed in *E. coli* M15 cells as a fused protein (rAST-RB1), sulfating activities toward several amines, phenols, and hydroxysteroids were determined (Table II). An alicyclic amine, PTHP, showed the highest activity among those examined. The rate of cytosolic sulfation was 4.4 nmol/mg of protein/min in rAST-RB1-expressed M15 cells, which was 4.4 times higher than that in male rabbit livers. The cDNA-expressed AST-RB1 also

TABLE II. **Sulfating activities of rAST-RB1 expressed in *E. coli* and of male rabbit livers.**

Substrate	rAST-RB1 (A)	Male rabbit liver (B)	Ratio (A/B)
	(nmol/mg of protein/min)		
PTHP[a]	4.4	1.0	4.4
Aniline[b]	0.81	0.30	2.7
4-Chloroaniline[c]	0.50	0.11	4.5
2-Naphthylamine[d]	1.92	0.27	7.1
Desipramine[e]	1.5	0.5	3.0
2-Naphthol[f]	0.19	4.5	0.042
4-Hydroxybiphenyl[g]	0.02	0.53	0.038
Dopamine[h]	<0.01	0.25	<0.04
Testosterone[i]	<0.01	0.06	<0.017
DHEA[j]	0.02	2.5	0.008

[a,b,e,f]The assays were performed at pH 10 (a and e) or 7.4 (b and f) and the concentrations of substrates were 2.0 mM (a), 5.0 mM (b), 0.5 mM (e), or 75 μM (f). After reactions, non-reacted substrates were removed by the extraction with organic solvent and the sulfates in the aqueous layer were measured by reverse-phase high performance liquid chromatography. [c,d,g,h,i]The assays were performed at pH 7.4 and the concentrations of substrates were 1.0 mM (c and d) or 0.2 mM (g, h, and i). After incubations, reaction mixtures were separated on cellulose thin layer plates. The radio active spots were scraped from the plates and quantified by liquid scintillation counting. [j]The assay was carried out with ^{14}C-DHEA (50 μM) at pH 6.8. After incubation the non-reacted DHEA was extracted with *n*-hexane and the product in the aqueous layer was measured by liquid scintillation counting.

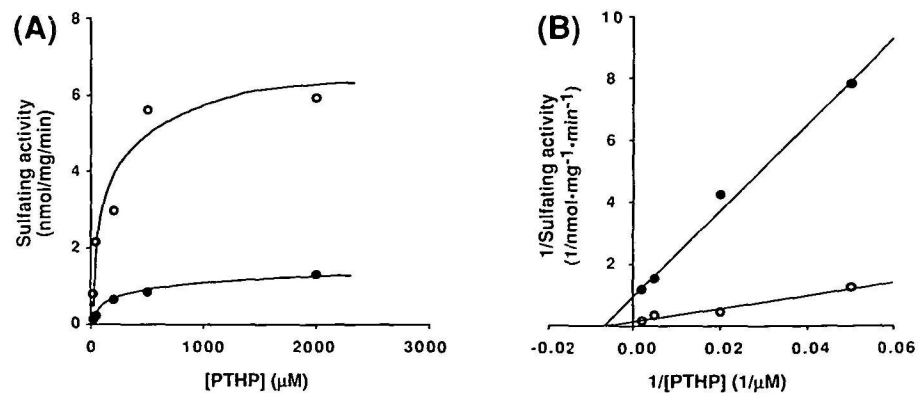

Fig. 4. **PTHP sulfation by cytosols from *E. coli* expressing rAST-RB1 and from male rabbit livers.** PTHP-sulfating activities were measured as described in "MATERIALS AND METHODS" with cytosols from M15 cells expressing rAST-RB1 (open circles) and from male rabbit livers (closed circles). Values shown are means of duplicate or triplicate samples at the substrate concentrations indicated. (A) PTHP-sulfating activity *versus* substrate concentration plots. (B) Corresponding double-reciprocal plots. K_m values were obtained by linear regression analysis: 149 μM (cytosols from *E. coli* expressing rAST-RB1) and 138 μM (cytosols from male rabbit livers).

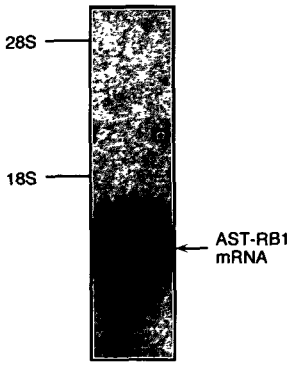

Fig. 5. **Northern blot analysis of mRNA from male rabbit livers.** Total RNA (20 μg) obtained from a male rabbit liver was subjected to electrophoresis on 1.2% formaldehyde-agarose gels and transferred to a nylon membrane. The membrane was hybridized with a ^{32}P-labeled full-length AST-RB1 cDNA overnight at 65°C, then washed twice with washing solution as described in "MATERIALS AND METHODS." Autoradiography was performed at −80°C for 48 h. Size markers are indicated as bars on the left.

mediated efficiently sulfations of aryl and alkyl amines, aniline (0.81 nmol/mg of protein/min), 4-chloroaniline (0.5 nmol/mg of protein/min), 2-naphthylamine (1.92 nmol/mg of protein/min), and desipramine (1.5 nmol/mg of protein/min). These rates were 2.7, 4.5, 7.1, and 3.0 times higher than those in cytosols from male rabbit livers, respectively. On the other hand, sulfating activities of rAST-RB1 expressed in *E. coli* toward phenols (2-naphthol, 4-hydroxybiphenyl, and dopamine) and hydroxysteroids (testosterone and DHEA) were much lower than those in rabbit liver cytosols, and the ratios of male rabbit livers to rAST-RB1 were 0.042, 0.038, <0.04, <0.017, and 0.008, respectively.

The apparent K_m values for PTHP sulfation by cytosols from M15 cells and male rabbit livers were estimated at 149 and 138 μM, respectively (Fig. 4).

Northern Blot Analysis of AST-RB1 mRNA—Northern blot analysis was carried out with total RNAs from male rabbit livers. A typical result is shown in Fig. 5. A single band at about 1.3 kbp hybridized with AST-RB1 cDNA probe was detected.

DISCUSSION

In the present study we have isolated a new sulfotransferase cDNA using polyclonal antibodies raised against AST-RB1 which preferentially catalyzes *N*-sulfation of amino compounds. The sequences of six polypeptide fragments from purified AST-RB1, which were determined by Edman amino acid sequencing, were consistent with the corresponding regions of the deduced amino acid sequence from the cDNA (Fig. 1). The expression of the sulfotransferase in male rabbit livers was confirmed by Northern blot analysis using the cDNA as a probe (Fig. 5). These results suggest that the newly isolated cDNA encodes a rabbit amine sulfotransferase, AST-RB1.

The amino acid sequence deduced from the AST-RB1 cDNA was compared with those of mammalian sulfotransferases reported previously (Table I). AST-RB1 showed only 36-38% identity with rat sulfotransferases (*11, 20-25*), suggesting that it constitutes a new gene family of sulfotransferases distinct from both ST1 and ST2 families in mammals. We arbitrarily named it the ST3 gene family, and AST-RB1 was termed ST3A1.

Although ST3A1 had only 36-38% sequence identity with seven rat sulfotransferases (Table I), most of the residues conserved in rat ST1 and ST2 families were also found in ST3A1 (Fig. 2). Hence these conserved amino acids throughout ST1, ST2, and ST3 gene families might be important for the essential functions of all sulfotransferases. A region containing the GXXGXXK motif in the C-terminal portion, a possible candidate for a PAPS-binding site (*31*), was also conserved in ST3A1 (the region is designated CR4 in this article). Moreover, we identified another three well-conserved regions (CR1-3) among the three sulfotransferase families with the help of the PROTOMAT system (Fig. 2). These regions and the GXXGXXK motif seems to be crucial for mammalian sulfotransferases. In particular, CR2, which could form a tandem β-strand as a possible secondary structure, is conserved in all sulfotransferase families including flavonol sulfotransferases (*32*), heparan sulfotransferases (*33*), chondroitin 6-sulfotransferase (*34*), and bacterial sulfotransferases (*35-37*). The latter bacterial form reported by Baek *et al.* (*37*) is not able to utilize PAPS as a sulfate donor. Therefore, this region may play an essential role common to all types of sulfotransferases, such as the transfer of sulfate from a donor to a substrate. Further studies are, of course, necessary to substantiate the structure and function relationship.

To date, two sulfotransferases have been isolated from livers of guinea pigs (*9*) and rats (*10*) as amine sulfotransferases. Both enzymes catalyzed *O*-sulfation as well as *N*-sulfation to similar extents. The rat form was likely to be included in the ST2 family because of the similarity of its N-terminal amino acid sequence to those of ST2 family enzymes. Moreover, rat ST1A1 (*12*) and human TS-PST (*38*) (arbitrarily named ST1A3 in Fig. 2) mediated sulfamate formation of 2-amino-3-methylimidazo[4,5-*f*]-quinoline and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, and 2-naphthylamine, respectively. These results give rise to the possibility that some sulfotransferases belonging to ST1 and ST2 gene families could catalyze *N*-sulfation of amines, and no rigid data on the existence of amine-specific sulfotransferases have been provided. In the present study, the catalytic properties of ST3A1 were determined using the enzyme expressed in *E. coli*. The protein expressed in this system had 17 additional amino acid residues at the N-terminal site of AST-RB1. It was demonstrated that an additional peptide fused to the N-terminus of sulfotransferases did not alter kinetic parameters drastically in the cases of flavonol 3-sulfotransferases (*39*) and human P-PST (*40*) (arbitrarily termed ST1A3). In addition, we have obtained similar results with rat sulfotransferases, ST1C1 and ST1B1. The histidine-tagged ST1C1 and the enzyme with the tag removed by enterokinase showed almost identical apparent K_m and V_{max} values for *p*-nitrophenol (unpublished data). Therefore, the additional peptide would not alter kinetic parameters of sulfotransferases. The recombinant protein showed a high selectivity toward amines and mediated only marginally *O*-sulfation of phenols and hydroxysteroids (Table II). It catalyzed PTHP-sulfation at low substrate concentrations (Fig. 4). As mentioned above, some forms

belonging to the ST1 and ST2 families were shown to have *N*-sulfation activity, but these enzymes rather showed high activities for *O*-sulfation. The K_m values for PTHP sulfation by cytosols from *E. coli* expressing rAST-RB1 and from male rabbit livers were of similar levels. These data indicate that ST3A1 is the first form catalyzing selectively amine *N*-sulfation among unequivocally characterized sulfotransferases. However, the physiological role of sulfamate formation and the endogenous substrates of ST3A form remain unclear.

In conclusion, we isolated a new cDNA encoding AST-RB1 purified from male rabbit livers. AST-RB1 was found to constitute a new gene family of sulfotransferases (ST3) by comparison of the deduced amino acid sequence of AST-RB1 cDNA with those of other cytosolic sulfotransferase cDNAs. Therefore, we arbitrarily termed AST-RB1 ST3A1. The distinct catalytic property of ST3A1 for amine substrates was shown using a bacterial expression system.

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