

Sulfotransferase (SULT) 1A1 Polymorphic Variants *1, *2, and *3 Are Associated with Altered Enzymatic Activity, Cellular Phenotype, and Protein Degradation

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ABSTRACT

The superfamily of sulfotransferase (SULT) enzymes catalyzes the sulfate conjugation of several pharmacologically important endo- and xenobiotics. SULT1A1 catalyzes the sulfation of small planar phenols such as neurotransmitters, steroid hormones, acetaminophen, and *p*-nitrophenol (PNP). Genetic polymorphisms in the human *SULT1A1* gene define three alleles, SULT1A1*1, *2, and *3. The enzyme activities of the SULT1A1 allozymes were studied with a variety of substrates, including PNP, 17 β -estradiol, 2-methoxyestradiol, catecholestrogens, the antiestrogen 4-hydroxytamoxifen (OHT), and dietary flavonoids. Using purified recombinant SULT1A1 protein, marked differences in *1, *2, and *3 activity toward every substrate studied were noted. Substrate inhibition was observed for most substrates. In general, the trend in V_{\max} estimates was *1 > *3 > *2; however, V_{\max}/K_m estimate trends

varied with substrate. In MCF-7 cells stably expressing either SULT1A1*1 or *2, the antiestrogenic response to OHT was found to be allele-specific: the cells expressing *2 exhibited a better antiproliferative response. The intracellular stability of the *1 and *2 allozymes was examined in insect as well as mammalian cells. The SULT1A1*2 protein had a shorter half-life than the *1 protein. In addition, the *2 protein was ubiquitinated to a greater extent than *1, suggesting increased degradation via a proteasome pathway. The results of this study suggest marked differences in activity of polymorphic SULT1A1 variants, including SULT1A1*3, toward a variety of substrates. These differences are potentially critical for understanding interindividual variability in drug response and toxicity, as well as cancer risk and incidence.

Cytosolic sulfotransferases (SULTs) are members of a superfamily of enzymes that catalyze the sulfate conjugation of various endobiotics and xenobiotics, such as steroid hormones, neurotransmitters, and therapeutic drugs. SULT1A1 is a phenol sulfotransferase that preferentially catalyzes the sulfation of small planar phenols such as *p*-nitrophenol (PNP) and acetaminophen (Coughtrie and Johnston, 2001; Coughtrie, 2002). In addition, SULT1A1 is known to catalyze the sulfation of not only dietary carcinogens such as the heterocyclic aromatic amines (HAAs), but also that of several dietary chemopreventives such as catechins (Coughtrie and

Johnston, 2001). SULT1A1 has been shown to bioactivate various procarcinogens and genotoxic agents (Glatt, 2000).

Common nucleotide polymorphisms have been reported for SULT1A1 that are associated with variation in activity and thermal stability (Jones et al., 1995; Raftogianis et al., 1997, 1999). The common SULT1A1 allozymes include *1, the *2 variant defined by an Arg213His amino acid change (G to A conversion at nucleotide 638), and the *3 variant defined by a Met223Val change (A to G conversion at nucleotide 667) (Table 1). It has been reported that these recombinant allozymes have variable activity toward PNP and certain catecholestrogens; *2 variant is associated with low enzyme activity (Raftogianis et al., 1999; Adjei and Weinsilboum, 2002). However, the functional significance of the *3 allozyme has not been thoroughly investigated, and the molecular mechanisms explaining variable activity of SULT1A1 allozymes have not been determined. Variable SULT1A1 allele frequencies have been reported in different populations, with *1 being the most frequent allele in white persons, followed by *2 and, with lower frequency in white persons, *3

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ABBREVIATIONS: SULT, sulfotransferase; PNP, *p*-nitrophenol; OHT, 4-hydroxytamoxifen; E2, β -estradiol; 2OHE2, 2-hydroxyestradiol; 2MeE2, 2-methoxyestradiol; HAA, heterocyclic aromatic amines; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; PAGE, polyacrylamide gel electrophoresis.

(Table 1) (Carlini et al., 2001). The *1 allele was the most common variant in Chinese subjects, a population with low *2 and *3 allele frequencies. In contrast, both the *2 and *3 alleles were common in African American subjects (Carlini et al., 2001; Coughtrie, 2002). These interpopulation differences in SULT1A1 allele frequencies might contribute to known variability in drug metabolism and disposition among different ethnic populations.

Because SULT1A1 catalyzes the sulfation of several carcinogens, mutagens, and hormones, genetic polymorphisms of SULT1A1 have been studied in the context of breast, lung, and esophageal cancer epidemiology (Seth et al., 2000; Wang et al., 2002; Wu et al., 2003). These studies reported significant association between SULT1A1 genotype and age of onset of disease, cancer risk, or cancer development. In addition, the role of SULT1A1 polymorphisms in the activation of HAA and increased risk for breast and colorectal cancer has been evaluated (Chou et al., 1995; Williams et al., 2001; Zheng et al., 2001; Nowell et al., 2002). Although these studies identified the role of SULT1A1 in HAA metabolism, a clear association between SULT1A1 genotype and cancer risk was not always established. Although SULT1A1*1 and *2 have been evaluated in detail in these epidemiological studies, the *3 allele has been generally ignored, possibly because of its low frequency in white persons, and the relative lack of information regarding its functional significance. However, it is important to note that the *3 allele is prevalent in the African American population (Table 1).

This study seeks to further our understanding of the functional significance of the SULT1A1*1, *2, and *3 allozymes. To this end, recombinant SULT1A1 proteins were expressed and purified from a baculovirus-insect cell system, and the enzyme kinetics toward several substrates were evaluated. Substrates included the model compound PNP, catecholestrogens, and the antiestrogen anticancer agent 4-hydroxy tamoxifen (OHT), and chemopreventive dietary flavonoids. The allele-specific antiestrogenic response of breast carcinoma cells to OHT was also evaluated. Finally, the cellular half-life of the polymorphic allozymes was evaluated to test the hypothesis that altered protein turnover might contribute to the mechanisms governing the functional variability of SULT1A1 allozymes.

Materials and Methods

Chemicals and Reagents. PNP, β -estradiol (E2), 2-hydroxyestradiol (2OHE2), 2-methoxyestradiol (2MeE2), OHT, chrysin, genistein, and quercetin were obtained from Sigma (St. Louis, MO). PAPS was obtained from Dr. H. Glatt (German Institute of Human Nutrition, Nuthetal, Germany). [35 S]PAPS and [35 S]methionine were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). All other buffers and reagents were analytical

TABLE 1

SULT1A1 alleles and allele frequencies in white, Chinese, and African-American subjects

Data are from Carlini et al. (2001).

SULT1A1 Allele	Amino Acid		Allele Frequency		
	213	223	White (n = 245)	African American (n = 70)	Chinese (n = 290)
*1	Arg	Met	0.656	0.477	0.914
*2	His	Met	0.332	0.294	0.080
*3	Arg	Val	0.012	0.229	0.006

grade. Sf-9 cells and related media were purchased from Invitrogen (Carlsbad, CA), whereas MCF-7 cells were obtained from American Type Culture Collection (Manassas, VA). All cell culture media and reagents were obtained from the Cell Culture Facility at the Fox Chase Cancer Center unless otherwise noted.

Generation of Recombinant SULT1A1*1, *2, and *3. SULT1A1*1, *2 and *3 constructs (Raftogianis et al., 1999) were cloned into the baculovirus expression vector pBLUEBac-His2A (Invitrogen), which encodes an amino-terminal hexahistidine (His₆) tag sequence to facilitate purification. The His₆-tagged SULT1A1 expression constructs were cotransfected with 1 μ g of BacVector-3000 viral DNA (Novagen, Madison, WI) through liposome-mediated transfection into Sf-9 insect cells. Individual viral clones were isolated to generate high-titer viral stocks, which were used to infect 1-liter Sf-9 cell cultures at 27°C for 48 h. The His₆-tagged proteins were purified with cobalt immobilized metal affinity chromatography using Talon resin (Clontech, Mountain View, CA), with imidazole as the elution agent. The purified proteins were dialyzed overnight against a Tris-NaCl buffer (50 mM Tris, pH 7.5, and 50 mM NaCl) to remove excess imidazole, and concentrated using Amicon Ultra 10,000 molecular weight cut-off centrifugal tubes (Millipore, Bedford, MA). Protein concentrations were determined using the Bradford protein assay (Bradford, 1976). Purified preparations were further analyzed with SDS-PAGE followed by Coomassie staining and Western blot with an anti-SULT1A1 antibody to ensure the purity of each product. The preparations were found to be highly purified, with a single SULT1A1 band detected for each preparation with Coomassie staining as well as Western blot analysis. Protein aliquots were stored at -80°C until further use.

Radiometric SULT Assays. Standard radiometric assays were performed for all substrates except flavonoids, with the following modifications (Anderson and Weinshilboun, 1980; Geese and Raftogianis, 2001). Initial experiments were performed to evaluate the linearity of each experiment with time and with protein content. Reactions were carried out in 0.2-ml, thin-walled polymerase chain reaction tubes with a final reaction volume of 30 μ l at 37°C for 30 min in a thermal cycler. The reaction buffer was 10 mM potassium phosphate buffer, pH 6.5. Each substrate was evaluated over a range of concentrations (0.01–2500 μ M). Total protein ranging from 1 to 2 μ g was used. The reaction was initiated with the addition of 10 μ M PAPS ([35 S]PAPS + unlabeled PAPS in the ratio 1:9 (v/v)). After an incubation period of 30 min, the reaction was quenched by the addition of 40 μ l each of 0.1 M barium acetate, 0.1 M barium hydroxide, and 0.1 M zinc sulfate. The resulting unreacted [35 S]PAPS precipitate was pelleted by centrifugation at 1800g for 3 min. The reaction product in the supernatant (100 μ l) was quantitated by liquid scintillation counting. Assays were performed in triplicate with appropriate blanks (no substrate). Three independent protein preparations were used with PNP as the substrate to ensure minimal interbatch variation. In the course of these experiments, it was found that the *2 protein was unstable upon multiple freeze-thaw cycles, and activity decreased with time; hence, all experiments were performed using preparations that had undergone a single freeze-thaw cycle. In addition, care was taken to use a batch of protein prepared not more than 3 months before use. Each batch of protein was tested with PNP to ensure comparable activity over time. All data are reported normalized to amount of purified SULT1A1 protein.

For the detection of flavonoid sulfates, the radiometric reactions were performed as described above, except that reactions were quenched by the addition of 3 μ l of 2.5% aqueous acetic acid, 6 μ l of fresh 0.1 M tetrabutylammonium dihydrogen phosphate, and 150 μ l of ethyl acetate (Varin et al., 1987). The tubes were vortexed and centrifuged to separate the aqueous and organic layers. The top organic layer (100 μ l) was added to scintillation fluid, and reaction products were quantitated.

Data Analysis for Enzyme Kinetics. Data from incubations with a broad substrate concentration range were evaluated by fitting

an equation describing enzymes that undergo partial substrate inhibition (Zhang et al., 1998): $v = V_1(1 + (V_2[S]/V_1K_i))/(1 + K_m/[S] + [S]/K_i)$, where v is the rate of reaction, V_1 is approximated by the maximum velocity (V_{max}) estimate, V_2 is the estimated activity plateau reached at high substrate concentrations in the presence of inhibition, K_m is the Michaelis-Menten constant, K_i is the inhibition constant, and $[S]$ is the substrate concentration.

This equation was fit to the data to obtain estimates of K_i whenever inhibition was observed. Subsequently, reactions were carried out with a narrow range of substrate concentrations; these were low concentrations where no inhibition was observed. Michaelis-Menten parameter estimates were obtained with the equation $v = V_{max} \times [S]/(K_m + [S])$.

Nonlinear regression was achieved using GraphPad Prism version 4.00 for Macintosh (GraphPad Software, San Diego, CA). Parameter estimates were statistically compared using a two-sided t test assuming normal distribution; a p value less than 0.01 was considered significant (Nagar et al., 2004).

Generation of MCF-7 Cells Stably Expressing SULT1A1*1, *2, or *3. Native SULT1A1*1, *2, and *3 cDNAs (Raftogianis et al., 1999) were cloned into the pCR3.1 expression vector (Invitrogen). MCF-7 cells were cultured in RPMI 1640 medium at 37°C overnight to 70% confluence. Cells were transfected with 5 μ g of control pCR3.1, pCR3.1/SULT1A1*1, pCR3.1/SULT1A1*2, or pCR3.1/SULT1A1*3 expression vectors using FuGene 6 transfection reagent (Roche Diagnostics, Indianapolis, IN), and cultured for 48 h. Cells were then cultured in the presence of 500 μ g/ml G418 (Geneticin; neomycin selectable marker) for 4 days, followed by 700 μ g/ml G418 until clones became visible to the eye. Multiple clones for each transfection were isolated and expanded. Clones expressing comparable RNA were used for the experiments described below.

Antiestrogenic Response of OHT in MCF-7 Cells Stably Expressing SULT1A1*1 or *2. MCF-7 stably expressing SULT1A1*1, SULT1A1*2 or the control vector pCR3.1 were plated in triplicate and cultured in RPMI 1640 medium with 5% charcoal-stripped fetal bovine serum at 37°C, 5% CO₂ for 48 h. Cells were washed with PBS, and cell culture medium was replaced every 12 h to remove endogenous estrogens. After 48 h, 0.25 to 2.5 μ M OHT was added to the culture medium along with 1 nM E2, and the proliferation of cells was monitored over 5 days. Medium, E2, and OHT were replaced on days 2 and 4. AlamarBlue (Biosource International, Camarillo, CA) was added to cells on day 4, and on day 5, the spectrophotometric measurement of absorbance at 570 nm (reduced reagent) and 600 nm (oxidized reagent) was monitored. The antiestrogenic response was calculated with the equation $AR = (PI_0 - PI_T)/PI_0$, where PI_0 is the proliferative index in the absence of an antiestrogen, and PI_T is the proliferative index in the presence of OHT. The responses were statistically compared using ANOVA.

Half-Life Determination. SULT1A1*1 and *2 protein turnover was evaluated in Sf-9 insect cells using [³⁵S]methionine-labeled pulse-chase experiments. Insect cells were infected with His₆-tagged SULT1A1*1 or *2 expressing virus as described above. A "mock"-infected group of cells was generated as a negative control. Infected cells were cultured in methionine-free medium for 2 h at 27°C. The cells were then pulsed with [³⁵S]methionine (95 μ Ci/plate; 0.08 μ M) for 1.5 h. After this labeling period, cells were washed to remove excess [³⁵S]methionine and cultured (chased) in complete culture medium containing 2.5 mM unlabeled methionine. Cells were collected over time, and cell pellets were lysed in a lysis buffer (50 mM Tris, pH 8.0, + 5 mM EDTA + 150 mM NaCl + 1% Nonidet P-40). The total protein concentration of the lysed supernatant was determined using the Bradford assay. Equal amounts of total protein for each sample were subjected to immunoprecipitation with an anti-His₆ antibody. After overnight incubation at 4°C with the antibody, the samples were incubated with a protein A Sepharose slurry for 2 h at 4°C. Samples were centrifuged and pellets were washed and resuspended in loading dye containing β -mercaptoethanol. Samples were heated at 98°C, and electrophoresed on a 10% NuPAGE SDS

gel (Invitrogen). Gels were dried and radiographic film was exposed to the dried gels. Next, ubiquitination was studied with the same cell lysates. Lysates expressing His₆-tagged SULT1A1*1 or *2 were immunoprecipitated with an anti-His₆ antibody, immunoprecipitate was separated by SDS-PAGE electrophoresis, and Western blot analysis was performed with an anti-ubiquitin antibody (Sigma).

In separate experiments, protein stability was evaluated by the addition of cycloheximide to MCF-7 cells stably expressing SULT1A1*1 or *2. Cells (1×10^6 per 60-mm culture dish) were allowed to grow for 24 h, after which cycloheximide at a final concentration of 40 μ g/ml was added to each culture dish. Cells were collected over 48 h, pellets were lysed with lysis buffer (radioimmunoprecipitation assay buffer containing Pefabloc), and total protein concentration was determined with the Bradford assay. Equal amounts of total protein were electrophoresed on an SDS-PAGE 10% NuPAGE gel and subjected to Western blot analysis with an anti-SULT1A1 antibody. For generation of the antibody, an 18-residue peptide representing amino acids 81 to 98 in the SULT1A1 protein (FLELKAPGIPSGMETLKD) was used to immunize New Zealand white rabbits (Research Genetics, Inc., Huntsville, AL). Antiserum from immunized rabbits was purified using the SulfoLink antibody purification kit (Pierce Biotechnology, Rockford, IL). The purified antibody exhibited no cross-reactivity with human recombinant SULTs 1A2 and 1A3 upon Western blot analysis.

Results

This study describes the biochemical characterization of SULT1A1*1, *2, and *3 allozymes. We first characterized the kinetics of the model SULT1A1 substrate PNP. Significant substrate inhibition was observed for SULT1A1*1 and *3 recombinant proteins at PNP concentrations above 100 μ M (Fig. 1a). Inhibition could not be modeled for the *2 allozyme because of very low activity; hence, only the Michaelis-Menten curve is shown (Fig. 1a, inset). The *1 allozyme exhibited the highest activity toward PNP, followed by *3 and *2. The V_{max} , K_m , V_{max}/K_m , and K_i parameter estimates are reported in Table 2. Michaelis-Menten curves for the cofactor PAPS are depicted in Fig. 1b. The K_m estimate for the *3 protein for PAPS was significantly lower than that for the *1 allozyme, indicating a higher affinity of *3 for PAPS (Table 2). This is in agreement with our previous report (Raftogianis et al., 1999), although that previous report suggested a 10-fold higher affinity of *3 for PAPS compared with *1, whereas the current data suggest a 2.5-fold higher affinity of *3 for PAPS.

Dietary flavonoids that are SULT1A1 substrates were characterized next. Enzyme kinetic curves for chrysin, genistein, and quercetin are depicted in Fig. 2 a, b, and c, respectively. The kinetics estimates are reported in Table 2. A significant difference was observed in the rate of flavonoid sulfation among the SULT1A1 polymorphic allozymes. The trend in V_{max} toward these substrates was *1 > *3 > *2. It is noteworthy that the V_{max}/K_m ratio (a common measure of intrinsic enzyme activity) was highest for chrysin with the SULT1A1*3 protein, whereas that for quercetin was highest with the *2 allozyme. This discordance is due to differences among the allozymes in K_m values. Inhibition was observed and modeled for genistein with SULT1A1*1 and *3 allozymes.

Next, E2, 2OHE2, and 2MeE2, as well as the antiestrogen drug OHT, were evaluated as SULT1A1 substrates. Very low activity toward E2 was observed with the *2 and *3 allozymes. The Michaelis-Menten estimates for *1 (Fig. 3a) are reported in Table 2. At 1 mM E2, the velocity of sulfation was

0.64 ± 0.07 nmol/min/mg (mean \pm S.D., $n = 3$), 0.04 ± 0.016 , and 0.06 ± 0.02 for *1, *2, and *3, respectively. Inhibition was not observed at the concentrations of E2 studied. The sulfation of 2OHE2 by the SULT1A1 allozymes is depicted in Fig. 3b. The *1 allozyme had the highest V_{\max} estimate toward 2OHE2, followed by *3 and *2 (Fig. 3b; Table 2). The V_{\max}/K_m ratio was highest with SULT1A1*3, followed by *1 and *2. Although inhibition was observed at higher 2OHE2 concentrations, the equation $v = V_{\max} \times [S]/(K_m + [S])$ could not adequately describe these data. Inhibition was observed and could be modeled for 2MeE2 with all three allozymes (Fig. 3c and Table 2). The Michaelis-Menten estimates for 2MeE2 sulfation were significantly different for both *2 and *3 compared with *1. SULT1A1*2 exhibited very low activity toward OHT; the velocity of OHT sulfation at 75 μ M OHT for *1, *2, and *3 was 3.49 ± 0.22 , 0.02 ± 0.01 , and 0.8 ± 0.2 nmol/min/mg (mean \pm S.D., $n = 3$), respectively. The curves for OHT sulfation by *1 and *3 are exhibited in Fig. 3d; V_{\max} , K_m , and K_i estimates are in Table 2.

The antiestrogenic response of cultured cells to OHT, defined by the rate of cell proliferation in the presence of E2 and OHT, was evaluated in MCF-7 cells stably expressing SULT1A1*1 or *2. As observed in Fig. 4, the cells responded to OHT in an allele-dependent manner. Thus, cells expressing SULT1A1*2 had a significantly higher antiestrogenic response than *1 ($p < 0.001$), consistent with the kinetic data shown in Fig. 3d, suggesting that OHT was sulfated readily by the *1 allozyme but no activity was detected with the *2 allozyme.

We observed consistently low yields of the SULT1A1*2 allozyme in multiple expression systems, whereas the *1 and *3 allozymes consistently yielded abundant expression. Therefore, we explored the possibility that the *2 allozyme might experience rapid cellular degradation—a common biological mechanism contributing to the low activity phenotype observed with several polymorphic variants (Tai et al., 1997; Wang et al., 2003, 2004). Protein half-life of the *1 and *2 allozymes was studied in Sf-9 insect cells. The results of

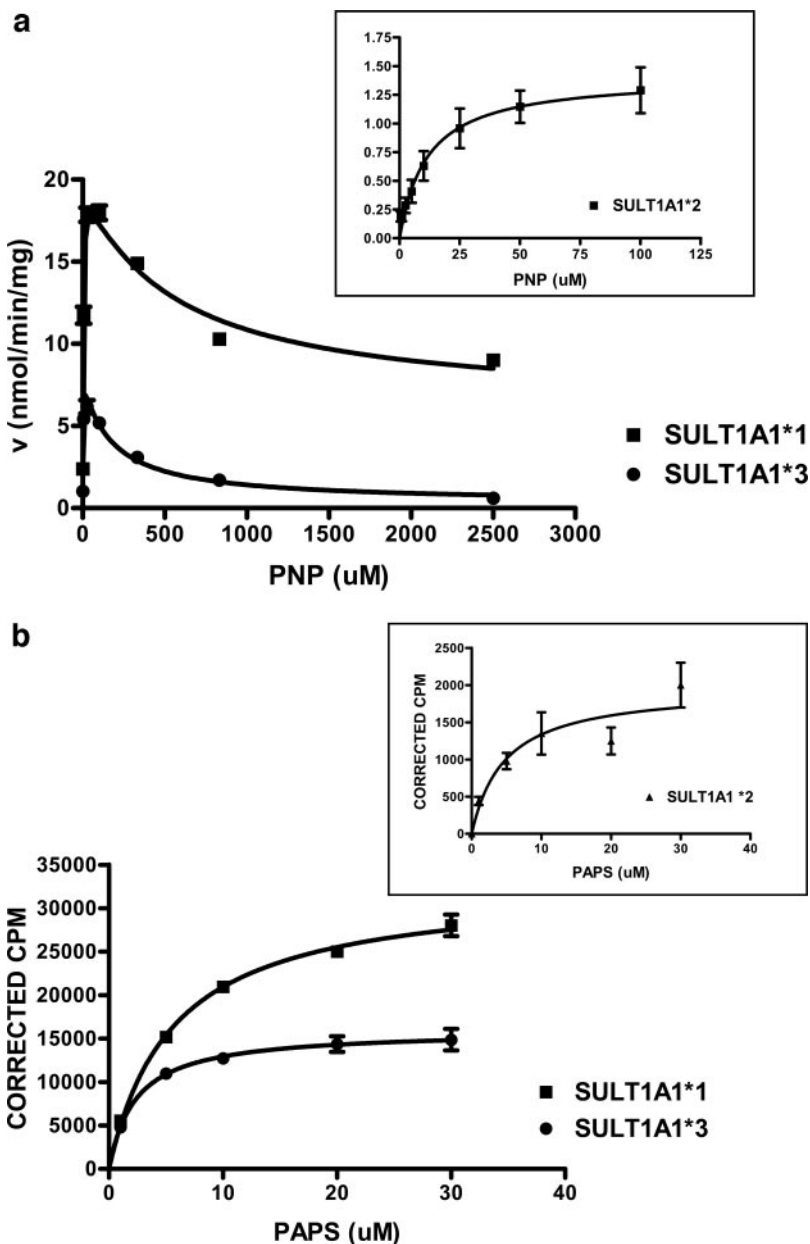


Fig. 1. Kinetics of PNP sulfation. Purified recombinant SULT1A1*1, *2, or *3 protein (75–200 ng) was incubated with substrate and [35 S]PAPS for 30 min at 37°C, and the formation of radioactive sulfated product was quantitated with a radiometric assay, as described under *Materials and Methods*. **a**, PNP sulfation by SULT1A1*1 and *3 purified recombinant protein, at PNP concentrations ranging from 0 to 2500 μ M. Inset, PNP sulfation by SULT1A1*2, with 0 to 100 μ M PNP. **b**, kinetics of PNP sulfation with varying PAPS concentration (0–30 μ M) by SULT1A1*1 and *3, using 10 μ M PNP as substrate. Inset, PNP sulfation by SULT1A1*2 with 0 to 30 μ M PAPS concentration. Data expressed as mean \pm S.D., $n = 3$. Representative fitted lines are depicted; parameter estimates were obtained by fitting the kinetic models to actual data replicates.

the pulse-chase experiments are exhibited in Fig. 5a. In this system, the *1 allozyme exhibited a cellular half-life of 7.4 h, versus 1.3 h for *2. This ~6-fold difference in cellular half-life was corroborated in studies with cycloheximide treatment of MCF-7 cells stably expressing SULT1A1*1 and *2. In that system, the cellular half-life for *1 was again 6-fold higher than *2 (18.9 h for *1 versus 3 h for *2; Fig. 5b). Next, we evaluated the ubiquitination of SULT1A1 allozymes in Sf-9 cell lysates. The results of this study established that no ubiquitinated products were observed from lysates expressing SULT1A1*1, but a typical "ubiquitination ladder" was observed in 24- and 36-h lysates expressing SULT1A1*2 (Fig. 5c).

Discussion

This study examined the phenotypic differences in SULT1A1 polymorphic variants. The allele frequencies of SULT1A1*1, *2, and *3 were found to vary in different ethnic populations (Coughtrie et al., 1999; Carlini et al., 2001). In previous studies, particular effort was spent characterizing the kinetics of the low activity and low thermal stability

TABLE 2

Kinetic parameter estimates for the sulfation by SULT1A1 allozymes
Data are expressed as estimate \pm S.E., $n = 3$, unless otherwise noted. Estimate units are as follows: V_{\max} , nanomoles per minute per milligram; K_m and K_i , micromolar; V_{\max}/K_m , milliliters per minute per milligram.

Substrate and Estimate	SULT1A1 Allozyme		
	*1	*2	*3
PNP			
V_{\max}	42.33 \pm 1.7	1.41 \pm 0.15 [†]	9.98 \pm 0.5 [†]
K_m	3.4 \pm 0.52	11.5 \pm 3.8	1.24 \pm 0.33 [†]
V_{\max}/K_m	12.45	0.12	8.05
K_i	526 \pm 99	N.E.	168 \pm 40 [†]
PAPS			
K_m	5.60 \pm 0.55	4.75 \pm 1.85	2.34 \pm 0.43 [†]
Chrysin			
V_{\max}	8.4 \pm 0.14	0.54 \pm 0.01 [†]	5.2 \pm 0.01 [†]
K_m	2.5 \pm 0.3	0.43 \pm 0.04 [†]	0.6 \pm 0.2 [†]
V_{\max}/K_m	3.36	1.26	8.67
Genistein			
V_{\max}	7.6 \pm 0.1	0.17 \pm 0.01 [†]	3.4 \pm 0.06 [†]
K_m	6.5 \pm 0.5	5.6 \pm 0.2	3.3 \pm 0.3 [†]
V_{\max}/K_m	1.17	0.03	1.03
K_i	166 \pm 39	N.E.	204 \pm 80
Quercetin			
V_{\max}	35.1 \pm 5.8	0.45 \pm 0.02 [†]	4.2 \pm 0.2 [†]
K_m	33.5 \pm 6.8	0.19 \pm 0.05 [†]	2.7 \pm 0.39 [†]
V_{\max}/K_m	1.05	2.37	1.56
E2 ^a			
V_{\max}	0.59 \pm 0.01	N.E.	N.E.
K_m	5.0 \pm 0.51	N.E.	N.E.
2OHE2			
V_{\max}	9.9 \pm 0.17	0.37 \pm 0.01 [†]	5.9 \pm 0.06 [†]
K_m	3.4 \pm 0.38	5.4 \pm 0.95	1.4 \pm 0.15 [†]
V_{\max}/K_m	2.91	0.07	4.21
2MeE2			
V_{\max}	16.5 \pm 0.2	0.86 \pm 0.08 [†]	10.2 \pm 0.12 [†]
K_m	11.0 \pm 0.5	38.0 \pm 8.5 [†]	6.5 \pm 0.4 [†]
K_i	328 \pm 99	235 \pm 60	122 \pm 33
V_{\max}/K_m	1.5	0.02	1.57
OHT			
V_{\max}	4.2 \pm 0.1	N.E.	1.3 \pm 0.09 [†]
K_m	13.6 \pm 1.05	N.E.	17.2 \pm 3.0
K_i	230 \pm 36	N.E.	261 \pm 86
V_{\max}/K_m	0.31	N.E.	0.08

N.E., not estimated.

^a Data expressed as estimate \pm S.E., $n = 6$.

[†] Estimate significantly different from *1 as determined by a two-sided *t* test, $P < 0.01$.

SULT1A1*2 protein. Relatively little has been reported regarding the functional significance of the *3 variant.

We report here significant differences among the three SULT1A1 variants, SULT1A1*1, *2 and *3, in their capacity to sulfate several substrates; allele-specific phenotypic differences in cultured cells; and a short cellular half-life of the *2 allozyme. In the present study, we report trends similar to those previously reported (Raftogianis et al., 1999). Previous studies were conducted using cell lysates as the source of allozyme, which complicated V_{\max} estimates. In our study, using purified protein, the V_{\max} estimates for PNP sulfation were dramatically different; *2 was significantly lower than either *1 or *3 (Table 2). In previous studies as well as the present study, the K_m estimate for PAPS for the *3 allozyme was the lowest among the three isozymes (Raftogianis et al., 1999). Together, these data support the notion that allozyme-specific differences occur between the three common SULT1A1 allozymes, that these differences are most pronounced with respect to the rate of the reaction, and that the *3 variant exhibits a higher affinity for the cosubstrate PAPS.

Dietary flavonoids may play a preventive role in some carcinogenesis processes, partially via interactions with estrogen receptors (Kuiper et al., 1998; Beecher, 2003). Flavonoids are sulfated by SULT1A1 and can result in drug interactions via enzyme inhibition (Mesia-Vela and Kauffman, 2003; Rossi et al., 2004). We observed significant differences in the capacity of SULT1A1 variants to sulfate chrysin, genistein, and quercetin. Allozyme-specific differences were noted in V_{\max} , with the *1 allozyme exhibiting the greatest activity, followed by *3 and *2 (Fig. 2, Table 2). Significant differences were also observed in the K_m estimates for these substrates. It is noteworthy that the V_{\max}/K_m ratio, an indicator of intrinsic clearance, exhibited varying trends with different flavonoids. Thus, The V_{\max}/K_m estimate was highest for SULT1A1*3 toward chrysin, whereas toward quercetin, *2 displayed higher V_{\max}/K_m than *1. Given that humans are commonly exposed to these chemopreventives, these results may contribute to our understanding of variation in the chemopreventive efficacy in the context of SULT1A1 pharmacogenetics. However, it is difficult to determine which parameter (V_{\max} or V_{\max}/K_m) will be most predictive of in vivo differences in flavonoid sulfation.

The allozyme-specific kinetics of E2 and 2OHE2 sulfation have been previously reported using SULT1A1 allozymes expressed in COS-1 cells (Adjei and Weinshilboum, 2002). These authors reported the K_m for SULT1A1*1 toward E2 to be $31.3 \pm 6.2 \mu\text{M}$, and we report here a K_m value of 5.0 ± 0.01 . Previous reports did not provide V_{\max} estimates. Herein, we report V_{\max} estimates (or lack of notable activity) for SULT1A1 allozymes that suggest striking differences among SULT1A1 allozymes toward sulfate estrogens (Table 2). We report very low activity of SULT1A1*2 and *3 toward E2. E2 has been shown to be mitogenic via estrogen-receptor mediated cellular events (Soderqvist, 1998). K_m estimates for the sulfation of 2OHE2 by SULT1A1*1 and *2 (Table 2) were similar to those reported previously (Adjei and Weinshilboum, 2002). However, we observed a significantly lower K_m estimate for SULT1A1*3 toward 2OHE2, resulting in a high V_{\max}/K_m ratio. The K_m reported here for SULT1A1*3 toward 2OHE2 was approximately 10-fold lower than that reported by Adjei and Weinshilboum (2002) (1.4 versus 17.3

μM). Differences between the two studies in the systems used to generate recombinant protein, and the fact that we analyzed purified protein rather than cell lysates, might account for the observed differences in kinetics. One limita-

tion of this study is the presence of a His₆ tag in the purified proteins, which may affect enzyme activity. The 2-methylation of E2 to yield 2MeE2 may be an anticarcinogenic pathway; 2MeE2 has antiproliferative and antiangiogenic effects

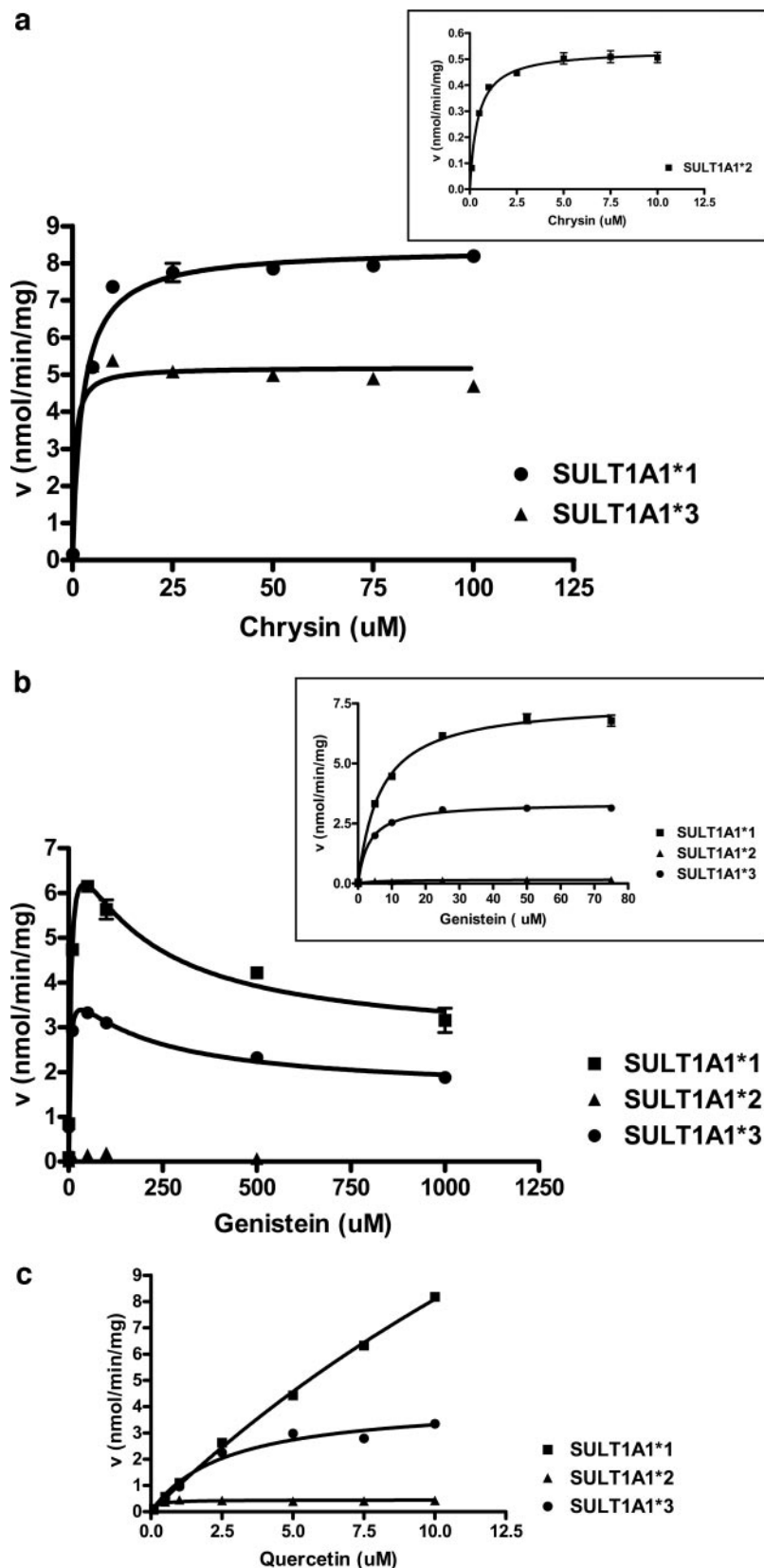


Fig. 2. Kinetics of sulfation of dietary flavonoids by SULT1A1 allozymes. Purified recombinant SULT1A1*1, *2, or *3 protein (1 μg) was incubated with substrate and 10 μM [³⁵S]PAPS for 30 min at 37°C, and the formation of radioactive sulfated product was quantitated with a radio-metric assay as described under *Materials and Methods*. a, Michaelis-Menten curves for chrysin sulfation by SULT1A1*1 and *3, at 0 to 100 μM chrysin. Inset, Michaelis-Menten curve for the *2 allozyme is depicted on an expanded y-axis scale. b, kinetic curves for genistein sulfation by SULT1A1*1, *2, and *3, at 0 to 1000 μM genistein. Inset: Michaelis-Menten curves at low substrate concentrations (0–75 μM) are depicted. c, Michaelis-Menten curves for quercetin sulfation by SULT1A1*1, *2, and *3, at 0 to 10 μM quercetin. Data expressed as mean \pm S.D., $n = 3$. Representative fitted lines are depicted; parameter estimates were obtained by fitting the kinetic models to actual data replicates.

in cells (Fotsis et al., 1994; Spink et al., 2000). In this study, significant differences were observed in Michaelis-Menten estimates for 2MeE2 sulfation among the SULT1A1 allozymes. Each of these estrogens is sulfated and inactivated by SULT1A1, among other SULTs. SULT1A1 is expressed in normal breast tissue as well as breast tumors (Falany and Falany, 1996, 1997). Our results indicate that SULT1A1 allozymes exhibit drastically different capacity to sulfate the carcinogen E2 and the anticarcinogens 2OHE2 and 2MeE2. It is possible that these differences contribute to physiologically significant differences in cellular metabolism of these molecules.

The crystal structure of human SULT1A1 has been resolved, and substrate- and PAPS- binding sites identified (Gamage et al., 2003). Based on crystal structure modeling, it has been proposed that the Arg213His amino acid substitution in SULT1A1*2 may exhibit altered interaction with

neighboring residues, leading to altered protein stability as well as cofactor binding (Gamage et al., 2003). Although amino acids at positions 213 and 223 are not thought to directly contribute to the substrate-binding site, they may interact with other residues and affect the substrate binding pocket. It is plausible that SULT1A1*2 and *3 variants exhibit altered kinetics toward different substrates because of structural alterations in the protein.

Several epidemiological studies have examined the role of SULT1A1 polymorphisms in the risk and incidence of hormone-dependent cancers (Nowell et al., 2000, 2002; Seth et al., 2000; Bamber et al., 2001; Zheng et al., 2001; Magagnotti et al., 2003; Tang et al., 2003). It is noteworthy that most population studies have focused on genotyping for SULT1A1*1 and *2 but not *3. When not specifically analyzed, the *3 allele is misclassified as *1 in most common genotyping assays. Given the marked differences in activity

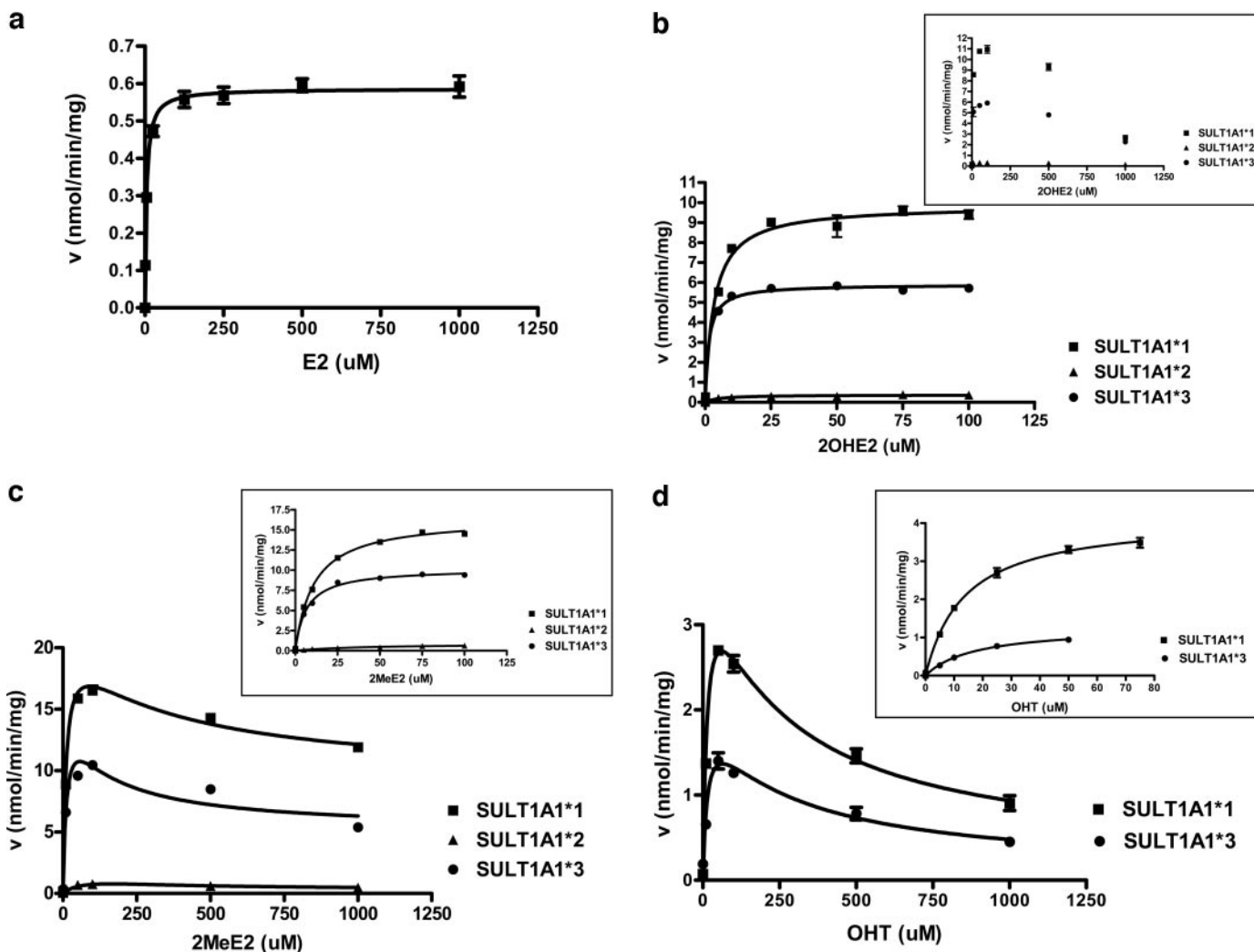


Fig. 3. Kinetics of sulfation of E2, 2OHE2, 2MeE2, and OHT by purified recombinant SULT1A1 protein. Purified recombinant SULT1A1*1, *2, or *3 protein (0.5–2 μ g) was incubated with substrate and 10 μ M [35 S]PAPS for 30 min at 37°C, and the formation of radioactive sulfated product was quantitated with a radiometric assay as described under *Materials and Methods*. **a**, Michaelis-Menten data for E2 sulfation by purified recombinant SULT1A1*1 protein, at 0 to 1000 μ M E2. Data expressed as mean \pm S.D., $n = 6$. Incubations were carried out with *2 and *3 proteins as well, but no activity was detected. **b**, Michaelis-Menten curves for 2OHE2 sulfation by recombinant SULT1A1*1, *2, and *3, at 0 to 100 μ M 2OHE2. Inset, 2OHE2 sulfation at high substrate concentrations (0–1000 μ M) are shown. **c**, enzyme kinetic curves for 2MeE2 sulfation by SULT1A1*1, *2, and *3, at 0 to 1000 μ M 2MeE2. Inset, Michaelis-Menten curves at low substrate concentrations (0–100 μ M) are shown. **d**, enzyme kinetic curves for OHT sulfation by SULT1A1*1 and *3, at 0 to 1000 μ M OHT. Incubations were carried out for *2, but no activity was detected. Inset, Michaelis-Menten curves at low substrate concentrations (0–75 μ M for *1 and 0–50 μ M for *3) are shown. Data represented as mean \pm S.D., $n = 3$. Representative fitted lines are depicted; parameter estimates were obtained by fitting the kinetic models to actual data replicates.

and the common frequency of the *3 allele in at least the African American population, it is important that the *3 allele be specifically typed in population studies.

An interesting observation in our study was the significant difference in OHT sulfation by SULT1A1*1, *2, and *3. OHT is a highly potent metabolite of the antiestrogen drug tamoxifen and is inactivated by sulfation (Shibutani et al., 1998). Variable activity of drug-metabolizing enzymes has been observed as a common mechanism of tumor drug resistance, and variable OHT sulfation might be a factor in tamoxifen resistance and variable patient response (Hayes and Pulford, 1995). Figure 3d depicts the kinetics of OHT sulfation by the purified allozymes. The *3 allozyme had a much lower V_{max} estimate for this reaction than *1, whereas V_{max} could not be estimated for *2 because of extremely low activity. We next evaluated the proliferative capacity of MCF-7 cells stably expressing SULT1A1*1 or *2 in the presence of OHT and E2 (Fig. 4). Consistent with the biochemical data, we observed an allele-specific antiestrogenic response to OHT. Cells expressing SULT1A1*1 exhibited a significantly poorer response (greater rate of proliferation) than those expressing *2. These data suggest that the cells expressing SULT1A1*2 possess diminished capacity to sulfate and inactivate OHT compared with cells expressing the *1 allozyme.

Because we consistently observed low cellular concentrations of SULT1A1*2, we suspected that this variant might undergo rapid cellular degradation. Protein degradation was examined as an additional factor contributing to polymorphic SULT1A1 activity. Pulse-chase and cycloheximide experiments suggested that the half-life of SULT1A1*1 was 6-fold longer than that of the *2 variant (Fig. 5). Further examination revealed that the *2 protein was highly ubiquitinated compared with *1 (Fig. 5c). Initial studies examining the degradation of the *3 allozyme revealed a turnover half-life similar to that of *1; hence, *3 degradation was not evaluated further. SULT1A3 is a polymorphic sulfotransferase, and it was reported that a variant SULT1A3 allozyme was degraded more rapidly by a ubiquitin-proteasome-dependent mechanism (Wang et al., 2004). Accelerated degradation has also been mechanistically implicated in the altered activity of the polymorphic enzyme thiopurine *S*-methyltransferase

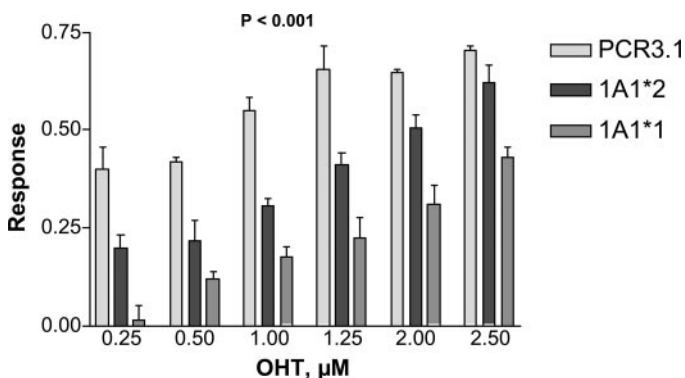


Fig. 4. Antiestrogenic response of OHT in MCF-7 cells stably expressing pCR3.1, SULT1A1*1, or SULT1A1*2. Cells were cultured in charcoal-stripped media for 48 h, followed by treatment with 1 nM E2 and 0.25 to 2.5 μ M OHT for 5 days. On day 5, proliferation was monitored using the alamarBLUE assay as described under *Materials and Methods*. Data expressed as mean \pm SD, $n = 3$. The response between cells expressing SULT1A1*1 and *2 was found to be statistically significant with ANOVA, $p < 0.001$.

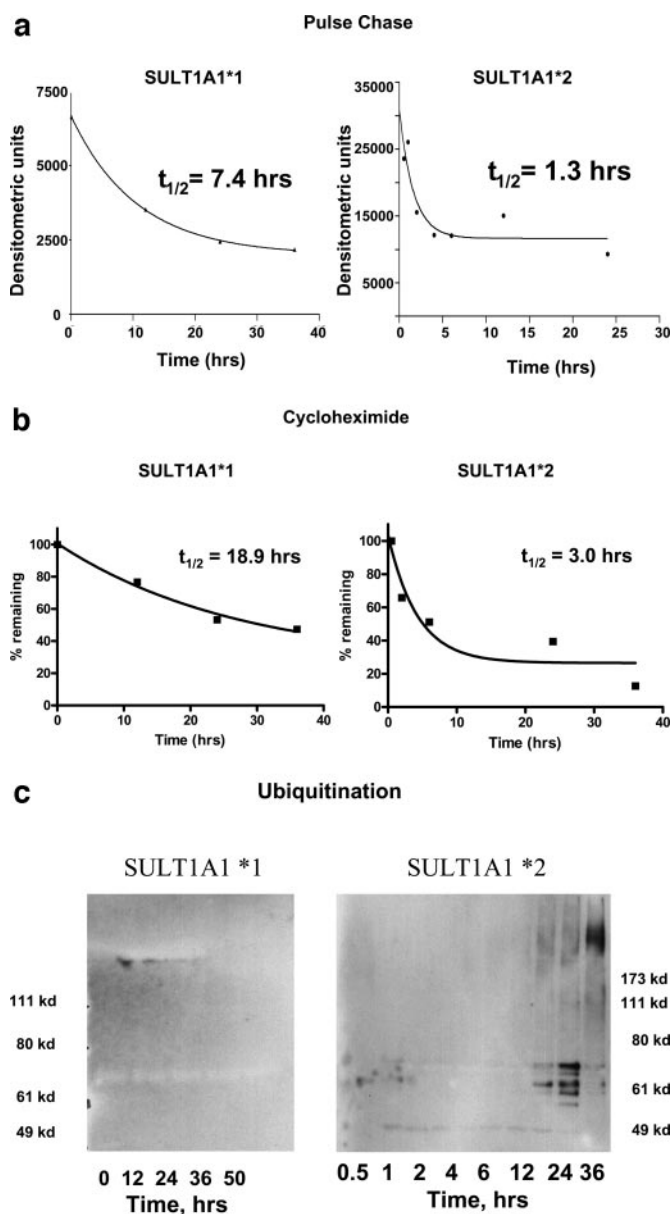


Fig. 5. Degradation of recombinant SULT1A1*1 and *2 protein. a, pulse-chase experiments were carried out with Sf-9 insect cells expressing SULT1A1*1 or *2. Recombinant human His₆-tagged SULT1A1*1 or *2 allozymes were expressed in Sf-9 insect cells. Cells were cultured in the absence of methionine and 'pulsed' with [³⁵S]methionine for 1.5 h. Cells were then "chased" with complete medium over 50 h for *1 and 36 h for *2, after which time no protein was detected. Aliquots were evaluated for SULT1A1 protein content over time by immunoprecipitation with an anti-His₆ antibody and gel electrophoresis. The data from the pulse-chase experiments were analyzed with densitometry, and the degradation half-life was calculated using a monoexponential equation to fit the data. b, protein stability was evaluated in MCF-7 cells stably expressing SULT1A1*1, *2, or *3. Cultured cells were treated with 40 μ g/ml cycloheximide and collected over 48 h. Cell lysates were prepared in radioimmunoprecipitation assay buffer, and equal amounts of total protein were subjected to SDS-PAGE and Western blot analysis with an anti-SULT1A1 antibody. The data from the Western blot were analyzed with densitometry, and the degradation half-life was calculated using a monoexponential equation to fit the data. c, Western blot analysis with an anti-ubiquitin antibody with the same samples from the pulse-chase experiment. Cell lysates from the pulse-chase experiments were subjected to immunoprecipitation with an anti-His₆ antibody, and after gel electrophoresis, Western blot analysis was performed with an anti-ubiquitin antibody. No ubiquitinated bands were observed for *1, whereas multiple ubiquitinated bands were observed in the *2 samples at the 12- and 24-h time points.

(Tai et al., 1997; Wang et al., 2003). Thus, there is ample precedence in the literature suggesting that altered degradation of polymorphic variants of drug-metabolizing enzymes is a common mechanism contributing to low activity.

In conclusion, this study characterized the functional significance and molecular mechanisms governing SULT1A1*1, *2, and *3 allozyme variation. These results clearly indicate significant differences in the catalytic activity of these proteins toward several different classes of substrates. The V_{\max} was in the order *1 > *3 > *2 for substrates including estradiol, 2OHE2, 2MeE2, OHT, and the dietary flavonoids chrysin, genistein, and quercetin. The intrinsic clearance as measured by the V_{\max}/K_m ratio varied with substrate among the SULT1A1 allozymes. MCF-7 cells stably expressing the *2 allele exhibited greater antiestrogenic response to OHT compared with cells expressing *1. The *2 allozyme exhibited a cellular half-life 6-fold lower than that of *1, via a mechanism that seems to involve a ubiquitin-mediated pathway. SULT1A1 polymorphic variants exhibit markedly different properties, and this is critical when evaluating the role of SULT1A1 as a xenobiotic and steroid hormone-metabolizing enzyme.

References

- Adjei A and Weinshilboum R (2002) Catecholestrogen sulfation: possible role in carcinogenesis. *Biochem Biophys Res Commun* **292**:402–408.
- Anderson R and Weinshilboum R (1980) Phenolsulphotransferase in human tissue: radiochemical enzymatic assay and biochemical properties. *Clin Chim Acta* **103**:79–90.
- Bamber D, Fryer A, Strange R, Elder J, Deakin M, Rajagopal R, Fawole A, Gilissen R, Campbell F, and Coughtrie M (2001) Phenol sulphotransferase SULT1A1*1 genotype is associated with reduced risk of colorectal cancer. *Pharmacogenetics* **11**:679–685.
- Beecher G (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. *J Nutr* **133**:3248S–3254S.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248–254.
- Carlini E, Raftogianis R, Wood T, Jin F, Zheng W, Rebbeck T, and Weinshilboum R (2001) Sulfation pharmacogenetics: SULT1A1 and SULT1A2 allele frequencies in Caucasian, Chinese and African-American subjects. *Pharmacogenetics* **11**:57–68.
- Chou H-C, Lang N, and Kadlubar F (1995) Metabolic activation of *N*-hydroxy arylamines and *N*-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res* **55**:525–529.
- Coughtrie M (2002) Sulfation through the looking glass—recent advances in sulfotransferase research for the curious. *Pharmacogenomics* **2**:297–308.
- Coughtrie M, Gilissen R, Shek B, Strange R, Fryer A, Jones P, and Bamber D (1999) Phenol sulfotransferase SULT1A1 polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations. *Biochem J* **337**:45–49.
- Coughtrie M and Johnston L (2001) Interactions between dietary chemicals and human sulfotransferases: molecular mechanisms and clinical significance. *Drug Metab Dispos* **29**:522–528.
- Falany JL and Falany CN (1996) Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. *Cancer Res* **56**:1551–1555.
- Falany JL and Falany CN (1997) Regulation of estrogen activity by sulfation in human MCF-7 breast cancer cells. *Oncol Res* **9**:589–596.
- Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, and Schweigerer L (1994) The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. *Nature (Lond)* **368**:237–239.
- Gamage NU, Duggleby RG, Barnett AC, Tresillian M, Latham CF, Liyou NE, McManus ME, and Martin JL (2003) Structure of a human carcinogen-converting enzyme, SULT1A1. Structural and kinetic implications of substrate inhibition. *J Biol Chem* **278**:7655–7662.
- Geese W and Raftogianis R (2001) Biochemical characterization and tissue distribution of human SULT2B1. *Biochem Biophys Res Commun* **288**:280–289.
- Glatt H (2000) Sulfotransferases in the bioactivation of xenobiotics. *Chem Biol Interact* **129**:141–170.
- Hayes JD and Pulford DJ (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* **30**:445–600.
- Jones AL, Hagen M, Coughtrie MW, Roberts RC, and Glatt H (1995) Human platelet phenolsulfotransferases: cDNA cloning, stable expression in V79 cells and identification of a novel allelic variant of the phenol-sulfating form. *Biochem Biophys Res Commun* **208**:855–862.
- Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, and Gustafsson JA (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139**:4252–4263.
- Magagnotti C, Pastorelli R, Pozzi S, Andreoni B, Fanelli R, and Airolidi L (2003) Genetic polymorphisms and modulation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-DNA adducts in human lymphocytes. *Int J Cancer* **107**:878–884.
- Mesia-Vela S and Kauffman FC (2003) Inhibition of rat liver sulfotransferases SULT1A1 and SULT2A1 and glucuronosyltransferase by dietary flavonoids. *Xenobiotica* **33**(12):1211–1220.
- Nagar S, Remmel R, Hebbel R, and Zimmerman C (2004) Metabolism of opioids is altered in liver microsomes of sickle cell transgenic mice. *Drug Metab Dispos* **32**:98–104.
- Nowell S, Ambrosone C, Ozawa S, MacLeod S, Mrackova G, Williams S, Plaxco J, Kadlubar F, and Lang N (2000) Relationship of phenol sulfotransferase activity (SULT1A1) genotype to sulfotransferase phenotype in platelet cytosol. *Pharmacogenetics* **10**:789–797.
- Nowell S, Coles B, Sinha R, MacLeod S, Ratnasinghe D, Stotts C, Kadlubar F, Ambrosone C, and Lang N (2002) Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: contribution of metabolic variation to risk. *Mutat Res* **506–507**:175–185.
- Raftogianis R, Wood T, Otterness D, Van Loon J, and Weinshilboum R (1997) Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. *Biochem Biophys Res Commun* **239**:298–304.
- Raftogianis R, Wood T, and Weinshilboum R (1999) Human phenol sulfotransferases SULT1A2 and SULT1A1. Genetic polymorphisms, allozyme properties and human liver genotype-phenotype correlations. *Biochem Pharmacol* **58**:605–616.
- Rossi AM, Maggini V, Fredianelli E, Di Bello D, Pietrabissa A, Mosca F, Barale R, and Pacifici GM (2004) Phenotype-genotype relationships of SULT1A1 in human liver and variations in the IC50 of the SULT1A1 inhibitor quercetin. *Int J Clin Pharmacol Ther* **42**:561–567.
- Seth P, Lunetta K, Bell D, Gray H, Nasser S, Rhei E, Kaelin C, Iglehart D, Marks J, Garber J, et al. (2000) Phenol sulfotransferases: hormonal regulation, polymorphism and age of onset of breast cancer. *Cancer Res* **60**:6859–6863.
- Shibutani S, Dasaradhi L, Terashima I, Banoglu E, and Duffel MW (1998) Alpha-hydroxytamoxifen is a substrate of hydroxysteroid (alcohol) sulfotransferase, resulting in tamoxifen DNA adducts. *Cancer Res* **58**:647–653.
- Soderqvist G (1998) Effects of sex steroids on proliferation in normal mammary tissue. *Ann Med* **30**:511–524.
- Spink BC, Katz BH, Hussain MM, Pang S, Connor SP, Aldous KM, Gierthy JF, and Spink DC (2000) SULT1A1 catalyzes 2-methoxyestradiol sulfonation in MCF-7 breast cancer cells. *Carcinogenesis* **21**:1947–1957.
- Tai H-L, Krynetski E, Scheutz E, Yanishevski Y, and Evans W (1997) Enhanced proteolysis of thiopurine S-methyltransferase (TPMT) encoded by mutant alleles in humans (TPMT*3A, TPMT*2): mechanisms for the genetic polymorphism of TPMT activity. *Proc Natl Acad Sci USA* **94**:6444–6449.
- Tang D, Rundle A, Mooney L, Cho S, Schnabel F, Estabrook A, Kelly A, Levine R, Hibshoosh H, and Perera F (2003) Sulfotransferase 1A1 (SULT1A1) polymorphism, PAH-DNA adduct levels in breast tissue and breast cancer risk in a case-control study. *Breast Cancer Res Treat* **78**:217–222.
- Varin L, Barron D, and Ibrahim RK (1987) Enzymatic assay for flavonoid sulfotransferase. *Anal Biochem* **161**:176–180.
- Wang L, Sullivan W, Toft D, and Weinshilboum R (2003) Thiopurine S-methyltransferase pharmacogenetics: chaperone protein association and allozyme degradation. *Pharmacogenetics* **13**:555–564.
- Wang L, Yee VC, and Weinshilboum RM (2004) Aggresome formation and pharmacogenetics: sulfotransferase 1A3 as a model system. *Biochem Biophys Res Commun* **325**:426–433.
- Wang Y, Spitz M, Tsou AM-H, Zhang K, Makan N, and Wu X (2002) Sulfotransferase (SULT) 1A1 polymorphism as a predisposition factor for lung cancer: a case-control analysis. *Lung Cancer* **35**:137–142.
- Williams J, Stone E, Fakis G, Johnson N, Cordell J, Meil W, Glatt H, Sim E, and Phillips D (2001) *N*-Acetyltransferases, sulfotransferases and heterocyclic amine activation in the breast. *Pharmacogenetics* **11**:373–388.
- Wu M-T, Wang Y-T, Ho C-K, Wu D-C, Lee Y-C, Hsu H-K, Kao E-L, and Lee J-M (2003) SULT1A1 polymorphism and esophageal cancer in males. *Int J Cancer* **103**:101–104.
- Zhang H, Varlamova O, Vargas FM, Falany CN, and Leyh TS (1998) Sulfuryl transfer: the catalytic mechanism of human estrogen sulfotransferase. *J Biol Chem* **273**:10888–10892.
- Zheng W, Xie D, Cerhan J, Sellers T, Wen W, and Folsom A (2001) Sulfotransferase 1A1 polymorphism, endogenous estrogen exposure, well-done meat intake and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **10**:89–94.

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