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Biochemical Characterization and Tissue Distribution of Human SULT2B1

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The human hydroxysteroid sulfotransferase (SULT) family is comprised of two subfamilies, SULT2A1 and SULT2B1. We characterized the substrate specificity, *in vitro* biochemical properties, and tissue distribution patterns of human SULT2B1a and SULT2B1b. In contrast to the wide substrate specificity of SULT2A1, SULT2B1a and SULT2B1b specifically catalyzed the sulfonation of 3β-hydroxysteroids with high catalytic efficiency. Both SULT2B1 enzymes also sulfonated dihydrotestosterone. *In vitro* studies revealed that the biochemical properties of SULT2B1a and SULT2B1b were not significantly different from each other. However, tissue expression analysis suggested that they are differentially regulated. In contrast to the limited tissue distribution of SULT2A1, SULT2B1 was detected in a variety of hormone-responsive tissues including placenta, ovary, uterus, and prostate. The catalytic activity toward dehydroepiandrosterone and dihydrotestosterone, biologically important androgens, coupled with expression in prostate suggests that SULT2B1 may play a novel regulatory role that protects against the mitogenic effects of androgens.

Key Words: **steroid; metabolism; SULT; sulfotransferase; androgens; DHEA; prostate; sulfonation; sulfation.**

Sulfate conjugation (sulfonation) is a major phase II biotransformation reaction in eukaryotes and is catalyzed by a superfamily of cytosolic sulfotransferases (SULTs) (1). This reaction plays a critical role in the metabolism of many drugs, hormones, neurotransmitters and other xenobiotics (2). Although a bioactivation reaction for some procarcinogens (3), sulfonation is typically an inactivation/detoxification reaction, increasing the water solubility of the parent compound and facilitating its renal or biliary excretion (1, 2). Sulfonation/desulfonation is also an important pathway for hormonal regulation (4). For example, the sulfonation of steroid hormones, such as dehydroepiandrosterone (DHEA), represents a fundamental mechanism for regulating androgen activity *in vivo*. In its sulfonated form, DHEA is quantitatively the most abundant circulating steroid hormone in humans (5). It is believed that the sulfonated moiety represents a readily accessible, yet biologically "inactive" storage form for the anabolism of estrogens and androgens. This storage form then becomes reactivated via hydrolysis of the sulfate group by steroid sulfatases (2, 4). The present study focuses on the biochemical characterization of a novel human SULT, SULT2B1, involved in steroid sulfonation in the prostate.

As many as 57 SULT genes have been identified from diverse organisms including plants, insects, and mammals. Eleven SULT genes have been identified in humans. Those genes span three families comprised of three phenol SULT subfamilies (SULT1A, SULT1B and SULT1C (1, 6, 7)), an estrogen SULT (SULT1E1 (8)) two hydroxysteroid SULTs (SULT2A1 and SULT2B1 (4, 9)) and an orphan SULT of unknown function (SULT4A1 (10–12)).

SULT isoforms often display broad, overlapping substrate specificities and tissue expression patterns. For example, human estrogen sulfonation is catalyzed by at least three SULT isoforms, SULT1A1, SULT1E1, and SULT2A1 (13). Furthermore, the genes that encode these enzymes are polymorphic, resulting in allozymes with altered biochemical properties (14–17). Consequently, the biochemical characterization of individual metabolic enzymes *in vivo* has remained a challenging and elusive area of pharmacogenetics.

Recently, the human (Hsa)SULT2B1 gene, encoding a member of the hydroxysteroid SULT family, has been cloned and mapped to the long arm of chromosome 19—approximately 500 kb telomeric to the only other known human hydroxysteroid sulfotransferase, (Hsa)SULT2A1 (9). Comparative sequence analysis between SULT2B1 and SULT2A1 genes revealed strik-

Abbreviations used: SULT, sulfotransferase; Hsa, *Homo sapians;* Cpo, *Cavia porcellus;* Mmu, *Mus musculus;* DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.

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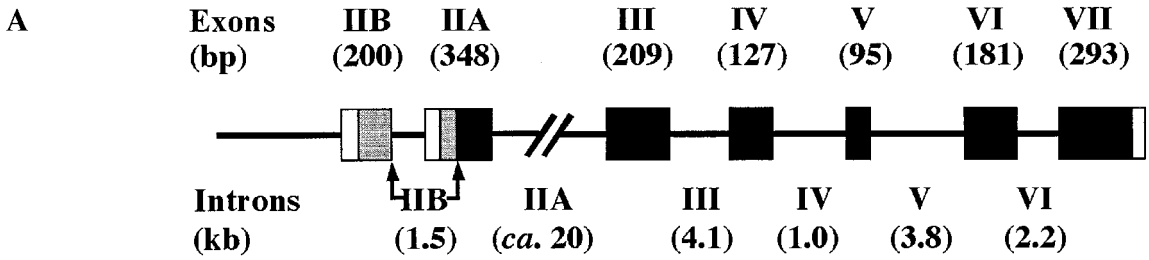

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                1          11         21         31         41         51         61
SULT2B1a        MASPPP----------------FHSQKLPGEYFRYKGVPFPVGLYSLESISLAENTQDVRDDDIFIITYPK
SULT2B1b        MDGPAEPQIPGLWDTYEDDISEISQKLPGEYFRYKGVPFPVGLYSLESISLAENTQDVRDDDIFIITYPK
SULT2A1         ------------------------MSDDFLWFEGIAFPTMGFRSETLRKVRDEFVIRDEDVIILTYPK
Consensus       m  p                     sqklpgeyfrykgvpfpvglyslesislaentqdvrdddifiitypk

                71         81         91         101        111        121        131
SULT2B1a        SGTTWMHEIICLILKEGDPSWIRSVPIWERAPWCETIVGAFSLPDQYSPRLMSSHLPIQIFTKAFFSSKA
SULT2B1b        SGTTWMHEIICLILKEGDPSWIRSVPIWERAPWCETIVGAFSLPDQYSPRLMSSHLPIQIFTKAFFSSKA
SULT2A1         SGTNWLAEILCLMHSKGDAKWIQSVPIWERSPWVESEIGYTALSETESPRLFSSHLPIQLFPKSFFSSKA
Consensus       sgttwmieiiclilkegdpswirsvpiwerapwcetivgafslpdqysprlmsshlpiqiftkaffsska

                141        151        161        171        181        191        201
SULT2B1a        KVIYMGRNPRDVVVSLYHYSKIAGQLKDPGTPDQFLRDFLKGEVQFGSWFDHIKGWLRMKGKDNFLFITY
SULT2B1b        KVIYMGRNPRDVVVSLYHYSKIAGQLKDPGTPDQFLRDFLKGEVQFGSWFDHIKGWLRMKGKDNFLFITY
SULT2A1         KVIYLMRNPRDVLVSGYFFWKNMKFIKKPKSWEEYFEWFCQGTVLYGSWFDHIHGWMPMREEKNFLLLSY
Consensus       kviymgrnprdvvvslyhyskiagqlkdpgtpdqflrdflkgevqfgswfdhikgwlrmkgkdnflfity

                211        221        231        241        251        261        271
SULT2B1a        EELQQDLQGSVERICGFLGRPLGKEALGSVVAHSTFSAMKANTMSNYTLLPPSLLDHRRGAFLRKGVCGD
SULT2B1b        EELQQDLQGSVERICGFLGRPLGKEALGSVVAHSTFSAMKANTMSNYTLLPPSLLDHRRGAFLRKGVCGD
SULT2A1         EELKQDTGRTIEKICQFLGKTLEPEELNLILKNSSFQSMKENKMSNYSLLSVDYVVDK-AQLLRKGVSGD
Consensus       eelqqdlqgsvericgflgrplgkealgsvvahstfsamkantmsnytllppslldhrrgaflrkgvcgd

                281        291        301        311        321        331        341
SULT2B1a        WKNHFTVAQSEAFDRAYRKQMRGMP--TFPWDEDPEEDGSPDPEPSPEPEPKPSLEPNTSLEREPRPNSS
SULT2B1b        WKNHFTVAQSEAFDRAYRKQMRGMP--TFPWDEDPEEDGSPDPEPSPEPEPKPSLEPNTSLEREPRPNSS
SULT2A1         WKNHFTVAQAEDFDKLFQEKMADLPRELFPWE-----------------------------------
Consensus       wknhftvaqseafdrayrkqmrgmp  tfpwdedpeedgspdpepspepepkpslepntslereprpnss

                351        361        371        381        391        401        411
SULT2B1a        PNPSPGQASETPHPRPS
SULT2B1b        PNPSPGQASETPHPRPS
SULT2A1         -----------------
Consensus       pspspgqasetphprps
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FIG. 1. (Hsa)SULT2B1 gene structure. (A) SULT2B1 gene structure. Open boxes indicate 5′ and 3′ UTRs. Gray boxes indicate gene-specific ORF sequence. Black boxes indicate common ORF sequence. Two distinct mRNA species, SULT2B1a and SULT2B1b, are transcribed from the human SULT2B1 gene as a result of alternate transcriptional initiation and mRNA splicing. (B) Amino acid alignment of human SULT2 enzymes using JellyFish software (BioWire). The putative residue conferring 3β-hydroxysteroid specificity is white on black. N-linked glycosylation consensus sequences are underlined. A myristoylation consensus sequence is in bold.

ing structural homology in their exon–intron organization, suggestive of a strong evolutionary link between these two genes.

Interestingly, (Hsa)SULT2B1 encodes two isoforms, SULT2B1a and SULT2B1b via alternative transcriptional initiation and alternative mRNA splicing (Fig. 1) (9). Multiple tissue Northern blot analysis revealed that SULT2B1 expression is limited to human placenta, prostate and trachea. By contrast, SULT2A1 is expressed in liver, adrenal and stomach, but not in prostate or placenta (18, 19).

As an initial step toward our understanding of the functional role played by SULT2B1 in human tissues, we conducted an *in vitro* biochemical characterization of recombinant SULT2B1a and SULT2B1b proteins expressed in a eukaryotic system. In a systematic fashion we studied the effects of temperature, Mg^{2+} and pH on SULT2B1 activity. We also determined the sub-

TABLE 1

Oligonucleotides Used in This Study

Construction of recombinant SULT2B1a and SULT2B1b	
SULT2B1aF(-23)/*Kpn*I	5′-GCTTAGGTACCATATGGCGTCTCCCCCACCTTTCC-3′
SULT2B1bF(-68)/*Kpn*I	5′-GCTTAGGTACCATATGGACGGGCCCGCCGAG-3′
SULT2B1abR1030/*Eco*RI	5′-GCTTAGAATTCTTATGAGGGTCGTGGGTGCGGGGTC-3′
Tissue expression analysis	
SULT2B1aF-23	5′-ATGGCGTCTCCCCCACCTTTCC-3′
SULT2B1bF-60c	5′-CCCGCCGAGCCCCAGATCC-3′
SULT2B1abR290	5′-GCGGGGGCTGTACTGGTC-3′
SULT2B1abR367	5′-GCCTTGGTGAAGATCTGGATGGGAAGATGGGAGC-3′
GAPDHF	5′-CCACATCGCTCAGACACCAT-3′
GAPDHR	5′-CCTCCGACGCCTGCTTCACCAC-3′

strate specificity of SULT2B1a and SULT2B1b by evaluating their ability to catalyze the sulfate conjugation of several biologically relevant steroid hormones, including androgens and estrogens. Furthermore, we determined the expression patterns of SULT2B1a and SULT2B1b using isoform-specific polymerase chain reaction (PCR) with a cDNA array consisting of 24 human tissues. The work described herein will provide critical insight in guiding future studies of the role of SULT2B1 in tissues where steroid bioavailability plays a clear and significant role in the etiology of steroid hormone-dependent disease.

MATERIALS AND METHODS

Generation of recombinant SULT2B1a and SULT2B1b. Purified, recombinant SULT2B1a and SULT2B1b proteins were used for all *in vitro* studies. SULT2B1a and SULT2B1b expression constructs were previously generated (9) in the eukaryotic expression vector pCR3.1 (Invitrogen, Carlsbad, CA) and were kindly provided by Dr. Richard Weinshilboum (Mayo Clinic, Rochester, MN). The SULT2B1a and SULT2B1b coding sequences were PCR amplified from the pCR3.1 expression constructs using gene-specific primers (Table 1). The amplified products were then subcloned into the baculovirus expression vector, pBLUEBac-His2A (Invitrogen), which encodes an amino-terminal hexahistidine (6×-His) tag sequence to facilitate purification. The nucleotide sequence of each construct was confirmed using dye-terminator sequencing (Fox Chase Cancer Center DNA Sequencing Facility). The 6×-His-tagged SULT2B1 expression constructs were cotransfected with 1 μg of BacVector-3000 viral DNA (Novagen, Madison, WI) through liposome-mediated transfection (Cellfectin, Invitrogen) into Sf-9 insect cells. Individual viral clones were isolated to generate high titer viral stocks. These were used to infect 1-liter cultures of Sf-9 cells which were incubated at 27°C for 48 h. The 6×-His-tagged proteins were purified using cobalt immobilized metal affinity chromatography (Talon resin, Clontech, Palo Alto, CA) with imidazole as the elution agent. The purified proteins were dialyzed overnight against enzyme storage buffer (50 mM potassium phosphate (pH 7.4), 50 mM NaCl, 50% glycerol). Purified proteins were analyzed by 10% SDS–polyacrylamide gel electrophoresis (PAGE) and were stained with Coomassie blue (GelCode, Bio-Rad, Hercules, CA). Protein concentrations were determined using the Bradford protein assay (Bio-Rad).

Radiometric sulfotransferase assays. Standard radiometric sulfotransferase assays (18) were performed with the following modifications: reactions were conducted in 0.2-ml thin-wall PCR tubes in a final reaction volume of 30 μl and were incubated at 37°C in a thermal cycler (Hybad) with no agitation for 15 min unless otherwise indicated. The standard reaction buffer consisted of the following final concentrations: 50 mM potassium phosphate (pH 7.4), 0.75 mg/ml BSA, 13 mM DTT, 7.5 mM $MgCl_2$. For reactions in which the pH was varied, the following buffers (each at 50 mM) were used over their respective buffering ranges: Mes (pH 5–6.4); Mops (pH 6.37–7.39); Tris–HCl (pH 7.0–8.62). Reactions were initiated with the addition of 0.4 μM ^{35}S-PAPS (NEN, Boston, MA). Following incubation at 37°C, all reactions were quenched with 20 μl of a 1:1 mixture of 50 mM barium hydroxide/barium acetate. Unincorporated ^{35}S-PAPS was precipitated from the reactions by adding, sequentially, 10 μl of 0.1 M $ZnSO_4$, 10 μl of 0.1 M $Ba(OH)_2$, 10 μl of 0.1 M $ZnSO_4$ and 80 μl of H_2O. The resulting precipitate was pelleted by centrifugation at 3220*g* for 10 min. Radiolabeled reaction products were detected in 100 μl of the supernatant by liquid scintillation counting. Each assay was performed in triplicate and "blank" samples utilized DMSO as the vehicle control. All hydroxysteroids utilized in this study were purchased from Sigma, reconstituted in DMSO and stored in the dark. For experiments performed to determine the substrate specificity, each substrate was evaluated at concentrations ranging from 1 pM to 3 mM. When activity was observed, the substrate was then assayed over a more narrow concentration range sufficiently high to observe potential substrate inhibition. If no substrate inhibition was observed, then the data were fit to the Michaelis–Menten equation to estimate V_{max} and K_m. When substrate inhibition was observed, the kinetic parameters V_{max} and K_m were approximated as above at low substrate concentrations. The entire data set was then fit to an equation that describes enzymes that undergo partial substrate inhibition (20). That equation is $v = V_1(1 + (V_2[S]/V_1K_i))/(1 + K_m/[S] + [S]/K_i)$, where v is the rate, V_1 is approximated by the V_{max} estimate, V_2 is the estimated activity plateau that is reached at high substrate concentrations (in the presence of inhibition), and K_m is approximated as above. V_{max} and K_m were then determined over substrate concentrations $\ll K_i$ such that substrate inhibition was negligible. All subsequent experiments (e.g., thermal stability and optima experiments) utilized 10 μM DHEA as the substrate.

PCR analysis of (Hsa)SULT2B1 expression in human tissues. Gene-specific oligonucleotide primers (Table 1) were used to PCR amplify SULT2B1a and SULT2B1b from a panel (Origene Technologies, Rockville, MD) consisting of 24 human tissue cDNAs of mixed ethnicity. Each 50 μl PCR contained 20 pmol (each) of forward and reverse primers, 2.5 U JumpStart *Taq* polymerase (Sigma), 2 mM $MgCl_2$ and 0.2 mM dNTPs. PCR parameters were as follows: 5 min at 95°C followed by 40 cycles of; 95°C for 30 s, 62°C for 30 s, 72°C for 45 s, followed by a final extension step for 10 min at 37°C. PCR-amplified products were resolved on 3% NuSieve (FMC) agarose gels.

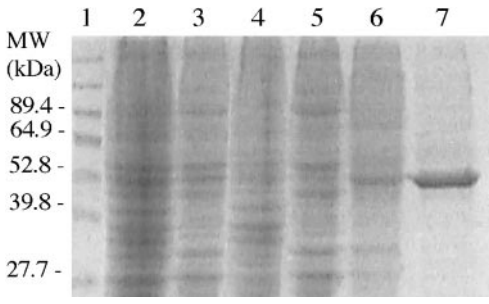

FIG. 2. Purification of recombinant SULT2B1. SULT2B1a and SULT2B1b were purified using affinity chromatography, separated by 10% SDS–PAGE, and visualized by Coomassie blue staining. SULT2B1a purification is shown here. Lanes are as follows: 1, protein MW standard; 2, soluble proteins; 3, insoluble proteins; 4, column flowthrough; 5, low stringency wash (5 mM imidazole); 6, moderate stringency wash (100 mM imidazole); 7, high stringency elution (1 M imidazole).

RESULTS AND DISCUSSION

Sulfonation of steroid hormones represents an important mechanism in the regulation of steroid bioavailability in target and non-target tissues (21, 22). However, direct *in vivo* evaluation of SULT activity toward hormones has proven difficult due to considerable overlap in tissue expression and in substrate specificity for several SULT isoforms. Consequently, over the past several years, recombinant DNA techniques have facilitated the functional *in vitro* analysis of SULT enzyme biochemistry.

The (Hsa)SULT2 family consists of two subfamilies, SULT2A1 and SULT2B1. SULT2A1 catalyzes the sulfonation of DHEA as well as androgens and estrogens and is expressed in liver and adrenal tissues (4, 18). While SULT2B1 was also shown to catalyze the sulfonation of DHEA, no activity was detected toward 17β-estradiol. Furthermore, multiple tissue Northern blot analysis revealed that SULT2B1 was expressed in the prostate (9), an androgen-responsive organ in which SULT2A1 expression is absent (16, 17). Taken together, these two observations suggest that SULT2B1 may play a novel functional role in abrogating androgen-dependent cellular processes in the prostate. To this end, we thoroughly characterized the *in vitro* biochemical properties, substrate specificity and distribution of SULT2B1a and SULT2B1b in human tissues. The results obtained will provide the foundation for future studies of SULT2B1 function in living cells.

Expression and Purification of Recombinant SULT2B1a and SULT2B1b

Recombinant SULT2B1a and SULT2B1b proteins were generated in an insect cell expression system and were purified by cobalt-immobilized agarose chromatography (Fig. 2). Both proteins were purified to >90% total detectable protein on Coomassie-stained polyacrylamide gels. Interestingly, after accounting for the 6×-His-tag (MW = 5.41 kDa), SULT2B1a and SULT2B1b migrated with an apparent molecular weight ca. 6 kDa greater than predicted. Sequence analysis (Jellyfish Software, BioWire) revealed several putative posttranslational modification consensus sequences. One carboxy-terminal myristoylation consensus sequence spans amino acids 255–259 and several N-linked glycosylation sequences are located near the carboxy-terminus (Fig. 1). Although the extent to which these account for the observed increase in apparent molecular weight is unknown at this time, those putative posttranslational modification consensus sequences provided the basis for our selection of a eukaryotic protein expression system to generate recombinant SULT2B1a and SULT2B1b.

The in Vitro Biochemical Properties of SULT2B1a and SULT2B1b

DHEA serves as the prototypic substrate for hydroxysteroid SULT enzymes. Therefore, the saturation kinetics of DHEA and the biological sulfate donor, PAPS, were evaluated for SULT2B1a and SULT2B1b (Fig. 3). Sulfotransferases often exhibit profound substrate inhibition, presumably due to low affinity allosteric substrate binding sites (20). Therefore, the apparent inhibition constant, K_i, was estimated (20) when substrate inhibition was observed. The apparent kinetic parameters V_{max} and K_m were then estimated over a range of concentrations where substrate inhibition was negligible (e.g., substrate concentration $\ll K_i$) (Fig 3, Table 2). DHEA inhibited the SULT2B1a and SULT2B1b-catalyzed reaction with K_i values of 48 and 22 μM, respectively. However, no inhibition was observed when the concentration of PAPS was varied (Fig. 3). Interestingly, V_{max}/K_m values for SULT2B1a were approximately 5-fold higher than for SULT2B1b. This trend was observed for all substrates evaluated in this study (Table 2).

Several parameters are known to influence SULT activity including temperature, pH, and $MgCl_2$. Therefore, these three parameters were varied systematically and were evaluated for their effects on SULT2B1 enzyme activity. For each of these experiments, a saturating concentration of substrate (10 μM DHEA) and cofactor (0.4 μM PAPS) was used (Fig. 3, Table 2), while a limiting concentration (0.07 μM, 100 ng) of purified, recombinant SULT2B1a or SULT2B1b was used as the enzyme source. Under these conditions, the reaction rate was protein concentration-dependent (data not shown).

With the exception of mouse (Mmu)SULT1E1, SULT enzymes are generally homodimers in solution (see (23) for review). The specific activity of purified, recombinant SULT2B1a and SULT2B1b was protein concen-

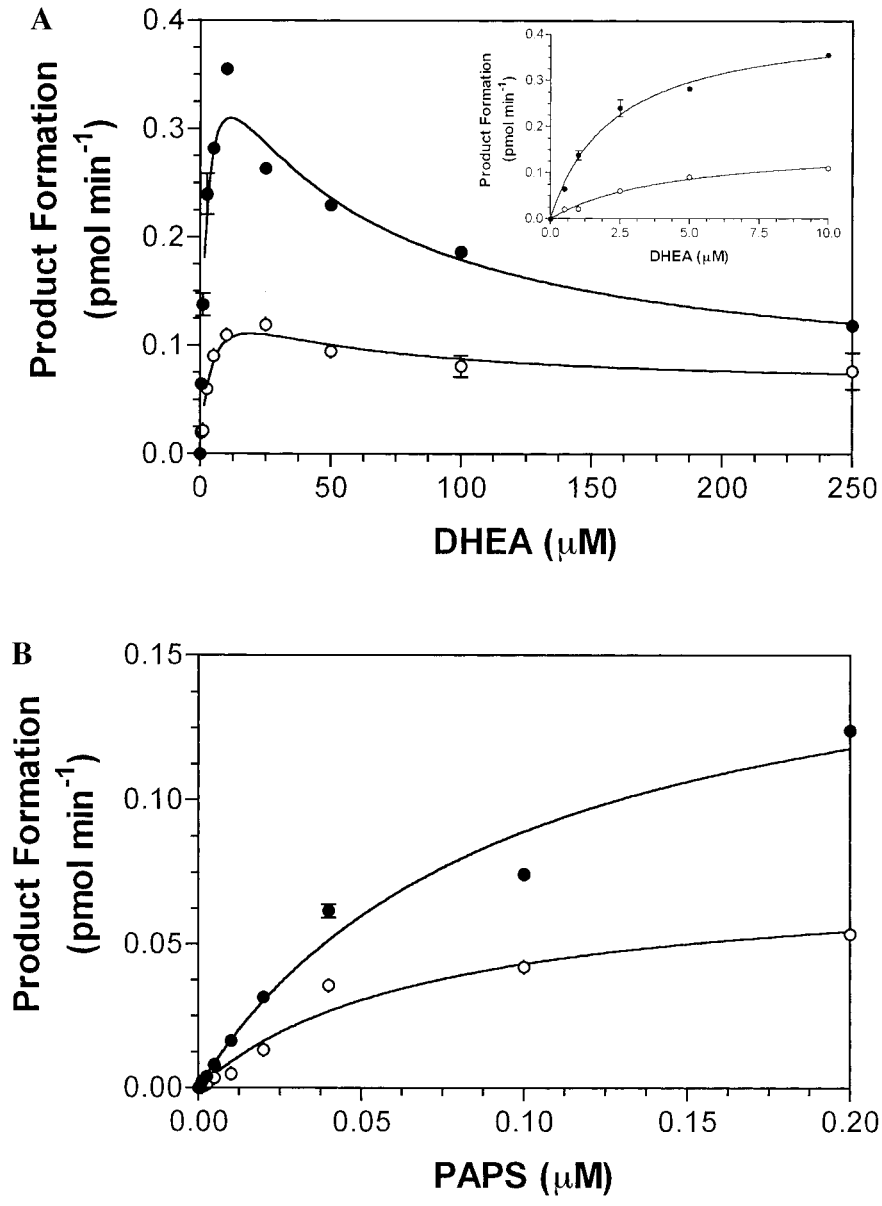

FIG. 3. Saturation kinetics of DHEA and PAPS. (A) 0.07 μM (100 ng) purified, recombinant SULT2B1a and SULT2B1b were incubated in the presence of varying concentrations of DHEA and 0.4 μM PAPS. The data were fit to an equation that describes enzymes that undergo partial substrate inhibition via an allosteric binding site to estimate the inhibition constant, K_i [20]. (Inset) The substrate was assayed at concentrations $\ll K_i$ and the resulting data were fit to the Michaelis–Menten equation to estimate V_{max} and K_m. (B) As in A, except with 10 μM DHEA and a varying concentration of PAPS. All of the data represent the mean of triplicate experiments $\pm$SEM. All kinetic constants are reported in Table 2. Closed symbols, SULT2B1a. Open symbols, SULT2B1b.

tration independent over a broad protein concentration range used in the standard assay (data not shown). Hence, the putative enzyme homo-dimers were stable at these low concentrations. Furthermore, SULT2B1a and SULT2B1b enzyme activity was robust under standard reaction conditions; the half-life for SULT2B1a and SULT2B1b enzyme activity in protein storage buffer at 37°C was approximately 14 h (data not shown). Thus, for all experiments discussed subsequently, no decay in SULT2B1 enzyme activity occurred over the assayed time period.

SULT isoform activity in human tissues is sometimes discernable by thermal stability characterization. In human blood platelet samples, (Hsa)SULT1A1 activity displays wide individual variation and the genetic basis underlying these activities have been characterized (15, 24, 25). One of the phenotypic changes that results from a common genetic polymorphism in SULT1A1 confers thermal lability to the variant allozyme (15). Thus, thermal stability assays have been used to indirectly determine amino acid differences among proteins. Therefore, we wished to determine

TABLE 2

SULT2B1a and SULT2B1b Preferentially Catalyze the Sulfonation of 3β-Hydroxysteroids

Substrate	Kinetic constants (SULT2B1a/SULT2B1b)			
	V_{max} (pmol min^{-1} μg^{-1})	K_m (μM)	V_{max}/K_m	K_i (μM)
PAPS	1.73 ± 0.14/0.73 ± 0.06	0.09 ± 0.02/0.07 ± 0.01	19.22/10.42	ND/ND
Dehydroepiandrosterone	4.31 ± 0.01/1.61 ± 0.01	2.27 ± 0.21/4.37 ± 0.59	1.90/0.37	48/22
Pregnenolone	2.78 ± 0.10/1.19 ± 0.04	0.20 ± 0.03/0.60 ± 0.05	13.90/1.98	103/12
17α-Hydroxypregnenolone	3.29 ± 0.06/1.29 ± 0.05	0.89 ± 0.06/0.97 ± 0.11	3.70/1.33	179/86
5-Androstene-3β,16α,17β-triol	1.01 ± 0.05/0.38 ± 0.01	19.70 ± 2.63/5.11 ± 0.80	0.05/0.08	ND/ND
Dihydrotestosterone	0.41 ± 0.02/0.32 ± 0.07	6.68 ± 1.07/15.14 ± 9.62	0.06/0.02	ND/ND
Etiocholan-3α-ol-17-one	0.29 ± 0.03/0.24 ± 0.01	50.02 ± 13.22/30.03 ± 8.00	$5.80 \times 10^{-3}/7.99 \times 10^{-3}$	ND/ND

Note. The kinetic constants reported in this table were derived from plots similar to Fig. 3. The data represent means ± SEM derived from triplicate experiments. No detectable activity was observed for the following steroids: 5α-androstane-3α,17β-diol; 19-hydroxytestosterone; 5β-pregnane-3α,20α-diol; 17α-hydroxyprogesterone; 17β-estradiol; testosterone; 16α-hydroxyestrone; androsterone; cortisol; cholesterol; estrone; 2-hydroxyestradiol; 2-hydroxyestriol; 2-methoxyestradiol; 2-methoxyestrone. K_i values were estimated as in Fig. 3. ND, means no detectable substrate inhibition.

whether the thermal stability profile of SULT2B1a was discernable from SULT2B1b.

Purified, recombinant SULT2B1a and SULT2B1b were individually pre-incubated for 15 min in protein storage buffer over a range of temperatures, followed by incubation on ice. Residual enzyme activity was then evaluated using the standard SULT assay. There was no striking difference in the thermal stability between SULT2B1a and SULT2B1b. The temperature at which 50% of enzyme activity was lost, T_{50}, was 46°C for SULT2B1a and 43°C for SULT2B1b (Fig. 4A).

As a follow-up to the thermal stability analysis, the effect of reaction temperature on SULT2B1a and SULT2B1b enzyme activity was determined. Recombinant SULT2B1a and SULT2B1b were evaluated in our standard SULT assay while the temperature of the reaction was varied over a similar range as the thermal stability assays. Both SULT2B1 isoforms displayed a broad temperature optimum (Fig. 4B). The greatest relative activity (>90%) was detected from 39 to 48°C. The temperature at which product formation was greatest for both enzymes was ca. 45°C. Incidentally, this was about the same temperature as the T_{50} for each SULT2B1 isoform (Fig. 4A). No activity was detected for SULT2B1a or SULT2B1b at temperatures >54°C, consistent with the <10% activity remaining at comparable temperatures observed in the thermal stability assay.

Sulfonation of hydroxysteroids by human SULT2A1 is differentially stimulated by $MgCl_2$ (26). Interestingly, the magnitude of the increase in activity depends on the substrate—with up to a twofold increase in activity toward estrone. Therefore the ability of Mg^{2+} to stimulate SULT2B1a- and SULT2B1b-catalyzed DHEA sulfonation was evaluated. Although modest in magnitude, SULT2B1a activity was maximally (ca. 10%) stimulated by 0.5 mM $MgCl_2$, while SULT2B1b activity was maximally (ca. 20%) stimulated by 0.05 mM $MgCl_2$. At concentrations >1 mM, Mg^{2+} was inhibitory toward sulfonation activity of both SULT2B1 isoforms, yielding ca. 15% activity at 100 mM (Fig. 4C). The pH of the reaction was also evaluated for its effect on SULT2B1a and SULT2B1b catalyzed sulfonation of DHEA (Fig. 4D). Both enzymes displayed a narrow range over which enzyme activity was within 90% of maximal activity (ca. pH 6.8–7.5). The pH at which enzyme activity was maximal (pH_{max}) for SULT2B1a was 7.08, while pH_{max} was 7.34 for SULT2B1b.

After the above parameters were evaluated, a standard reaction buffer was created to maximize HsaSULT2B1 activity, yet approximate *in vivo* conditions. This buffer consisted of the following components listed with their final concentrations: 50 mM potassium phosphate (pH 7.4), 3.75 mM $MgCl_2$, 0.75 mg/ml BSA, 13 mM DTT. The pH of the final reaction mixture at 37°C was 7.28.

SULT2B1 Is Stereoselective for 3β-Hydroxysteroids

The related human hydroxysteroid sulfotransferase, SULT2A1, like many SULT enzymes has broad substrate specificity, catalyzing the sulfonation of many hydroxysteroids, including DHEA, testosterone (27) and 17β-estradiol among others (4, 26, 27). By contrast, two related guinea pig hydroxysteroid sulfotransferases, (Cpo)SULT2A2 and (Cpo)SULT2A3, are stereospecific for either 3α or 3β hydroxysteroids respectively (28). Site-directed mutagenesis studies revealed that amino acid 51 plays a critical role in determining the stereoselectivity of each guinea pig enzyme, where N51 confers 3α selectivity and I51 confers 3β selectivity. Although only 38% identical at the amino acid level, comparative sequence analysis between SULT2B1a, SULT2B1b and (Cpo)SULT2A3 suggested that SULT2B1 might also be stereoselective for 3β hydroxysteroids; SULT2B1a and SULT2B1b contain

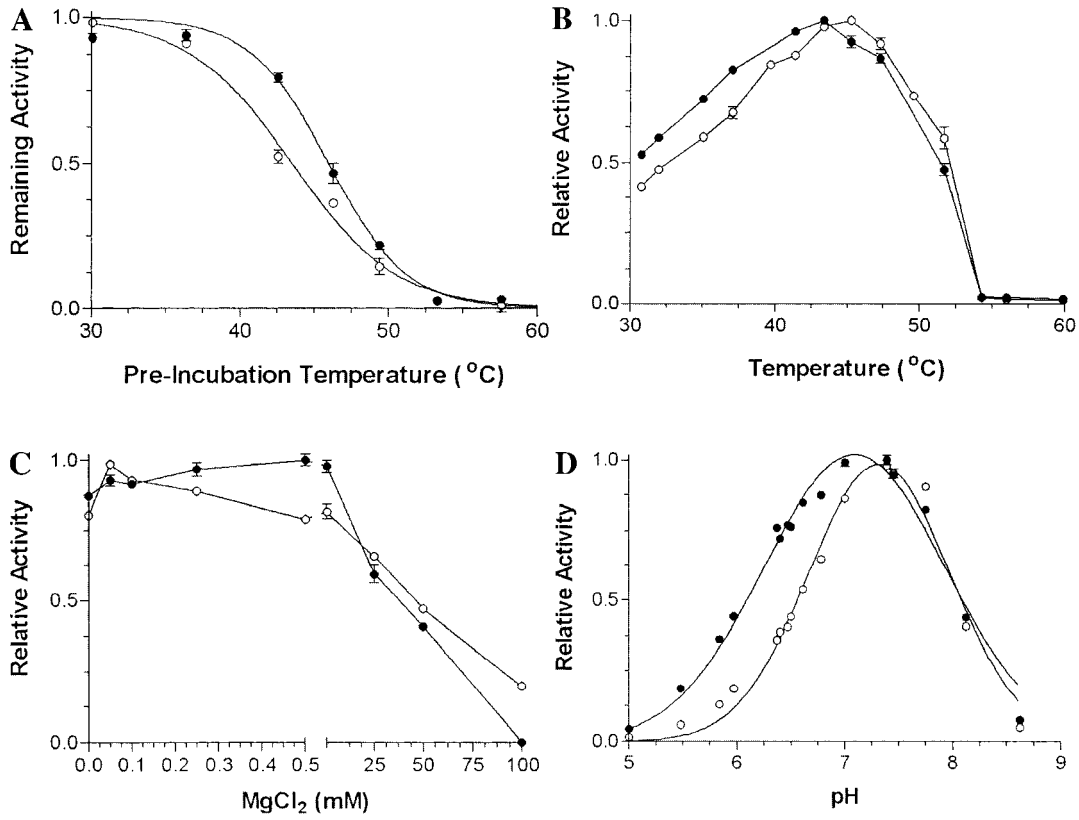

FIG. 4. Analysis of the *in vitro* biochemical properties of SULT2B1. 0.07 μM purified, recombinant SULT2B1a and SULT2B1b were assayed with 10 μM DHEA and 0.4 μM PAPS in the standard reaction buffer (Materials and Methods) at 37°C for 15 min with the following exceptions: in A, each SULT2B1 isoform was preincubated in enzyme dilution buffer (Materials and Methods) at each indicated temperature for 15 min. Residual catalytic activity was measured at 37°C as indicated above. The data are normalized to a reaction that was not preincubated. In B, C, and D the assay temperature, $MgCl_2$ concentration, and pH were systematically varied, respectively, as indicated. The data are normalized to the reaction showing the greatest amount of activity. The data in all panels represent the average of triplicate determinations ±SEM.

I62 and I77, respectively, which map to the equivalent amino acid as (Cpo)SULT2A3 I51 (Fig. 1). By contrast, (Hsa)SULT2A1 contains alanine at this position and apparently displays no stereoselectivity. We therefore determined SULT2B1 substrate specificity.

The hydroxysteroid SULT prototypic substrate, DHEA, is a 3β-hydroxysteroid and serves as a precursor for cellular estrogen and androgen biosynthesis (27). Therefore, putative substrates were selected based on the androgen and estrogen biosynthetic pathways, with particular consideration directed toward the position and stereochemistry of hydroxyl groups at position 3 as well as other positions on the steroid backbone. Using these criteria we studied 20 additional putative substrates (Table 2) and compared their activity to that of DHEA.

To determine whether the selected putative substrates were sulfonated by SULT2B1, steroid hormone concentrations were evaluated over nine orders of magnitude (10^{-12}–3×10^{-3} M) to ensure that activity would be detected for each putative substrate as well as to determine the concentration range over which substrate inhibition was apparent. When substrate inhibition was detected, the respective K_i value was estimated as discussed in the previous section. Subsequently, the respective kinetic parameters V_{max} and K_m were estimated over substrate concentrations $\ll K_i$.

Similar to (Cpo)SULT2A3, both SULT2B1 isoforms displayed remarkable substrate stereoselectivity as well as site-selectivity with respect to the positioning of the hydroxyl (sulfate acceptor) group on the steroidal ring. Only the 3β-hydroxysteroids, pregnenolone and 17α-hydroxy-pregnenolone were efficiently sulfonated, reflected by V_{max}/K_m values, comparable to DHEA (Table 2). The 3β-hydroxysteroid, 5-androstene-3β, 16α, 17β-triol, however, was not efficiently sulfonated. When 5-androstene-3β, 16α, 17β-triol was assayed as the substrate, V_{max}/K_m was reduced (compared to DHEA) by ca. 40-fold for SULT2B1a, while it was reduced by only ca. 5-fold for SULT2B1b. It is conceivable that the presence of the additional 16α-hydroxyl group and/or presence of the 17-hydroxyl group interfere with substrate binding. Indeed, comparing the apparent K_m values for 17α-hydroxy-pregnenolone to that of preg-

nenolone for both enzymes revealed that the 17α-hydroxyl group lowered the affinity for SULT2B1a and SULT2B1b by ca. 4- and 2-fold, respectively.

Regiospecific substrate binding has been demonstrated in (Mmu)SULT1E1, (29) which preferentially catalyzes the sulfonation of 17β-estradiol, but remains inactive toward DHEA. Mutational analysis of amino acid residues surrounding the substrate binding cleft revealed that (Mmu)SULT1E1 contains a gate-like structure. This structure functions by positioning 17β-estradiol between the side chains of Y81 and F142 which permits substrate access into the active site. DHEA, by contrast, is not positioned in this way because of steric interference of the C19 methyl group of DHEA with the side chain of Y81. An analysis of the SULT2A1 crystal structure, solved at 2.4 Å resolution, suggested the presence of a similar gate-like structure (30). The organization of that structure differs considerably, however, leading the authors to speculate that, depending on the orientation of DHEA binding, the mechanism by which this putative gate confers specificity is likely to be different from (Mmu)SULT1E1. We generated a molecular model (SCWRL Software) of SULT2B1a based on an alignment against the SULT2A1 crystal structure (Psi-Blast) (31). This model revealed that a similar gate-like structure maps to the equivalent position in SULT2B1a (data not shown) as in SULT2A1. It is currently not known if this putative gate confers the regio-specific binding properties observed with SULT2B1.

Two additional hydroxysteroids were identified as substrates in this study: DHT and etiocholan-3α-ol-17-one. When DHT served as the substrate, V_{max}/K_m was reduced by ca. 30- and 20-fold (compared to DHEA) for SULT2B1a and SULT2B1b respectively (Table 2). The catalytic activity toward such an important androgen receptor ligand suggests that SULT2B1 might play a protective role in androgen-responsive target tissues as steroid hormone-sulfates do not bind the androgen receptor.

Taken together, the data suggest that SULT2B1 activity is stereoselective for 3β-hydroxysteroids, notwithstanding the low activity toward 5-androstene-3β, 16α, 17β-triol. Although SULT stereoselectivity has been demonstrated in guinea pigs, this is the first stereospecific SULT enzyme identified in humans. Based on kinetic characterization, it is evident that SULT2B1a and SULT2B1b are distinct from one another in their binding surfaces and/or in their chemistry. In particular, V_{max}/K_m values for DHEA, pregnenolone and 17α-hydroxypregenenolone were consistently higher (two to sevenfold) for SULT2B1a than for SULT2B1b. This was reproducible over several recombinant protein preparations. Given the sequence identity shared between these two enzymes (Fig. 1) it is difficult to reconcile the biochemical basis for the disparity observed in their activities. It is conceivable that the 23 unique SULT2B1b N-terminal amino acids interfere with substrate and/or PAPS binding. Based on our preliminary molecular modeling studies, the amino-terminus is positioned close to the substrate binding site (data not shown). Alternatively, residues within this N-terminal domain may interfere with the catalytic step itself, possibly through hydrophobic interactions (as much as 50% of this domain consists of hydrophobic residues, Fig. 1). Indeed, the lower V_{max}/K_m values observed with SULT2B1b (compared to SULT2B1a) for each substrate were largely attributed to greater reductions in V_{max} than increases in K_m (Table 2). Mutational analysis of the SULT2B1b amino terminus is warranted to resolve these issues.

SULT2B1 Expression Is Widespread in Humans and Both Isoforms Are Regulated Differently from Each Other

SULT2B1 was previously shown to be expressed in human prostate, placenta, small intestine and trachea (9). These observations, however, were based on multiple tissue Northern blots using a SULT2B1a ORF cDNA as a probe. As SULT2B1a and SULT2B1b are 94% identical (Fig. 1), no conclusions were drawn about the isoform-specific expression in these tissues. Therefore, we evaluated the expression patterns of SULT2B1a and SULT2B1b in human tissues using isoform-specific PCR.

In agreement with previous data (9), SULT2B1 expression was detected in prostate, placenta and small intestine, but was also detected in a variety of additional human tissues (Fig. 5). SULT2B1a expression is limited in human tissues; transcripts were detected in colon, ovary and fetal brain. SULT2B1b expression, by contrast, is widespread and was detected in brain (trace), liver, colon, small intestine, placenta, ovary, uterus and prostate.

SUMMARY AND CONCLUSION

(Hsa)SULT2B1 is unique among the eleven human cytosolic SULTs due to its strong stereoselectivity for 3β-hydroxysteroids. The broad overlap in the *in vitro* biochemical properties between SULT2B1a and SULT2B1b, however, suggests that they may be biologically redundant *in vivo*. Based on the kinetic characterization presented in this study, we suspect that SULT2B1a is likely to be the biologically relevant enzyme in tissues where both isoforms are expressed (e.g., colon and ovary). The modest biochemical redundancy despite strong genetic linkage between SULT2A1 and SULT2B1 raises fundamental questions regarding the functional relevance of a stereoselective hydroxysteroid SULT over one that is promiscuous in its catalytic activity (SULT2A1). Based on comparative amino acid sequence analysis among all human

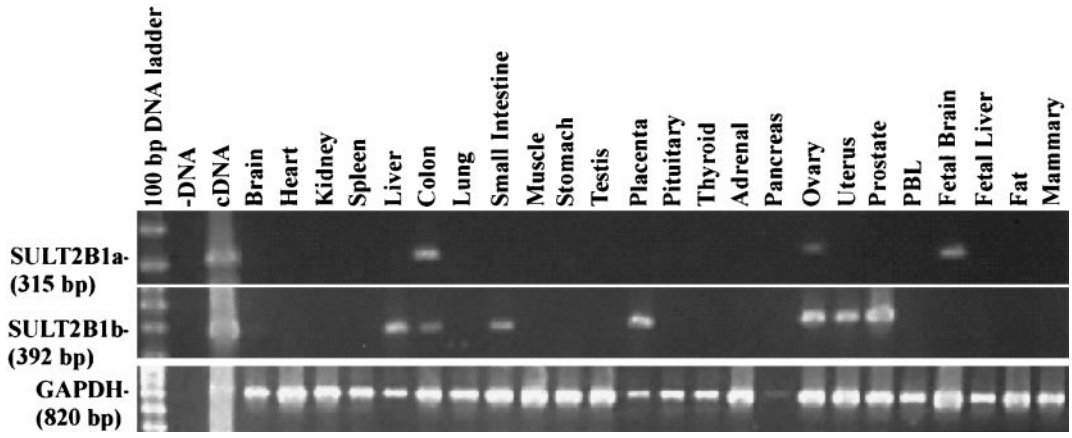

FIG. 5. SULT2B1a and SULT2B1b exhibit differential expression patterns in human tissues. Isoform-specific primers (Table 1) were used to amplify SULT2B1a and SULT2B1b from a cDNA array of pooled human tissues of mixed ethnicity (Origene Technologies). PCR products were resolved on a 3% NuSieve agarose gel.

SULTs, it is plausible that SULT2B1 arose from SULT2A1 via gene duplication. However, following that event it is evident that SULT2B1 acquired a prostate-specific role that is expected to be protective against the pro-mitogenic effects of androgens. This putative role could regulate intracellular levels of androgens not only at the androgen receptor level (via DHT sulfonation) but also at the anabolic level via sulfonation of DHEA and its precursors (e.g., pregnenolone and 17-hydroxy-pregnenolone). The interplay between this pathway and other competing metabolic pathways catalyzed by UDP-glucuronosyltransferases and steroid sulfatases must be addressed to elucidate the biological role of SULT2B1 in humans.

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REFERENCES

1. Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C., and Raftogianis, R. B. (1997) Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. *FASEB J.* **11**(1), 3–14.
2. Falany, C. N. (1997) Enzymology of human cytosolic sulfotransferases. *FASEB J.* **11**(4), 206–216.
3. Glatt, H. (2000) Sulfotransferases in the bioactivation of xenobiotics. *Chem.-Biol. Interact.* **129**(1–2), 141–170.
4. 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**(5), 865–872.
5. Nieschlag, E., Loriaux, D. L., Ruder, H. J., Zucker, I. R., Kirschner, M. A., and Lipsett, M. B. (1973) The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. *J. Endocrinol.* **57**(1), 123–134.
6. Fujita, K., Nagata, K., Ozawa, S., Sasano, H., and Yamazoe, Y. (1997) Molecular cloning and characterization of rat ST1B1 and human ST1B2 cDNAs, encoding thyroid hormone sulfotransferases. *J. Biochem. (Tokyo)* **122**(5), 1052–1061.
7. Freimuth, R. R., Raftogianis, R. B., Wood, T. C., Moon, E., Kim, U. J., Xu, J., Siciliano, M. J., and Weinshilboum, R. M. (2000) Human sulfotransferases SULT1C1 and SULT1C2: cDNA characterization, gene cloning, and chromosomal localization [in press citation]. *Genomics.* **65**(2), 157–165.
8. Aksoy, I. A., Wood, T. C., and Weinshilboum, R. (1994) Human liver estrogen sulfotransferase: Identification by cDNA cloning and expression. *Biochem. Biophys. Res. Commun.* **200**(3), 1621–9.
9. Her, C., Wood, T. C., Eichler, E. E., Mohrenweiser, H. W., Ramagli, L. S., Siciliano, M. J., and Weinshilboum, R. M. (1998) Human hydroxysteroid sulfotransferase SULT2B1: Two enzymes encoded by a single chromosome 19 gene. *Genomics* **53**(3), 284–95.
10. Walther, S., Dunbrack, R., and Raftogianis, R. (1999) Cloning, expression and characterization of a human sulfotransferase, SULT4A1, that represents a novel SULT family. *ISSX Proc.* **15,** 195.
11. Walther, S., Dunbrack, R., and Raftogianis, R. (2000) Molecular and biochemical characterization of human SULT4A1, a novel sulfotransferase. *Drug Metab. Rev.* **32,** 26.
12. Falany, C. N., Xie, X., Wang, J., Ferrer, J., and Falany, J. L. (2000) Molecular cloning and expression of novel sulphotransferase-like cDNAs from human and rat brain. *Biochem. J.* **346**(Pt 3), 857–864.
13. Raftogianis, R., Creveling, C., Weinshilboum, R., and Weisz, J. (2000) Estrogen metabolism by conjugation. *J. Natl. Cancer Inst. Monogr.* **27,** 113–124.
14. Wood, T. C., Her, C., Aksoy, I., Otterness, D. M., and Weinshilboum, R. M. (1996) Human dehydroepiandrosterone sulfotransferase pharmacogenetics: Quantitative Western analysis and gene sequence polymorphisms. *J. Steroid Biochem. Mol. Biol.* **59**(5–6), 467–478.

15. Raftogianis, R. B., Wood, T. C., Otterness, D. M., Van Loon, J. A., and Weinshilboum, R. M. (1997) Phenol sulfotransferase pharmacogenetics in humans: Association of common SULT1A1 alleles with TS PST phenotype. *Biochem. Biophys. Res. Commun.* **239**(1), 298–304.
16. Raftogianis, R. B., Wood, T. C., and Weinshilboum, R. M. (1999) Human phenol sulfotransferases SULT1A2 and SULT1A1: Genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. *Biochem. Pharmacol.* **58**(4), 605–616.
17. Her, C., Raftogianis, R., and Weinshilboum, R. M. (1996) Human phenol sulfotransferase STP2 gene: Molecular cloning, structural characterization, and chromosomal localization. *Genomics* **33,** 409–20.
18. Otterness, D. M., Her, C., Aksoy, S., Kimura, S., Wieben, E. D., and Weinshilboum, R. M. (1995) Human dehydroepiandrosterone sulfotransferase gene: Molecular cloning and structural characterization. *DNA Cell Biol.* **14**(4), 331–341.
19. Luu-The, V., Dufort, I., Paquet, N., Reimnitz, G., and Labrie, F. (1995) Structural characterization and expression of the human dehydroepiandrosterone sulfotransferase gene. *DNA Cell Biol.* **14**(6), 511–518.
20. Zhang, H., Varlamova, O., Vargas, F. M., Falany, C. N., Leyh, T. S., and Varmalova, O. (1998) Sulfuryl transfer: The catalytic mechanism of human estrogen sulfotransferase. *J. Biol. Chem.* **273**(18), 10888–10892.
21. Roy, A. K., Lavrovsky, Y., Song, C. S., Chen, S., Jung, M. H., Velu, N. K., Bi, B. Y., and Chatterjee, B. (1999) Regulation of androgen action. *Vit. Horm.* **55,** 309–352.
22. Strott, C. A. (1996) Steroid sulfotransferases. *Endocr. Rev.* **17**(6), 670–697.
23. Negishi, M., Pedersen, L. G., Petrotchenko, E., Shevtsov, S., Gorokhov, A., Kakuta, Y., and Pedersen, L. C. (2001) Structure and function of sulfotransferases. *Arch. Biochem. Biophys.* **390**(2), 149–157.
24. Price, R. A., Spielman, R. S., Lucena, A. L., Van Loon, J. A., Maidak,B. L., and Weinshilboum, R. M. (1989) Genetic polymorphism for human platelet thermostable phenol sulfotransferase (TS PST) activity. *Genetics* **122**(4), 905–914.
25. Van Loon, J., and Weinshilboum R. M. (1984) Human platelet phenol sulfotransferase: Familial variation in thermal stability of the TS form. *Biochem. Genet.* **22**(11–12), 997–1014.
26. Hernandez, J. S., Watson, R. W., Wood, T. C., and Weinshilboum, R. M. (1992) Sulfation of estrone and 17β-estradiol in human liver. Catalysis by thermostable phenol sulfotransferase and by dehydroepiandrosterone sulfotransferase. *Drug Metab. Dispos.* **20**(3), 413–422.
27. Falany, C. N., Vazquez, M. E., and Kalb, J. M. (1989) Purification and characterization of human liver dehydroepiandrosterone sulphotransferase. *Biochem. J.* **260**(3), 641–646.
28. Park, B. C., Lee, Y. C., and Strott, C. A. (1999) Identification by chimera formation and site-selected mutagenesis of a key amino acid residue involved in determining stereospecificity of guinea pig 3-hydroxysteroid sulfotransferase isoforms. *J. Biol. Chem.* **274**(31), 21562–21568.
29. Petrotchenko, E. V., Doerflein, M. E., Kakuta, Y., Pedersen, L. C., and Negishi, M. (1999) Substrate gating confers steroid specificity to estrogen sulfotransferase. *J Biol Chem.* **274**(42), 30019–30022.
30. Pedersen, L. C., Petrotchenko, E. V., and Negishi, M. (2000) Crystal structure of SULT2A3, human hydroxysteroid sulfotransferase. *FEBS Lett.* **475**(1), 61–64.
31. Dunbrack, R. L., Jr. (1999) Comparative modeling of CASP3 targets using PSI-BLAST and SCWRL. *Proteins* (Suppl. 3), 81–87.