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Expression and Characterization of a Novel Thyroid Hormone-Sulfating Form of Cytosolic Sulfotransferase from Human Liver

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ABSTRACT

Sulfation is an important conjugation reaction for a wide range of endogenous and exogenous compounds in humans, including steroids, bile acids, catecholamine neurotransmitters and thyroid hormones. The cDNA for a distinct human cytosolic sulfotransferase (ST), hST1B2, has been isolated from a human liver λZap cDNA library. The hST1B2 cDNA consists of 1144 bp and contains the coding region for a novel human cytosolic ST that has been termed hST1B2 on the basis of its sequence similarity to a rat sulfotransferase, ST1B1. The hST1B2 cDNA contains an 888-bp open reading frame that encodes a 296-amino acid protein with a calculated molecular mass of 34,897 Da. The hST1B2 cDNA also has a 127-bp 5′ untranslated region (UTR) and a 129-bp 3′-UTR, including a 22-bp poly(A)$^+$ tract. The amino acid sequence of hST1B2 is 74%, 53%, 53%, 52%, 56%, and 34% identical to the amino acid sequences of rat ST1B1 and human P-PST-1, P-PST-2, M-PST, EST, and DHEA-ST, respectively. Enzymatically active hST1B2 was expressed in the bacterial expression vector pKK233–2 for kinetic characterization and in the bacterial expression vector pQE-31, which generates a histidine-tagged fusion protein for the generation of antibodies. Expressed hST1B2 sulfates small phenols such as 1-naphthol and *p*-nitrophenol and thyroid hormones, including 3,3′-diiodothyronine, triiodothyronine, reverse triiodothyronine, and thyroxine. No activity was detected when several steroids or dopamine were tested as substrates. High levels of hST1B2 message were detected by Northern blot analysis in RNA isolated from human liver, colon, small intestine, and blood leukocytes. Immunoblot analysis detected a protein with the same mass as expressed hST1B2 in several human tissues that also possessed hST1B2 message. These results indicate that a novel cytosolic ST is present in human tissues, which may have an important role in thyroid hormone and xenobiotic metabolism.

Sulfation is a major conjugation reaction in human tissues involved in the biotransformation of a large number of endogenous compounds such as catecholamine neurotransmitters, steroids, bile acids, and thyroid hormones, as well as a wide variety of drugs and xenobiotics. Sulfation involves the transfer of the sulfonate group from PAPS to an acceptor compound, usually to form a sulfate ester or sulfamate. The sulfation reaction is catalyzed by a superfamily of enzymes termed STs (Mulder and Jakoby, 1990; Falany and Roth, 1993; Falany, 1997). Five human cytosolic STs have been cloned and characterized from human tissues; this number most likely will increase.

In adults, thyroid hormones have an important function in maintaining and regulating metabolic processes in most tissues. To achieve this function, the levels of active T3 are regulated tightly in the body. The sulfation of thyroid hormones has been proposed to have an important role in stimulating the metabolism and inactivation of thyroid hormones. Under normal conditions, the prohormone T4 is secreted from the thyroid glands and is converted in peripheral tissues to biologically active T3 or biologically inactive rT3 by type ID-I (Visser, 1996). Sulfation of the phenolic hydroxyl group of T4 inhibits the formation of T3 by ID-I and stimulates the synthesis of rT3. The sulfation of T3 stimulates its conversion to inactive 3,3′-T2 (Visser, 1994, 1996). Thyroid hormone sulfation has been proposed as a rapid and irreversible mechanism for the inactivation and degradation of thyroid hormones in the presence of ID-I activity (Visser, 1996).

To date, five different human cytosolic STs have been identified and cloned from human tissues; these cytosolic STs are a hydroxysteroid ST, hDHEA-ST (Adams and McDonald,

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ABBREVIATIONS: PAPS, 3′-phosphoadenosine-5′-phosphosulfate; ST, sulfotransferase; hDHEA-ST, human dehydroepiandrosterone sulfotransferase; PST, phenol sulfotransferase; hEST, human estrogen sulfotransferase; hP-PST-1, human phenol-sulfating form of phenol sulfotransferase; hM-PST, human monoamine-sulfating form of phenol sulfotransferase; E1, estrone; E2, β-estradiol; DHEA, dehydroepiandrosterone; T4, 3,3′,5,5′-tetraiodothyronine (thyroxine); T3, 3,5,3′-triiodothyronine; rT3, 3,3′,5′-triiodothyronine; 3,5-T2, 3,5-diiodothyronine; 3,3′-T2, 3,3′-diiodothyronine; ID-I, type I iodothyronine deiodinase; PCR, polymerase chain reaction; pfu, *Pyrococcus furiosus*; bp, base pair(s); UTR, untranslated region; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TLC, thin layer chromatography; MBP, maltose binding protein.

1979; Falany *et al.*, 1989; Comer *et al.*, 1993); an estrogen ST, hEST (Aksoy *et al.*, 1994; Falany *et al.*, 1995); and three forms of PST: the monoamine-sulfating form of PST (hM-PST) (Heroux and Roth, 1988; Wood *et al.*, 1994; Ganguly *et al.*, 1995) and two forms of the phenol-sulfating form of PST (hP-PST1 and hP-PST2) (Falany *et al.*, 1990; Wilborn *et al.* 1993; Veronese *et al.*, 1994). Young *et al.* (1988) reported that T3 is sulfated by both hM-PST and hP-PST activities in human liver, although these authors also suggest that another thyroid hormone-sulfating activity may be present.

In this report, we describe the isolation and expression of a novel cytosolic ST cDNA from a human liver cDNA library that has the capability of sulfating thyroid hormones and small phenolic compounds. The cDNA was expressed in two bacterial expression systems for characterization of the kinetic properties of the enzyme and preparation of pure enzyme for the generation of antibodies. Comparison of the translation of this novel ST with the sequences of other STs indicates that hST1B2 is a member of the PST gene family and is similar to a rat sulfotransferase termed ST1B1 (Yamazoe *et al.*, 1994). Because of its similarity to ST1B1, this new human ST has been referred to as hST1B2, or thyroid hormone ST, until a standardized nomenclature is adopted for the ST gene family. Thyroid hormones are the major endogenous substrates for hST1B2 identified to date and may represent characteristic identifying activities for this enzyme. Characterization of the kinetic and physical properties of hST1B2 is important in understanding the metabolism of thyroid hormones as well as the metabolism of many drugs and xenobiotics in human tissues.

Materials and Methods

Oligonucleotide primers were synthesized in the Molecular Biology Core Facility of the Comprehensive Cancer Center (University of Alabama at Birmingham, Birmingham, AL). PCR reagents and Elongase were purchased from GIBCO BRL (Gaithersburg, MD). Restriction enzymes were obtained from New England Biolabs (Beverly, MA). The TA-Cloning kit was purchased from InVitrogen (San Diego, CA). The λUni-Zap XR human liver cDNA library was purchased from Stratagene (La Jolla, CA). [^{35}S]PAPS, [1,2,6,7-^{3}H]DHEA (79 Ci/mmol), [6,7-^{3}H]E2 (45 Ci/mmol), [2,4,6,7-^{3}H]E1 (96 Ci/mmol), [7-^{3}H]pregnenolone (25 Ci/mmol), [1,2,3-^{3}H]cortisol (54.4 Ci/mmol), and [2,4-^{3}H]dihydroequilenin (34.5 Ci/mmol) were purchased from New England Nuclear Research Products (Boston, MA). Nonradiolabeled PAPS was obtained from Dr. Sanford Singer (Dayton, OH). *p*-Nitrophenol, 1-naphthol, 3,5-T2, and DEAE-Sepharose CL-6B were obtained from Sigma Chemical (St. Louis, MO). T4 and T3 were purchased from Aldrich Chemical (Milwaukee, WI). rT3 was obtained from Calbiochem (La Jolla, CA). 3,3′-T2 was a gift from Dr. T. J. Visser (Erasmus University, Rotterdam, The Netherlands). Minoxidil was a gift from the Upjohn Co. (Kalamazoo, MI). Affinity-purified goat anti-rabbit horseradish peroxidase conjugate was purchased from Southern Biotechnology (Birmingham, AL). The Luminol chemiluminescence substrate kit was obtained from Pierce Chemical (Rockford, IL). The QIA Expression System was purchased from Qiagen (Chatsworth, CA). Human multiple-tissue RNA blot membranes were from Clontech (Palo Alto, CA). All other chemicals were of reagent grade quality.

Isolation of human liver hST1B2 cDNA. The sequence of a novel form of human cytosolic ST related to rat ST1B1 was presented previously (poster format; Fourth International ISSX Meeting; 1995 Aug 27–31; Seattle, WA) (Yamazoe *et al.*, 1995). This sequence was used to design oligonucleotide primers to facilitate isolation of the hST1B2 cDNA from a human liver λZAP cDNA library using PCR, which was were carried out using pfu polymerase (Stratagene) as the DNA polymerase to increase fidelity of the reactions. In the first PCR, an aliquot of the human liver λZAP cDNA library (2×10^6 pfu) was used as the DNA template with an oligonucleotide primer incorporating the termination codon of the hST1B2 sequence (5′-TTTAAATCTCTGTGCGGAATTGAAGTG-3′) as an antisense primer and T3 primer as a sense primer. After 30 cycles of PCR (42° annealing), an aliquot of the reaction mix was used as the template for an additional 30 cycles of PCR with an oligonucleotide incorporating the initial ATG of the putative hST1B2 sequence (5′-ACAATCTGGTATTAAATGCTTTCC) as the sense primer and with the same antisense primer. A 908-bp product was generated by the second PCR; this fragment was subcloned into the pCR II vector for characterization and sequence analysis. The hST1B2 cDNA was subjected to double-stranded sequencing by the dideoxynucleotide chain termination method using Sequenase 2.0 and α-^{35}S-dATP to label the newly synthesized strands. The ^{35}S-labeled products were resolved in 6% polyacrylamide urea sequencing gels. The sequences were read manually and analyzed using MacVector 6.0 sequence analysis software (Oxford Molecular, Campbell, CA). The cDNA sequence of hST1B2 coding region was obtained using the T7 and M13 reverse primers in the pCRII vector and oligonucleotide primers synthesized to the internal sequence of hST1B2.

To isolate the 5′ and 3′ untranslated sequences of hST1B2 from the λZAP human liver cDNA library, an inverse PCR procedure was used (Li and Nichols, 1997). This method combines the utility of inverse PCR with the convenience of *in vivo* mass excision of λZAP cDNA libraries. *In vivo* mass excision of the human liver λZAP cDNA library in pBluescript 7zf was carried out using 2.0×10^7 pfu to ensure statistical representation of the excised clones in the library. Inverse PCR of the excised library was performed using Elongase (Life Technologies, Grand Island, NY) and two cDNA-specific primers: an antisense primer, 5′-GGTACCAGGAAAAGGCTGTA (bp 573–592), and a sense primer, 5′-CTAGAGAAGAACCTGAATGATGAG (bp 761–784). The primers were selected to amplify the 5′ and 3′ flanking sequences of the hST1B2 cDNA as well as the pBluescript vector. The annealing temperature was 55° and the extension time was 10 min to allow for amplification of the complete plasmid with Elongase. To amplify adequate amounts of the 5′ and 3′ nontranslated regions of the hST1B2 cDNA as well as the 5′ and 3′ coding regions of the hST1B2 cDNA for subcloning, a second round of nested PCR was carried out using Elongase and the products of the initial PCR as a template. Two nested PCR reactions were performed to separately generate the 5′- and 3′-UTRs. The T3 primer and an antisense hST1B2 primer 5′-CACTAACCCAAGTAGTACCTGATTTAGG (bp 266–293) generated the 5′ nontranslated region. The T7 primer and a sense primer 5′-CACATCTACCAACTACAGTGATGG (bp 852–87) was used to generate the 3′-nontranslated region. The amplified products obtained from the nested PCR reactions were subcloned into pCR2.1 and sequenced.

Bacterial expression of HST1B2. The hST1B2 cDNA was expressed in two different bacterial expression systems. The QIA expression system was used to generate pure protein to raise specific polyclonal antibodies. The QIA expression system generates a histidine-tagged fusion protein that can be purified by affinity chromatography on an Ni-NTA resin. However, the presence of the added histidine residues may alter the kinetic properties of the enzyme (Lewis *et al.* 1997). The pKK233–2 expression system was used to generate kinetically active hST1B2 without a histidine tag for comparison with other human STs expressed in the same system (Falany *et al.*, 1994; Ganguly *et al.*, 1995; Falany *et al.*, 1995).

To insert the hST1B2 cDNA into the pQE-31 vector, PCR was used to generate an hST1B2 cDNA with a *Bam*HI site at the 5′ end and a *Hin*dIII site at the 3′ end. The sequence of the 5′-sense primer was 5′-ACCGGGATCCCGAAATGCTTTCCCCAAAAGA-3′, and the sequence of the 3′-antisense primer was 5′-TACCCAAGCTTGGTTTAAATCTCTGTGC GGAATTG. The pCRII/hST1B2 cDNA was used as a template, and the purified PCR fragment was subcloned into the

*Bam*HI and *Hin*dIII sites of the pCRII vector. The pCRII/hST1B2(*Bam*HI/*Hin*dIII) plasmid was digested with *Bam*HI and *Hin*dIII, and the hST1B2 cDNA fragment was ligated into the *Bam*HI and *Hin*dIII sites of the pQE-31 vector. The pQE/hST1B2 expression construct then was transformed into M15 bacteria carrying the pREP4 repressor plasmid. Transformants were selected on Luria broth agar plates containing both ampicillin (100 μg/ml) and kanamycin (25 μg/ml). To express the pQE/hST1B2 fusion protein, M15 cells containing the pQE/hST1B2 construct were grown in Luria broth medium containing both ampicillin and kanamycin to an A_{600nm} value of 0.7. IPTG was added to a final concentration of 2 mM, and the culture was grown for an additional 5 hr. Cells were pelleted by centrifugation and cell pellets were resuspended in sonication buffer (50 mM Na-phosphate, pH 8.0, 300 mM NaCl) at 2.5 vol/g of wet weight. Cell pellets were frozen in dry ice/ethanol, thawed in cold water, and then sonicated on ice three times in 30-sec bursts with a 30-sec cooling period between each burst. The supernatant fraction containing hST1B2 activity was recovered after centrifugation at 10,000 $\times$ *g* for 20 min.

The pQE/hST1B2 fusion protein was purified by Ni-NTA affinity chromatography according the manufacturer's instructions (Qiagen). After Ni-NTA affinity chromatography, the purified hST1B2 fraction was analyzed by SDS-PAGE, and the purity was estimated at 90%. Before injection into the rabbits for antibody production, hST1B2 was purified further by electroelution from the polyacrylamide gels using a BioRad (Hercules, CA) model 422 electroelution unit. The eluted hST1B2 protein was concentrated in an Amicon (Beverly, MA) model 30 microconcentrator, washed three times with phosphate-buffered saline, and used to raise antibodies in rabbits.

The hST1B2 cDNA also was expressed in the pKK233–2 bacterial expression vector for generation of native protein for kinetic characterization (Falany *et al.*, 1994; Ganguly *et al.*, 1995; Falany *et al.*, 1995). To position the initial ATG of hST1B2 close to the ribosome binding site in pKK233–2, a PCR procedure was used to generate an hST1B2 cDNA with a *Bsph*I site incorporating the initial ATG and a *Hin*dIII site after the termination codon. The sense oligonucleotide primer was (5′-CGGTCATGATTTCCCCAAAAGATATTCTGCG-3′), which included a single nucleotide difference (underlined) from hST1B2. This resulted in the incorporation of an isoleucine residue instead of a leucine residue as the second amino acid of the expressed protein. The sequence of the antisense primer was (5′-TAC-CCAAGCTTGGTTTAAATCTCTGTG CGGAATTG-3′). Using the pCRII/hST1B2 cDNA as a template, the hST1B2 cDNA with the *Bsph*I and *Hin*dIII sites was amplified and subcloned into pCRII. The modified pCRII/hST1B2 was digested with *Bsph*I and *Hin*dIII and ligated into the *Nco*I and *Hin*dIII sites of pKK233–2. *Escherichia coli* XL1-Blue cells were transformed with the hST1B2/pKK233–2 vector. Enzymatically active hST1B2 was expressed and purified from bacterial cytosol by DEAE-Sepharose CL-6B chromatography as described previously for four other human cytosolic STs (Falany *et al.*, 1994, 1995; Ganguly *et al.*, 1995).

Northern blot analysis of human tissues. The expression of hST1B2 message in different human tissues was investigated using commercially available human multiple tissue RNA blots (Clontech). Each Northern blot contains 2 μg of poly(A)$^+$ RNA isolated from different human tissues. For use as a probe, the hST1B2 cDNA (1×10^9 cpm/μg) was labeled with [^{32}P]dCTP by random-priming using an Oligolabeling Kit (Pharmacia, Piscataway, NJ). Hybridizations were carried out for 2 hr at 65° in Quickhyb (Stratagene) containing 1.25×10^6 cpm/ml [^{32}P]hST1B2. The blots were washed twice for 15 min at 22° in 2× standard saline citrate containing 0.1% SDS and then twice for 15 min in 0.1× standard saline citrate containing 0.1% SDS at 60°. Autoradiography was performed at −70° with an intensifying screen.

Immunoblot analysis. To raise polyclonal antibodies, pure His-tagged hST1B2 protein (100 μg) was mixed 1:1 with Freund's complete adjuvant and injected subcutaneously at several sites along the back of a female New Zealand White rabbit. Two weeks later, the rabbit received a booster injection of 100 μg of hSULT1B2 in Freund's incomplete adjuvant in a similar manner. Two weeks after the second injection, the rabbit was bled, and the serum was tested for the presence of antibodies to hSULT1B2 by immunoblot analysis of human tissues as described previously (Falany *et al.*, 1995; Ganguly *et al.*, 1995). After resolution of human cytosolic proteins by SDS-PAGE, the proteins were electrotransferred to nitrocellulose paper. Rabbit anti-hSULT1B2 antisera was diluted 1:150,000 and incubated with the nitrocellulose filter for 1 hr. Goat anti-rabbit IgG horseradish peroxidase conjugate was used as the secondary antibody, and immunoconjugates were visualized by chemiluminescence using a Luminol/peroxide chemiluminescence substrate kit.

ST assays. Sulfation activity with nonradiolabeled acceptor compounds was determined using [^{35}S]PAPS as described previously (Falany *et al.*, 1994). The reactions contained 50 mM Tris·HCl, pH 7.4, 7 mM $MgCl_2$, 20 μM [^{35}S]PAPS, and varying concentrations of substrates in a final volume of 62.5 μl. Control reactions were identical except no substrates were added. Reactions were incubated for 20 min at 37° and terminated by spotting a 55-μl aliquot of each reaction onto a silica gel F-250 TLC plate. For *p*-nitrophenol, 1-naphthol, and diethylstilbestrol, the TLC plate was developed in methylene chloride/methanol/ammonium hydroxide (85:15:5, v/v/v). Sulfation of thyroid hormones was determined as described previously (Sekura *et al.*, 1980; Sekura *et al.*, 1981) except the TLC plates were developed in isopropanol/chloroform/methanol/water (40:40:20:8, v/v/v/v). The ^{35}S-labeled products were localized by autoradiography and then scraped into scintillation vials. The radioactivity was determined by scintillation spectroscopy.

Sulfation assays using radiolabeled substrates were determined as described previously (Falany *et al.*, 1990; Falany *et al.*, 1994; Ganguly *et al.*, 1995). Tritiated steroid substrates included DHEA, E_1, E_2, pregnenolone, testosterone, cortisol, and dihydroequilenin. Minoxidil sulfation activity was assayed as described by Falany and Kerl (1990). The STs frequently show substrate inhibition with increasing concentrations of substrates; therefore, for comparison of sulfation rates between substrates, each substrate is assayed at the concentration that gives maximal activity.

The apparent K_m values for the sulfation of several thyroid hormones by hST1B2, hP-PST-1, and hM-PST were determined using the enzymes expressed and purified from *E. coli* as described previously (Falany *et al.*, 1994; Ganguly *et al.*, 1995). Because the STs display substrate inhibition with increasing concentrations of thyroid hormones, the apparent K_m values were determined using thyroid hormone concentrations in the ascending portions of their concentration-versus-activity curves. For T4 and T3, the concentrations in the reactions were 1–20 μM. For rT3, the concentration range was 1–40 μM. For 3,3′-T2, the concentration range used for hST1B2 and hM-PST was 1–30 μM, whereas for hP-PST-1, 3,3′-T2 concentrations of 0.2–2 μM were used. The apparent K_m values were calculated using EnzymeKinetics (Trinity Software, Campton, NH).

Results

Isolation and characterization of the hST1B2 cDNA. The hST1B2 cDNA was isolated from a human liver λZap cDNA library using a PCR procedure. The human liver cDNA library has been used previously in our library for the isolation of hP-PST-1, hDHEA-ST, and hEST cDNAs (Comer *et al.*, 1993; Wilborn *et al.*, 1993; Falany *et al.*, 1995). The oligonucleotide primers used to generate the hST1B2 cDNA were designed to a cDNA sequence reported previously (abstract form; Fourth International ISSX meeting) (Yamazoe *et al.*, 1995). The PCR procedure generated a 908-bp fragment with an 888-bp open reading frame encoding a 296-amino acid protein with a molecular mass of 34,896 Da (Fig. 1). The cDNA was termed hST1B2 on the basis of its sequence sim-

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hST1B2                                                GAACCACCACCGTTTCCCATGGTTACTCTACAGCAGCTTCACTGCCTG   48
AAAATGAAAACTGAACAAAGGGATTAAATTGTGAGAACAACTGTCAATCTATTATATATTTGTACAATCTGGTATTAAA  127

ATG CTT TCC CCA AAA GAT ATT CTG CGA AAA GAT CTG AAG TTG GTC CAT GGT TAT CCC ATG  187
Met Leu Ser Pro Lys Asp Ile Leu Arg Lys Asp Leu Lys Leu Val His Gly Tyr Pro Met   20

ACC TGT GCT TTT GCA AGC AAC TGG GAA AAA ATT GAA CAG TTC CAT AGC AGA CCA GAT GAC  247
Thr Cys Ala Phe Ala Ser Asn Trp Glu Lys Ile Glu Gln Phe His Ser Arg Pro Asp Asp   40

ATT GTG ATA GCC ACT TAT CCT AAA TCA GGT ACT ACT TGG GTT AGT GAA ATT ATA GAC ATG  307
Ile Val Ile Ala Thr Tyr Pro Lys Ser Gly Thr Thr Trp Val Ser Glu Ile Ile Asp Met   60

ATT CTA AAT GAT GGA GAT ATT GAA AAA TGT AAG CGA GGT TTT ATT ACT GAA AAA GTT CCA  367
Ile Leu Asn Asp Gly Asp Ile Glu Lys Cys Lys Arg Gly Phe Ile Thr Glu Lys Val Pro   80

ATG TTG GAA ATG ACT CTC CCT GGA TTA AGA ACA TCA GGT ATA GAA CAA TTG GAG AAG AAT  427
Met Leu Glu Met Thr Leu Pro Gly Leu Arg Thr Ser Gly Ile Glu Gln Leu Glu Lys Asn  100

CCA TCA CCC CGG ATT GTG AAA ACA CAT CTA CCG ACT GAT CTT CTT CCT AAA TCT TTC TGG  487
Pro Ser Pro Arg Ile Val Lys Thr His Leu Pro Thr Asp Leu Leu Pro Lys Ser Phe Trp  120

GAA AAC AAT TGC AAG ATG ATT TAT CTG GCT CGT AAT GCC AAG GAT GTT TCA GTC TCA TAT  547
Glu Asn Asn Cys Lys Met Ile Tyr Leu Ala Arg Asn Ala Lys Asp Val Ser Val Ser Tyr  140

TAC CAT TTT GAC TTA ATG AAT AAT TTA CAG CCT TTT CCT GGT ACC TGG GAA GAA TAT CTG  607
Tyr His Phe Asp Leu Met Asn Asn Leu Gln Pro Phe Pro Gly Thr Trp Glu Glu Tyr Leu  160

GAG AAA TTC TTA ACT GGA AAA GTG GCC TAT GGT TCC TGG TTT ACT CAT GTT AAA AAC TGG  667
Glu Lys Phe Leu Thr Gly Lys Val Ala Tyr Gly Ser Trp Phe Thr His Val Lys Asn Trp  180

TGG AAG AAA AAG GAA GAA CAC CCA ATA CTT TTT TTG TAC TAT GAA GAT ATG AAA GAG AAT  727
Trp Lys Lys Lys Glu Glu His Pro Ile Leu Phe Leu Tyr Tyr Glu Asp Met Lys Glu Asn  200

CCA AAG GAG GAA ATC AAG AAG ATC ATT AGA TTT CTA GAG AAG AAC CTG AAT GAT GAG ATC  787
Pro Lys Glu Glu Ile Lys Lys Ile Ile Arg Phe Leu Glu Lys Asn Leu Asn Asp Glu Ile  220

TTG GAT AGG ATC ATC CAT CAC ACC TCA TTT GAA GTG ATG AAG GAC AAT CCT TTG GTA AAT  847
Leu Asp Arg Ile Ile His His Thr Ser Phe Glu Val Met Lys Asp Asn Pro Leu Val Asn  240

TAT ACA CAT CTA CCA ACT ACA GTG ATG GAT CAT AGC AAA TCC CCT TTT ATG CGT AAA GGG  907
Tyr Thr His Leu Pro Thr Thr Val Met Asp His Ser Lys Ser Pro Phe Met Arg Lys Gly  260

ACG GCT GGT GAC TGG AAG AAT TAC TTC ACC GTG GCC CAA AAT GAG AAA TTT GAT GCT ATT  967
Thr Ala Gly Asp Trp Lys Asn Tyr Phe Thr Val Ala Gln Asn Glu Lys Phe Asp Ala Ile  280

TAT GAG ACA GAA ATG TCC AAA ACT GCA CTT CAA TTC CGC ACA GAG ATT TAA AGT GTC TAA 1027
Tyr Glu Thr Glu Met Ser Lys Thr Ala Leu Gln Phe Arg Thr Glu Ile ***              296

ATCACACATCTGAAGAAATAAGAGATTGTCTGTAGTTGATTGAAACGAGGGCAGTTATGAATTGATTTGGGCAATCAAA 1106
TGAATTTATAAAGGAGAAAAAAAAAAAAAAAAAAAAAA                                          1144
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Fig. 1. *Numbers on the right*, nucleotide sequence and derived translation of hST1B2. *, Stop codon. *Underlined sequences*, primers used in the initial PCR to generate the coding region of hST1B2 cDNA (sense, bp 112–136; antisense, bp 993-1019) and primers used in the inverse PCR procedure (antisense, bp 573–592; sense, bp 761–784; antisense, bp 266–293; sense, bp 852–875).

ilarity to the previously reported rat ST sequence ST1B1 (Yamazoe *et al.*, 1994, 1995). The amino acid sequence of hST1B2 is 74% identical and 85.5% similar to the amino acid sequence of ST1B1 (Fig. 2).

The 5′- and 3′-UTRs of the hST1B2 cDNA were isolated from the human liver cDNA library using an inverse PCR procedure (Li and Nichols, 1997). The initial pair of primers were oriented to amplify all the 5′ and 3′ regions of the hST1B2 cDNA in the cDNA library. A second nested PCR was carried out with hST1B2-specific primers and pBluescript-specific primers to generate sufficient 5′- and 3′-UTRs for subcloning and sequencing. This procedure produced only one major band for both the 5′- and 3′-UTRs. Both bands contained 293 bp of hST1B2 sequence. The sequences of the

FIGURE 2. AMINO ACID SEQUENCES OF HUMAN CYTOSOLIC SULFOTRANSFERASES

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                    .         .         .         .         .         .   60
  hST1B2 MLSPKDILRKDLKLVHGYPMTCAFASNWEKIEQFHSRPDDIVIATYPKSGTTWVSEIIDM
  rST1B1  GTAE VF     II     VY   LG     E Q   C    P          L   V
hP-PST-1  ELIQ TS PP EY K V LIKY  EALGPLQS QA    LL S            Q
hP-PST-2  ELIQ IS PP EY K V LIKY  EALGPLQS QA    LL S            Q L
  hM-PST  ELIQ TS PP EY K VPLIKY  EALGPLQSSQA    LL N            Q L
    hEST  N EL YYE .FEE   IL YKD VKY DNV A QA    L                 VY
hDHEA-ST  SDDFLWFEGIAFPTM FRSETLRKVRD .... VI DE VI L       N LA  LCL

                    .         .         .         .         .         .  120
  hST1B2 ILNDGDIEKCKRGFITEKVPMLEMTLPGLRTSGIEQLEKNPSPRIVKTHLPTDLLPKSFW
  rST1B1 V    NV     DV  S      QNV  A R  V L K T     I     I
hP-PST-1  YQG  L   H AP FMR  F  FKA  I.P  M T KDT A  LL     LA   QTLL
hP-PST-2  YQG  L   H AP FMR  F  FKV  I.P  M T KN  A  LL     LA   QTLL
  hM-PST  YQG  L   N AP YVR  F  VND  E.P  L T KDT P  LI S   LA   QTLL
    hEST  YKE  V    EDV FNRI F  CRKEN MN. VK  DEMN          PE   A
hDHEA-ST MHSK  A. WIQSVPIWERSPWVESEI..... YTA SESE   LFSS   IQ F    F

                    .         .         .         .         .         .  180
  hST1B2 ENNCKMIYLARNAKDVSVSYYHFDLMNNLQPFPGTWEEYLEKFLTGKVAYGSWFTHVKNW
  rST1B1 D K         G   A           I  L            A N       D   S
hP-PST-1 DQKV VV V       A      YH AKVH E    DSF    MV E S    YQ  QE
hP-PST-2 DQKV VV V       A      YH AKVY H     SF    MA E S    YQ  QE
  hM-PST DQKV VV V   P   A      HR EKAH E    DSF    MA E S    YQ  QE
    hEST  KD  I   C      A  F Y F  VAGH N  SFP FV   MQ Q P    YK   S
hDHEA-ST SSKA V   M  PR  L  G F WKNMKFIKK KS    F W CQ T L     D IHG

                    .         .         .         .         .         .  240
  hST1B2 WKKKEEHPILFLYYEDMKENPKEEIKKIIRFLEKNLNDEILDRIIHHTSFEVMKDNPLVN
  rST1B1  E R G          L K   K     AN  D T DEHT E  V
hP-PST-1  ELSRT  V Y F         R  Q  LE VGRS PE TV FMVQ    KE  K  MT
hP-PST-2  ELSRT  V Y F         R  Q  LE VGRS PE TV LMVE    KE  K  MT
  hM-PST  ELSRT  V Y F         R  Q  LE VGRS PE TM FMVQ    KE  K  MT
    hEST  E GKSPRV   F   L  DIRK VI L H   RKPSE LV        QE  N  ST
hDHEA-ST MPMR  KNF L S  EL QDTGRT E  CQ  G T EP E NL LKNS  QS  E KMS

                    .         .         .         .         .         .  296
  hST1B2 YTHLPTTVMDHSKSPFMRKGTAGDWKNYFTVAQNEKFDAIYETEMSKTALQFRTEI*
  rST1B1       EI            VV        MT S       KKKL G T E C D QSA*
hP-PST-1   TV QEF    I       M     TT       R   D AKK AGCS T  S L*
hP-PST-2   TVRREF    I       M     TT       R   D AEK AGCS S  S L*
  hM-PST   TV QEL    I       M     TT       R   D AEK AGCS S  S L*
    hEST   T  DEI NQKL       IT     H    L     KH  QQ KEST K     *
hDHEA-ST  SL SVDYVVDK.AQLL   VS     H     A D  KLFQEK ADLPRELFPWE*
```

Fig. 2. Comparison of amino acid sequence of hST1B2 with the sequences of rat ST1B1 and other human cytosolic STs. The sequences used in the comparison were hST1B2 (see Fig. 1), rat ST1B1 (Yamazoe *et al.*, 1994), hP-PST-1 (Wilborn *et al.*, 1993), hP-PST-2 (Her *et al.*, 1996), hM-PST (Ganguly *et al.*, 1995), hDHEA-ST (Comer *et al.*, 1993), and hEST (Falany *et al.*, 1995). The amino acid sequences of the STs were aligned using MacVector Sequence analysis software. The initial methionine residue of the hST1B2 sequence is denoted as the first amino acid. Short gaps (*dots*) have been inserted into the sequence comparisons to optimize the alignments.

inverse PCR products were identical to those of the initial hST1B2 sequence in the regions of overlap. The 3′-UTR terminated in a poly(A)$^+$ tract (Fig. 1). Compilation of these sequences generated a 1144-bp cDNA with 127 bp of 5′ untranslated sequence and 129 bp of 3′ untranslated sequence.

Fig. 2 also shows the comparison of the translation of hST1B2 to the amino acid sequences of five other human cytosolic STs: hEST (Falany *et al.*, 1995), hP-PST-1 (Wilborn *et al.*, 1993), P-PST-2 (Her *et al.*, 1996), hM-PST (Ganguly *et al.*, 1995), and hDHEA-ST (Comer *et al.*, 1993). The amino acid sequence of hST1B2 is 74.9%, 73.6%, 73.6%, and 74.2% similar and 56.3%, 52.9%, 53.2%, and 51.5% identical to the sequences of hEST, hP-PST-1, hP-PST-2, and hM-PST, respectively. In comparison, hST1B2 is only 53.9% similar and 33.9% identical to the sequence of hDHEA-ST. The sequence of hST1B2 is more similar to the sequence of the members of the PST gene family than to the human hydroxysteroid ST hDHEA-ST.

Northern blot analysis of the expression of hST1B2. The expression of the hST1B2 message in different human tissues was investigated by Northern blot analysis of poly(A)$^+$ RNA isolated from different human tissues using

the hST1B2 cDNA as a probe and commercially prepared multiple-tissue RNA blots. Fig. 3 shows the results of the hybridization of poly(A)$^+$ RNA isolated from 16 different human tissues. hST1B2 hybridized relatively strongly with poly(A)$^+$ RNA isolated from liver, peripheral blood leukocytes, colon (mucosal lining), small intestine/jejunum, and spleen. A lesser degree of hybridization was observed with poly(A)$^+$ RNA isolated from lung, placenta, and thymus. There are at least four bands present in each poly(A)$^+$ RNA sample, and the relative intensities of the bands in each sample were consistent. The largest and most intense band was ~7900 nucleotides. The smallest and second most intense band was ~1300 nucleotides. All of the bands were larger than the coding region of hST1B2. Identical results were obtained using the 588-bp *Nco*I/*Xho*I restriction fragment isolated from the coding region of hST1B2 as a probe and a different lot number of human multiple-tissue RNA membranes (Clontech).

Immunoblot analysis of rabbit anti-hST1B2 antibodies. Polyclonal antibodies were raised in rabbits to the pure histidine-tagged hST1B2 fusion protein. Fig. 4 (*1*) shows that the rabbit anti-hST1B2 antibodies did not cross-react with bacterially expressed hP-PST-1, hM-PST, or hDHEA-ST. However, the antibodies showed some cross-reactivity with bacterially expressed hEST. To generate specific antibodies, the anti-hST1B2 antibody was incubated overnight at 4° with pure hEST-MBP fusion protein (Falany *et al.*, 1995) and then tested for reactivity with hST1B2 and hEST (Fig. 4, *2*). Preabsorption of the anti-hST1B2 antibody with pure hEST/MBP effectively removed the antibodies, which recognized both ST proteins, and generated an antibody preparation that is specific for hST1B2. Even if the antibody reacts with both proteins, the migration of hST1B2 and hEST during SDS-PAGE is sufficiently different to discriminate between the two proteins during immunoblot analysis of tissue cytosols.

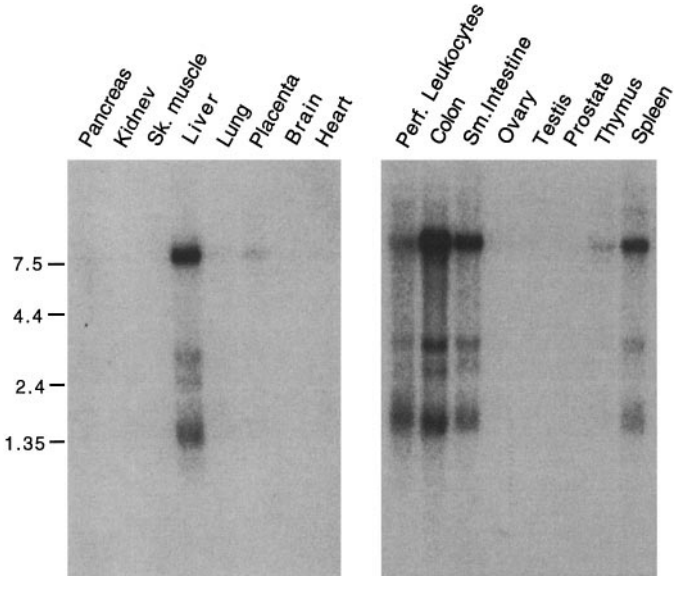

Fig. 3. Northern blot analysis of RNA isolated from different human tissues using the hST1B2 cDNA as a probe. Commercial multiple-tissue RNA blots (Clontech) containing RNA isolated from 16 different human tissues were probed with ^{32}P-labeled hST1B2 cDNA. Each lane contained ~2 μg of poly(A)$^+$ RNA. *Left*, *Lane 1*, pancreas. *Lane 2*, kidney. *Lane 3*, skeletal (*Sk.*) muscle. *Lane 4*, liver. *Lane 5*, lung. *Lane 6*, placenta. *Lane 7*, brain. *Lane 8*, heart. *Right*, *Lane 1*, peripheral (*Perf.*) blood leukocytes. *Lane 2*, colon (mucosal lining). *Lane 3*, small (*Sm.*) intestine. *Lane 4*, ovary. *Lane 5*, testis. *Lane 6*, prostate. *Lane 7*, thymus. *Lane 8*, spleen.

Immunoblot analysis of cytosol prepared from several different human tissues is shown in Fig. 5. hST1B2 immunoreactivity was readily detectable in the colon and small intestine, and a relatively lower level of reactivity was present in liver cytosol. No immunostaining was observed in cytosol prepared from lung, kidney, or endometrium. The expression of hST1B2 protein in tissues is consistent with the expression of its message in these tissues (Fig. 3). The immunoreactivity is specific for hST1B2 because the hEST-preabsorbed anti-hST1B2 antibody did not react with an aliquot of expressed hEST included with the protein samples.

Substrate reactivity of expressed hST1B2. Enzymatically active hST1B2 was expressed in bacteria using the hST1B2/pKK233–2 construct and purified for kinetic characterization. The expressed enzyme was capable of sulfating thyroid hormones, several small phenols, and minoxidil (Table 1). Because substrate inhibition frequently is observed with the cytosolic STs (Falany *et al.*, 1989, 1994, 1995), increasing concentrations of substrates were assayed to determine the concentration of substrate giving maximal activity. hST1B2 sulfated small phenols such as 1-naphthol, 4-nitrophenol, and diethylstilbestrol; however, no activity was detected with any of the steroids tested as substrates (Table 1). As observed with other human STs, substrate inhibition was not observed with increasing concentrations of PAPS in the reactions (Falany *et al.*, 1990; Falany *et al.*, 1995; Ganguly *et al.*, 1995). The apparent K_m value of expressed hST1B2 for PAPS was ~1.2 μM (data not shown), which is similar to the values reported for other bacterially expressed human STs (Falany *et al.*, 1990, 1995; Ganguly *et al.*, 1995). The apparent K_m values for the sulfation of 1-naphthol, *p*-nitrophenol, and diethylstilbestrol by hST1B2 were 5, 42, and 60 μM, respectively.

Human liver P-PST and M-PST activities have been reported to sulfate T3 (Young *et al.*, 1988); however, the ability of these enzymes to sulfate other thyroid hormones was not reported. For comparative purposes, the apparent K_m values for several thyroid hormones were determined using bacterially expressed hST1B2, hP-PST-1, and hM-PST activities. Table 2 shows that both hM-PST and hP-PST-1 do not significantly sulfate T4 and rT3, although both PSTs readily sulfated T3 and 3,3′-T2. hST1B2 sulfated both T4 and rT3 with apparent K_m values of 23 and 141 μM, respectively. hP-PST-1 and hM-PST sulfated 3,3′-T2 with apparent K_m values of 2.3 and 26 μM, respectively, compared with an apparent K_m value of 51 μM for sulfation by hST1B2. T3 was conjugated with apparent K_m values of 15 and 20 μM by P-PST-1 and M-PST, respectively. hST1B2 also rapidly sulfated T3; however, a reliable apparent K_m value for T3 could not be calculated from the concentration-versus-activity curves.

Discussion

This study describes the isolation, expression, and characterization of a novel form of human liver cytosolic ST that is capable of conjugating thyroid hormones and small phenolic compounds. This ST tentatively has been termed hST1B2, or thyroid hormone ST, until the adoption of a formal ST nomenclature. The cloning, expression, and characterization of

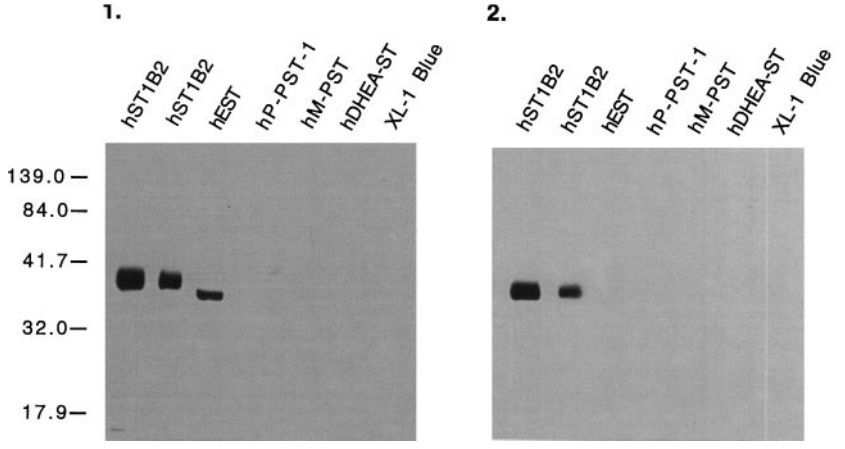

Fig. 4. Immunoblot analysis of hST1B2. Polyclonal antibodies were raised in rabbits against pure hST1B2. The specificity of the anti-hST1B2 antiserum was tested using the human STs expressed in *E. coli* XL1-Blue cells. The expressed STs were resolved by SDS-PAGE in 12.5% gels and then transferred to nitrocellulose membranes as described by Towbin *et al.* (1979). After incubation with a 1/150,000 dilution of the rabbit anti-hST1B2 IgG, goat anti-rabbit IgG horseradish peroxidase conjugate was used as the secondary antibody and immunoconjugates were visualized by chemiluminescence using a Luminol/peroxide chemiluminescence substrate kit (Pierce Chemical). *Lanes* contained DEAE-Sepharose Cl-6B purified STs. *1*: *Lane 1*, 0.5 μg of hST1B2. *Lane 2*, 0.2 μg of hST1B2. *Lane 3*, 20 μg of hEST. *Lane 4*, 20 μg of hP-PST. *Lane 5*, 20 μg of hM-PST. *Lane 6*, 20 μg of DHEA-ST. To remove the cross-reactivity of the rabbit anti-hST1B2 antiserum with hEST, the antibody was preincubated with pure hEST/MBP fusion protein (Falany *et al.*, 1995) 16 hr at 4° before use of the antiserum in the immunoblot of the DEAE-Sepharose Cl-6B purified STs shown in *2*. *2*: *Lane 1*, 0.5 μg of hST1B2. *Lane 2*, 0.2 μg of hST1B2. *Lane 3*, 20 μg of hEST. *Lane 4*, 20 μg of hP-PST. *Lane 5*, 20 μg of hM-PST. *Lane 6*, 20 μg of DHEA-ST. *Lane 7*, 20 μg of control *E. coli* XL1-Blue cytosol.

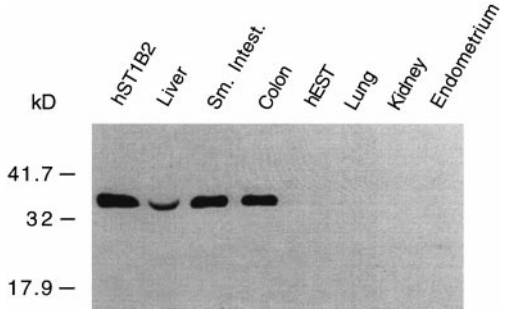

Fig. 5. Immunoblot analysis of hST1B2 in human tissues. Cytosol was prepared from several human tissues, and the cytosolic proteins were resolved by SDS-PAGE in 12.5% gels. The proteins were electrotransferred to nitrocellulose membranes. After incubation with a 1/10,000 dilution of the rabbit anti-hST1B2 preabsorbed against hEST, goat anti-rabbit IgG horseradish peroxidase conjugate was used as the secondary antibody and immunoconjugates were visualized by chemiluminescence. *Lane 1*, 0.1 μg of DEAE-Sepharose Cl-6B purified expressed hST1B2. *Lane 2*, 300 μg of human liver cytosol (from 55-year-old man). *Lane 3*, 50 μg of human small intestine (*Sm. Intest.*) cytosol (from 32-year-old man). *Lane 4*, 50 μg of human colon cytosol (from 17-year-old girl). *Lane 5*, 5 μg of DEAE-Sepharose Cl-6B purified expressed hEST. *Lane 6*, 100 μg of human lung cytosol (from 55 year-old woman). *Lane 7*, 100 μg of human kidney cytosol (from 59 year-old woman). *Lane 8*, 100 μg of human endometrium cytosol (from 29-year-old woman). The human tissues were obtained through the Tissue Procurement Service of the Comprehensive Cancer Center at the University of Alabama at Birmingham (Birmingham, AL).

TABLE 1

Substrate reactivity of hST1B2

hST1B2 was expressed in *E. coli,* partially purified by DEAE-Sepharose Cl-6B chromatography (Falany *et al.*, 1994), and used to assay the ability of the enzyme to sulfate a number of possible substrates. Because substrate inhibition is a characteristic of the STs (Falany, 1997), each substrate was assayed using a range of concentrations to determine the concentration generating maximal activity. The steroids were tested as substrates at several concentrations from 1 to 25 μM. The reaction rates for each substrate then were compared using the concentration generating maximal activity. The activity of hST1B2 with 3,3′,5-T3 is chosen as 100%.

Substrate	Activity		[Substrate]
	nmol/min/mg	%	μM
3,3′,5-T3	1.11	100	50
3,3′,5,5′-T4	0.05	5	200
3,3′,5′-rT3	0.67	61	200
3,3′-T2	18.9	1628	150
3,5-T2	0.01	1	200
1-Naphthol	74.4	6705	10
4-Nitrophenol	11.4	1030	100
Diethylstilbestrol	0.82	74	100
E2	N.D.		1–25
E1	N.D.		1–25
DHEA	N.D.		1–25
Cortisol	N.D.		1–25
Pregnenolone	N.D.		1–25
Testosterone	N.D.		1–25
Dihydroequilenin	N.D.		1–25

N.D., no detectable activity.

hST1B2 indicate that at least six distinct forms of cytosolic ST are expressed in human tissues. Comparison of the amino acid sequence of hST1B2 with the sequences of other human STs demonstrates that hST1B2 is a member of the PST gene family, which includes the PSTs, ESTs, and ST1B1 (Yamazoe *et al.*, 1994).

Northern blot analysis of poly(A)$^+$ RNA from different human tissues with the hST1B2 cDNA detected RNAs of at least four different sizes in each tissue under high stringency conditions (Fig. 3). The relative intensities of the bands were constant between poly(A)$^+$ RNA isolated from different tissues, suggesting that the bands are not regulated independently. Identical hybridization patterns were observed with different hST1B2 probes and different multiple-tissue Northern blots. To investigate the nature of the multiple RNA bands in the Northern blots, we conducted PCR analysis of the hST1B2 coding region using the λZAP cDNA library as a template. Only a single major product was produced, which was the hST1B2 cDNA with no unspliced intronic sequence. Analysis of the 5′- and 3′-UTRs of the hST1B2 cDNAs in the λZAP cDNA library by inverse PCR generated only one major band for both regions, indicating that the major hST1B2 cDNA in the library represented the smallest of the bands on the Northern blots. No large 3′ untranslated sequences were generated, indicating that hST1B2 cDNAs containing alternate sites of polyadenylation are not abundant in the cDNA

TABLE 2

Apparent K_m values for thyroid hormone sulfation by hST1B2, hP-PST-1, and hM-PST

Each value represents the average ± standard deviation of three separate determinations of the apparent K_m using the EnzymeKinetics programs. The human ST activities were expressed in *E. coli* XL1-Blue cells using the pKK233-2 vector as described in the text.

Expressed ST	Thyroid hormone apparent K_m			
	T4	T3	rT3	3,3′-T2
		μM		
ST1B2	23 ± 9	NC[a]	141 ± 28	51 ± 14
P-PST-1	Trace[b]	15 ± 8	Trace[b]	2.3 ± 0.5
M-PST	Trace[b]	20 ± 3	Trace[b]	26 ± 4

[a] An apparent K_m value could not be calculated from the ascending portion of the concentration curve (0.5–10 μM) using the EnzymeKinetics programs.

[b] The levels of activity were too low to be reliably measured (<0.025 pmol sulfated/min).

library. Hybridization of the same multiple-tissue RNA blots with the hEST and hP-PST-1 cDNAs did not show this same hybridization pattern, indicating that the hST1B2 cDNA is not hybridizing to other known ST messages. Analysis of the relationship between the different sizes of poly(A)$^+$ RNAs that hybridize with the hST1B2 cDNA will require further investigation of these RNAs and of the hST1B2 structural gene.

Immunoblot analysis of different human tissue cytosols shows that the hST1B2 protein is expressed in several tissues that express the hST1B2 message. hST1B2 message is most highly expressed in the gastrointestinal tract, liver, and blood leukocytes (Fig. 3). Lung and kidney did not possess either hST1B2 message or protein. Immunoreactive hST1B2 also was readily detectable in cytosol from small intestine, colon, and liver (Fig. 5). The presence of high levels of hST1B2 in leukocytes may be responsible for the low levels of detection of hST1B2 message in the thymus and placenta, as well as relatively high levels in spleen. High levels of hST1B2 in blood leukocytes may be related to a role in the inactivation of circulating T3 and T4.

hST1B2 was capable of sulfating all the thyroid hormones tested, although with greatly differing efficiencies (Table 1). The highest levels of sulfation activity were observed with 3,3′-T2, although maximal activity was observed at a concentration of ~150 μM. In contrast, 3,5-T2 was conjugated at a much lower rate, suggesting that an outer- ring iodine increases enzyme binding or activity. The biologically active form of thyroid hormone, T3, was sulfated relatively rapidly by hST1B2 and displayed maximal activity at the lowest concentration of the thyroid hormones tested. However, an apparent K_m value could not be calculated reliably for T3 using low concentrations of the hormone. This inability to calculate a reliable K_m value with the use of five different algorithms in the EnzymeKinetics program may be due to allosteric regulation of hST1B2 activity by T3. This aspect of the regulation of ST activity by allosteric binding of substrates is under investigation. hST1B2 also displays substrate inhibition with increasing thyroid hormone concentrations, which adds to the complexity of determination of the kinetic properties of the enzyme.

For comparative purposes, the ability of human P-PST-1 and M-PST to sulfate several thyroid hormones was investigated. Neither human PST sulfated T4 or rT3 significantly, suggesting that two iodines on the outer ring inhibit sulfation by the PSTs. hP-PST-1 and hM-PST displayed similar apparent K_m values for the sulfation of T3, although hP-PST-1 had a 10-fold lower K_m value for the sulfation of 3,3′-T2 than hM-PST. Young *et al.* (1988) reported K_m values for T3 sulfation by partially purified human liver P-PST and M-PST activities of 81–217 μM. The sulfation of other thyroid hormones by these authors was not reported. These observations suggest that T3 sulfation is catalyzed by several ST isoforms in human tissues, whereas T4 may be sulfated primarily by hST1B2. The relatively low level of expression of hST1B2 in liver compared with that in small intestine and colon suggests that T4 sulfation occurs primarily in extrahepatic tissues. A more detailed analysis of the expression of hST1B2 and thyroid hormone sulfation in human tissues will be required to resolve the role of the individual STs in thyroid hormone sulfation.

hST1B2 represents the fifth cytosolic ST present in human liver cytosol capable of sulfating small phenols such as 1-naphthol and *p*-nitrophenol (Veronese *et al.*, 1994; Wood *et al.*, 1994; Falany *et al.*, 1995; Zhu *et al.*, 1996). 1-Naphthol was sulfated maximally by hST1B2 at the lowest concentration (10 μM) and apparent K_m value (5 μM) of any of the small phenolic substrates assayed. *p*-Nitrophenol, which has been used as a model substrate to discriminate between P-PST and M-PST activities in human tissues (Reiter *et al.*, 1983; Weinshilboum, 1986, 1990) was maximally sulfated at 100 μM with an apparent K_m value of ~60 μM. The presence of multiple STs with different kinetic properties in a tissue capable of sulfating the same substrate such as *p*-nitrophenol emphasizes the necessity for evaluating the presence of a given ST in a tissue preparation by several different methods including activity, immunoreactivity, and Northern blot analysis.

Of the human STs examined, hST1B2 has the most immunological and sequence similarity with hEST. However, no steroid sulfation activity was observed with expressed hST1B2 using several different steroids as substrates. Preliminary mapping results indicate that the hST1B2 gene is located close to the hEST gene on human chromosome 4q13 (Falany and Wang, 1997). In the PST family, hM-PST is incapable of sulfating steroids, whereas both human P-PSTs are capable of sulfating the phenolic hydroxyl group of estrogens but not of hydroxysteroids such as DHEA and pregnenolone (Falany *et al.*, 1990; Hernandez *et al.*, 1992; Forbes-Bamforth and Coughtrie, 1994; Ganguly *et al.*, 1995).

Thyroid hormones modulate a large number of metabolic processes by regulating the production and activity of many enzyme systems; production and metabolism of other hormones; and utilization of substrates, vitamins, and minerals. In adult animals, thyroid hormones help maintain metabolic stability in tissues. This requires that the circulating levels of thyroid hormones be maintained within relatively narrow limits. Thyroid hormones are metabolized primarily by deiodination catalyzed by several deiodinases (Visser, 1994, 1996) and by conjugation with either glucuronic acid or sulfate (Visser *et al.*, 1993; Moreno *et al.*, 1994; Visser, 1994, 1996). Several human glucuronyltransferases are capable of conjugating different thyroid hormones with differing efficiencies (Visser *et al.*, 1993; Burchell *et al.*, 1994); however, the kinetic parameters of the glucuronidation of thyroid hormones in human tissues have not been reported. The glucuronide conjugates are excreted in the bile, and the thyroid hormone glucuronides are available for deglucuronidation to

regenerate the hormones. In contrast to sulfation, glucuronidation does not stimulate the deiodination of the thyroid hormones, so it does not have the potent inactivation effect of sulfation.

Sulfation of thyroid hormones greatly stimulates their irreversible inactivation via deiodination by ID-I. Sulfation of T4 and T3 increases their inner-ring deiodination ~200- and ~40-fold compared with that of the nonsulfated hormones (Visser, 1994, 1996). Inner-ring deiodination of T4-sulfate and T3-sulfate is considered an inactivation process because rT3 and 3,3′-T2 have little biological activity even if they are desulfated. In addition, the rapid deiodination of T4 and T3 prevents their regeneration by desulfation. Outer-ring deiodination of T4 to T3 leads to enhanced biological activity, but all the other metabolites of T4 have little biological activity. Thus, if the bioactivity of the thyroid hormone, essentially T3, is controlled by competing activation (T4 to T3) and inactivation pathways (metabolism of T3), the ability of sulfation to inactivate T3 and stimulate the metabolism of T4 and T3 to inactive metabolites may be important in regulating the plasma levels of free thyroid hormones.

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