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EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF HUMAN CYTOSOLIC SULFOTRANSFERASE (SULT) 1C4

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Abstract

Human cytosolic sulfotransferase 1C4 (hSULT1C4) is a dimeric Phase II drug-metabolizing enzyme primarily expressed in the developing fetus. SULTs facilitate the transfer of a hydrophilic sulfonate moiety from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) onto an acceptor substrate altering the substrate's biological activity and increasing the compound's water solubility. While several of the hSULTs' endogenous and xenobiotic substrates have been identified, the physiological function of hSULT1C4 remains unknown. The fetal expression of hSULT1C4 leads to the hypothesis that the function of this enzyme may be to regulate metabolic and hormonal signaling molecules, such as estrogenic compounds, that may be generated or consumed by the mother during fetal development. Human SULT1C4 has previously been shown to sulfonate estrogenic compounds, such as catechol estrogens; therefore, this study focused on the expression and purification of hSULT1C4 in order to further characterize this enzyme's sulfonation of estrogenic compounds. Molecular modeling of the enzyme's native properties helped to establish a novel purification protocol for hSULT1C4. The optimal activity assay conditions for hSULT1C4 were determined to be pH 7.4 at 37°C for up to 10 min. Kinetic analysis revealed the enzyme's reduced affinity for PAPS compared to PAP. Human SULT1C4 sulfonated all the estrogenic compounds tested, including dietary flavonoids and environmental estrogens; however, the enzyme has a higher affinity for sulfonation of flavonoids. These results suggest hSULT1C4 could be metabolizing and regulating hormone signaling pathways during human fetal development.

Introduction

Cytosolic sulfotransferases (SULTs), a superfamily of Phase II drug-metabolizing enzymes, catalyze the transfer of a sulfonate moiety ($-SO_3$) from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to the hydroxyl or primary amine group of the acceptor substrate [1, 2]. The 14 human SULT isoforms are involved in the metabolism of many endogenous compounds such as bile acids, steroids, thyroid hormones and neurotransmitters [3]. Addition of the sulfonate moiety makes compounds more water soluble and often leads to the renal and biliary excretion of the sulfonated compound. However, detoxification is just

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one of the several functions of these conjugation reactions; sulfonation also results in the bioactivation of procarcinogens and prodrugs [4–7].

Of the four human SULT families (hSULT1, hSULT2, hSULT4 and hSULT6), relatively little is known about the SULT1C subfamily (hSULT1C2, hSULT1C3a, hSULT1C3d and hSULT1C4) [8]. Several studies on the hSULT1C subfamily have shown that these isoforms convert various xenobiotics, such as procarcinogens, into reactive metabolites; however, their function in endogenous metabolism and physiology has not yet been well characterized [9–11]. Although the hSULT1C subfamily's substrate specificity remains unclear, hSULT1C4 (previously referred to as SULT1C2) is highly expressed in the fetal lung and fetal kidney, with lower expression in fetal heart, adult kidney, ovary, brain and spinal cord [12–14]. Due to hSULT1C4's fetal expression, one of the physiological functions of this enzyme may be to metabolize and regulate endogenous and exogenous signaling molecules during fetal development.

The SULTs are involved in the sulfonation of various hormones, including endogenous estrogens, environmental estrogens, and estrogen metabolites, which are all present throughout pregnancy [15–18]. Although hSULT1E1 is the major enzyme responsible for the sulfonation of estrogens, hSULT1C4 can also sulfonate estrogenic compounds, such as the catechol estrogens; these estrogenic compounds can bind to the estrogen receptor (ER) and alter ER signaling [17, 19]. During pregnancy, the fetus is exposed to a large variety of endogenous estrogens from the mother as well as environmental estrogens, including flavonoids and bisphenol A (BPA), from the mother's diet. These dietary estrogenic compounds are potential substrates and/or inhibitors of the SULTs; therefore, they can influence the bioavailability and metabolism of endogenous estrogens [20, 21]. Tagged hSULT1C4 has been expressed and purified in previous studies in which a few of the endogenous estrogens were screened; however, there is little information on hSULT1C4's regulation of estrogen signaling and its potential role in sulfonating environmental estrogens [22]. One of the current limitations in deciphering the physiological functions of hSULT1C4 is the lack of research studying the native protein directly from human tissues or cells.

The purpose of this study was to express and purify untagged hSULT1C4 to characterize this isoform's enzymatic activity. The kinetic properties of pure hSULT1C4 were evaluated using activity assays, molecular modeling, and binding studies. Characterization of hSULT1C4's physical and kinetic properties is important to gain a more thorough understanding of this isoform's role in the sulfonation of exogenous estrogenic compounds in human adult and fetal tissues.

Materials and methods

Materials

A Molecular Operating Environment (MOE) license was purchased from Chemical Computing Company (Montréal, QC, Canada). Ni-NTA resin was obtained from Qiagen (Hilden, Germany). DEAE-Sepharose CL-6B was purchased from GE Healthcare Bio-Sciences (Pittsburgh, PA, USA). SDS (sodium dodecyl sulfate) running buffer, Precision Plus Protein Dual Color Standards, and Poly-Prep Chromatography Columns were

purchased from Bio-Rad (Hercules, CA, USA). Genistein, daidzein, apigenin, 17β-estradiol (E2), and Coomassie blue stain were acquired from Sigma-Aldrich (St. Louis, MO, USA). 6,4′-Dihydroxyflavone was purchased from INDOFINE Chemical Company (Belle Mead, NJ, USA). [^{35}S]PAPS (1.4 Ci/mmol) and [^{3}H]17β-estradiol (60 mCi/mmol) were purchased from PerkinElmer Life and Analytical Sciences, respectively (Waltham, MA, USA). [^{3}H]1-Naphthol (40 Ci/mmol) and [^{14}C]1-naphthol (55 mCi/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). PAPS was purchased from R&D Systems (Minneapolis, MN, USA). Silica gel thin-layer chromatography (TLC) plates (250 mm) were obtained from Analtech, Inc. (Newark, DE, USA). All other reagents were reagent grade and purchased from Thermo-Fisher Scientific (Waltham, MA, USA).

Expression and purification of native hSULT1C4

The hSULT1C4 cDNA was ligated into the BamHI and HindIII sites in the pKK233-2 bacterial expression vector (Pharmacia, Piscataway, NJ, USA) using T4 DNA Ligase. The DNA sequence was verified by the UAB Heflin Center for Genomic Sciences DNA Sequencing Core Facility. BL21-DE3-RIL competent *Escherichia coli*, optimized for the expression of human proteins, were transformed with the pKK233-2-hSULT1C4 vector.

A glycerol stock containing the pKK233-2-hSULT1C4 expression vector was used to create overnight cultures [LB broth medium with 100 μg/mL ampicillin (Amp +) and 170 μg/mL chloramphenicol (Chlo +)], and each 20 mL culture was used to inoculate 500 mL of LB-Amp +-Chlo + in a 2L Erlenmeyer flask. *Escherichia coli* were grown at 37 °C with shaking at 225 rpm until an optical density at 600 nm (OD_{600}) of 0.5–0.7 was reached. Isopropyl β-D-thiogalactoside (IPTG) was added to a final concentration of 0.5 mM to induce expression of the hSULT1C4 protein followed by culturing at 20 °C for 4 h. The cultures were centrifuged, and the bacterial pellets resuspended in Nickel (Ni) column wash buffer (25 mM Tris, 5% glycerol, 10 mM imidazole, 2 mM β-ME, 300 mM NaCl, pH 8.8) with 0.1 mM phenylmethanesulfonylfluoride (PMSF). To induce lysis, cells were sonicated six times for 15 s with 30 s rest on ice between each sonication. To isolate the cytosolic fraction, the cell lysate was centrifuged at 100,000 × g for 1 h at 4 °C.

Cytosol was then passed over a HisPur Ni-NTA column at 4 °C. The column was washed with 20 bed volume (BV) of Ni column wash buffer, and the bound protein eluted from the column using a gradient from 10 mM to 300 mM imidazole buffer. The eluate was collected in fractions and analyzed using SDS-gel electrophoresis and sulfonation activity assays. Pooled fractions with high sulfonation activity were dialyzed for 2 h in 1L buffer (25 mM Tris, 5% glycerol, 2 mM β-ME, pH 8.8) followed by a second dialysis overnight at 4 °C in 1 L fresh buffer.

Dialyzed protein was passed over a DEAE-Sepharose column. The column was washed with 1 BV of DEAE column wash buffer (25 mM Tris, 5% glycerol, 2 mM β-ME, pH 8.8) and then washed with 2.5 BV of DEAE column wash buffer containing 10 mM NaCl. The protein was eluted from the column using a gradient from 10 mM to 300 mM NaCl. The purity of the eluted fractions was determined using SDS-PAGE stained with Coomassie. The pure fractions exhibiting sulfonation activity were pooled, aliquoted, and stored at − 80 °C.

Sulfotransferase activity assays

To evaluate hSULT1C4's sulfonation activity, all radiolabeled compounds ([^{3}H]1-naphthol, [^{14}C]1-naphthol, and [^{3}H]E2) were evaluated across a range of concentrations (0–50 μM). The compound, enzyme, and reaction buffer (50 mM Tris-HCl pH 7.4, 1 mM $MgCl_2$, and 1.75 mM BSA) were preincubated for 2 min at 37 °C before starting the reactions. Reactions were initiated with PAPS (10 μM final concentration) and incubated at 37 °C for 5 min. When using radiolabeled substrates, the reaction was quenched with 3 mL chloroform, and the product was extracted with 375 μL product extraction buffer (250 mM Tris-HCl pH 8.5) [23]. The quenched reactions were centrifuged at 1500 rpm for 5 min to enhance phase separation. A portion of the aqueous layer was removed and quantified by liquid scintillation counting. Every reaction, including the control (denatured hSULT1C4), was tested in two independent sets of triplicate reactions.

For non-radiolabeled compounds, the reactions were performed under the same conditions as described above except using [^{35}S]PAPS [24]. After a reaction was complete, a portion of the reaction was spotted onto a TLC plate and immediately dried. TLC plates were placed into an 85 : 15 : 5 (v/v/v methylene chloride : methanol : ammonium hydroxide) solvent system for daidzein, genistein and BPA or a 20 : 20 : 30 : 10 (v/v/v/v n-butanol : isopropanol : 90% formic acid : water) solvent system for apigenin, chrysin, and 6,4′-dihydroxyflavone. Product bands were visualized on a Storm 865 phosphorimager (GE Healthcare; Pittsburgh, PA, USA) after a 1 h exposure to the storage phosphor screen. The TLC product bands were scraped into scintillation vials for quantification by scintillation spectroscopy. The kinetic parameters were determined using Microsoft Excel and Visual Enzymics 2010-Igor Pro 6.3.6.4 (Michaelis-Menten model and substrate inhibition model) (Softzymics, Inc., Princeton, NJ, USA).

K_d determination of PAPS and PAP for hSULT1C4

To determine the K_d of PAPS's binding, the intrinsic fluorescence of pure SULT1C4 was measured at room temperature with continuous stirring using a Fluromax-4 spectrofluorometer (Horiba Scientific, Kyoto, Japan) with the excitation wavelength at 282 nm and the emission wavelength at 342 nm. PAPS was titrated into the cuvette, which contained the enzyme (200 nM) and buffer (10 mM sodium phosphate pH 7.8, 10% glycerol, and 150 mM NaCl). Increasing concentrations of PAPS were added in 2 μL aliquots (five titrations of 100 μM, five titrations of 500 μM, four titrations of 2500 μM, and two titrations of 8100 μM) and allowed to equilibrate for 15 s before monitoring the fluorescence for 15 s (0.1 s increments). Due to the adenosine ring's absorption of the protein's emission at high PAPS concentrations, the same experiment was performed with AMP as a blank control [25, 26]. The K_d values were calculated by plotting the change in fluorescence versus substrate concentration using Microsoft Excel and Visual Enzymics 2010-Igor Pro 6.3.6.4 [one site binding (quadratic) model]. Each experiment, including the control, was conducted in triplicate.

Molecular modeling of hSULT1C4

MOE software was used to visualize hSULT1C4's 3D protein structure. The crystal structure of hSULT1C4, co-crystallized with PAP and pentachlorophenol, (PDB 2GWH) has several

unresolved amino acids in the crystal structure; therefore, the homology model function in MOE was used to thread these missing residues onto the existing crystal structure while preserving the location of all the other atoms [18, 22]. The hSULT1C4 homology model was then energy minimized using the Amber99 force field and protonated with the MOE 3D Protonate function set to physiological conditions (pH of 7.4 and 150 mM NaCl) [27, 28]. The last step was to confirm the quality of our structure by uploading the structure to the NIH Structure Analysis and Verification Server (NIH-SAVES, Los Angeles, CA, USA) for analysis. A score of 90% or higher is considered an acceptable structure.

Visual Molecular Dynamics 1.9.1 (VMD, Champaign, IL, USA) was used to visualize hSULT1C4's acidic and basic residues [29]. The isoelectric points of the hSULTs were calculated using the MacVector (Oxford Molecular Group, Inc., Oxford, UK) sequence analysis software 10.0 (Genetics Computer Group, Madison, WI, USA) [30].

Results

Purification of hSULT1C4

Previously published purification methods for hSULT1C4 involved the use of an affinity tag (i.e. glutathione S-transferase or polyhistidine); however, these tagged purification approaches cannot be used to purify the enzyme out of human tissues [12, 22]. We used the natural features of hSULT1C4 to develop a new purification protocol that can be applied to potentially isolate SULT1C4 from human tissues. We utilized chelated metal's affinity for histidines to bind bacterially expressed native hSULT1C4 to Ni-NTA resin [31]. Figure 1A shows two adjacent histidines (His148 and His274) exposed on the surface of hSULT1C4. The SULTs exist as naturally occurring homodimers, so dimerization results in four adjacent histidines on the protein's surface [32]. Active partially pure protein eluted off the Ni-NTA column with approximately 100–200 mM imidazole (Figure 1B and C).

Comparison of all the human SULT isoforms' isoelectric points revealed that hSULT1C4 has the highest calculated isoelectric point (pI), 8.42, of all the hSULT isoforms, which have an average pI of 6.31 (Table 1). Molecular modeling of hSULT1C4 displayed more basic functional groups compared to acidic functional groups contributing to the protein's relatively high isoelectric point (Figure 2A). When a protein is at a pH higher than its pI, the protein becomes an anion and can bind to positively charged resin; therefore, anion exchange chromatography (DEAE-sepharose) at pH 8.8 was performed to further purify the pooled enzyme fractions from the Ni-NTA column with the highest activity [33]. Pure hSULT1C4 eluted from the DEAE-sepharose with approximately 150–200 mM NaCl (Figure 2B and C). Table 2 summarizes the purification of bacterially expressed native hSULT1C4. The purification procedure resulted in a 115-fold purification of hSULT1C4 activity as compared to cytosol. Pure hSULT1C4 had a specific activity of 26.0 nmol of 1-naphthol sulfated per min per milligram protein (nmol/[min * mg]) and a yield of 34.5%.

Optimization of kinetic assay conditions

To begin kinetic characterization and optimization of enzyme assays for hSULT1C4, we utilized the small phenolic compound 1-naphthol, a hSULT1C4 substrate [22]. The

calculated K_m for 1-naphthol sulfonation by hSULT1C4 was 1.1 ± 0.2 μM (Figure 3A). All of the subsequent optimization reactions were tested using 10μM 1-naphthol (Figure 3). The optimal conditions for hSULT1C4 enzyme assays were identified as pH of 7.4 at a temperature of 37 °C for up to ten min (Figure 3B and C). Initial kinetic observations and variability with hSULT1C4 (data not shown) suggested the protein was binding to the borosilicate glass tube, a complication that often leads to miscalculations and inaccurate estimations of protein concentration [34]. Bovine serum albumin (BSA) is commonly used to help reduce non-specific protein-surface binding and ensure the protein remains in solution [35]. Increasing the concentration of BSA in the reactions resulted in approximately an 18-fold increase in the specific activity of hSULT1C4 (Figure 3D). The same range of BSA concentrations was tested with different substrates to confirm that the BSA does not affect other substrates' availability to interact with the enzyme (data not shown). Fifty micrograms of BSA was added to all subsequent reactions to inhibit interactions between the enzyme and the tube's glass surface.

PAP and PAPS interaction with hSULT1C4

PAPS and the product PAP both have essential interactions with the SULTs; to characterize these interactions, the Michaelis constant (K_m) of PAPS and the dissociation constant (K_d) of PAP and PAPS were measured [36]. In the presence of daidzein, an estrogenic substrate (Figure 6), the apparent K_m of PAPS for hSULT1C4 was 7.0 ± 1.0 μM (Figure 4A). This K_m is higher than expected when compared to the K_m of PAPS for the other SULTs, which can range from 0.2 to 1.5 μM; therefore, we determined the affinity for binding PAPS or PAP to the enzyme alone by monitoring changes in the protein's intrinsic fluorescence [26, 37, 38]. The K_d of PAPS for hSULT1C4 was determined to be 4.8 ± 0.8 μM (Figure 4B), and the K_d of PAP for hSULT1C4 was 2.8 ± 0.2 μM (Figure 4C).

Sulfonation of estrogenic compounds by hSULT1C4

Previous studies, as well as substrate screens in our lab, have shown that hSULT1C4 can sulfonate various estrogenic compounds [17]. Based on hSULT1C4's expression in the fetus and the importance of estrogen signaling during development, hSULT1C4's ability to sulfonate several environmental and dietary estrogenic compounds was analyzed (Figure 5) [12, 39]. Using a standard activity assay for non-radiolabeled compounds, genistein, daidzein, chrysin, apigenin, and 6,4′-dihydroxyflavone were identified as substrates for hSULT1C4 (Figure 6). The two isoflavones, genistein and daidzein, had K_ms of 3.3 μM ± 0.3 μM and 10.5 μM ± 1.1 μM, respectively, for sulfonation by hSULT1C4; at concentrations above 10 μM, genistein displayed substrate inhibition, which was not observed with daidzein (Figure 6A and B). The three flavones (apigenin, chrysin, and 6,4′-dihydroxyflavone) also had different, but relatively good rates of sulfonation by the enzyme. Chrysin and apigenin had the lowest K_ms (0.9 μM ± 0.4 μM and 1.7 μM ± 1.0 μM, respectively) of the flavones (Figure 6C and D), while 6,4′-dihydroxyflavone had a higher K_m (10.1 μM ± 2.1 μM) for sulfonation by hSULT1C4 (Figure 6E). Both chrysin and apigenin caused substrate inhibition at concentrations above 10 μM but this effect was not observed with 6,4′-dihydroxyflavone; however, due to poor solubility, 6,4′-dihydroxyflavone was not screened at concentrations higher than 20 μM.

Based on hSULT1C4's high affinity for estrogenic compounds, we determined the K_m (36.2 μM ± 2.6 μM) for E2's sulfonation (Figure 6F). Certain industrial chemicals, such as BPA, act as endocrine disruptors by interacting with human ERs and disrupting the receptor's ability to bind E2 [15, 40]. Activity assays determined that hSULT1C4 can also sulfonate synthetic estrogens, such as BPA, with a K_m of 32.8 μM ± 3.2 μM (Figure 6G).

Discussion

Human SULT1C4 was identified in 1998; however, the physiological function of this drug metabolizing enzyme, along with the rest of the SULT1C subfamily (hSULT1C2, hSULT1C3a, hSULT1C3d), remains poorly understood [12]. Previous studies have shown that the SULT1Cs are predominantly expressed in the human fetus; therefore, researchers in the field hypothesize that the primary role of these enzymes is to metabolize endogenous and exogenous signaling molecules involved in growth and differentiation during human fetal development [12–14, 41]. Sulfonation and desulfonation pathways are involved in the regulation of hormones (e.g. estrogen) during various stages of human development, such as estrogen sulfonation in the placenta [19, 39, 42]. Initial substrate screens using tagged hSULT1C4 identified catechol estrogens as substrates for this isoform, but few studies have been performed to fully characterize hSULT1C4 and evaluate if the enzyme is involved in the sulfonation of endogenous and environmental estrogens [17]. Further investigation of hSULT1C4 will provide new information about drug metabolism during critical time points in development that could be used to help protect the human fetus. In this study, we expressed, purified, and characterized untagged human SULT1C4; furthermore, we identified several estrogenic compounds as hSULT1C4 substrates.

To overcome the limitations of tagged protein purification methods that can alter protein folding as well as activity and not be representative of the native enzyme, we utilized hSULT1C4's biochemical properties to develop a novel purification protocol for the untagged protein [12, 22, 43]. Molecular modeling software aided in the visualization of four adjacent histidines on the surface of the hSULT1C4 dimer (Figure 1). As anticipated, bacterially expressed native hSULT1C4 bound with a high affinity to Ni-NTA resin [31]. Comparison of the other SULTs' amino acid sequences revealed four other hSULTs (1C2, 1C3a, 2B1a, and 2B1b) with histidines in the same locations suggesting that these SULTs may also have a similar affinity for Ni-NTA resin [44]. Further analysis of hSULT1C4 exposed its unusually high pI (8.42) compared to the other SULTs' average pI of 6.31 (Figure 2). With the pH of the column's buffer higher than protein's pI, hSULT1C4 bound to DEAE-sepharose resulting in pure, active enzyme following elution [33].

Sakakibara et al. [12] performed the initial cloning, expression, and purification of hSULT1C4; however, detailed characterization of the enzyme's kinetic properties has not yet been reported. The small phenolic compound 1-naphthol, a known substrate for hSULT1C4, was utilized throughout our study in SULT activity assays for enzyme characterization; the size of this small phenol allows it to easily access the SULT's active site. Human SULT1C4's K_m for 1-naphthol (1.1 μM) is comparable to the other SULTs' average K_m for 1-naphthol (2.2 μM) [22, 45]. The optimal pH (7.4), temperature (37 °C), and time (10 min) for hSULT1C4 activity are also similar to the other SULTs (Figure 3). One unexpected

observation during hSULT1C4's kinetic characterization was that the highly purified protein has a high affinity at low concentrations for borosilicate glass. Tagged protein purifications often leave small traces of other proteins that can also bind to the glass making the effects less noticeable; however, our optimized purification protocol for untagged hSULT1C4 produced pure protein with very few impurities. The addition of BSA to the reactions, which binds to the surface of the glass allowing hSULT1C4 to stay in solution, resulted in an 18-fold increase in the specific activity of hSULT1C4.

Human SULT1C4's interactions with the obligate sulfonate donor PAPS and inactive product PAP have not been reported. Our findings suggest that hSULT1C4 has a reduced affinity for PAPS (K_m ~ 7.0 μM) compared to the other hSULTs (K_m ~ 1.0 μM) [26, 37, 38]. An alternative explanation for this difference is the possibility of hSULT1C4 exhibiting two different affinities for PAPS due to the SULT's dimerization domain facilitating communication between the two monomeric subunits resulting in half-site reactivity, as has been previously reported for hSULT1A1, hSULT1E1, and hSULT2A1 [38, 46, 47]. This hypothesis was tested extensively, and the data (Figure 4A and B) appear to have two different affinities. However, the data fit a one-site model significantly better than a two-site model when analyzed in Visual Enzymics 2010-Igor Pro 6.3.6.4, and two different affinities could not be extrapolated. The affinity for PAPS binding (K_d ~ 4.8 μM) to hSULT1C4 was also reduced, supporting the validity of the PAPS K_m data. PAP has a better hSULT1C4 binding affinity (K_d ~ 2.8 μM) compared to PAPS; this classic preference for the SULTs to bind PAP rather than PAPS often results in the formation of dead-end product (enzyme-PAP-substrate complex), which limits the rate of the reaction [48, 49]. Human SULT1C4's affinities for PAPS and PAP are physiologically important in respect to intracellular concentrations of PAPS, which can range from 15 to 80 nmol/g tissue and impact enzyme activity in different tissues [50–52]. There are very few studies on PAPS concentrations in various tissues, especially human fetal tissues, which are the primary tissues for hSULT1C4 expression; early estimates of PAPS concentrations in fetal tissues found approximately 10 nmol/g tissue in human fetal liver and approximately 4 nmol/g tissue in the placenta [53, 54].

Pregnant mothers ingest considerable amounts of dietary flavonoids, or phytoestrogens that can transfer from the mother to the fetus; consequently, since hSULTs sulfonate these dietary compounds, certain levels of these environmental estrogens may affect the bioavailability of endogenous hormones by competing as substrates and/or inhibitors of hSULTs potentially altering estrogen metabolism in the developing fetus [55–57]. All of the estrogenic compounds screened in this study are substrates for hSULT1C4; however, the flavonoids are better substrates for hSULT1C4 than E2 or BPA (Figure 6). This high affinity for sulfonating the flavonoids has also been observed with other hSULTs, such as hSULT1E1 and hSULT1A1 [15]. Human SULT1C4 exhibited substrate inhibition, which is usually the result of dead-end product formation, for three of the flavonoids, but detailed characterization of the different flavonoids' binding affinities for the hSULT1C4-PAP complex could not be performed due to the overlapping intrinsic fluorescence of hSULT1C4 and the flavonoids. Docking studies with the flavonoids and hSULT1C4-PAPS or -PAP complex was also limited due to the inability of the docking studies to replicate the in vitro kinetic data. This weak correlation between the structural and biochemical data suggests that

the structure is not representative of the active enzyme. However, substrate inhibition of the hSULTs (e.g. hSULT1A1 and hSULT1E1) by the flavonoids (daidzein and genistein or quercetin and chrysin, respectively) has been reported numerous times and is not unexpected [58, 59]. Similar to our findings, a recent study found hesperetin, another dietary flavonoid, is also a substrate (K_m ~ 0.1 μM) for hSULT1C4 and undergoes substrate inhibition at concentrations > 1 μM [60].

In summary, this study describes a novel purification technique for bacterially expressed native hSULT1C4 as well as characterization of the enzyme's kinetic properties. Analysis of hSULT1C4's interactions with the obligate sulfonate donor PAPS and the inactive cofactor PAP revealed the enzyme's reduced affinity for these nucleotides. Our data suggest that hSULT1C4 has a high affinity for the sulfonation of estrogenic compounds, including phytoestrogens and industrial chemicals; however, several of the phytoestrogens can also inhibit hSULT1C4's activity primarily through the mechanism of competitive inhibition. Further characterization of the biochemical and molecular properties of hSULT1C4 will provide critical insights into its role in human physiology and drug metabolism.

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References

1. Strott CA. Sulfonation and molecular action. Endocr Rev. 2002; 23:703–32. [PubMed: 12372849]
2. Falany CN. Enzymology of human cytosolic sulfotransferases. FASEB J. 1997; 11:206–16. [PubMed: 9068609]
3. Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, Raftogianis RB. Sulfation and sulfotransferases 1: sulfotransferase molecular biology: cDNAs and genes. FASEB J. 1997; 11:3–14. [PubMed: 9034160]
4. Meinl W, Tsoi C, Swedmark S, Tibbs ZE, Falany CN, Glatt H. Highly selective bioactivation of 1- and 2-hydroxy-3-methylcholanthrene to mutagens by individual human and other mammalian sulphotransferases expressed in Salmonella typhimurium. Mutagenesis. 2013; 28:609–19. [PubMed: 23894158]
5. Glatt H. Sulfotransferases in the bioactivation of xenobiotics. Chem Biol Interact. 2000; 129:141–70. [PubMed: 11154739]
6. Goldstein DS, Swoboda KJ, Miles JM, Coppack SW, Aneman A, Holmes C, Lamensdorf I, Eisenhofer G. Sources and physiological significance of plasma dopamine sulfate. J Clin Endocrinol Metab. 1999; 84:2523–31. [PubMed: 10404831]
7. Visser TJ. Pathways of thyroid hormone metabolism. Acta Med Aust. 1996; 23:10–6.
8. Blanchard RL, Freimuth RR, Buck J, Weinshilboum RM, Coughtrie MW. A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. Pharmacogenetics. 2004; 14:199–211. [PubMed: 15167709]
9. Glatt H. Sulfation and sulfotransferases 4: bioactivation of mutagens via sulfation. FASEB J. 1997; 11:314–21. [PubMed: 9141497]
10. Glatt H, Schneider H, Murkovic M, Monien BH, Meinl W. Hydroxymethyl-substituted furans: mutagenicity in Salmonella typhimurium strains engineered for expression of various human and rodent sulphotransferases. Mutagenesis. 2012; 27:41–8. [PubMed: 21825114]
11. Glatt H, Pabel U, Meinl W, Frederiksen H, Frandsen H, Muckel E. Bioactivation of the heterocyclic aromatic amine 2-amino-3-methyl-9H-pyrido [2,3-b]indole (MeAalphaC) in

recombinant test systems expressing human xenobiotic-metabolizing enzymes. Carcinogenesis. 2004; 25:801–7. [PubMed: 14729582]

12. Sakakibara Y, Yanagisawa K, Katafuchi J, Ringer DP, Takami Y, Nakayama T, Suiko M, Liu MC. Molecular cloning, expression, and characterization of novel human SULT1C sulfotransferases that catalyze the sulfonation of N-hydroxy-2-acetylaminofluorene. J Biol Chem. 1998; 273:33929–35. [PubMed: 9852044]
13. Stanley EL, Hume R, Coughtrie MW. Expression profiling of human fetal cytosolic sulfotransferases involved in steroid and thyroid hormone metabolism and in detoxification. Mol Cell Endocrinol. 2005; 240:32–42. [PubMed: 16024168]
14. Runge-Morris M, Kocarek TA. Expression of the sulfotransferase 1C family: implications for xenobiotic toxicity. Drug Metab Rev. 2013; 45:450–9. [PubMed: 24028175]
15. Nishiyama T, Ogura K, Nakano H, Kaku T, Takahashi E, Ohkubo Y, Sekine K, Hiratsuka A, Kadota S, Watabe T. Sulfation of environmental estrogens by cytosolic human sulfotransferases. Drug Metab Pharmacokinet. 2002; 17:221–8. [PubMed: 15618673]
16. Aksoy IA, Wood TC, Weinshilboum R. Human liver estrogen sulfotransferase: identification by cDNA cloning and expression. Biochem Bioph Res Commun. 1994; 200:1621–9.
17. Hui Y, Yasuda S, Liu MY, Wu YY, Liu MC. On the sulfation and methylation of catecholestrogens in human mammary epithelial cells and breast cancer cells. Biol Pharm Bull. 2008; 31:769–73. [PubMed: 18379081]
18. Rohn KJ, Cook IT, Leyh TS, Kadlubar SA, Falany CN. Potent inhibition of human sulfotransferase 1A1 by 17alpha-ethinylestradiol: role of 3′-phosphoadenosine 5′-phosphosulfate binding and structural rearrangements in regulating inhibition and activity. Drug Metab Dispos. 2012; 40:1588–95. [PubMed: 22593037]
19. Tong MH, Jiang H, Liu P, Lawson JA, Brass LF, Song WC. Spontaneous fetal loss caused by placental thrombosis in estrogen sulfotransferase-deficient mice. Nat Med. 2005; 11:153–9. [PubMed: 15685171]
20. Harris RM, Wood DM, Bottomley L, Blagg S, Owen K, Hughes PJ, Waring RH, Kirk CJ. Phytoestrogens are potent inhibitors of estrogen sulfation: implications for breast cancer risk and treatment. J Clin Endocrinol Metab. 2004; 89:1779–87. [PubMed: 15070945]
21. Harris RM, Waring RH. Sulfotransferase inhibition: potential impact of diet and environmental chemicals on steroid metabolism and drug detoxification. Curr Drug Metab. 2008; 9:269–75. [PubMed: 18473744]
22. Allali-Hassani A, Pan PW, Dombrovski L, Najmanovich R, Tempel W, Dong A, Loppnau P, Martin F, Thornton J, Edwards AM, Bochkarev A, Plotnikov AN, Vedadi M, Arrowsmith CH. Structural and chemical profiling of the human cytosolic sulfotransferases. PLoS Biol. 2007; 5:e97. [PubMed: 17425406]
23. Falany CN, Krasnykh V, Falany JL. Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. J Steroid Biochem. 1995; 52:529–39.
24. Meloche CA, Falany CN. Expression and characterization of the human 3 beta-hydroxysteroid sulfotransferases (SULT2B1a and SULT2B1b). J Steroid Biochem. 2001; 77:261–9.
25. Rens-Domiano SS, Roth JA. Inhibition of M and P phenol sulfotransferase by analogues of 3′-phosphoadenosine-5′-phosphosulfate. J Neurochem. 1987; 48:1411–5. [PubMed: 3470439]
26. Tibbs ZE, Falany CN. An engineered heterodimeric model to investigate SULT1B1 dependence on intersubunit communication. Biochem Pharmacol. 2016
27. Bower MJ, Cohen FE, Dunbrack RL Jr. Prediction of protein sidechain rotamers from a backbone-dependent rotamer library: a new homology modeling tool. J Mol Biol. 1997; 267:1268–82. [PubMed: 9150411]
28. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a general amber force field. J Comput Chem. 2004; 25:1157–74. [PubMed: 15116359]
29. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graphics. 1996; 14:33–8. 27–8.
30. Rastogi PA. MacVector. Integrated sequence analysis for the Macintosh. Meth Mol Biol. 2000; 132:47–69.

31. Hochuli E, Dobeli H, Schacher A. New metal chelate adsorbent selective for proteins and peptides containing neighbouring histidine residues. J Chromatogr. 1987; 411:177–84. [PubMed: 3443622]
32. Petrotchenko EV, Pedersen LC, Borchers CH, Tomer KB, Negishi M. The dimerization motif of cytosolic sulfotransferases. FEBS Lett. 2001; 490:39–43. [PubMed: 11172807]
33. Peterson EA, Sober HA. Chromatography of proteins. I. Cellulose ion-exchange adsorbents. J Am Chem Soc. 1956; 78:751–5.
34. Macritchie, F. Proteins at Interfaces. In: Anfinsen, CB., Frederic, JTEMR., editors. Advances in protein chemistry. Vol. 32. Cambridge, Massachusetts: Academic Press; 1978. p. 283-326.
35. Goebel-Stengel M, Stengel A, Tache Y, Reeve JR Jr. The importance of using the optimal plasticware and glassware in studies involving peptides. Anal Biochem. 2011; 414:38–46. [PubMed: 21315060]
36. Klaassen CD, Boles JW. Sulfation and sulfotransferases 5: the importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation. FASEB J. 1997; 11:404–18. [PubMed: 9194521]
37. Cook IT, Leyh TS, Kadlubar SA, Falany CN. Lack of substrate inhibition in a monomeric form of human cytosolic SULT2A1. Horm Mol Biol Clin Invest. 2010; 3:357–66.
38. Wang T, Cook I, Leyh TS. 3′-Phosphoadenosine 5′-phosphosulfate allosterically regulates sulfotransferase turnover. Biochemistry. 2014; 53:6893–900. [PubMed: 25314023]
39. Bondesson M, Hao R, Lin CY, Williams C, Gustafsson JA. Estrogen receptor signaling during vertebrate development. Biochim Biophys Acta. 2015; 1849:142–51. [PubMed: 24954179]
40. Takayanagi S, Tokunaga T, Liu X, Okada H, Matsushima A, Shimohigashi Y. Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor gamma (ERRgamma) with high constitutive activity. Toxicol Lett. 2006; 167:95–105. [PubMed: 17049190]
41. Her C, Kaur GP, Athwal RS, Weinshilboum RM. Human sulfotransferase SULT1C1: cDNA cloning, tissue-specific expression, and chromosomal localization. Genomics. 1997; 41:467–70. [PubMed: 9169148]
42. Suiko M, Sakakibara Y, Liu MC. Sulfation of environmental estrogen-like chemicals by human cytosolic sulfotransferases. Biochem Bioph Res Commun. 2000; 267:80–4.
43. Pina AS, Lowe CR, Roque AC. Challenges and opportunities in the purification of recombinant tagged proteins. Biotechnol Adv. 2014; 32:366–81. [PubMed: 24334194]
44. Tibbs ZE, Rohn-Glowacki KJ, Crittenden F, Guidry AL, Falany CN. Structural plasticity in the human cytosolic sulfotransferase dimer and its role in substrate selectivity and catalysis. Drug Metab Pharmacokinet. 2015; 30:3–20. [PubMed: 25760527]
45. Falany CN, Strom P, Swedmark S. Sulphation of o-desmethylnaproxen and related compounds by human cytosolic sulfotransferases. Br J Clin Pharmaco. 2005; 60:632–40.
46. Sun M, Leyh TS. The human estrogen sulfotransferase: a halfsite reactive enzyme. Biochemistry. 2010; 49:4779–85. [PubMed: 20429582]
47. Wang T, Cook I, Falany CN, Leyh TS. Paradigms of sulfotransferase catalysis – the mechanism of SULT2A1. J Biol Chem. 2014; 289:26474–80. [PubMed: 25056952]
48. Tyapochkin E, Cook PF, Chen G. Isotope exchange at equilibrium indicates a steady state ordered kinetic mechanism for human sulfotransferase. Biochemistry. 2008; 47:11894–9. [PubMed: 18928301]
49. Tyapochkin E, Kumar VP, Cook PF, Chen G. Reaction product affinity regulates activation of human sulfotransferase 1A1 PAP sulfation. Arch Biochem Biophys. 2011; 506:137–41. [PubMed: 21111704]
50. Cook IT, Leyh TS, Kadlubar SA, Falany CN. Structural rearrangement of SULT2A1: effects on dehydroepiandrosterone and raloxifene sulfation. Horm Mol Biol Clin Invest. 2010; 1:81–7.
51. Brzeznicka EA, Hazelton GA, Klaassen CD. Comparison of adenosine 3′-phosphate 5′-phosphosulfate concentrations in tissues from different laboratory animals. Drug Metab Dispos. 1987; 15:133–5. [PubMed: 2881750]
52. Kim HJ, Rozman P, Madhu C, Klaassen CD. Homeostasis of sulfate and 3′-phosphoadenosine 5′-phosphosulfate in rats after acetaminophen administration. J Pharmacol Exp Ther. 1992; 261:1015–21. [PubMed: 1602369]

53. Cappiello M, Franchi M, Giuliani L, Pacifici GM. Distribution of 2-naphthol sulphotransferase and its endogenous substrate adenosine 3′-phosphate 5′-phosphosulphate in human tissues. Eur J Clin Pharmacol. 1989; 37:317–20. [PubMed: 2612547]

54. Cappiello M, Franchi M, Rane A, Pacifici GM. Sulphotransferase and its substrate: adenosine-3′-phosphate-5′-phosphosulphate in human fetal liver and placenta. Dev Pharmacol Therap. 1990; 14:62–5. [PubMed: 2311482]

55. Adlercreutz H, Yamada T, Wahala K, Watanabe S. Maternal and neonatal phytoestrogens in Japanese women during birth. Am J Obstet Gynecol. 1999; 180(3 Pt 1):737–43. [PubMed: 10076156]

56. Schroder-van der Elst JP, van der Heide D, Rokos H, Morreale de Escobar G, Kohrle J. Synthetic flavonoids cross the placenta in the rat and are found in fetal brain. Am J Physiol. 1998; 274(2 Pt 1):E253–6. [PubMed: 9486155]

57. Waring RH, Ayers S, Gescher AJ, Glatt HR, Meinl W, Jarratt P, Kirk CJ, Pettitt T, Rea D, Harris RM. Phytoestrogens and xenoestrogens: the contribution of diet and environment to endocrine disruption. J Steroid Biochem. 2008; 108:213–20.

58. Nakano H, Ogura K, Takahashi E, Harada T, Nishiyama T, Muro K, Hiratsuka A, Kadota S, Watabe T. Regioselective monosulfation and disulfation of the phytoestrogens daidzein and genistein by human liver sulfotransferases. Drug Metab Pharmacokinet. 2004; 19:216–26. [PubMed: 15499189]

59. Ung D, Nagar S. Variable sulfation of dietary polyphenols by recombinant human sulfotransferase (SULT) 1A1 genetic variants and SULT1E1. Drug Metab Dispos. 2007; 35:740–6. [PubMed: 17293380]

60. Brand W, Boersma MG, Bik H, Hoek-van den Hil EF, Vervoort J, Barron D, Meinl W, Glatt H, Williamson G, van Bladeren PJ, Rietjens IM. Phase II metabolism of hesperetin by individual UDP-glucuronosyltransferases and sulfotransferases and rat and human tissue samples. Drug Metab Dispos. 2010; 38:617–25. [PubMed: 20056724]

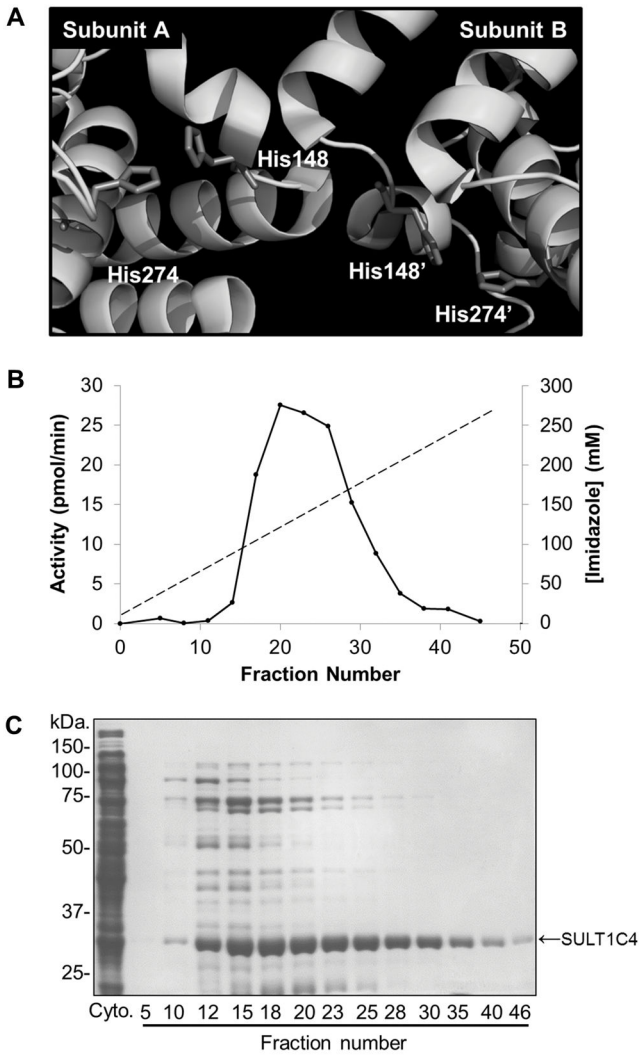

Figure 1. Untagged hSULT1C4 binds with a high affinity to Ni-NTA resin

(A) Using MOE, two adjacent histidine (His148 and His274) clusters (blue) were identified near hSULT1C4's dimerization domain, which functions as an interface for the dimerization of subunit A and subunit B (green). (B) The elution of 1-naphthol (10 μM 1-naphthol, 10 μM PAPS) sulfonation activity from the Ni-NTA column. The volume of each fraction was 0.5 mL. (C) Fractions (5 μL) from the Ni-NTA column were resolved by SDS-PAGE and stained with Coomassie. The molecular weight size markers are indicated to the left of the image.

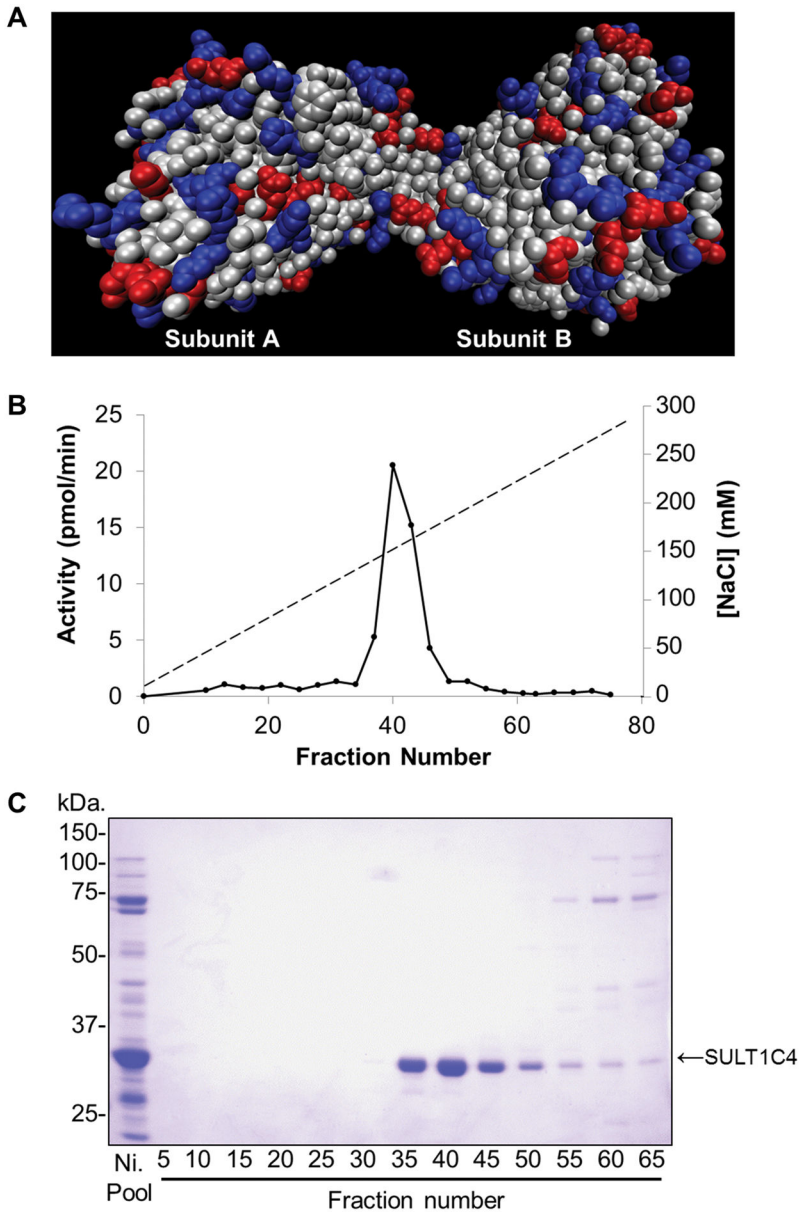

Figure 2. DEAE-sepharose chromatography results in highly pure, active hSULT1C4

(A) VMD was used to visualize the basic (blue) and acidic (red) charges on the surface of hSULT1C4 (PDB 2GWH) [29]. (B) DEA-sepharose elution profile of hSULT1C4 activity assayed with 10 μM 1-naphthol in the presence of 10 μM PAPS. The volume of each fraction was 2.3 mL; each lane was loaded with 7.5 μL of the fraction. (C) Protein fractions from the DEAE-sepharose column were resolved by SDS-PAGE and stained with Coomassie. The molecular weight size markers are indicated to the left of the image.

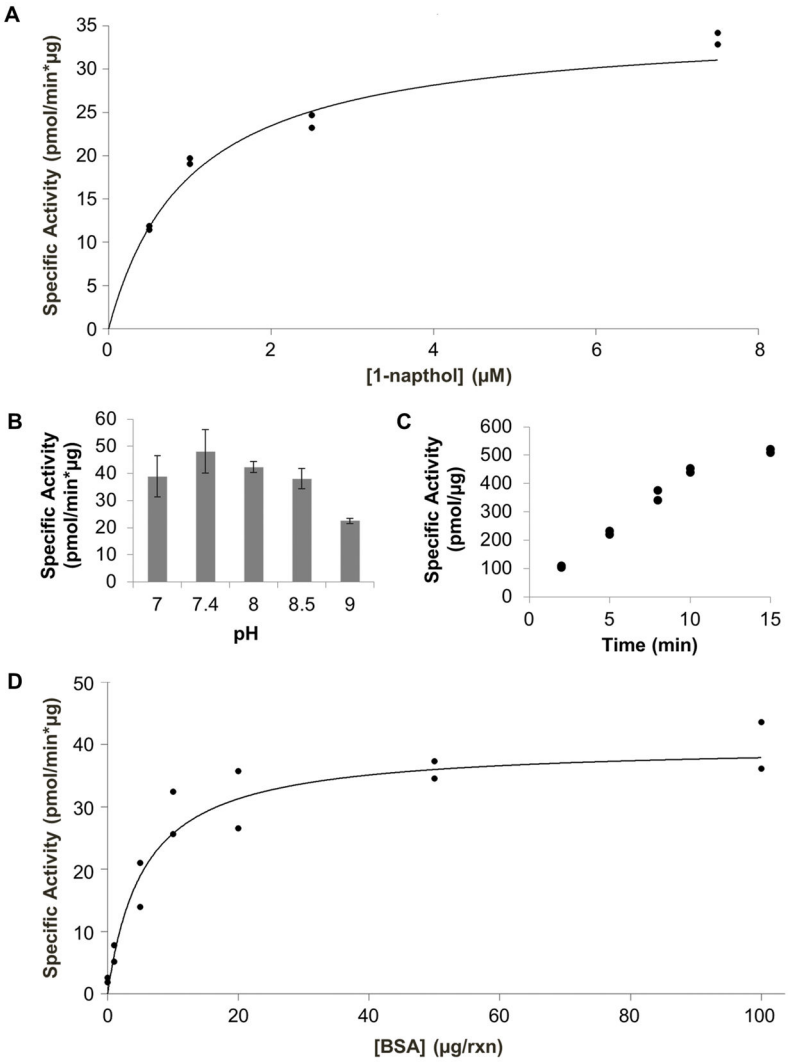

Figure 3. Native hSULT1C4 kinetic assay optimization

(A) Sulfonation of 1-naphthol by hSULT1C4 with increasing concentrations of 1-naphthol and 10 µM PAPS. (B) Optimization of pH for hSULT1C4 activity using 1-naphthol as a substrate and varying the pH from 7.0 to 9.0. (C) Human SULT1C4 activity was assessed while the reaction time was varied between 2 and 15 min at 37 °C with 10 µM 1-naphthol and 10 µM PAPS. (D) The optimal BSA concentration in hSULT1C4 activity assays was determined by varying BSA concentrations from 0 to 100 µg/reaction (rxn) in the presence of 10 µM 1-naphthol and 10 µM PAPS.

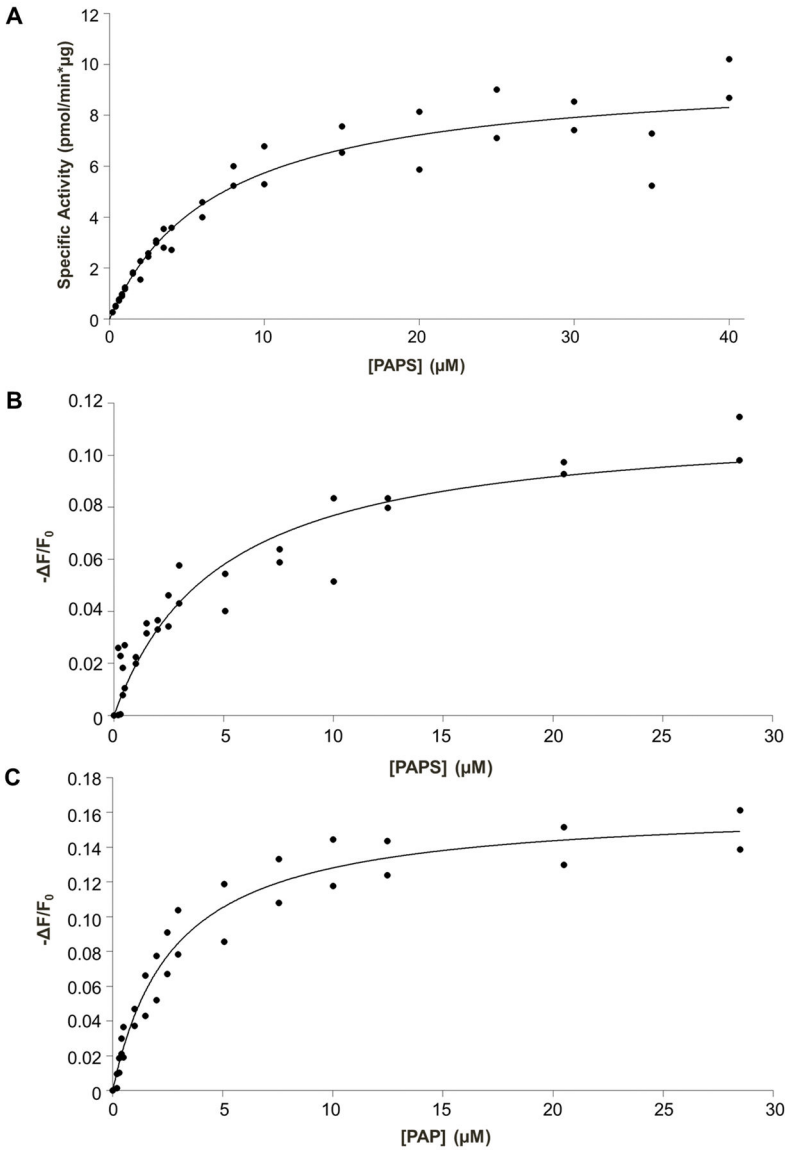

Figure 4. Comparison of hSULT1C4's interactions with PAPS and PAP

(A) Specific activity of hSULT1C4 with increasing concentrations of PAPS and 30 μM daidzein. (B) Change in hSULT1C4 (λ_{ex} = 282 nm, λ_{em} = 342 nm) intrinsic fluorescence (F) upon PAPS binding. (C) Change in hSULT1C4 intrinsic fluorescence upon PAP binding.

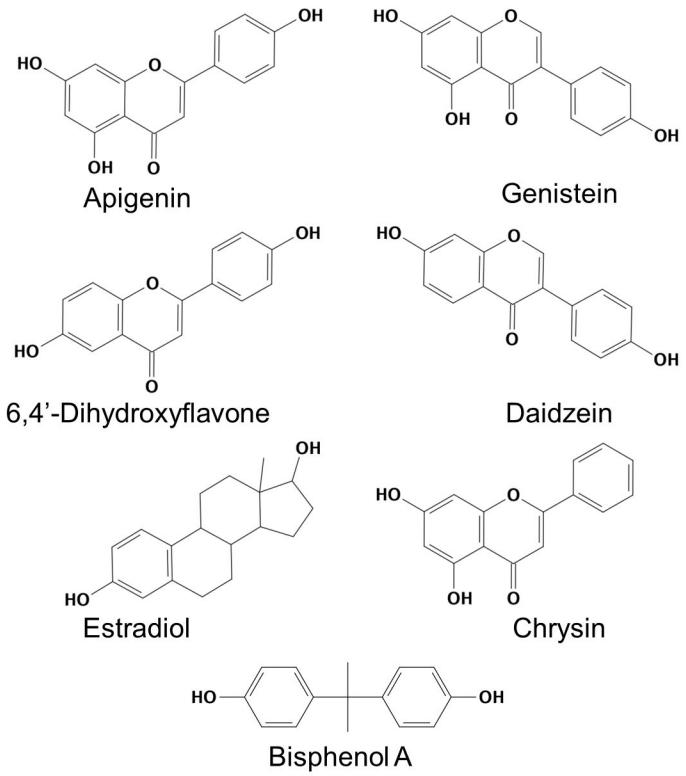

Figure 5.
Chemical structures of estrogenic compounds.

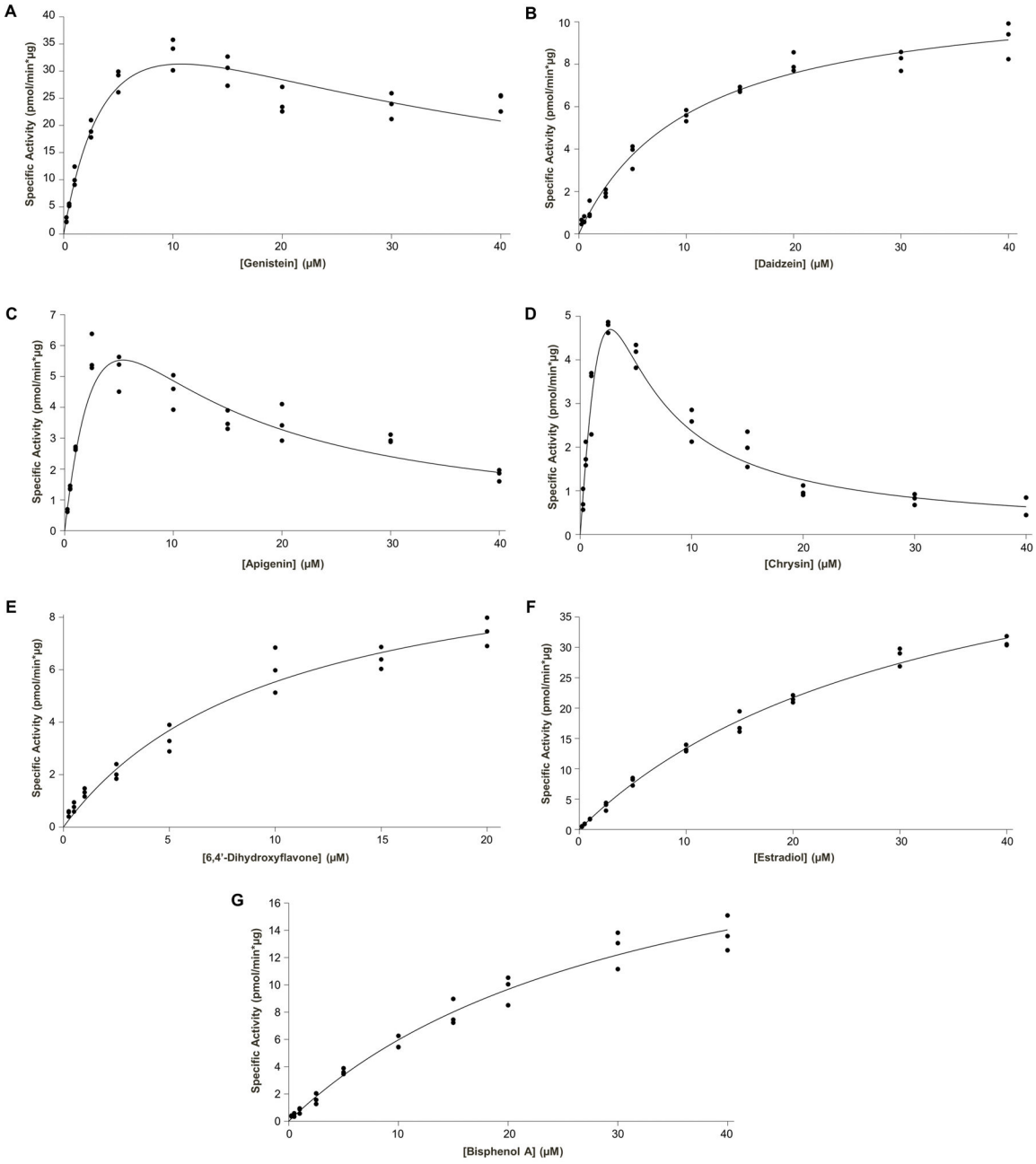

Figure 6. Sulfonation of estrogenic compounds by hSULT1C4

Increasing concentrations of (A) genistein, (B) daidzein, (C) apigenin, (D) chrysin, (E) 6,4-dihydroxyflavone, (F) estradiol, and (G) bisphenol A were assayed for sulfonation by hSULT1C4 with 10 µM PAPS.

Table 1

Table of the human cytosolic sulfotransferases' isoelectric points. The isoelectric points of the hSULTs were calculated using the MacVector sequence analysis software v. 10.0.

Protein Name	Pl
SULT1A1	6.16
SULT1A2	8.07
SULT1A3	5.6
SULT1B1	6.62
SULT1C2	7.43
SULT1C3a	7.16
SULT1C3d	6.46
SULT1C4	8.42
SULT1E1	6.17
SULT2A1	5.59
SULT2B1a	6.09
SULT2B1b	5.08
SULT4A1	5.3

Table 2

Purification of untagged human SULT1C4 from *E. coli* cytosol.

Purification Step	Protein (mg)	Total Activity (nmol/min)	Specific Activity (nmol/(min*mg))	% Yield	Purification
Cytosol	810	183.6	0.2	100.0	1
Ni-NTA	36	83.0	2.3	45.2	10
DEAE-Sepharose	2.4	63.4	26.0	34.5	115

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