Modification of the Catalytic Function of Human Hydroxysteroid Sulfotransferase hSULT2A1 by Formation of Disulfide Bonds

Xiaoyan Qin, Lynn M. Teesch, and Michael W. Duffel

Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy (X.Q., M.W.D.), High Resolution Mass Spectrometry Facility (L.M.T.), and Interdisciplinary Graduate Program in Human Toxicology (X.Q., M.W.D.) The University of Iowa, Iowa City, Iowa

Received December 3, 2012; accepted February 26, 2013

ABSTRACT
The human cytosolic sulfotransferase hSULT2A1 catalyzes the sulfation of a broad range of xenobiotics, as well as endogenous hydroxysteroids and bile acids. Reversible modulation of the catalytic activity of this enzyme could play important roles in its physiologic functions. Whereas other mammalian sulfotransferases are known to be reversibly altered by changes in their redox environment, this has not been previously shown for hSULT2A1. We have examined the hypothesis that the formation of disulfide bonds in hSULT2A1 can reversibly regulate the catalytic function of the enzyme. Three thiol oxidants were used as model compounds to investigate their effects on homogeneous preparations of hSULT2A1: glutathione disulfide, 5,5′-dithiobis(2-nitrobenzoic acid), and 1,1′-azobis[N,N-dimethylformamide] (diamide). Examination of the effects of disulfide bond formation with these agents indicated that the activity of the enzyme is reversibly altered. Studies on the kinetics of the hSULT2A1-catalyzed sulfation of dehydroepiandrosterone (DHEA) showed the effects of disulfide bond formation on the substrate inhibition characteristics of the enzyme. The effects of these agents on the binding of substrates and products, liquid chromatography-mass spectrometry identification of the disulfides formed, and structural modeling of the modified enzyme were examined. Our results indicate that conformational changes at cysteines near the nucleotide binding site affect the binding of both the nucleotide and DHEA to the enzyme, with the specific effects dependent on the structure of the resulting disulfide. Thus, the formation of disulfide bonds in hSULT2A1 is a potentially important reversible mechanism for alterations in the rates of sulfation of both endogenous and xenobiotic substrates.

Introduction
The superfamily of mammalian cytosolic sulfotransferases (SULTs) contributes to the metabolism of a wide range of xenobiotics that includes drugs, carcinogens, and environmental contaminants, as well as endogenous steroid hormones, bile acids, catecholamines, and iodothyronines (Falany, 1997; Glatt et al., 2000; Duffel et al., 2001; Coughtrie, 2002; Duffel, 2010). Although the various isoforms often have distinct, but overlapping, specificities for substrates and inhibitors, it is becoming increasingly apparent that other factors, such as the redox environment of these enzymes, can have additional effects on catalysis. For example, several family 1 SULTs are sensitive to oxidants that cause the formation of disulfide bonds (Marshall et al., 1997, 2000; Duffel et al., 2001; Maiti et al., 2005, 2007; Liu et al., 2011; Dammanahalli and Duffel, 2012). Moreover, it is clear that, in addition to the rates of catalysis, substrate specificity may be altered by disulfide bond formation in family 1 SULTs (Marshall et al., 2000; Duffel et al., 2001; Liu et al., 2011).

Although the effects of thiol oxidants on the catalytic function of family 1 SULTs have been studied, the potential for alteration in the catalysis of family 2 enzymes has not received an appropriate amount of attention. We have hypothesized that a family 2 SULT, such as the human hydroxysteroid sulfotransferase hSULT2A1, might also be subject to alteration of its catalytic function by thiol oxidants. The hSULT2A1 isomorph is a major human SULT that catalyzes the sulfation of endogenous alcohol–containing steroids such as dehydroepiandrosterone (DHEA), pregnenolone, androstenedione, bile acids, and others. Moreover, this enzyme is also of interest for its roles in the bioactivation of carcinogens that contain, or are metabolized to, benzylic or allylic alcohols (Watabe et al., 1982; Surh and Miller, 1994; Glatt et al., 1997).

An examination of the crystal structure of hSULT2A1 [e.g., protein data bank (PDB) ID 1999] indicates that there are no cysteine residues within the sulfuryl acceptor (e.g., DHEA) binding site of the protein. However, it should be noted that this is also the case in rSULT1A1, where there is pronounced regulation of catalytic rate and specificity through conformational alterations in the binding site for adenosine 3′-phosphate 5′-phosphosulfate (PAPS) and adenosine 3′,5′-diphosphate sodium salt (PAP) (Duffel et al., 2001). When the structure of hSULT2A1 is examined with respect to the PAPS/PAP binding site (e.g., PDB ID 1EHF), it is clear that two cysteine residues, Cys55 and Cys199, are near this nucleotide binding site in the protein. This location of cysteines in relation to the PAPS/PAP binding site led us to

ABBREVIATIONS: ANS, 8-anilinonaphthalene-1-sulfonic acid; DHEA, dehydroepiandrosterone; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; GSH, reduced glutathione; GSSG, glutathione disulfide; PAP, adenosine 3′,5′-diphosphate; LC-MS, liquid chromatography-mass spectrometry; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; PDB, Protein Data Bank; SULT, sulfotransferase.
investigate the effect of disulfide bond formation on the catalysis of hSULT2A1.

Materials and Methods

Chemicals. DHEA, 1,1’-azobis(N,N-dimethylformamide) (diadime), t-glutathione (GSH), t-glutathione disulfide (dissodium salt) (GSSG), 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB), PAP, methylene blue, 2-mercaptopethanol, and 4-vinlypyridine were purchased from Sigma-Aldrich (St. Louis, MO). 8-Anilinonaphthalene-1-sulfonic acid ammonium salt (ANS) was purchased from Fluka (Steinheim, Germany). PAPS was purchased from Sigma-Aldrich and was further purified by anion-exchange chromatography (Sekura, 1981) to reach at least 98% purity (as determined by high-performance liquid chromatography analysis). Modified trypsin and Glu-C (both sequencing grade) were purchased from Promega (Madison, WI). Precast 12% SDS-PAGE gels were purchased from Bio-Rad Laboratories (Hercules, CA). Radiolabeled [3H]-DHEA was purchased from PerkinElmer (Waltham, MA). All other chemicals used in this study were of the highest chemical purity commercially available.

Expression and Purification of Recombinant hSULT2A1. Recombinant human SULT2A1 was expressed in Escherichia coli BL21 (DE3) cells (Sheng and Duffel, 2003). The cells were lysed and extracted, and the enzyme was purified as previously described (Gulcan et al., 2008). After each step in the purification procedure, hSULT2A1 was initially monitored for sulfation activity, with the methylene-blue assay using dehydroepiandrosterone (DHEA) as substrate (Nose and Lipmann, 1958; Sheng et al., 2001). Protein content was determined by the modified Lowry procedure with bovine serum albumin as standard (Bensadoun and Weinstein, 1976). Following purification, SDS-PAGE with Coomassie brilliant blue staining yielded a single band with a 34-kDa relative molecular mass, and this was consistent with the previously reported mass of hSULT2A1 (Falany et al., 1989).

Pretreatment of hSULT2A1 with Thiol-Oxidants. Before treatment of hSULT2A1 with various oxidants, the diithiothreitol (DTT) that remained from purification of the enzyme was removed by chromatography on a PD-10 column (1.45 × 5.0 cm; GE Healthcare, Pittsburgh, PA). After 1 hour equilibration of the PD-10 column at 4°C, it was washed with four column volumes of 50 mM Tris-HCl, pH 7.4, containing 0.25 M sucrose, 10% (v/v) glycerol, and 0.05% (v/v) Tween 20 (buffer A). A volume of 1 ml of purified hSULT2A1 (1 mg/ml) was added onto the column, followed by elution with 5 ml of buffer A. The eluate was collected at the first increase in UV absorbance at 280 nm. The eluted hSULT2A1 was concentrated using a 10-ml Amicon stirred cell with PM-10 membrane (Millipore Corporation, Billerica, MA) to a protein concentration of at least 1 mg/ml (Liu et al., 2009). A standard assay for thiols (Jocelyn, 1987) was used to determine the residual concentration of DTT, which was in all cases less than 0.01 mM. Incubations of the resulting hSULT2A1 were carried out in buffer A with appropriate concentrations of reduced GSH, oxidized GSSG, diadime, or DTNB at 25°C for 1 hour. Solutions were saturated with argon before the incubations, and these reactions were conducted in sealed tubes with an argon atmosphere above the solution.

Kinetics of DHEA-Sulfation Catalyzed by hSULT2A1. The kinetics of the hSULT2A1-catalyzed reaction were determined by quantitation of the formation of DHEA sulfate (DHEA-S) from DHEA as described previously (Gulcan and Duffel, 2011). After treatment of the enzyme with various oxidants, assays for the rate of sulfation of DHEA catalyzed by hSULT2A1 were carried out in a total volume of 0.2 ml of 0.25 M potassium phosphate at pH 7.4 containing 0.2 mM PAPS and the indicated concentration of a mixture of [3H]-DHEA and unlabeled DHEA. This reaction mixture was incubated in a 37°C water bath for 2 minutes before the addition of 30 ng (in a 2-μl volume) of the oxidant-treated hSULT2A1 (final concentration of the enzyme was 2.2 mM based on the relative molecular mass (Mm) of the dimeric protein). This reaction was carried out at 37°C for either 3 or 4 minutes (incubation time adjusted to maintain initial velocity conditions). The reaction was terminated by addition of 0.8 ml of 50 mM potassium hydroxide, and analysis of the DHEA-sulfate formed was carried out as previously described (Gulcan and Duffel, 2011). Relative IC50 values were obtained by fitting data to a sigmoidal dose-response curve using SigmaPlot 11.0 (Systat Software, Chicago, IL). The mean ± S.D. of three replicates was determined for each assay.

Ligand-Binding Studies. ANS was used as a fluorescent probe for determination of the binding of ligands (e.g., DHEA and PAP) to both the unmodified and the oxidized hSULT2A1 using the previously described procedure for the study of rSULT1A1 (Marshall et al., 1997; Liu et al., 2011) with a modification wherein concentrations of ANS were selected by determining conditions of saturation under the conditions that were used in the assay (Supplemental Fig. 1). Binding of ligands to the enzyme was determined at 37°C in a 1.0-ml assay containing 0.25 M potassium phosphate, pH 7.4, with a 200 μM final concentration of ANS and 2 μg of either oxidant-treated or untreated hSULT2A1 (i.e., 30 μM enzyme concentration based on the Mm of dimeric hSULT2A1). Fluorescence was determined using a quartz cuvette (1.0-cm excitation path length and 0.4-cm emission path length) in a PerkinElmer LS55 luminescence spectrometer (PerkinElmer). After incubation at 37°C for 2 minutes, various volumes of either a 1.0 mM or a 10 mM solution of DHEA in absolute ethanol were added to the enzyme-ANS mixture to make the final concentration of DHEA in the solution ranging from 0.2 to 60 μM. After each addition of DHEA, the solution was mixed well, and a 10-second incubation time was allowed before determining the fluorescence emission (excitation wavelength at 410 nm and emission wavelength at 480 nm, with entrance and the exit slits both set at 5 nm). The shutters for the fluorimeter were closed between fluorescence measurements. A dilution factor for each addition of DHEA was applied in the final calculation of the change in fluorescence. All solutions were filtered with a Millex-GS 0.22-μm filter before use. Each concentration of DHEA in the titration was done in duplicate. The means and standard errors of Kd values were calculated by nonlinear regression fitting of the data to a two-site binding equation corrected for nonspecific binding (SigmaPlot 11.0; Systat Software).

Conformational alterations in hSULT2A1 on interaction with ligands were also determined by changes in the intrinsic fluorescence of tryptophan residues as described previously (Beechem and Brand, 1985; Gulcan and Duffel, 2011). Both kinetic and crystallographic studies have shown that PAP and PAPS bind at the same binding site in each SULT (Yoshinari et al., 2001; Chapman et al., 2004; Wang and James, 2006), and we determined binding at this site by titration with PAP. These studies were conducted in 0.25 M potassium phosphate, pH 7.4, with an excitation wavelength of 290 nm and an emission wavelength of 347 nm (entrance and the exit slits were set at 5 and 7 nm, respectively). Pretreated or untreated hSULT2A1 (0.12 μM final concentration based on the Mm of the dimeric enzyme) was mixed with DHEA (0 μM, 0.5 μM, or 50 μM final concentration) in 0.25 M potassium phosphate buffer and incubated for 20 minutes at 37°C. Titration was then carried out by addition of aliquots of PAP, with final concentrations of PAP ranging from 0.5 to 300 μM. After each addition of PAP, the solution was mixed and incubated in the chamber for 10 seconds before determining the fluorescence. The change in fluorescence was determined after applying a dilution factor for each addition of PAP. All solutions used in this assay were filtered with a Millex-GS 0.22-μm filter before the experiment. PAP titrations were carried out in duplicate, and the means and standard errors of dissociation constant (Kd) values were calculated by nonlinear regression fitting of the data to a two-site binding equation (SigmaPlot 11.0, Systat Software). For all ligand-binding studies, the statistical significance of changes in equilibrium dissociation constants was estimated with an unpaired t test using the calculated Kd values and standard errors.

Protein Conformational Changes upon Oxidation of hSULT2A1. hSULT2A1 was diluted to a concentration 0.25 μM (based on dimeric Mm) in 0.25 M potassium phosphate buffer, pH 7.4. Fluorescence changes on the addition of oxidants were determined at 25°C with a Spectra Max M5 spectrometer (Molecular Devices, Downingtown, PA) using an excitation wavelength of 290 nm and an emission wavelength of 347 nm. Varying concentrations of diazomethane (i.e., 20, 40, and 80 μM), as well as 1 mM GSH and 1 mM GSSG were added to the enzyme-containing buffer after the cell had been incubated in the chamber for 5 minutes. Fluorescence values were recorded at 5 minutes, at 10 minutes, and then at 10-minute intervals up to 90 minutes. Immediately after the 90-minute determination, 5 mM DTT or 2-ME was added into the enzyme mixture. After the addition of DTT, measurements were made at 95 minutes, 100 minutes, 110 minutes, and 120 minutes. Since minor light-absorption properties of diazomethane close to the excitation and emission wavelength of tryptophan cause a filter effect in the fluorescence measurement, a correction equation (F0 = Fr * C) was applied. In this

Catalytic Regulation of hSULT2A1 by Disulfide Bond Formation 1095

Downloaded from dmd.aspetjournals.org on July 7, 2017
equation, $F_{obs}$ is the observed fluorescence in the presence of filtration effect, and $F_r$ is the original fluorescence. The value of $C$ in this equation represents the correction factor, and it is determined from the absorption at 290 nm or 347 nm by the equation ($C = (1 - 10^{-A})/2.303A$) (Birdsall et al., 1983).

**Structural Characterization of Oxidatively Modified hSULT2A1.** The structural modification of hSULT2A1 by thiol oxidants was detected and characterized by liquid chromatography-mass spectrometry (LC-MS). A PD-10–size exclusion column was used to remove the DTT that remained from the purification of hSULT2A1, and this column was eluted with 50 mM Tris-HCl buffer, pH 8.0. The resulting enzyme was concentrated to approximately 2 μg/μl, and the removal of DTT was verified using the assay described previously herein. Aliquots of hSULT2A1, each containing 15 μg of enzyme in a 25-μl volume, were incubated for 1 hour at 25°C with various thiol oxidants followed by addition of 4-vinylpyridine (45-mM final concentration of 4-vinylpyridine). After incubation for another 1 hour, proteolytic digestion was carried out by adding 0.6 μg of sequencing-grade trypsin in 50 mM Tris-HCl buffer (pH 8.0) and acetonitrile (final 10% v/v) in a total volume of 0.1 ml and incubating at 37°C for 16 hours. In cases where a secondary digestion was performed, 1 μg of sequencing grade Glu-C was added immediately after the 16-hour tryptic digestion, and the mixture was incubated at 25°C for another 16 hours. Proteolytic reactions were terminated by adding 2 μl of concentrated acetic acid, and samples were subsequently analyzed with a Thermo LCQ Deca quadrupole ion trap mass spectrometer (ThermoElectron, San Jose, CA) interfaced with a Dionex LC (Dionex, Sunnyvale, CA). Data were collected using positive electrospray ionization (ESI) over the mass range of 300–2000. A Supelco Discovery Bio Wide Pore C18 (2.1 × 150 mm) column (Sigma-Aldrich) was used with a 10-μl injection volume. The LC mobile phase was 0.1% (v/v) formic acid in water (A) and acetonitrile with 0.1% (v/v) formic acid (B). The gradient conditions for elution of the column were 5% B, which was held for 2 minutes, and then the solvent composition was increased linearily to 60% B over 80 minutes at a flow rate of 200 μl/min.

**Computational Modeling Studies on hSULT2A1.** Sybyl X (Tripos, St. Louis, MO) was used to suggest potential conformational changes in hSULT2A1 after oxidative modifications. The previously reported x-ray crystal structure of hSULT2A1 with the cofactor PAP bound (Pedersen et al., 2000) was obtained from the Protein Data Bank (PDB ID 1EFH). PAP was extracted from the original structure, and the conformation of the resulting structure was optimized using the Prepare Structure application in Sybyl X. The optimization included fixing the terminal ends by adding charges at the terminus, adding back missing hydrogens on the side chain of the amino acid residues, as well as fixing the side-chain steric interactions in the structure, followed by energy minimization. The Powell method was used for the initial optimization with a termination gradient of 0.5 kcal/(mol*Å). The force field was set at the Tripos default value with Gasteiger–Hückel charges using an 8.00 Å nonbonding interaction cutoff. The maximum number of iterations was set at 15000 for the calculation.

A disulfide bond was constructed between Cys55 and Cys199, and the resulting structural model was minimized as noted previously herein. For modeling the effect of mixed disulfide formation with GSH on the conformation of the protein, the structure of GSH was constructed with the build-protein apparatus in the biopolymer suite, and it was merged into the optimized structure of hSULT2A1 with the addition of a covalent bond to link the cysteine in GSH to the Cys55 or Cys199 of the protein via disulfide bonds. Bond angles were adjusted to avoid any side-chain bumps, and a regional minimization was performed using a terminal gradient of 0.1 kcal/(mol*Å) and the same force field as previously described. Overall stage energy minimization was conducted with same setting as for the original structure, with the exception of allowing C–α changes. The energy-minimized modified hSULT2A1 was saved as a PDB file and aligned with the optimized unmodified structure of hSULT2A1 that had been developed based on PDB ID 1EFH. The structural model with the disulfide bond between Cys55 and Cys199 was subjected to analysis using Verify_3D and Procheck (NIH Structural Analysis and Validation Server, SAVeS). Although, as expected, a localized higher energy was noted for the disulfide bond, the overall protein structure of the model was acceptable when related to similar analysis of the crystal structure of the reduced protein (PDB ID 1EFH). The protein backbone of the two models were compared with the alterations in conformation and depicted using PyMol (v. 1.5.0.4; Schrödinger, LLC, New York, NY).

**Results**

**Regulation of the Catalytic Activity in hSULT2A1 by Disulfide Bond Formation.** Previous reports have indicated that oxidants capable of forming disulfide bonds with cysteines (e.g., diamide, DTNB, and GSSG) can regulate the catalytic activity of rSULT1A1, the major family 1 SULT in rat liver (Marshall et al., 1997; Duffel et al., 2001). Either the formation of a Cys66-glutathione disulfide or an intramolecular disulfide bond, Cys66-Cys232, can alter the protein conformation near the PAPS/PAP binding site in rSULT1A1 and lead to catalytic alterations (Marshall et al., 1997; Duffel et al., 2001).

We used diamide and DTNB in preliminary experiments to examine both the ability of hSULT2A1 to form intramolecular disulfides and the effect of this alteration on catalytic function. The results indicated a decrease in the catalytic activity of hSULT2A1 after incubation with either diamide or DTNB for periods up to 1 hour, but no significant change was observed after additional exposure to these oxidants (Fig. 1). Thus, a 1-hour incubation with oxidants was applied as a pretreatment throughout subsequent studies.

In addition to diamide and DTNB, GSSG was used as a model oxidant for hSULT2A1, and this was compared with catalysis in the presence of GSH at varied concentrations of DHEA as substrate. As shown in Fig. 2, the effects of 1 mM GSSG or 1 mM GSH on catalysis were examined by coincubation with hSULT2A1 for 1 hour at 25°C. After this incubation, the enzyme was diluted 100-fold into a standard assay for determination of hSULT2A1 activity at 37°C. DHEA (concentrations from 0.25 to 10 μM) was used as a sulfuryl acceptor substrate for the catalytic reaction in the presence of 0.2 mM PAPS. Significant differences between the catalytic activities of the GSSG- and GSH-retreated hSULT2A1 were observed only at DHEA concentrations below 1 μM. Based on this finding, 0.5 μM DHEA was used as the standard substrate concentration in all subsequent experiments.

**Reversibility of Oxidative Modifications of hSULT2A1.** Since the reversibility of oxidative modification of family 1 SULTs has been previously demonstrated (Maïtï et al., 2007), we sought to determine the reversibility of disulfide bond formation in hSULT2A1. Following a 1-hour treatment of hSULT2A1 with 0.5 mM diamide or 0.5 mM DHEA and acetonitrile (final 10% v/v) in a total volume of 0.1 ml and incubating at 37°C for 16 hours. In cases where a secondary digestion was performed, 1 μl of concentrated acetic acid, and samples were subsequently analyzed with a Thermo LCQ Deca quadrupole ion trap mass spectrometer (ThermoElectron, San Jose, CA) interfaced with a Dionex LC (Dionex, Sunnyvale, CA). Data were collected using positive electrospray ionization (ESI) over the mass range of 300–2000. A Supelco Discovery Bio Wide Pore C18 (2.1 × 150 mm) column (Sigma-Aldrich) was used with a 10-μl injection volume. The LC mobile phase was 0.1% (v/v) formic acid in water (A) and acetonitrile with 0.1% (v/v) formic acid (B). The gradient conditions for elution of the column were 5% B, which was held for 2 minutes, and then the solvent composition was increased linearily to 60% B over 80 minutes at a flow rate of 200 μl/min.

A disulfide bond was constructed between Cys55 and Cys199, and the resulting structural model was minimized as noted previously herein. For modeling the effect of mixed disulfide formation with GSH on the conformation of the protein, the structure of GSH was constructed with the build-protein apparatus in the biopolymer suite, and it was merged into the optimized structure of hSULT2A1 with the addition of a covalent bond to link the cysteine in GSH to the Cys55 or Cys199 of the protein via disulfide bonds. Bond angles were adjusted to avoid any side-chain bumps, and a regional minimization was performed using a terminal gradient of 0.1 kcal/(mol*Å) and the same force field as previously described. Overall stage energy minimization was conducted with same setting as for the original structure, with the exception of allowing C–α changes. The energy-minimized modified hSULT2A1 was saved as a PDB file and aligned with the optimized unmodified structure of hSULT2A1 that had been developed based on PDB ID 1EFH. The structural model with the disulfide bond between Cys55 and Cys199 was subjected to analysis using Verify_3D and Procheck (NIH Structural Analysis and Verification Server, SAVeS). Although, as expected, a localized higher energy was noted for the disulfide bond, the overall protein structure of the model was acceptable when related to similar analysis of the crystal structure of the reduced protein (PDB ID 1EFH). The protein backbone of the two models were compared with the alterations in conformation and depicted using PyMol (v. 1.5.0.4; Schrödinger, LLC, New York, NY).

**Fig. 1.** The alteration in catalytic function of hSULT2A1 by pretreatment with either DTNB or diamide was complete after 1 hour. The enzyme was treated with oxidant for the indicated time at 25°C, followed by 100- fold dilution into a standard assay for sulfation of 0.5 μM DHEA as described in Materials and Methods.
DTNB, 5 mM DTT was added, and the mixture was further incubated at 25°C for another 1 hour before conducting an assay for catalytic activity with DHEA as substrate. As seen in Fig. 3A, recovery of catalytic activity was observed for diamide-pretreated hSULT2A1 after restoring the reducing environment. Likewise, the addition of DTT into the DTNB-pretreated hSULT2A1 resulted in the recovery of approximately 80% of the value seen with untreated hSULT2A1 (Fig. 3B). When using GSH-treated hSULT2A1 as control, the GSSG-treated enzyme showed a statistically significant decrease in the rate of DHEA-sulfation, and this effect was reversed by addition of DTT (Fig. 3C).

Alteration in Kinetic Characteristics of the hSULT2A1-Catalyzed Sulfation of DHEA. The kinetic characteristics of oxidized as well as untreated hSULT2A1 were examined for the sulfation of DHEA at substrate concentrations ranging from 0.25 to 10 μM. As noted previously (Falany et al., 1989; Gulcan and Duffel, 2011), hSULT2A1 that had not been subjected to treatment with oxidants displayed substrate inhibition kinetics (Fig. 4). Based on this characteristic kinetic profile, we applied an equation for substrate inhibition kinetics (shown in the equation below) to calculate the kinetic constants for untreated as well as oxidant-treated hSULT2A1.

\[
V = \frac{V_{\text{max}}[\text{DHEA}]}{K_m + \left(\frac{[\text{DHEA}]}{K_i} + \frac{[\text{DHEA}]}{K_i} + 1\right)}
\]

As seen in Table 1, values for \(K_m\), \(V_{\text{max}}\) and inhibition constant (\(K_i\)) with the untreated hSULT2A1 were 178 ± 35 nmol/min per milligram, 1.3 ± 0.4 μM, and 6.2 ± 2.4 μM, respectively. After treatment with GSSG, a slight increase in \(V_{\text{max}}\) value was observed compared with the GSH-pretreated enzyme; however, \(K_i\) and \(K_m\) values were not significantly changed (Table 1). A similar effect on the \(V_{\text{max}}\) value was observed for 0.5 mM DTNB-pretreated hSULT2A1 (268 ± 14 nmol/min per milligram) (Fig. 4; Table 1), whereas a decrease in \(V_{\text{max}}\) value was observed for 0.5 mM diamide-pretreated hSULT2A1 at 145 ± 5 nmol/min per milligram (Fig. 4; Table 1). As shown in Table 1, no significant differences were found in the \(K_m\) values for DHEA observed in all the oxidative pretreatment cases compared with the untreated enzyme. Although no significant change was seen in the calculated \(K_i\) value for the GSSG-pretreated enzyme compared with untreated hSULT2A1, both diamide- and DTNB-pretreated hSULT2A1 exhibited a loss of substrate inhibition (Fig. 4; Supplemental Fig. 2; Table 1).

Binding of Substrate and Products after Disulfide Bond Formation in hSULT2A1. The loss of substrate inhibition on oxidation of hSULT2A1 indicated the potential for changes in the binding of DHEA, PAPS, or PAP to the enzyme. The binding of these substrates and products to hSULT2A1 is essential in the formation of a dead-end complex that causes substrate inhibition (Zhang et al., 1998; Gulcan and Duffel, 2011). Binding of DHEA to untreated and oxidant-treated hSULT2A1 was determined by displacement of the fluorescent probe ANS. The change in fluorescence on displacement of ANS by DHEA was determined, and the results were fit to a two-site binding equation with a nonspecific binding term. The \(K_a\) values for DHEA bound to the untreated hSULT2A1 (Supplemental Fig. 3; Table 2) were consistent with those previously determined by monitoring the intrinsic fluorescence of the protein (Gulcan and Duffel, 2011). As seen in Table 2, pretreatment of the enzyme with 1 mM GSSG changed the binding of DHEA, as seen in the loss of a second dissociation constant. The \(K_{d1}\) for the GSSG-treated enzyme was 1.0 ± 0.1 μM, and this was only slightly different from that seen for the untreated enzyme. Pretreatment of hSULT2A1 with 0.5 mM diamide also resulted in the loss of the second binding constant, with the \(K_{d1}\) value similar to both the GSSG-treated and the untreated enzyme. When the enzyme was pretreated with 0.5 mM DTNB, no significant change was observed in terms of DHEA binding.

Binding at the nucleotide binding site (i.e., binding site for PAPS and PAP) in hSULT2A1 was determined using PAP as described in Materials and Methods. Values are the means ± S.D. of three determinations. *Statistical significance by t test (P < 0.05).
The binding of PAP to the oxidant-treated hSULT2A1 was also determined in the presence of either 0.5 or 50 μM DHEA in the final assay mixture (Table 3). In the case of the untreated enzyme, $K_{d1}$ and $K_{d2}$ values were not significantly different from those obtained without DHEA present (Table 3). Pretreatment of hSULT2A1 with 1 mM GSH resulted in a small increase in the value of $K_{d1}$ for PAP when 0.5 μM DHEA was present and an increase in $K_{d2}$ when 50 μM DHEA was present (Table 3). Pretreatment with 1 mM GSSG increased the $K_{d2}$ for PAP-binding in the presence of 50 μM DHEA (Table 3); however, this was not the case in the presence of 0.5 μM DHEA (Table 3). Treatment of hSULT2A1 with DTNB had no effect on the binding of PAP in the presence of 50 μM DHEA (Table 3), but significant increases in both $K_{d1}$ and $K_{d2}$ occurred in the presence of 0.5 μM DHEA (Table 3). Pretreatment with diamide yielded no significant effect on the binding of PAP in the presence of either 0.5 or 50 μM DHEA (Table 3).

Oxidative Modifications of hSULT2A1 Identified by LC-MS. Structural modifications of hSULT2A1 after pretreatment with oxidants were analyzed by proteolytic digestion, followed by LC-MS using a quadrupole ion trap mass spectrometer. After treatment with each oxidant, 4-vinylpyridine (45 mM) was added to the hSULT2A1 to alkylate remaining free cysteine thiols and, thereby, preclude any disulfide exchange during the later digestion steps. Following tryptic digestion, peptides with pretreatments were analyzed by LC-ESI-MS. Ions formed from peptides in the ESI process can be multiply charged, which allows us to detect peptides that have molecule weights higher than 2000 within the mass range of the mass spectrometer. The presence of those tryptic peptides that bear masses higher than 2000 could be identified by searching for their corresponding doubly or triply charged ions in the mass spectral data. In control experiments,
Catalytic Regulation of hSULT2A1 by Disulfide Bond Formation

hSULT2A1 that had not been treated with oxidants was subjected to reaction with 45 mM vinylpyridine. Three 4-vinylpyridine-added cysteines, including Cys55, Cys199, and Cys154, were found in tryptic peptides (unpublished data). Reaction of hSULT2A1 with GSSG resulted in two additional chromatographic peaks, and these corresponded to glutathione mixed disulfides at Cys199 and Cys55, respectively (Table 4). Proteolytic cleavage with Glu-C after the tryptic digestion of the enzyme confirmed the findings from the single reaction of the modified hSULT2A1 with trypsin. After digestion with both trypsin and Glu-C, an additional Cys154-glutathione mixed disulfide adduct was identified by LC-MS (Table 4). After the reaction of hSULT2A1 with diamide, tryptic digestion LC-MS analysis showed a loss of all three 4-vinylpyridine-modified cysteines, and the formation of a disulfide bond between Cys55 and Cys199 was observed, although (M + 4H)4+ ion was in low abundance (Table 4). A secondary digestion of the tryptic peptide with Glu-C confirmed the findings that diamide pretreatment resulted in a disulfide bond between Cys55 and Cys199 (Table 4). As also seen in Table 4, LC-MS analysis of hSULT2A1 after the reaction with DTNB, and subsequent proteolytic digestion, showed that all three cysteines in hSULT2A1 were modified by formation of a thionitrobenzoic acid adduct.

Conformational Alterations in hSULT2A1 after Oxidative Modification. Since the formation of disulfide bonds in hSULT2A1 would likely lead to conformational changes in the protein structure, we examined changes in the intrinsic fluorescence of the enzyme as a sensitive indicator of these alterations in structure on formation of disulfide bonds. The fluorescence of tryptophan residues in the protein exhibited a statistically significant change only after the first 60-minute time period after the addition of 20 μM diamide. Statistically significant (P < 0.05) time-dependent changes in the conformation of hSULT2A1 were observed for all time points after the addition of either 40 or 80 μM diamide (Fig. 5). On the addition of the reducing reagent DTT, the diamide-treated hSULT2A1 exhibited an immediate return of the intrinsic fluorescence back to the original level seen for the enzyme that had not been subjected to reaction with diamide (Fig. 5). Thus, the reaction of the oxidized protein with DTT restored both the conformation of the protein and the catalytic activity.

After reaction with 1 mM GSSG, there was only a small time-dependent decrease in the intrinsic fluorescence of hSULT2A1, and this was reversed by addition of 2-mercaptoethanol (Supplemental Fig. 4). A control experiment using 1 mM GSH yielded no significant change in the intrinsic fluorescence of hSULT2A1 (unpublished data). It was not possible to use this method of analysis for treatment of the enzyme with DTNB because of its high absorption at a wavelength overlapping with the excitation wavelength of tryptophan.

Modeling of Conformational Alterations in hSULT2A1 after Disulfide Bond Formation. Energy-minimized structural models of an intramolecular disulfide bond between Cys55 and Cys199 revealed an overall alteration in the C-backbone structure of hSULT2A1. Key components of the enzyme, including regions near the PAPS/PAP binding site and the DHEA binding site, exhibited small conformational changes (Fig. 6). Such structural modifications seen in the model were consistent with the changes in structure and catalytic function of hSULT2A1 that were observed on formation of this disulfide bond. An alteration in the position of the His99 side chain (an essential residue in catalytic transfer of a sulfuryl group) was also observed in the model following disulfide bond formation. However, Lys44 and Ser129 did not show significant changes in their side-chain positions in the model structure (Fig. 6).

The modeled structures of hSULT2A1 were also compared both before and after formation of a mixed disulfide with glutathione at Cys55 and Cys199, and small changes in the C-alpha backbone near the positions of modification were observed (Supplemental Fig. 5; Supplemental Material Structure File for S3.). These conformational changes included the binding region for PAPS and PAP in the enzyme. It is also noteworthy that the glutathione tripeptide side chain that is bound to Cys55 in the energy-minimized model is pointing out toward the exterior of the globular structure of hSULT2A1, and this may limit the conformational change in the backbone structure.

### Table 1

<table>
<thead>
<tr>
<th>DHEA Binding</th>
<th>Untreated</th>
<th>GSH Treatment</th>
<th>GSSG Treatment</th>
<th>Diamide Treatment</th>
<th>DTNB Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{max} (nmol/min/mg)</td>
<td>178 ± 35</td>
<td>191 ± 15</td>
<td>278 ± 33</td>
<td>145 ± 5</td>
<td>268 ± 14</td>
</tr>
<tr>
<td>$K_m$ (μM)</td>
<td>1.3 ± 0.4</td>
<td>0.3 ± 0.07</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>$K_i$ (μM)</td>
<td>6.2 ± 2.4</td>
<td>10 ± 2.2</td>
<td>5.5 ± 1.2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>DHEA Binding</th>
<th>Untreated</th>
<th>Pretreatment 1 mM GSSG</th>
<th>Pretreatment 0.5 mM Diamide</th>
<th>Pretreatment 0.5 mM DTNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{d1}$ (μM)</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.1$^a$</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>$K_{d2}$ (μM)</td>
<td>12 ± 5.0</td>
<td>N/A</td>
<td>N/A</td>
<td>11 ± 8.0</td>
</tr>
</tbody>
</table>

$^a$ $K_d$ value was different (P < 0.05) from the corresponding dissociation constant for untreated hSULT2A1.

### Discussion

Oxidation of cysteine residues significantly alters the function of many proteins, and this has been particularly of interest with respect to cellular oxidative stress (O’Brian and Chu, 2005; Biswas et al., 2006; Mieyal et al., 2008; Dalle-Donne et al., 2009; Jones and Go, 2010). Oxidative regulation of the catalytic activity of cytosolic sulfotransferases has been demonstrated in family 1 SULTs, including members of both SULT1A, as well as SULT1E subfamilies (Marshall et al., 1997; Maiti et al., 2005, 2007). Our results from the present study indicate that the catalytic activity of hSULT2A1, an important member of the SULT2 family in humans, can also be modulated through oxidation at cysteine residues.
Previous studies on the rat hepatic rSULT1A1 showed that the oxidation of Cys66 and Cys232 could reversibly alter the catalytic activity of the enzyme through conformational changes affecting the PAPS/PAP binding site (Marshall et al., 1997; Duffel et al., 2001). This change in conformation and activity occurred as a result of the formation of either a Cys66-glutathione disulfide or an intramolecular disulfide bond between Cys66 and Cys232 (Marshall et al., 1997; Duffel et al., 2001). By virtue of their proximity to the PAPS/PAP binding site, Cys55 and Cys199 represent the analogous cysteines in hSULT2A1. Our finding that reaction of hSULT2A1 with GSSG, DTNB, or diamide reversibly decreased the catalytic activity of hSULT2A1 indicates that this SULT is also subject to catalytic modification by formation of disulfide bonds.

In the case of GSSG, we observed that the effects on hSULT2A1-catalyzed sulfation of DHEA occur only at low concentrations of DHEA (i.e., \(1 \mu M\) in the assay). This suggests that at lower, more physiologically relevant concentrations of DHEA, the effect of disulfide bond formation may be more significant than under conditions of higher substrate concentration. Indeed, our results at higher concentrations of DHEA are consistent with a previous report where no effect of GSSG treatment on hSULT2A1 was found (Chen et al., 2006; Maiti et al., 2007). This dependence on substrate concentration is most likely linked to the substrate inhibition of hSULT2A1 by DHEA at concentrations higher than 1.0 to 1.5 \(\mu M\).

In our results, a mixed disulfide with glutathione was found at both Cys55 and Cys199 of hSULT2A1, although LC-MS analysis indicated that GSSG only caused a partial oxidation of hSULT2A1 under the conditions used. Ligand-binding studies indicated, however, that structural modifications after GSSG-treatment did alter the binding characteristics of both DHEA and PAP. A decrease in the \(K_d\) value for PAP after GSSG treatment and an increase in \(K_d\) values for DHEA-binding were observed. Our results showed that without DHEA present, there was only a small difference in the \(K_d\) value for PAP after GSSG-pretreatment. However, when 0.5 \(\mu M\) DHEA (i.e., the

**TABLE 3**

<table>
<thead>
<tr>
<th>PAP Binding</th>
<th>Untreated</th>
<th>PreTreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM GSH</td>
<td>1 mM GSSG</td>
</tr>
<tr>
<td>No DHEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K_a) ((\mu M))</td>
<td>1.9 ± 0.2</td>
<td>0.9 ± 0.3*</td>
</tr>
<tr>
<td>(K_{d1}) ((\mu M))</td>
<td>540 ± 100</td>
<td>661 ± 194</td>
</tr>
<tr>
<td>0.5 (\mu M) DHEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K_a) ((\mu M))</td>
<td>0.9 ± 0.2</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>(K_{d1}) ((\mu M))</td>
<td>248 ± 92</td>
<td>328 ± 94</td>
</tr>
<tr>
<td>50 (\mu M) DHEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K_a) ((\mu M))</td>
<td>1.1 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>(K_{d2}) ((\mu M))</td>
<td>264 ± 36</td>
<td>453 ± 63*</td>
</tr>
</tbody>
</table>

\*\(K_a\) value was different (\(P < 0.05\)) from the corresponding dissociation constant for untreated hSULT2A1.

**TABLE 4**

<table>
<thead>
<tr>
<th>Modified Cysteine</th>
<th>Peptide Structure</th>
<th>Predicted Mass (M + H)(^+)</th>
<th>Mass Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSSG treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cys199-GS</td>
<td>GS-(ICQFLGK)</td>
<td>1113.94</td>
<td>1113.41 (M + H)(^+)</td>
</tr>
<tr>
<td>Cys55-GS</td>
<td>GS-(SGTNWLAELCLMHK)</td>
<td>2108.39</td>
<td>1054.73 (M + 2H)(^+)</td>
</tr>
<tr>
<td>Cys199-GS*</td>
<td>GS-(ICQFLGK)</td>
<td>1113.94</td>
<td>1113.43 (M + H)(^+)</td>
</tr>
<tr>
<td>Cys55-GS*</td>
<td>GS-(ILCLMHK)</td>
<td>1249.57</td>
<td>1249.41 (M + H)(^+)</td>
</tr>
<tr>
<td>Cys154-GS*</td>
<td>GS-(WFCQGTVLYGSFWDHIHGMPMR)</td>
<td>3159.35</td>
<td>1580.19 (M + 2H)(^+)</td>
</tr>
<tr>
<td>Diamide treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C199-C55</td>
<td>(TIEKICQFLGK)-(SGTNWLAELCLMHK)</td>
<td>4746.47</td>
<td>1119.82 (M + 4H)(^+)</td>
</tr>
<tr>
<td>C199-C55*</td>
<td>(TIEKICQFLGKTLPEELNLK)-(SGTNWLAELCLMHK)</td>
<td>4474.38</td>
<td>1119.82 (M + 4H)(^+)</td>
</tr>
<tr>
<td>C199-C55*</td>
<td>(ICQFLGK)-(ILCLMHK)</td>
<td>1749.94</td>
<td>1749.2 (M + H)(^+)</td>
</tr>
<tr>
<td>DTNB treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cys199-TNB</td>
<td>TNB-(ICQFLGK)</td>
<td>1005.62</td>
<td>1005.33 (M + H)(^+)</td>
</tr>
<tr>
<td>Cys55-TNB</td>
<td>TNB-(DEDVIHTYPSGTNWLAELCLMHK)</td>
<td>3286.75</td>
<td>1096.51 (M + 3H)(^+)</td>
</tr>
<tr>
<td>Cys55-TNB</td>
<td>TNB-(SGTNWLAELCLMHK)</td>
<td>2000.07</td>
<td>1999.51 (M + H)(^+)</td>
</tr>
<tr>
<td>Cys199-TNB*</td>
<td>TNB-(ICQFLGKTLPEELNLKQSSMQK)</td>
<td>3307.81</td>
<td>1103.27 (M + 3H)(^+)</td>
</tr>
<tr>
<td>Cys154-TNB</td>
<td>TNB-(SWEFYEFWFCQGTLYGSFWDHIHGMPMR)</td>
<td>4021.84</td>
<td>1341.28 (M + 3H)(^+)</td>
</tr>
</tbody>
</table>

\(M + H\) singly protonated molecular ion.

*A peptide resulting from the double-digestion procedure using both trypsin and Glu-C.*
same concentration as in our kinetic assays) was added into the PAP-binding assay, an increase in the $K_{d2}$ value was observed, and this became larger in the presence of 50 $\mu$M DHEA. A lowered binding affinity indicated by increases in $K_d$ values would then lead to destabilization of the PAP-enzyme-substrate ternary complex, a key component of substrate inhibition (Gulcan and Duffel, 2011). X-ray crystal structures (Pedersen et al., 2000; Rehse et al., 2002) indicate that Cys55 and Cys199 are in close vicinity to the PAPS/PAP binding pocket, and changes here may cause differences in the binding of the nucleotide substrate and product. Indeed, our modeling studies are consistent with small conformational changes in the PAPS/PAP binding site occurring on formation of mixed disulfides with glutathione at these two residues.

On reaction with DTNB, all three cysteines in hSULT2A1 formed mixed disulfides with TNB that were detected by LC-MS. As with the alteration of Cys55 and Cys199 near the PAPS/PAP site with GSSG, one would expect conformational changes that would affect binding at this site. Indeed, formation of TNB-cysteine adducts caused increases in the values of both $K_{d1}$ and $K_{d2}$ for the binding of PAP in the presence of 0.5 $\mu$M DHEA. Such changes in the binding of PAP are consistent with the elimination of a substrate inhibition complex, as evident from the increased $K_i$ in the kinetic assays. These conformational changes, however, had no effect on DHEA binding. It is also evident that differences in the effects of DTNB on substrate binding and catalysis compared with the other oxidants may relate to the very different structures of the cysteine disulfides/adducts formed.

LC-MS structural analysis showed the formation of a disulfide bond between Cys55 and Cys199 in hSULT2A1 after the reaction of the protein with diamide. When the LC-MS results were combined with our fluorescence studies, it was clear that the disulfide bond between Cys55 and Cys199 in hSULT2A1 resulting from diamide treatment caused an alteration in the conformation of the protein. The overall result of this modification in the structure of hSULT2A1 was a loss of substrate inhibition. When this result was combined with the effects of treatment of the enzyme with GSSG, where there was a loss of $K_{d2}$ for DHEA binding, we considered a previously proposed mechanism for substrate inhibition in hSULT1A1, where a second substrate binds at the same active site (Gamage et al., 2006). However, in the case of reaction of hSULT2A1 with DTNB, there are no significant changes in the $K_{d2}$ for DHEA binding. This finding indicates that there are either multiple mechanisms for substrate inhibition in hSULT2A1 that depend on the structure of the modified cysteine residue(s) or that the effects are due primarily to increases in the $K_d$ values for binding of PAPS/PAP.

Such changes in substrate inhibition and $K_d$ values highlight the complexity of protein conformational changes occurring in hSULT2A1.
The first studies on the crystal structure of hSULT2A1 (Pedersen et al., 2000) suggested that the possibility of conformational changes related to the dimeric structure could regulate enzyme activity. Indeed, an important dimer interface sequence in the active hSULT2A1 was identified by site-directed mutagenesis and kinetic studies (Petrochenko et al., 2001). Interestingly, there is a significant discrepancy between this dimeric interface and the interface formed between monomers in the crystals of hSULT2A1 (Petrochenko et al., 2001). Even though the crystal forms of the enzyme display varied dimeric interactions, the available crystal structures of hSULT2A1 indicate protein structural differences between ligand-free enzyme, DHEA-bound enzyme, and PAP-bound enzyme (Zhou et al., 2001). Zhou and coworkers also observed significant conformational changes in their attempts to add PAP to crystals containing the DHEA-bound hSULT2A1, wherein these crystals were destroyed by binding PAP (Zhou et al., 2001). Kinetic and ligand-binding studies on the substrate inhibition by DHEA and the formation of nonproductive inhibitory complexes have also pointed to conformational changes that give rise to more than one dissociation constant for substrates and products, depending on the complex formed (Gulcan and Duffel, 2011). Studies on a model of a monomeric hSULT2A1 (i.e., a fusion protein of monomeric hSULT2A1 and maltose-binding protein) have also indicated a loss of substrate inhibition in the monomer (Cook et al., 2010). More recent molecular modeling and molecular dynamics studies based on the crystal structures of hSULT2A1 suggest important conformational changes in the protein on the binding of PAPS/PAP that alter interactions with DHEA (Cook et al., 2012, 2013).

Additional mechanisms for catalytic regulation of hSULT2A1 could come from conformational alterations that interfere with the key amino acids involved in the catalytic step. Modifications of the cysteines in hSULT2A1 may have conformational effects on the binding cavity of PAPS/PAP as suggested by computational analysis in which the loops surrounding PAPS/PAP were shifted to a small extent after modification. According to these models, an alteration in the side-chain position was also observed for His99 after disulfide formation between Cys55 and Cys199. Despite the lack of changes in Lys44 and Ser129, small alterations in the position of His99 might decrease the efficiency of sulfuryl group transfer during catalysis.

A full interpretation of the significance of disulfide bond formation in hSULT2A1 must consider its applicability to intact cells and tissues. Previous studies with rSULT1A1 have shown that oxidative effects on the catalytic function of the purified enzyme (Marshall et al., 1997, 2000; Duffel et al., 2001) are consistent with effects seen in precision cut tissue slices (Dammanahalli and Duffel, 2012). Similarities in the mechanisms of catalytic alterations seen with the two SULTs suggest that regulation by disulfide bond formation may occur within intact cells; however, the response of hSULT2A1 to disulfide bond formation within intact cells remains to be determined. Our findings with the purified enzyme, however, are critical to the design of experimental approaches necessary to study the effects of disulfide bond formation on the enzyme within cells and tissues. The need for development of methods for isolating and characterizing hSULT2A1 from cells and tissues under conditions to preserve disulfide bonds and the importance of substrate concentration in studies of oxidized and reduced states of the enzyme will be key elements in developing experiments to examine these effects in cells and tissues. Those future studies will, however, be essential to assess more fully the potential impact of oxidative and reductive changes to hSULT2A1 and the impact of those changes on metabolism of endogenous hydroxysteroids, as well as drugs and other xenobiotics.

Authorship Contributions
Participated in research design: Qin, Teesch, Duffel.
Conducted experiments: Qin, Teesch.
Performed data analysis: Qin, Teesch, Duffel.
Wrote or contributed to writing of the manuscript: Qin, Teesch, Duffel.

References


Address correspondence to: Dr. Michael W. Duffel, College of Pharmacy, University of Iowa, 115 S. Grand Ave., Iowa City, IA 52242. E-mail: michael-duffel@uiowa.edu