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Celecoxib influences steroid sulfonation catalyzed by human recombinant sulfotransferase 2A1

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Abstract

Celecoxib has been reported to switch the human SULT2A1-catalyzed sulfonation of 17 β estradiol (17 β -E2) from the 3- to the 17-position. The effects of celecoxib on the sulfonation of selected steroids catalyzed by human SULT2A1 were assessed through *in vitro* and *in silico* studies. Celecoxib inhibited SULT2A1-catalyzed sulfonation of dehydroepiandrosterone (DHEA), androst-5-ene-3 β , 17 β -diol (AD), testosterone (T) and epitestosterone (Epi-T) in a concentration-dependent manner. Low μ M concentrations of celecoxib strikingly enhanced the formation of the 17-sulfates of 6-dehydroestradiol (6D-E2), 17 β -dihydroequilenin (17 β -Eqn), 17 β -dihydroequilin (17 β -Eq), and 9-dehydroestradiol (9D-E2) as well as the overall rate of sulfonation. For 6D-E2, 9D-E2 and 17 β -Eqn, celecoxib inhibited 3-sulfonation, however 3-sulfonation of 17 β -Eq was stimulated at celecoxib concentrations below 40 μ M. Ligand docking studies *in silico* suggest that celecoxib binds in the substrate-binding site of SULT2A1 in a manner that prohibits the usual binding of substrates but facilitates, for appropriately shaped substrates, a binding mode that favors 17-sulfonation.

Keywords

Sulfotransferase; Dehydroepiandrosterone; Estradiol; Steroids; Celecoxib; SULT2A1

1. INTRODUCTION

Sulfonation has evolved as a key step in xenobiotic metabolism, but also plays a critical role in steroid biosynthesis and in modulating the biological activity and facilitating the inactivation and elimination of potent endogenous chemicals including thyroid hormones, steroids, bile acids and catecholamines [1-3]. Steroid-sulfates are not always metabolic end

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products destined only for excretion, but also serve as storage and transport forms of steroid hormones such as estrone (E1), 17 β -estradiol (17 β -E2) and dehydroepiandrosterone (DHEA) [4-6]. These “inactive” sulfates are highly protein bound and can be readily transported in the blood around the body and taken to the site of action to be desulfated by sulfatase enzymes [7-9]. Formation of the sulfate conjugates of small molecules is catalyzed by a family of enzymes with overlapping substrate selectivities termed the cytosolic sulfotransferases (SULTs).

Celecoxib, a specific cyclooxygenase-2 (COX-2) inhibitor, is used clinically to relieve arthritic pain and to treat familial adenomatous polyposis [10]. Unrelated to its effect on COX-2, celecoxib has been found to alter the preferred position of ethynylestradiol and 17 β -E2 sulfonation from the 3- to the 17- hydroxy group and to increase total sulfonation in a concentration-dependent manner with SULT2A1 [11, 12]. The celecoxib effect of altering the preferred position of sulfonation of 17 β -E2 and stimulating overall sulfonation is specific for SULT2A1, and is not observed with other major SULTs including SULT1A1, SULT1A3, SULT1B1 and SULT1E1 [12]. The binding site of celecoxib with SULT2A1 was inferred to be different from that of 17 β -E2, as there was no inhibition of 17 β -E2 sulfate conjugate formation, which might be expected if 17 β -E2 competed for the same binding site, and it was proposed that a conformational change of the SULT2A1 enzyme upon binding celecoxib favored greater production of 17-sulfates [11, 12]. To the contrary, computational analyses of protein-ligand docking suggested that celecoxib occupied the same binding region as 17 β -E2 and modulated SULT2A1-catalyzed 17 β -E2 sulfonation by inducing a conformational change that favored docking of 17 β -E2 into an alternative binding region such that the 17 β -hydroxy group was sulfonated in preference to the 3-OH group [13].

SULT1E1 and SULT1A1 have been shown to sulfonate 17 β -E2 with higher affinity and capacity than SULT2A1 [14, 15]. SULT1E1 particularly has very high affinity for 17 β -E2 and is thought to play the most important role in its sulfonation at physiological low nM concentrations [16]. Even though the concentration of 17 β -E2 required for SULT2A1 to play a physiological role is high, the greater susceptibility of SULT1A1 and SULT1E1 to dietary and environmental chemical inhibition [17] necessitates a holistic view of its sulfonation. Celecoxib in therapeutic doses can reach hepatic concentrations that have been shown to be inhibitory to 17 β -E2 sulfonation by SULT1A1 (unpublished).

This work aimed to yield further insight into the influence of celecoxib on the catalytic activity of SULT2A1 with a variety of steroid substrates and to examine the possible mechanism of the effects through computer modeling. The study determined the effect of celecoxib on three different concentrations of 17 β -E2 (0.05–0.4 μ M) to augment earlier studies [12]. DHEA sulfonation in the presence of celecoxib was measured, as DHEA is the main physiological substrate of SULT2A1 and DHEA–sulfate is a major circulating steroid–sulfate [18]. DHEA and its sulfate are known to influence a variety of physiological processes as well as pathophysiological conditions [19-22]. Testosterone (T) and epitestosterone (Epi-T) were chosen to test whether celecoxib generally stimulates steroid 17-sulfate formation. Androst-5-ene-3 β , 17 β -diol (AD), a DHEA metabolite, was selected for determining whether celecoxib would switch the position of sulfonation of AD in the absence of an aromatic A-ring. An important objective of the study was to further

investigate the effect of celecoxib on the sulfonation of estrogens. SULT2A1 as well as other SULTs metabolize 17 β -E2 and other estrogens [11, 12, 14]. The compounds selected were 17 β -estradiol (17 β -E2), E1 and several 17 β -E2 analogs as well as catechol estrogens (CEs). The 17 β -E2 analogs were the 3-methyl ether of 17 β -estradiol (3Me-E2), 6-dehydroestradiol (6D-E2), 9-dehydroestradiol (9D-E2), 17 β -dihydroequilenin (17 β -Eqn) and 17 β -dihydroequilin (17 β -Eq). Several of these analogs are present in hormone replacement therapy for post-menopausal women [23]. The CEs were 2-hydroxyestradiol (2-OHE2) and 4-hydroxyestradiol (4-OH-E2), metabolic products of 17 β -E2 produced in the human body that are in turn metabolized to quinones and semi-quinones capable of forming carcinogenic DNA adducts [24-26]. O-methylation of the CEs by catechol-O-methyltransferase (COMT) is the major pathway for detoxification apart from sulfonation [27-30]. Conflicting reports have suggested that an inherited defect of the polymorphic COMT enzyme [31-33] may or may not put the subject under increased risk for breast cancer. Insight into the mechanism of the effect of celecoxib on positional sulfonation of steroids was sought by modeling the interaction of celecoxib, human SULT2A1 and substrates whose sulfonation position was markedly affected by celecoxib, as well as substrates that were not affected.

The structures of 17 β -E2 and celecoxib are shown in Figure 1 and the structures of the steroids used in this study are shown in Figure 2. Recombinant human SULT2A1 was used as the enzyme source.

2. MATERIALS AND METHODS

2.1 Reagents

The [³⁵S]-3'-phosphoadenosine-5'-phosphosulfate (PAPS) (1.86Ci/mmol) was obtained from PerkinElmer Life Sciences, Inc (Boston, MA). Unlabeled steroids were obtained from Steraloids (Newport, RI). The PAPS was purchased from Dr. Sanford S. Singer (University of Dayton, OH) or from Sigma-Aldrich (St. Louis, MO) and purified as described [34]. Celecoxib was obtained via extraction from a 100 mg commercial capsule formulation as described previously [12]. The 17 β -E2-3-sulfate standard was obtained from Sigma-Aldrich (St. Louis, MO). The 17 β -E2-17-sulfate, disulfate of 17 β -E2, DHEA sulfate and E1 sulfate standards were obtained from Steraloids (Newport, RI). Other reagents were the highest grade available from Fisher Scientific (Atlanta, GA) and Sigma-Aldrich (St. Louis, MO).

2.2 Expression of Sulfotransferase

Expression in *E. coli* of SULT2A1 has been described previously [14]. Briefly, *E. coli* cells containing the SULT2A1 gene were grown to late log phase (OD₆₀₀ = 0.5) in Luria broth (LB) with ampicillin (200 μ g/mL) and induced overnight with 0.5 mM isopropyl-beta-D-thiogalactopyranoside. Cells were pelleted and resuspended in bacterial lysis buffer (75 mM Tris-Cl, pH 8.0, 0.25 M sucrose, 0.25 mM EDTA, 0.02 mg/mL lysozyme) and incubated 20 min on ice. Cells were repelleted at 3000 g, resuspended in 10 mM triethanolamine buffer, pH 7.5, which contained 10% glycerol, 1.5 mM dithiothreitol, and 10 μ g/mL phenylmethylsulfonylfluoride (PMSF), and sonicated 4 $\times$ with 10 s bursts and 30 s cooling between each burst. A final centrifugation at 100,000 g for 60 min was performed and the supernatant fraction was used for the assays.

2.3 Sulfotransferase Assay

Assay conditions with all substrates were optimized in such a manner that the rate of reaction was linear with protein and time, and was saturating for PAPS. Incubation mixtures contained 100 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 0.07–21 µg SULT2A1 cytosolic protein, 2.0 µM ³⁵S-PAPS (diluted with unlabeled PAPS to a specific activity of 1169 mCi/mmol) or 20 µM PAPS, and 0.4 µM substrate (DHEA, AD, Epi-T, T, 17α-E2, E1, 17β-E2, 3Me-E2, 6D-E2, 9D-E2, 17β-Eqn, 17β-Eq, 2-OH-E2 and 4-OH-E2) in a total volume of 0.25 mL. The steroid substrates were added to incubation tubes in ethanol and the ethanol removed under nitrogen before adding the other components. In studies with 17β-E2, additional concentrations of 0.05 µM and 0.2 µM were studied. Stock solutions of celecoxib were prepared in DMSO, such that the DMSO concentration did not exceed 0.5% (v/v). At this concentration, DMSO did not affect activity. In most studies, reaction was initiated with the addition of PAPS after a 3 min preincubation at 37 °C. After 10–30 min incubation, the reaction was stopped with 0.3 mL methanol followed by vortex-mixing and centrifugation. The resultant supernatant was transferred into new tubes and analyzed by HPLC or LC-MS/MS as described below. Studies were conducted to determine if the order of addition of PAPS (20 µM) and celecoxib (50 µM) affected the pattern of sulfonation of 17β-E2. In these studies, three sets of tubes were prepared. In two sets, 100 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 1.5 µg SULT2A1 and PAPS were pre-incubated at 37°C for 1 minute followed by addition of DMSO or a solution of celecoxib in DMSO (50 µM celecoxib), pre-incubation for another minute then addition of the incubation mixture to tubes containing 17β-E2, 0.16 or 0.4 µM and incubation for 15 min. The third set of tubes was a positive control in which the celecoxib and 17β-E2 were pre-incubated with other assay components as described above prior to starting the reaction with PAPS.

2.4 HPLC Analysis

HPLC analyses were conducted on a Beckman Gold Nouveau system, equipped with UV and fluorescence detectors and an IN/US (β-ram, IN/US systems, Inc., Tampa, FL) radiochemical detector. Separation of parent substrate and its sulfate conjugates was achieved on a C18 reverse-phase column (4.6 mm × 25 cm) with a C18 pre-column (Discovery system, Supelco, Bellefonte, PA) at a constant flow of 1 mL/min with 5 mM tetrabutylammonium sulfate in 50% methanol for the sulfonation of 6D-E2 and in 55 % methanol for 17α-E2. The flow of scintillation cocktail (In-flow 2, IN/US systems, Inc., Tampa, FL) was maintained at 3 mL/min. The retention times for the sulfates measured by HPLC are as follows: 6D-E2 (for 17S, 14.4 min; for 3S, 16.2 min) and for 17α-E2 (for 17S, 12.1 min; for 3S, 14.6 min).

2.5 LC-MS/MS analysis

The liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for simultaneous analysis of steroid-sulfates (isomers steroid-3-sulfates and steroid-17-sulfates, as well as steroid-disulfates) derived from sulfonation of the selected steroids was reported previously [35, 36]. LC-MS/MS analyses were performed using the Accela high speed LC system (Thermo Electron Corporation, San Jose, CA) coupled on-line with a Finnigan TSQ Quantum Ultra triple-quadrupole mass spectrometer (Thermo Electron

Corporation, San Jose, CA) equipped with a heated electrospray ionization (H-ESI) interface operated in negative-ion mode of detection. The liquid chromatography system comprised a thermostatted autosampler, a column temperature controller, a solvent delivery system and a pump. Chromatographic separation was achieved on a BETASIL Phenyl (150 mm × 2.1 mm, 5 μm) column (Thermo Electron), by using an isocratic mobile phase consisting of acetic acid-ammonium acetate buffer (50%) and methanol (50%) at a constant flow rate of 0.2 mL/min. The injection volume was 25 μL. In the Finnigan TSQ Quantum Ultra instrument, high pressure nitrogen (Airgas, Gainesville, FL) was used as both the sheath and the auxiliary gases. The vaporizer temperature was kept at 100 °C, and the ion transfer heated capillary temperature was maintained at 350 °C. The spray voltage was set at –2.5 kV in the negative-ion mode. The second quadrupole of the mass spectrometer was used as a collision cell with ultra-high purity argon (Airgas, Radnor, PA) as a collision gas at a pressure of 1.5 mTorr. Measurement of steroid–sulfates was performed in selected reaction monitoring (SRM) mode, monitoring the appropriate fragmentation transition of each target compound. The most sensitive and isomer-specific SRM transitions for the isomeric steroid–3-sulfates and steroid–17-sulfates respectively were as follows: $[M - H]^- \rightarrow [M - H - SO_3]^-$ (loss of 80 u) and $[M - H]^- \rightarrow [HSO_4]^-$ (monitoring of the ions with m/z 97). The optimal collision energy for each SRM transition was determined from collision-induced dissociation and energy-resolved mass spectrometry experiments. The H-ESI interface operational parameters were optimized to achieve maximum sensitivity for $[M - H]^-$ of studied compounds. The LC-MS/MS system was controlled by Xcalibur (v. 2.0) software, a flexible Windows NT PC-based data acquisition system that allows complete instrument control. The retention times for steroid estrogen sulfates measured by LC-MS/MS are as follows: E1-3S (12.9 min), 17β-E2 (for 3S, 10.2 min; for 17S, 9.1 min), 9D-E2 (for 3S, 8.8 min; for 17S, 9.0 min), 17β-Eq (for 3S, 9.5 min; for 17S, 8.5 min), 17β-Eqn (for 3S, 7.8 min; for 17S, 6.9 min), 3Me-E2-17S (15 min), 2-OH-E2 (for 17S, 8.6 min; for 3S, 11.0 min; for 2S, 14.3 min), 4-OH-E2 (for 17S, 6.3 min; for 4S, 10.1 min; for 3S, 11.5 min).

DHEA, AD, Epi-T and T sulfates were analyzed by means of LC-MS/MS using LC-20AD (Shimadzu, Durham, NC) liquid chromatography system coupled on-line with a 3200 Q TRAP® mass spectrometer (AB Sciex, Foster city, CA). A Kinetex (Phenomenex) C8 column (50 mm × 4.6 mm, 2.6 μm) was used with the same mobile phase described above at a 0.2 mL/min flow rate, coupled to an electrospray ionization (ESI) interface operated in negative-ion mode of detection. The injection volume was 25 μL. Nitrogen generated by SOURCE LC/MS TriGas Generator Series Model LC-MS 5000 (Parker Balston, Haverhill, MA) was used as both the nebulizer and the auxiliary gases. The source temperature was maintained at 500 °C and the ion spray voltage was set at –4.5 kV. The most sensitive transition was $[M - H]^- \rightarrow [HSO_4]^-$ and the ions with m/z 97 were monitored. The LC-MS/MS system was controlled by Analyst (v. 1.4.2) software. The retention times for non-aromatic steroid sulfates are as follows: DHEA (12.1 min), AD (10.2 min), Epi-T (10.1 min) and T (8.7 min).

2.6 Data Analysis

Results for the expressed enzymes are from the mean of duplicate determinations. IC₅₀ values were obtained by fitting the data for percentage of control activity in the absence of

celecoxib and log celecoxib concentration to a sigmoidal dose-response curve using Graph-Pad software (v6, La Jolla, CA).

2.7 Molecular modeling studies

Three crystal structures of human hydroxysteroid sulfotransferase have been reported, one co-crystallized with PAP [37] and the others with the endogenous substrate DHEA [38] and androsterone [39]. There is a crucial difference between the first two structures pertaining to the access to the substrate binding site. The loop of amino acids from Tyr-231 to Glu-244 (loop 3) in the former truncates the substrate binding site whereas in the latter this loop is moved away from the opening of the binding site. It has been observed that the order of binding of the substrate and PAPS will have an effect on the sulfonation behavior of the enzyme [40]. Whereas DHEA, the principal endogenous substrate of SULT2A1, can bind to the substrate binding site productively independent of PAPS binding, a larger molecule like raloxifene must bind prior to the PAPS for the full binding pocket to be available and for the sulfonation to proceed [40]. SiteID analyses of both the available SULT2A1 crystal structures using Sybyl-X 2.0 showed that only the substrate and PAPS binding sites are available for docking celecoxib. Of the solved crystal structures for SULT2A1, only that with DHEA has a binding pocket that can accommodate both celecoxib and a sulfonate accepting substrate such as 17 β -E2. The truncated binding site in the other structures would not allow modeling of celecoxib in it, as reported earlier [13].

All the available crystal structures of sulfotransferases co-crystallized with PAPS or PAP in the Protein Data Bank (with PDB IDs 3U3J, 3U3K, 3U3M, 3U3O, 3F3Y, 3CKL, 3BFX, 2REO, 2Z5F, 2H8K, 2GWH, 2DO6, 2A3R, 1Q1Q, 1Q1Z, 1Q20, 1Q22, 1LS6, 1G3M and 1EFH) have near identical conformation of PAPS (Figure 3). This is true despite the fact that the data set compared contains enzyme models that are less than 40% identical like SULT1A1 and SULT2A1. An attempt at modeling the PAPS molecule into the co-factor binding site of the SULT2A1 model (PDB ID 1J99, used in the ligand-docking studies) to mimic the conformation identical to the one in the crystal structure with PAP showed that it would clash with more than one residue in the pocket. A change in the conformation of the enzyme is needed for the sulfonation to proceed and for the PAPS to bind in catalytic mode as seen in Figure 3. Thus, it appears the substrate and celecoxib bind SULT2A1 before PAPS. Celecoxib could however bind in the co-factor binding site of not only SULT2A1 but also those of SULT1A1 and SULT1E1. Such an interaction, if pursued further in future, could not only explain inhibition of the latter two SULTs by celecoxib but can also provide an explanation for SULT2A1 modulation. Dimers of SULTs could, upon docking of celecoxib in one of the co-factor binding sites, change the conformation of the substrate binding site of the second monomer unit to affect positional sulfonation as observed with SULT2A1.

The crystal structure of SULT2A1 with bound DHEA, when used for the docking experiments, gave satisfactory explanations for the behavior of various substrates in the presence and absence of celecoxib. This crystal structure, PDB ID 1J99 (resolution of 1.99 Å), was used to generate a homology model with the help of the SWISS-MODEL [41, 42] server to construct missing residues from the protein sequence under the ID NP_003158.2 in

the NCBI database. Ligand docking studies along with receptor and ligand preparation and energy calculations were conducted on Sybyl-X 2.0 program running on a Linux operating system. The Flexidock utility in Sybyl-X 2.0 was used for ligand docking. The SULT2A1 structure file was prepared for docking using the Biopolymer utility in Sybyl. The various steps carried out in the preparation of the protein included termini treatment to add charges to the terminal amino acid residues, adding hydrogen atoms, adding Amber7 FF99 charges, choosing the side chain amide orientations that have most hydrogen bonding interactions with the surrounding residues and performing staged minimization in Amber7 FF99 force field [43] in 100 steps. The non-aromatic steroids were generated in Sybyl from the template of docked DHEA in PDB ID 1J99 and the aromatic ones from 17 β -E2 from PDB ID 1AQY. They were prepared by checking their Amber atom types, adding Gasteiger-Huckel charges and minimization steps. The crystal structure 1J99 has the ligand, DHEA, with its 3-OH group in catalytic position, i.e. it is in hydrogen bonding distance from the conserved catalytic residue His (His-99 in human SULT 2A1), i.e. between 0.8 and 2.8 Angstrom units and with a minimum angle between donor and acceptor atoms of 120° [38]. The ligand molecules were placed in the binding pocket before the docking as Flexidock needs the ligand to be roughly in position in the active site to be able to generate the results. For docking, a 10 Å area around the DHEA molecule in the crystal structure was selected as the active site. The various steps involved in preparing to run Flexidock include removing the water in the active site, checking the atom types and adding hydrogen atoms and charges to the ligands and binding site residues. Only the bonds in the ligands were allowed to be rotated. The binding site residues were left rigid in both backbone and side chains. The results generated (about 20 for each ligand) by Flexidock were examined visually and the catalytic conformation with least energy was chosen as the solution. These steps were repeated several times to ensure the reproducibility of the results. All the molecular modeling figures were generated in Chimera 1.6.2.[44, 45]

3. RESULTS

3.1 Effect of celecoxib on the sulfonation of 17 β -E2

Celecoxib switched the major position of sulfonation and altered the extent of sulfonation with all three concentrations of 17 β -E2 (0.05, 0.2 and 0.4 μ M) tested, with two different patterns (Figure 4). The stimulation of 17 β -E2–17-S formation did not saturate even with 80 μ M celecoxib for 0.05 μ M 17 β -E2, whereas with 0.2 and 0.4 μ M 17 β -E2, it peaked at 50 μ M celecoxib. The concentration of 17 β -E2–3-S decreased with higher concentrations (50 μ M) of celecoxib for all three substrate concentrations. The total sulfate formed closely followed the pattern of 17 β -E2–17-S, as there was significant stimulation of the formation of this sulfate compared to the controls (0 μ M celecoxib).

Table 1 shows the results of studies in which PAPS was added to SULT2A1 prior to addition of celecoxib or 17 β -E2. The effect of celecoxib on position of sulfonation was as shown in Figure 4, however compared with a positive control in which the reaction was started with PAPS as was done for all other experiments, the activity was lower (Table 1).

3.2 Effects of celecoxib on the sulfonation of non-aromatic steroids

SULT2A1 showed higher enzyme activity toward DHEA, AD and Epi-T (8–9 nmol sulfate/min/mg protein) than with other steroids (Figure 5). Celecoxib inhibited the sulfonation of DHEA in a concentration-dependent manner with an IC_{50} of 82 μ M (Figure 5A). Sulfonation of AD with SULT2A1 produced only one metabolite, which was assigned as AD-3-sulfate based on previous reports because AD-3-sulfate is unavailable commercially [46, 47]. Celecoxib inhibited the production of AD-3-sulfate with an IC_{50} of 88 μ M celecoxib (Figure 5A). Increasing celecoxib concentrations resulted in a concentration-dependent inhibition of T sulfonation (Figure 5B). The IC_{50} value for inhibition of T sulfonation by celecoxib was 59 μ M. Epi-T was sulfonated more readily than T, but celecoxib was a less potent inhibitor with an IC_{50} of 97 μ M.

3.3 Effect of celecoxib on the sulfonation of 17 β -E2 analogs

Celecoxib altered the major position of sulfonation and the extent of sulfonation of several 17 β -E2 analogs. When using 6D-E2 as a substrate, the rate of 3-sulfate formation was 51 pmol/min/mg protein and was higher than that of 17-sulfate formation, 2 pmol/min/mg protein. In the presence of increased concentrations of celecoxib (1–80 μ M), the 6D-E2-3-sulfate formation was gradually reduced, while the 17-sulfate formation was greatly stimulated (Figure 6A). The product ratio of 6D-E2-3-sulfate/6D-E2-17-sulfate reached 1 at 2.5 μ M celecoxib. At 50 μ M celecoxib, the rate of 6D-E2-17-sulfate formation was 200 fold higher than the rate in the absence of celecoxib. As a result, the total sulfate product formation was enhanced over 8-fold by 50 μ M celecoxib.

For 17 β -Eqn, the rate of formation of 17 β -Eqn-3-sulfate (56 pmol/min/mg protein) was comparable to that of 6D-E2 in the absence of celecoxib, while the rate of formation of 17 β -Eqn-17-sulfate was higher (11.4 pmol/min/mg protein). As seen for 6D-E2 in the presence of celecoxib, the formation of the 17-sulfate of 17 β -Eqn was greatly stimulated, and seemed to saturate only at 80 μ M celecoxib (Figure 6B). The product ratio of 17 β -Eqn-3-sulfate/17 β -Eqn-17-sulfate reached 1 at a celecoxib concentration of 1.5 μ M. At 80 μ M celecoxib, there was a 34-fold stimulation of 17 β -Eqn-17-sulfate formation compared with rates in the absence of celecoxib.

In the absence of celecoxib, 9D-E2 formed 3-sulfate as its major product at 14 pmol/min/mg protein (Figure 6C). The total product formation increased with increasing celecoxib concentration (by 9.4 times at 80 μ M), due to the stimulation of 9D-E2-17-sulfate formation. Formation of 9D-E2-17-sulfate at 80 μ M was 34 times higher than that at 0 μ M celecoxib. The ratio of 9D-E2-3-sulfate/9D-E2-17-sulfate was 1 at 5 μ M celecoxib.

With 17 β -Eq (Figure 6D), the rate of 3-sulfate formation (46 pmol/min/mg protein) in the absence of celecoxib was comparable to that of 17 β -Eqn and 6D-E2. In the presence of increasing concentrations of celecoxib, the formation of 17 β -Eq-17-sulfate was stimulated, but to a smaller extent than that observed with 17 β -E2, 6D-E2 17 β -Eqn and 9D-E2 i.e. at 80 μ M celecoxib, the rate of 17 β -Eq-17-sulfate was only 11-fold higher than that found without celecoxib. At 80 μ M celecoxib, 17 β -Eq-3-sulfate formation was comparable to and the total product formation was double that at 0 μ M celecoxib (Figure 6D). No inhibition of 17 β -

Eq-3-sulfate formation was observed in the celecoxib range studied. The product ratio of 17 β -Eq-3-sulfate/17 β -Eq-17-sulfate reached 1 at around 50 μ M celecoxib.

As shown in Figure 6E, sulfonation of E1 was not affected significantly ($p > 0.05$, ANOVA) with an IC_{50} value higher than the range of celecoxib tested (0–80 μ M). Celecoxib stimulated the formation of 3Me-E2-17 β -sulfate in a concentration dependent manner. The increase in sulfotransferase activity was not saturated at the highest concentration of celecoxib tested (80 μ M) (Figure 6F).

In the absence of celecoxib, sulfonation of 17 β -E2 with SULT2A1 produced around 76 times more 17-sulfate than of 3-sulfate in contrast to 17 β -E2, which formed 3-sulfate as the major product. Formation of 17 β -E2-17-sulfate was not stimulated by celecoxib (Figure 6G), rather 17-sulfate formation was inhibited with an increase in celecoxib concentration, with an IC_{50} of greater than 80 μ M. The small amount of 3-sulfate formed was inhibited by celecoxib to a negligible rate (<1 pmol/min/mg protein, data not shown).

3.4 Effects of celecoxib on the sulfonation of catechol estrogens

There were significant differences in the sulfonation of the two CEs (Figure 7A and Figure 7B). The sulfates formed at 17-hydroxy for 2-OH-E2 and 4-OH-E2 had retention times of 8.6 and 6.3 min, respectively and these could be differentiated from the sulfates at phenolic hydroxy groups as the most sensitive SRM transition for the 17-sulfates is different from that of the 3-sulfates as described in the Materials and Methods section. SULT2A1 formed two sulfates with the phenolic hydroxy groups of 2-OH-E2, which had retention times of 11.0 and 14.3 min. Of the two, the former was the major product and was assumed to be 2-OH-E2-3-sulfate as the analytical method employed could not distinguish between the sulfates at the phenolic hydroxy group. Similarly, 4-OH-E2 formed two sulfates at the phenolic hydroxyl groups with retention times of 11.5 and 10.1 min. Again, the former was the major product and was assumed to be 4-OH-E2-3-sulfate. Molecular modeling data presented in the following section supports the assumption that both 2-OH-E2 and 4-OH-E2 formed 3-sulfates as their major products upon sulfonation by SULT2A1 in the absence of celecoxib.

In the absence of celecoxib, 4-OH-E2 formed more of the major phenolic metabolite, 3-sulfate (15 pmol/min/mg protein), whereas the 3-sulfonation of 2-OH-E2 was far lower (2 pmol/min/mg protein). With the increase in celecoxib concentrations a resulting increased formation of the 17-sulfates of both the CEs was observed. Much of the increase in 17-sulfate in the case of 4-OH-E2 was accompanied by decreasing 3-sulfate formation, with the curves crossing at approximately 30 μ M celecoxib. The overall sulfonation was nearly constant up to 30 μ M celecoxib and reduced beyond that (Figure 7B). With 2-OH-E2, the overall sulfonation was considerably stimulated by celecoxib. The amount of 2-OH-E2-17-sulfate formed in the presence of 50 μ M celecoxib was about 3.5 times the total sulfonation in the control (Figure 7A).

3.5 Dockings in the Absence of Celecoxib

It was observed previously [38] in a SULT2A1 crystal co-crystallized with DHEA that DHEA can bind to SULT2A1 in two alternate binding modes, one roughly horizontal to the

opening of the binding pocket and the other vertical. In the horizontal binding mode, the 3-hydroxy group is in hydrogen bonding distance to the conserved His-99 residue, a feature necessary for catalysis. The 3-OH group of DHEA was too far from His-99 to form a hydrogen bond in the vertical binding mode.

When celecoxib was not present, DHEA preferentially docked into the substrate-binding site of SULT2A1 such that the 3-hydroxy group was within hydrogen bonding distance to His-99 (Figure 8A), a conformation of DHEA that was similar to the horizontal binding mode reported in the published crystal structure [38]. The ligands tested in this study that assumed conformations roughly similar to either the horizontal or the vertical mode of DHEA bindings described above were good substrates of SULT2A1. DHEA and AD docked very similarly with their 3 β -OH group in horizontal position and their C-18 and C-19 groups facing the opening of the binding site so as to avoid the clash of C-19 with Trp-134. The 17-hydroxy group of AD was not in a position to be sulfonated (Figure 8B). Epi-T and T docked in conformations dissimilar to each other, reflecting their isomeric difference with respect to the 17-hydroxy group (Figure 8C). Epi-T with the 17 α -OH assumed a relatively vertical orientation whereas T with its 17 β -OH was relatively horizontal. These two orientations were more similar to the vertical and horizontal bindings of DHEA in 1J99 crystal, respectively, than to each other, however in both cases the -OH group was within hydrogen-bonding distance to His-99, i.e. between 0.8 and 2.8 Angstrom units.

The 17 β -E2, 9D-E2 6D-E2, 17 β -Eqn, and 17 β -Eq docked in conformations that favored 3-sulfonation. Except for the fact that the molecule itself is flipped by 180° allowing the C-18 group to face away from the opening of the binding site, 17 β -E2 approximates the horizontal binding mode of DHEA, the best among the estrogens with two hydroxy groups (Figure 8A). This match is reflected in it being the most sulfonated of the estrogen analogs in the absence of celecoxib. The 9D-E2 was a less preferred substrate, even though it has a conformation similar to that of the horizontal DHEA, as it goes too deeply into the binding site compared to 17 β -E2 and DHEA (Figure 8D). The 6D-E2, 17 β -Eqn, and 17 β -Eq were also poor substrates in the absence of celecoxib and their docked conformations do not lie in the zone of the substrate binding pocket occupied by the horizontal orientation of DHEA (Figure 8D).

The catechol estrogens were not good substrates of SULT2A1, as reflected by their control activities (Figure 7). The presence of the additional phenolic group in these molecules hindered them from assuming the binding configuration of 17 β -E2. His-99 in the binding pocket is flanked by two tyrosine residues, Tyr-160 and Tyr-231 whose side chain phenolic oxygen atoms are at distances of 5.4 and 6.0 Å, respectively, from the NC2 atom of His-99, respectively. The dockings of 4-OH-E2 and 2-OH-E2 in the absence of celecoxib (Figure 8E) had to avoid the clash of the additional phenolic groups with these tyrosine residues and hence are different from that of 17 β -E2 (Figure 8A). Nevertheless, for both CEs, the 3-hydroxy groups were closest to the His-99, an interaction required for sulfonation, supporting our hypothesis that the major metabolite of both CEs was 3-sulfate.

Both 17 α -E2 and E1 seem to assume conformations similar to the vertical conformation of DHEA (Figure 8F). Whereas the 3-hydroxy group of the non-catalytic conformation goes

too deep into the pocket and is closer to the 5'-phosphate group of the inactive co-factor PAP (3'-phosphoadenosine-5'-phosphate) than to His-99, the 17 α -hydroxy group of 17 α -E2 is perfectly positioned to be sulfonated, as its axial oxygen atom is in hydrogen bonding distance to His-99. In the case of E1, an additional hydrogen bond between the side chain of Ser-80 and the acceptor 17-keto group keeps it in position for catalysis.

3.6 Dockings in the Presence of Celecoxib

The docked conformation of celecoxib in the binding site occupied the same binding site of DHEA in the crystal structure, as previously predicted by Yalcin et al [13]. The trifluoromethyl group on the pyrazole ring goes farthest into the pocket leaving the sulfonamide on the outer side. One of the fluorine atoms of the trifluoromethyl group and the nitrogen of the sulfonamide group are in hydrogen bonding distance to the side chain amine of Lys-44 and the backbone carbonyl group of Met-16, respectively (Figure 9). These interactions anchor the celecoxib molecule to the side of the pocket leaving the methyl-phenyl group floating in the middle, thus, the substrate binding pocket is truncated and the substrates can only assume a vertical orientation. The celecoxib binding mode is shown in Figure 10A along with docked 17 β -E2, which is in a vertical position that puts its 17-hydroxy group adjacent to His-99 in the catalytic position. The aromatic interaction between the A-ring of 17 β -E2 and the methyl-phenyl group of celecoxib along with the interactions with Pro-14, Phe-18, Trp-77, and Tyr-231 favor this binding.

Figure 10B shows the overlap of the docked conformations of AD and DHEA in the presence of celecoxib with the 17 β -E2 docking shown in Figure 10A. The lack of the aromatic interaction with the celecoxib and their molecular shapes that are far from being as flat as 17 β -E2 render them incapable of forming the essential hydrogen bond with the His-99. T and Epi-T both have a non-aromatic A-ring and shapes more similar to DHEA than to 17 β -E2: the presence of celecoxib directs these substrates into conformations not ideal for hydrogen bonding with His-99 (Figure 10C).

The docked conformations of 9D-E2, 6D-E2, 17 β -Eqn and 17 β -Eq, shown in Figure 10D, are similar to that of 17 β -E2 in Figure 10A. 17 β -Eqn and 6D-E2, the flattest of the set, having double bonds in B ring that are conjugated to their A rings, are very similar to each other and to 17 β -E2 in their conformations. The estrogen 9D-E2, with unsaturation in C-ring conjugated with the aromatic A-ring is flat enough to assume a conformation that allows 17-hydroxy sulfonation even though its A-ring is tilted away from the methyl-phenyl group of celecoxib. The compound that showed the smallest amount of product switching and stimulation of overall sulfonation among the analogs of 17 β -E2 was 17 β -Eq and its docked conformation helps rationalize these results. The unsaturation at the 7-position in the B-ring twists this molecule with respect to the other three molecules in such a way that while it can interact well with the methyl-phenyl group of celecoxib, its 17-hydroxy group is farther away from His-99. Moreover, 17 β -Eq is the only analog of 17 β -E2 that showed a docking conformation in the presence of celecoxib that allowed 3-sulfonation. This interaction could explain the stimulation of 17 β -Eq 3-sulfonation in the presence of low concentrations of celecoxib.

The catechol estrogens also show the switching of the major product from 3-sulfate to 17-sulfate in the presence of celecoxib and they dock in configurations (Figure 10E) similar to 17 β -E2. The stimulation of 17-sulfate formation was also almost identical for these two compounds (Figure 10).

E1 and 3Me-E2 have similar docked conformations in the presence of celecoxib. Both molecules have their aromatic rings in close proximity to celecoxib for the π stacking interaction with the methyl-phenyl ring of celecoxib (Figure 8F). This leaves the 17-keto group of E1 and the 17-hydroxy group of 3Me-E2 in hydrogen binding distance to His-99, a scenario that suggests inhibition of E1 3-sulfonation (when celecoxib concentration is high enough to displace E1 from its catalytic position) and stimulation of 3Me-E2 17-sulfonation will be observed in the presence of celecoxib. The 17 α -E2 has a conformation similar to 17 β -E2, E1 and 3Me-E2 but its axial hydroxy group at the 17-position is turned away from the His-99 making the hydrogen bonding interaction difficult to achieve (Figure 10F).

4. DISCUSSION

The effects of xenobiotics on steroid sulfonation have received considerable attention during the past decade because of the importance of this pathway in steroid homeostasis [3, 48, 49]. This study showed that celecoxib interacted with SULT2A1 and affected sulfonation of DHEA, its physiologically important substrate, and other steroid and sterol substrates with hydroxy groups at different carbon locations and with different spatial orientations. There are reports that celecoxib and its analogues have anti-cancer effects in breast cancer [50-53] and colorectal cancer [54], two cancers whose growths may be stimulated by estrogen. The effect of celecoxib on regulation of the availability of unconjugated (active) estrogen may in part explain the protective effects observed in these cancers. The stimulation of 17-sulfate generation may be important because estrogen-17-sulfates are reported to be resistant to sulfatase hydrolysis and may therefore be more readily eliminated [55].

In vitro experiments showed that celecoxib at low concentrations (0–40 μ M) did not affect the sulfonation of DHEA or AD, however inhibition was observed at higher concentrations, with IC₅₀ values of 82 and 87 μ M respectively (Figure 5A). Other steroids with no aromatic A-ring, T and Epi-T, have 17 β - and 17 α -hydroxyl groups, respectively and were readily sulfonated with SULT2A1 (Figure 5B). Like DHEA, their sulfonation was inhibited by celecoxib, with IC₅₀ values of 59 and 97 μ M, respectively. The maximum liver concentration of celecoxib following a therapeutic dose of 200 mg was calculated to be 230 μ M [12], suggesting the possibility of inhibition of sulfonation of these steroids in patients taking celecoxib. Further studies will be needed to determine if celecoxib affects sulfonation of physiologically relevant steroids, DHEA and T *in vivo*.

Results with AD, which was sulfonated exclusively at the 3 β -position and not the 17-position with expressed SULT2A1, even in the presence of celecoxib, considered together with published results for 17 β -E2 and ethylnestradiol [11, 12] and our findings in this study, suggest that an aromatic A-ring is required for celecoxib to alter the preferred position of sulfonation.

To gain further insight into the effects of celecoxib on positional sulfonation of SULT2A1 substrates with an aromatic A-ring, several 17 β -E2 analogs were investigated. It was shown that a 17 β -hydroxy group was required, because sulfonation of 17 α -E2 was not stimulated, but was inhibited by celecoxib (Figure 6G). However, all steroids with an aromatic A-ring and a 17 β -hydroxy group exhibited stimulation of sulfonation at the 17 β -hydroxy group (Figures 4, 6 and 7). Demonstrating enhanced sulfonation of the 17 β -hydroxy group of 3-methyl-E2 showed that the 3-phenolic group was not required for the celecoxib effect. Steroids with a B-ring double bond that was conjugated with the aromatic A-ring, namely 6D-E2, 17 β -Eqn and 9D-E2, were similar to each other and to 17 β -E2 in that sulfonation at the 17-position was greatly stimulated by celecoxib at low μ M concentrations. Although 3-sulfonation of 6D-E2, 17 β -Eqn and 9D-E2 was inhibited by celecoxib concentrations above 20 μ M, the effect on 17-sulfonation was such that total sulfonation increased over 8-fold. Sulfonation of 17 β -Eq, which has a double bond in the B-ring that is not conjugated with the A-ring, was also affected by the presence of celecoxib, but with a different pattern. Sulfonation at both the 3- and the 17-positions was stimulated at celecoxib concentrations of 30 μ M or less, and the overall stimulation of 17-sulfonation was not as great as with 17 β -E2, 6D-E2, 17 β -Eqn or 9D-E2. Celecoxib stimulated 17-sulfonation of both catechol estrogens, but had a greater overall effect on sulfonation of 2-OH-E2 than 4-OH-E2, mainly because 2-OH-E2 was a very poor substrate in the absence of celecoxib. Other SULTs, notably SULT1E1 and SULT1A1 catalyze the sulfonation of estrogens [12, 14], however the overall stimulation by celecoxib suggests SULT2A1 may play a role in estrogen sulfonation in women taking this drug.

In efforts to better understand how celecoxib affects SULT2A1, molecular docking studies were conducted, using known crystal structures as templates. Analysis of the crystal structure of SULT2A1 in complex with DHEA showed that His-99 formed a hydrogen bond to the steroid O-3. This histidine is conserved among several SULTs and is considered to play a catalytic role [56]. In SULT2A1, there are a large number of hydrophobic residues surrounding the active site (6 Å), and several hydrophobic residues are in proximity to the substrates. These include Trp-77, Phe-18, Tyr-231, Leu-234 and Met-137. In addition, the side chains of residues Tyr-160, Trp-72, Pro-14, Pro-43, Phe-133, Tyr-238, Trp-134 and Met-16 contribute to the hydrophobic nature of the active site. Interaction of these groups with the bulky steroid substrates appear to be important for orientating the 3- or 17-hydroxy groups in a favorable position for sulfonation. B-ring unsaturation changes the shape of the steroid substrate, and is likely to alter the substrate-binding to the highly hydrophobic SULT2A1 binding site and influence the ease of formation of the sulfated forms. *In silico* studies described in the results section support these ideas.

Steroids with a non-aromatic A-ring are the best substrates of SULT2A1 as evidenced by the high control activities. The order of sulfonating efficiency of these compounds is Epi-T $\approx$ AD $\approx$ DHEA > T. Flexidock scores for docking of the sulfonated hydroxy group of each studied substrate to SULT2A1 reflects their relative affinities (Supplementary Table 1). For those steroids whose sulfonation at the 17-position is increased by celecoxib, the scores become more favorable for catalysis when celecoxib is present. The 17 α -OH isomer Epi-T is sulfonated 7 times more rapidly than the 17 β -OH isomer T, whereas DHEA and AD, both

with 3 β -OH have similar activities. Also interesting is the comparison between 17 α -E2 and 17 β -E2, and between 17 β -E2 and E1. In the absence of celecoxib, 17 β -E2 and other compounds with a 3-phenolic group and a 17 β -hydroxy group are predominantly sulfonated at the 3- position, suggesting that they bind to SULT2A1 with the 3-hydroxy group located close to the sulfate group of PAPS. However, for 17 β -E2, the favored sulfonation position was the 17 β -position, the phenolic group was sulfonated at a very low rate and 17-sulfonation was inhibited, not enhanced by the presence of celecoxib. The *in silico* conformations of these two molecules suggest that they bind to SULT2A1 in very different ways (Figure 8A and Figure 8F). The vertical conformation of 17 α -E2 is catalytic because its axial 17-hydroxy group is close to His 99, whereas the equatorial hydroxy group of 17 β -E2 is away from the catalytic His (Figure 8F). When celecoxib is present, however, the preferred orientation of the 17 β -E2 hydroxy group is near His 99 and the sulfate group of PAPS, while the 17 α hydroxy group of 17 α -E2 is away from His 99.

In the absence of celecoxib 17 α -E2 is a better SULT2A1 substrate than 17 β -E2, while E1 had similar activity to 17 β -E2 (Figures 4 and 5). Considered in conjunction with a previous report that the k_{cat}/K_m values for the Z-enantiomers of α -hydroxytamoxifen were higher than those for the E-enantiomers [57], the comparisons between 17 α - and 17 β -E2 throw more light on the isomer/enantiomer selectivity of SULT2A1.

In the presence of celecoxib the flatter molecules that could slide into the truncated binding pocket showed enhanced activity. Substrates similar to 17 β -E2 in 3D-structure, i.e., those with a double bond conjugated to the aromatic ring, 6D-E2, 17 β -Eqn and 9D-E2, showed the highest stimulation of overall sulfonation. The increase in sulfonation was entirely due to the facilitation of 17-sulfonation by celecoxib modulation as the presence of celecoxib inhibited the 3-sulfonation. Absence of the conjugated double bond and the consequential twist in the molecular shape seems to have hindered the stimulation of 17 β -Eq sulfonation. In the case of 3Me-E2, celecoxib appears to eliminate unproductive binding of the substrate in the binding site, as 3Me-E2 along with 17 β -Eqn showed no signs of plateauing of the 17-sulfonation within the celecoxib concentration range tested (0–80 μ M).

The sulfonation of non-aromatic steroids tested such as DHEA, AD, Epi-T and T was inhibited by the celecoxib binding, as these molecules are not flat enough to reach the His-99 for catalysis in the vertical conformations. It is interesting that inhibition of these compounds by celecoxib was not very potent. This may be due to the difficulty of displacing these compounds from the active site: they all have good affinities to the enzyme as evidenced by their control activities.

In summary, this paper describes the effects of celecoxib on sulfonation of a broad range of steroid substrates by SULT2A1. Celecoxib inhibited the sulfonation of DHEA, AD, T, Epi-T and 17 β -E2 albeit with relatively low potency. The effects of celecoxib on 17 β -E2 and its analogs were more potent and suggested the following: 1) an aromatic A-ring was essential for switching; 2) a 17 β -hydroxyl group was critical for the stimulation and switching; 3) the conjugated double bond(s) in B- and C-rings decreased the formation of 3-sulfate, but stimulated the formation of 17-sulfate, increasing the ratio of 17-sulfate to 3-sulfate. Furthermore, the stimulation of 17-sulfate generation by celecoxib could be a potentially

useful therapeutic approach as this conjugate is resistant to sulfatase hydrolysis and is thus more amenable to elimination [55].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

17β-E2	17 β -estradiol
17S	17-sulfate
17α-E2	17- α -estradiol
2-OH-E2	2-hydroxyestradiol
3Me-E2	3-methyl ether of estradiol
3S	3-sulfate
4-OH-E2	4-hydroxyestradiol
6D-E2	6-dehydroestradiol
9D-E2	9-dehydroestradiol
AD	5-androsten-3 β ,17 β -diol
CE	catechol estrogen
COMT	catechol-O-methyltransferase
COX-2	cyclooxygenase-2
DHEA	dehydroepiandrosterone
DMSO	dimethyl sulfoxide
Epi-T	epi-testosterone
17β-Eq	17 β -dihydroequilin
17β-Eqn	17 β -dihydroequilenin
H-ESI	heated electrospray ionization
IC₅₀	concentration required to achieve 50 % inhibition
k_{cat}/K_m	catalytic efficiency
PAPS	3'-phosphoadenosine-5'-phosphosulfate
PDB	protein data bank

SRM	selected reaction monitoring
SULT	sulfotransferase
T	testosterone

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HIGHLIGHTS

- Modulation of steroid sulfonation by celecoxib was investigated
- *In vitro* and *in silico* approaches were used
- Celecoxib inhibited DHEA and testosterone sulfonation with IC₅₀ of 60-80 μ M
- Celecoxib greatly increased 17-sulfonation of 17- β -estradiol analogs
- Modeling studies suggest celecoxib docks in the substrate-binding site of SULT2A1

Figure 1.

Structure of 17 β -Estradiol with carbon atoms numbered and rings named according to steroid nomenclature. Also shown in the figure is the structure of celecoxib.

Figure 2.

The chemical structures of the 14 steroids used in this study. The structural differences from 17β-E2 are highlighted in red.

Figure 3.

An overlap showing the binding of PAP or PAPS molecules in sulfotransferase crystal structures available in the Protein Data Bank. (PDB IDs represented are: 3U3J, 3U3K, 3U3M, 3U3O, 3F3Y, 3CKL, 3BFX, 2REO, 2Z5F, 2H8K, 2GWH, 2DO6, 2A3R, 1Q1Q, 1Q1Z, 1Q20, 1Q22, 1LS6, 1G3M and 1EFH). For all the structures oxygen atoms are shown in red, nitrogen atoms in blue and phosphorus atoms in orange. Also shown is the structure of PAP.

Figure 4. Celecoxib alters the rate and position of SULT2A1-catalyzed sulfonation of 17β-E2. Second order polynomial plots of rates of formation of the 3-sulfate (●), 17-sulfate (○) and both sulfates (▼) are shown for A) 0.05 μM 17β-E2, B) 0.2 μM 17β-E2 and C) 0.4 μM 17β-E2.

Figure 5.

Androgen sulfonation is inhibited by celecoxib in a concentration-dependent manner. SULT2A1 activities were measured with 0.4 μM concentrations of A) DHEA (●) and AD (○); B) T (○) and Epi-T (●) in the presence of 0 to 200 μM celecoxib as described in the Experimental section.

Figure 6.

Rates of SULT2A1-catalyzed sulfonation of the 17 β -hydroxy group of estrogens were increased in the presence of several concentrations of celecoxib. Results for formation of 3-sulfates (●), 17-sulfates (○) and both 3- and 17- sulfates (▼) are shown for incubations with 0.4 μ M concentrations of A) 6D-E2; B) 17 β -Eqn; C) 9D-E2; D) 17 β -Eq; E) E1; F) 3Me-E2; and G) 17 α -E2 with SULT2A1. Assays were conducted with 2 μ M 35 S-PAPS for 6D-E2 and 17 α -E2 and 20 μ M PAPS for all the other substrates.

Figure 7.

The 17β-sulfonation of each catechol derivative of 17β-E2 was increased by celecoxib. SULT2A1 was incubated with 0.4 μM concentrations of A) 2-OH-E2 and B) 4-OH-E2 in the presence of 20 μM PAPS and 0 to 80 μM celecoxib. Two individual experiments were conducted for every celecoxib concentration and each of these reactions was analyzed by LCMS/MS in triplicate. Data points in the plots are Mean ± SD for all six measurements. Closed circles represent 3-sulfates, the open ones represent 17-sulfates and the 2- and 4-sulfates are shown as open and closed diamonds respectively.

Figure 8.

Docking conformations of SULT2A1 substrates in the absence of celecoxib. A) 17 β -E2 (magenta) and DHEA (green). B) DHEA (green) and AD (blue). C) Epi-T (purple) and T (green). D) 6D-E2 (magenta), 17 β -Eqn (purple), 17 β -Eq (green) and 9D-E2 (gold). E) 2-OH-E2 (light green) and 4-OH-E2 (cyan). F) E1 (purple), 17 α -E2 (green) and 3Me-E2 (salmon). The ligands and His-99 (orange) are shown as sticks and the residues in the protein as wires. The enzyme is colored by atom type, with oxygens in red, nitrogens in blue and carbons in cyan.

Figure 9. Celecoxib docked in the substrate binding site of SULT2A1. The moieties within hydrogen bonding distance of celecoxib, Lys-44 and Met-16, are shown along with His-99.

Figure 10.

Docking conformations of SULT2A1 substrates in the presence of celecoxib. A) 17 β -E2 (magenta). B) DHEA (green), AD (blue) and 17 β -E2 (magenta). C) Epi-T (purple) and T (green). D) 6D-E2 (magenta), 17 β -Eqn (purple), 17 β -Eq (green) and 9D-E2 (gold). E) 2-OH-E2 (light green) and 4-OH-E2 (cyan). F) E1 (purple), 17 α -E2 (green) and 3Me-E2 (salmon). Celecoxib molecules corresponding to the substrate in the docked structure are shown in color matching the substrate. The ligands and His-99 (orange) are shown as sticks and the residues in the protein as wires. The enzyme is colored by atom type, with oxygens in red, nitrogens in blue and carbons in cyan.

Table 1

The order of addition of celecoxib (50 μM) and PAPS (20 μM) to assay tubes affects the total sulfonation activity of SULT2A1 with 17 β -E2 but not the positional switching effect.

Assay Conditions ^a		[E2], μM	Product formed, pmol/min/mg protein		
			17-sulfate	3-sulfate	Total sulfates
No celecoxib		0.16	Not detected	150	150
Celecoxib after PAPS	added	0.16	199	85	284
Celecoxib before PAPS	added	0.16	432	155	587
No celecoxib		0.4	Not detected	306	306
Celecoxib after PAPS	added	0.4	387	177	564
Celecoxib before PAPS	added	0.4	1076	363	1439

^a See the materials and methods section for complete assay conditions. Results shown are the mean of duplicate determinations.