In Vivo and In Vitro Oxidative Regulation of Rat Aryl Sulfotransferase IV (AST IV)

Smarajit Maiti, Sangita Maiti Dutta, Sharon M. Baker, Jimei Zhang, Telugu Narasaraju, Lin Liu, and Guangping Chen

Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA; E-mail: *cguang@okstate.edu*

Received 1 October 2004; revised 14 December 2004; accepted 17 December 2004

ABSTRACT: Sulfotransferase catalyzed sulfation is important in the regulation of different hormones and the metabolism of hydroxyl containing xenobiotics. In the present investigation, we examined the effects of hyperoxia on aryl sulfotransferase IV in rat lungs in vivo. The enzyme activity of aryl sulfotransferase IV increased 3- to 8-fold in >95% O_2 treated rat lungs. However, hyperoxic exposure did not change the mRNA and protein levels of aryl sulfotransferase IV in lungs as revealed by Western blot and RT-PCR. This suggests that oxidative regulation occurs at the level of protein modification. The increase of nonprotein soluble thiol and reduced glutathione (GSH)/oxidized glutathione (GSSG) ratios in treated lung cytosols correlated well with the aryl sulfotransferase IV activity increase. In vitro, rat liver cytosol 2-naphthol sulfation activity was activated by GSH and inactivated by GSSG. Our results suggest that Cys residue chemical modification is responsible for the in vivo and in vitro oxidative regulation. The molecular modeling structure of aryl sulfotransferase IV supports this conclusion. Our gel filtration chromatography results demonstrated that neither GSH nor GSSG treatment changed the existing aryl sulfotransferase IV dimer status in cytosol, suggesting that oxidative regulation of aryl sulfotransferase IV is not caused by dimer–monomer status change. © 2005 Wiley Periodicals, Inc. J Biochem Mol Toxicol 19:109–118, 2005; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jbt.20064

KEYWORDS: **Hyperoxia; Oxidative Regulation; Glutathione; Sulfotransferase; AST IV**

INTRODUCTION

Sulfotransferases (SULTs) catalyze the sulfuryl group transfer from the universal sulfuryl group donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a wide range of nucleophiles including endobiotics (i.e., hormones, monoamines, and bile acids) or xenobiotics [1–6]. Sulfation of hormones primarily results in their inactivation. Sulfation of xenobiotics leads to their detoxification or bioactivation depending on the nature of the metabolites. In rat liver, aryl SULT IV (AST IV or SULT1A1) and hydroxysteroid SULT a (STa) are expressed in greater quantities [7,8]. Adult rat lungs express a moderate amount of AST IV [7]. Reports on STa expression in adult lungs are contradictory [7,8], though neonatal rat lungs express STa protein. Extensive studies on SULT hormonal regulation [3,9] and a few studies on its xenobiotic regulation [10–12] have shown SULT regulation either at the translational and/or transcriptional level. Different nuclear hormone receptors and orphan nuclear receptors have been shown to be involved in some SULT induction processes [13–19].

The cytoplasm is a highly reducing environment (millimolar GSH) in which protein cysteine residues are maintained primarily in their thiol state [20]. Redox modification of Cys residues provides a mechanism for protein regulation. Proteins can be *S*-glutathionylated [21,22] or *S*-nitrosylated [23,24], especially during oxidative stress. Oxidative stress is involved in the pathogenesis of various degenerative diseases including cancer [25]. Many factors such as clinical oxygen treatment, chemical (toxicants) stress, physical stress, aging, virus infection, and different pathological conditions can cause oxidative stress [26–30]. The reactive oxygen species (ROS) formed can modify thiol bonds affecting the protein's function [20]. It is well known that oxidative stress can cause changes in the cellular thiol pool and GSH/GSSG ratios and levels in vivo

Correspondence to: Guangping Chen.
Contract Grant Sponsor: NIH.
Contract Grant Number: GM59873, HL052146, HL071628.

[31]. *S*-glutathionylation has been reported to regulate activity of various enzymes [32–39], although no data has been reported regarding in vivo SULT regulation. Only in vitro redox regulation using *E. coli* expressed AST IV has been reported [2,40–43]. Redox regulation of recombinant AST IV has been shown to include the modification of cysteine residues [42]. The ratio of the oxidized to reduced form of recombinant AST IV protein has been linked to bacterial culture conditions, i.e., growth temperature and oxygen supply [44]. This suggests that intracellular redox status, which can be altered by external factors, may regulate AST IV activity under different conditions. Though several studies explain the oxidative regulation of recombinant AST IV, to the best of our knowledge, no investigation has been carried out to demonstrate the behavior of this enzyme in its comparatively native state in the in vivo experimental model.

In the present investigation, we studied the expression and regulation of SULTs in lung and liver tissues of male rats exposed to >95% oxygen. Our results suggest that in vivo oxidative stress can regulate lung AST IV activity at the protein modification level. Understanding how SULTs are oxidatively regulated could be important in understanding their role in normal and abnormal biological processes. Changes in SULT activities can have either a positive or negative impact on human health through hormone regulation/metabolism and xenobiotic metabolism/detoxification.

MATERIALS AND METHODS

Materials

2-Naphthol, [^{14}C] 2-naphthol (4.7 mCi/mmol), *p*-nitro-phenyl sulfate (PNPS), 3′-phosphoadenosine 5′-phosphosulfate (PAPS), and [1,2,6,7-^{3}H(*N*)] dehydroepiandrosterone ([^{3}H] DHEA, 60 Ci/mmol) were purchased from Sigma-Aldrich (St. Louis, MO). SDS-polyacrylamide gel electrophoresis reagents were obtained from Bio-Rad (Hercules, CA). Western blot chemiluminescence reagent kits (Super Signal West Pico Stable Peroxide and Super Signal West Pico Luminol/Enhancer solutions) were purchased from Pierce Chemical (Rockford, IL). Nitrocellulose membrane (Immobilon-P; Millipore Corporation, Bedford, MA) used during Western blot procedure was purchased from Fisher Scientific Co. (Fair Lawn, NJ). Total RNA extraction kit (RNeasy mini protection kit) was supplied by QIAGEN (Valenica, CA). One-step RT-PCR kit was purchased from Promega (Madison, WI). Antibodies against AST IV and STa [45] were generously provided by Dr Michael W. Duffel (Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA). Protein assay reagent was purchased from Bio-Rad. All other reagents and chemicals were of the highest analytical grade available.

Animals and Drug Treatment

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) 250–275 g body weight were used in this investigation. Rats were housed in the Oklahoma State University laboratory animal resource facility and provided with rodent chow and water ad lib for at least 1 week before use. Two different sets of experiments were conducted. In the first set, rats were divided randomly into five groups with three in each. Rats of a single group were exposed to >95% O_2 for a given time period, i.e., 0, 3, 6, 12, or 24 h. In the second set, rats were divided randomly into four groups with three in each. Rats of a single group were exposed to >95% O_2 for a given time period, i.e., 0, 48, 60, or 72 h. Rats were treated with >95% O_2 in a sealed chamber connected to an oxygen cylinder as previously described [24]. The animals were sacrificed after the scheduled time period. Livers and lungs were collected, washed with sterile, ice-cold NaCl (0.9%, w/v), and snap frozen. Samples were stored at −80°C until use.

Cytosol Preparation

Tissues were homogenated in 50 mM Tris buffer containing 0.25 M sucrose, 0.01 mg/mL trypsin inhibitor and 10 μg/mL phenylmethylsulfonyl fluoride, pH 7.5. All homogenates were centrifuged at 100,000*g* for 1 h at 4°C [12]. Cytosol aliquots were collected and preserved at −80°C for enzymatic assay and Western blot.

Enzyme Assays

Two different enzyme assay methods were used.

PNPS Assay

AST IV (2-naphthol sulfation) activity from liver cytosol was determined as previously described [46,47]. Briefly, sulfation activity was determined in a reaction mixture containing 50 mM Tris buffer, pH 6.2, 5 mM PNPS, 20 μM PAPS, and 0.1 mM 2-naphthol. Rat liver cytosols (50 μg protein) were used as the enzyme source in a total reaction volume of 250 μL. After 30-min incubation at 37°C in a shaking water bath, the reaction was stopped by adding 250 μL of 0.25 M Tris, pH 8.7. The reaction mixtures were read at 401 nm in a spectrophotometer. Specific activity (SA) was expressed as nmol per minute per milligram of protein.

Radioactive Assay

AST IV activity in lung and STa activity in lung and liver cytosol were determined using the radioactive assay method as previously described [11,12,48]. Two hundred micrograms protein from lung cytosol was used to assay the AST IV activity. Fifty micrograms protein from liver cytosol and 200 μg protein from lung cytosol were used to assay the STa activity. For radioactive 2-naphthol sulfation activity, [^{14}C] 2-naphthol (4.7 mCi/mmol; 0.1 mM final concentration) was used as substrate. To determine STa (DHEA sulfation) activity, [^{3}H] DHEA (diluted to 0.4 Ci/mmol; 2 μM final concentration) was used as substrate. For all assays, 20 μM PAPS was used. All enzymatic reactions were performed in a total reaction volume of 250 μL. After 30-min incubation at 37°C in a shaking water bath, the reaction was stopped by adding 250 μL of 0.25 M Tris, pH 8.7. Extraction was performed twice by addition of 0.5 mL of water-saturated chloroform. After the final extraction, 50 μL of aqueous phase was used for scintillation counting. For the controls, PAPS was omitted in both assays. Assays were run in duplicate and the average of the results was used for enzyme activity calculations. The data are expressed as means ± SEM from three rats.

Western Blot Analysis

Cytosol proteins from lung (50 μg) and liver (10 μg) were separated on a 12% (w/v) polyacrylamide gel in an electrophoresis system (Novex, San Diego, CA) [45]. After running at 200 V, the protein bands were transferred overnight at 40 V onto a nitrocellulose membrane. All membranes were blocked in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, 0.05% (v/v) Tween-20) containing 5% (w/v) dry milk for 1 h on a shaker at room temperature. Membranes were incubated with either rabbit anti-AST IV or rabbit anti-STa (1:5000) in TBST containing 5% (w/v) dry milk for 2 h on a shaker at room temperature. After incubation, membranes were washed with TBST for 4 × 15 min and incubated with secondary antibody (horseradish peroxidase-conjugated immuno-pure goat anti-rabbit IgG; H+L) at 1:5000 dilutions in the same buffer for 2 h. The membranes were washed with TBST for 4 × 15 min and then with Tris buffered saline (TBS) 3 × 5 min. Fluorescent bands were developed with 1 mL of substrate containing same volume of each Super Signal West Pico Luminol Enhancer solution and Super Signal West Pico Stable Peroxidase solution at room temperature for 5 min. The X-ray films were exposed to the membrane and then developed. Films were scanned and the densitometry analysis was performed with AAB software in a Gel Documentation and Analysis System from Advanced American Biotechnology (Fullerton, CA).

Extraction of Total RNA and RT-PCR

Total RNA was extracted from lung and liver tissues (~20 mg) using RNeasy mini protection kit from QIAGEN according to the supplier's guidelines [12]. The concentration and purity of the extracted RNA were checked spectrophotometrically by measuring 260/280 absorption ratios. The primer pair for AST IV was designed in our laboratory using the Gene Fisher primer designing and Multialignment software. Using the forward primer (FP) 5′-GTGTCCTATGGGTCGTGGTA-3′/reverse primer (RP) 5′-TTCTGGGCTACAGTGAAGGTA-3′ (*GenBank accession no. X52883*), the 299- bp AST IV cDNA was synthesized. The 264-bp STa cDNA was synthesized using the primer pair FP 5′-TCCTCAAAGGATATGTTCCG-3′/RP 5′-CAGTTCCTTCTCCATGAGAT-3′ (*GenBank accession no. M33329*) [49]. The specificity of all primers was tested using the BLAST from the National Center for Biotechnology Information Open Reading Frame software. cDNA synthesis from total 1 μg of liver and 2 μg of lung RNA was performed in a 50 μL reaction mixture. Concentrations of the different ingredients used followed the supplier's guidelines. For internal control, 500-bp cDNA of rat β-actin was synthesized from the same amount of RNA from respective sources. The primer pair FP 5′-GATGTACGTAGCCATCCA-3′/RP 5′-GTGCCAACCAGACAGCA-3′ for the synthesis of rat β-actin cDNA was designed in our laboratory using the software mentioned above.

Determination of Nonprotein Soluble Thiol (NPSH)

Total nonprotein soluble thiol in lung and liver cytosol was determined by the standard DTNB (Ellman's reagent, 5,5′-dithiobis-2-nitrobenzoic acid) method [50] with a slight modification. Lung and liver tissues were homogenized with 50 mM Tris buffer, pH 7.5, containing 250 mM sucrose and 5 mM EDTA. Homogenates were centrifuged at 100,000*g* for 1 h. Equal volume of 5% (w/v) sulfosalisialic acid was used to precipitate all proteins from the tissue cytosols. After mild vortexing and centrifugation at 4000*g*, 80 μL of transparent sample supernatant was added to 720 μL of 0.1 M potassium phosphate buffer containing 5 μM DTNB. The absorbance was measured after 5 min at 412 nm in a UV-spectrophotometer. The steady state of the reaction kinetics was checked up to 7 min. A standard curve was generated using GSH and individual sample values were determined from this standard curve.

Determination of GSH/GSSG Ratios

GSH/GSSG ratios were determined by assaying separately the total GSH and GSSG in rat liver and lung with the glutathione assay kit (Cat #: 703002, Cayman Chemical Company, Ann Arbor, MI). The recommended protocol was followed. The recycling method was utilized using glutathione reductase for the quantification of GSH.

GSH Effect on 2-Naphthol Sulfation Activity in Rat Liver Cytosol

Rat liver cytosol was dialyzed for over 48 h in an air-saturated buffer without reducing reagent (50 mM Tris, 0.25 M sucrose, pH 7.4) in a cold room. This cytosol was treated with different concentrations of GSH (1, 2, 4, and 8 mM) for 1 h at room temperature. 2-Naphthol sulfation activity in the GSH treated cytosols (50 μg) was determined in the presence of varied concentrations of 2-napthol (1 μM–2 mM). The PNPS assay was employed to determine the enzyme activity.

Time- and Concentration-Dependent GSSG Inactivation of 2-Naphthol Sulfation Activity in Rat Liver Cytosol

Rat liver cytosol was incubated with 10 mM of GSH for 2 h at room temperature. It was then dialyzed (SPECTRA/POR, molecularporous membrane, mw cut off: 12–14 kDa) for 48 h in buffer (50 mM Tris, 0.25 M sucrose, pH 7.4) with a continuous supply of N_2 gas to keep proteins in the reduced state. This reduced cytosol was treated with different concentrations of GSSG (0.1, 0.2, 0.4, 0.8, and 1.6 mM) for different times (0,5,10,20,40,60 min) at room temperature. 2-Naphthol (0.1 mM) sulfation activities from GSSG treated cytosols (50 μg) were determined. The PNPS assay was employed to determine the enzyme activity. Triplicate experiments were done for each experimental data point.

Gel Filtration Chromatography

Rat liver cytosol (2.5 mg of protein) was incubated with either 10 mM GSH or 4 mM of GSSG for 1 h at room temperature. The cytosolic proteins were separated on a column (10 × 1000 mm) of Sephadex G-75 (Pharmacia fine chemicals) equilibrated with buffer (50 mM Tris, 250 mM sucrose, pH 7.4) containing either 0.5 mM GSH (in case of GSH treated cytosol) or 0.5 mM GSSG (in case of GSSG treated cytosol). Untreated cytosolic proteins were separated in the same column equilibrated with the same buffer without GSH or GSSG. Fractions were collected in tubes on a fraction collector at the rate of 1 mL/3 min. 2-Naphthol sulfation activity (PNPS assay, 5 mM GSH was added to reaction mixture for GSSG treated cytosol column fractions) was measured from different tubes and plotted against corresponding V_e/V_0. The respective V_e/V_0 from each treatment group at the peak enzyme activity was used in the standard curve to calculate the molecular weight of the protein responsible for 2-naphthol sulfation activity. V_0 of the column was determined using blue dextran. Standard curve for the plot of Log (MW) versus V_e/V_0 was determined using Molecular Weight Marker Kit For Gel Filtration Chromatography (MW-GF-200) from Sigma Chemical Company (cytochrome C 12.4 kDa, carbonic anhydrase 29 kDa, bovine serum albumin 66 kDa, alcohol dehydrogenase 150 kDa, and β-amylase 200 kDa).

Statistical Analysis

Student's t test was performed for the statistical significance between control and treated samples. Data presented in the figures denotes the mean ± SEM of the data collected separately from three individual animals.

RESULTS

Hyperoxic (>95% O_2) In Vivo Effect on Rat Liver and Lung 2-Naphthol and DHEA Sulfation Activities

Results in Figure 1A show that 2-naphthol sulfation activity (AST IV) increased slightly in liver of male rats treated with >95% oxygen for 48 h or more. High concentration of oxygen treatment did not alter DHEA sulfation activity (STa) in liver (Figure 1B). 2-Naphthol sulfation activity in the lung cytosol significantly increased from 12 to 48 h oxygen treatment (Figure 1C). It remained constant after 48 h. At 12 and 24 h, activity increased ~3-fold ($p < 0.05$) and ~5-fold ($p < 0.01$), respectively. From 48 through 72 h, activity increased ~8-fold ($p < 0.001$). DHEA sulfation activity in lung cytosol was undetectable. 4-Nitrophenol and 4-phenylphenol were also used to test the changes in enzyme activity of hyperoxia treated rat liver and lung cytosols. The results were similar to 2-naphthol (data not shown).

Figure 2 demonstrates the Western blot results from liver AST IV (2A), liver STa (2B) and lung AST IV (2C) proteins. Their densitometry analysis is shown in Figure 2D. Two different sets of experiments were conducted. In one set, rats were exposed to >95% oxygen for 0–24 h and in the other set time periods were 0, 48, 60, and 72 h. Two separate Western blot experiments were conducted. Figure 2D was plotted as relative densities. The results show that AST IV in liver (Figures 2A

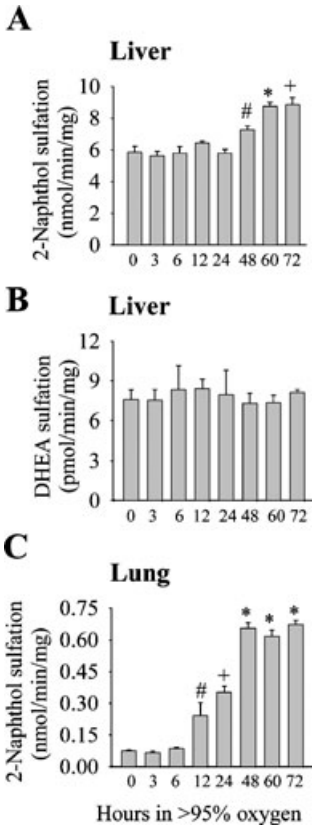

FIGURE 1. Effect of hyperoxia on 2-naphthol and DHEA sulfation activities in rat liver and/or lung. The PNPS assay method was employed to measure 2-naphthol sulfation in liver. Radioactive assay methods were used to determine liver DHEA and lung 2-naphthol sulfation activities. Liver cytosol (50 μg protein) and lung cytosol (200 μg protein) were used to assay the activities ($n = 3$). Data are mean ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$; $*p < 0.001$.

and 2D) began to increase slightly from 48 through 72 h, which can be correlated to the corresponding enzyme activity (Figure 1A). Western blot results of lung AST IV (Figures 2C and 2D) showed no significant alteration in protein amount in any of the treatment groups. Our present Western blot results of lung AST IV protein (Figure 2C) do not correlate with the corresponding enzyme activity results (Figure 1C). Lung AST IV activity increased 3- to 8-fold from 12 to 72 h treatment, whereas Western blot results (Figure 2C) and densitometry analysis (Figure 2D) of the protein showed no significant change after oxygen exposure. Rat liver STa protein levels (Figures 2B and 2D) did not change significantly with different time treatments. This is in agreement with DHEA sulfation activity results (Figure 1B).

AST IV and STa mRNA expression in liver (Figure 3A) and AST IV mRNA expression in lung (Figure 3B) did not change significantly in rats exposed to >95% oxygen. No detectable STa mRNA expression was observed in lung tissue in the present investigation. These results are in basic agreement with Western blot results.

Hyperoxic (>95% O_2) In Vivo Effect on Rat Liver and Lung Nonprotein Thiol

Nonprotein soluble thiol (NPSH) was measured in liver (Figure 4A) and lung (Figure 4B) cytosols. The GSH/GSSG ratios have been presented in Figures 4C and 4D in the same tissues, respectively. NPSH in liver did not change significantly after >95% oxygen treatment. NPSH in lung increased significantly from 12 to 48 h and then remained almost at the same level up to 72-h treatment. The increase paralleled the increase in AST IV activity. GSH/GSSG ratios in the liver tissue did not change significantly after oxygen treatment, but did change significantly in lung tissue from 24 to 72 h. This increase can be correlated with the increase in NPSH and 2-naphthol sulfation activity in lung.

GSH and GSSG In Vitro Effect on Rat Liver Cytosol 2-Naphthol Sulfation Activity

Rat liver cytosol was dialyzed at 4°C in air-saturated buffer (50 mM phosphate, 0.25 M sucrose, pH 7.4) for more than 48 h. This treatment (without reducing agent in the buffer) decreased 2-naphthol sulfation activity in the cytosol. The treated cytosol was used to investigate the GSH effect on 2-naphthol sulfation activity. The cytosol was incubated with different concentrations of GSH for 1 h at room temperature, and then 2-naphthol sulfation activities were determined at various 2-naphthol concentrations. Results shown in Figure 5 demonstrate that the liver cytosol 2-naphthol sulfation activity was activated by GSH in a concentration dependent manner. This suggests that the reduced form of AST IV is more active. Results in Figure 5 show that GSH activates AST IV in a wide substrate concentration range, suggesting that GSH activation of AST IV does not influence the enzyme's substrate inhibition properties. Rat lung cytosol showed a similar GSH activation property for 2-naphthol sulfation, although with much lower (100-fold) specific activity compared to liver cytosol (data not shown).

Figure 6 shows that reduced AST IV (rat liver cytosol) can be inactivated by GSSG in a time- and concentration-dependent manner. In these experiments, rat liver cytosol was first treated with 10 mM GSH for 2 h at room temperature. After treatment, the cytosol was dialyzed in the same way as described in the previous paragraph except that the buffer was continuously saturated with nitrogen. This treated cytosol was used for GSSG inactivation experiments.

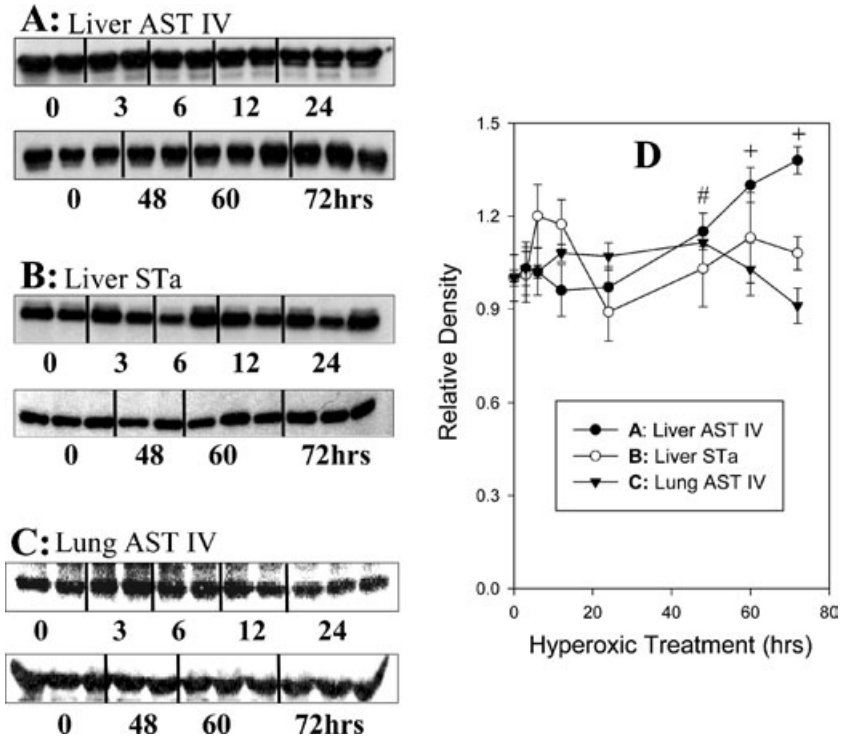

FIGURE 2. Western blot and densitometry analysis of hyperoxic liver AST IV, liver STa, and lung AST IV. Ten micrograms of liver cytosol and 50 μg of lung cytosol were used to run 12% bis-acrylamide SDS gels. Densitometry data are calculated as relative to the control of corresponding set of experiment. Values of the graph denote mean ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$; $*p < 0.001$.

The time- and concentration-dependent inactivation (Figure 6) suggests that the inactivation is specific. The oxidized form of AST IV is inactive. These results support the hypothesis that Cys residue modification of AST IV is responsible for the oxidative regulation of this enzyme.

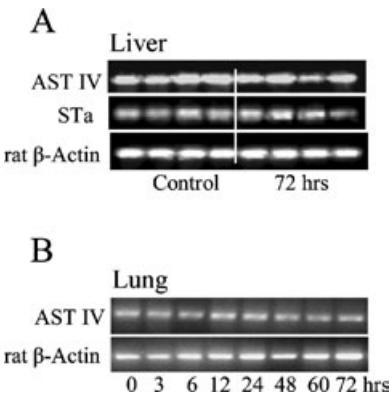

FIGURE 3. Effect of hyperoxia on mRNA expression of liver AST IV, liver STa, and lung AST IV. cDNA was synthesized from 1 μg of total RNA from liver and 2 μg of total RNA from lung in the presence of the gene-specific primer pairs. The reaction was conducted in a total volume of 50 μL reaction mixture and 10 μL of product was run in each lane of a 2% agarose gel.

Computer Modeling Structures Support Cys Residue Modification for Oxidative Regulation of AST IV

The on-line program, Swiss-Model, was used for the construction of model structures of AST IV and

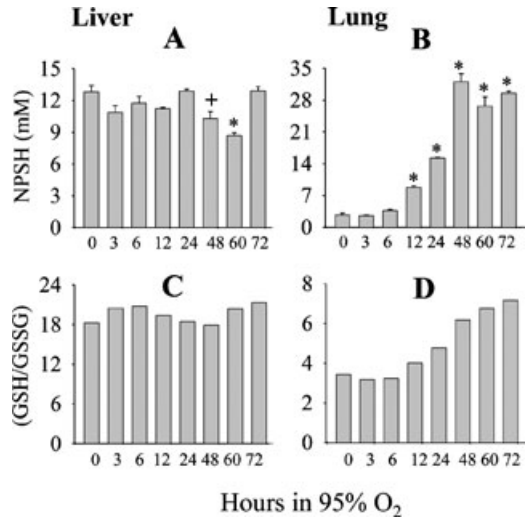

FIGURE 4. Effect of hyperoxia on nonprotein soluble thiol (NPSH) level and GSH/GSSG ratio in rat liver and lung. Values of the bars denote mean ± SEM. $^{\#}p < 0.05$; $^{+}p < 0.01$; $*p < 0.001$.

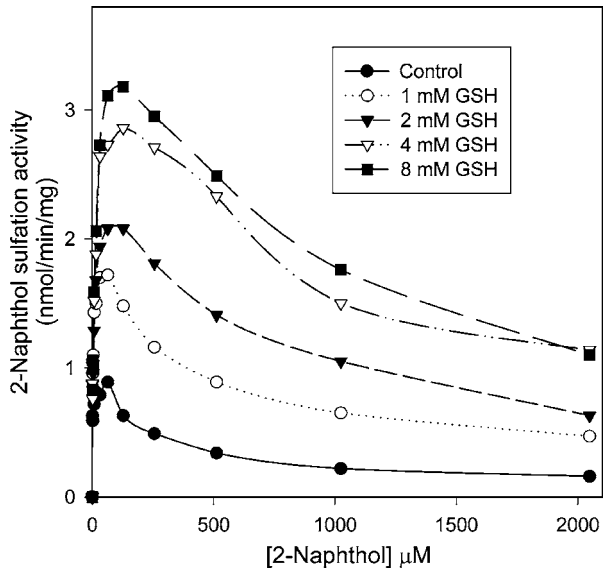

FIGURE 5. Effect of GSH on rat liver cytosol 2-naphthol sulfation activity. Fresh rat liver cytosol was dialyzed for 48 h in air saturation condition. Sulfation activity (50 μg) was assayed by the PNPS assay method in the presence of different concentration of 2-naphthol (1 μM–2 mM).

STa (Figure 7) based on all known SULT crystal structures. Computer modeling structures support the fact that AST IV can be oxidatively regulated while STa cannot be oxidatively regulated. We found that incubation of rat liver cytosol with 2 mM GSH or GSSG did not change DHEA sulfation activity (data not shown). The three Cys residues (Cys26, Cys54, and Cys198) in STa are distant (>13 Å) from the active site and buried in the internal structure. They are unlikely to be modified by GSSG. The chemical modification of these residues is unlikely to inactivate the enzyme. For AST IV, there are five Cys residues in the structure. Cys66, Cys232, Cys283, and Cys289 are located more than 11 Å from PAPS or substrate in the active site. On the other hand, Cys82 is in direct contact with substrate (3 Å) and is exposed on the substrate binding site surface. Cys82 should be easily chemically modified by GSSG. This modification would prevent the binding of substrate or releasing of product therefore inactivating the enzyme.

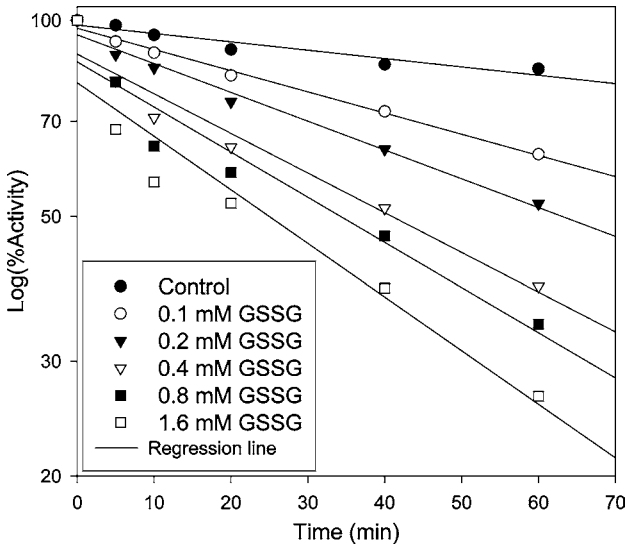

FIGURE 6. Time- and concentration-dependent GSSG inactivation of rat liver cytosol 2-naphthol sulfation activity. Rat liver cytosol was treated with 10 mM GSH for 2 h at room temperature. The reduced cytosol was dialyzed for 48 h with a constant supply of N_2. PNPS assay was used to determine 2-naphthol sulfation activity.

GSH and GSSG In Vitro Effect on Rat Liver AST IV Dimer–Monomer Status

In vitro, neither GSH nor GSSG changed the dimer–monomer status of AST IV in rat liver cytosol. Gel filtration experiments demonstrated that the enzyme activity peaks for untreated, 10 mM GSH-treated, and 4 mM GSSG-treated cytosols were very close in terms of V_e/V_0. The determined native molecular weight of AST IV is similar in untreated (65.8 kDa), GSH-treated (64.5 kDa), and GSSG-treated (65.0 kDa) cytosols (dimer). This suggests that GSH or GSSG cannot change the dimer–monomer status of AST IV in cytosol.

DISCUSSION

In the present investigation, we demonstrate that AST IV activity in lung increased in a time-dependent manner from 12 to 72 h with >95% oxygen exposure. This activity increase correlated well with NPSH levels and GSH/GSSG ratios, but did not correlate with the enzyme's protein (Western blot) or mRNA levels (RT-PCR). The changes seen in NPSH and GSH/GSSG are consistent with previous reports that a high concentration of oxygen exposure increases NPSH and GSH along with an increase in GSH/GSSG in lung tissue [51]. These results strongly suggest that the increase in rat lung AST IV activity seen with oxygen treatment is caused by protein modification. Our in vitro GSH activation and GSSG inactivation results demonstrate that Cys residue chemical modification can regulate AST IV activity significantly. Computer-based modeling of AST IV shows that Cys82 is present in close proximity to the substrate-binding site of the enzyme, suggesting the possible involvement of this residue in the redox dependent modification process. Our results indicate that oxidative regulation of AST IV is at the protein modification level rather than the gene transcription level.

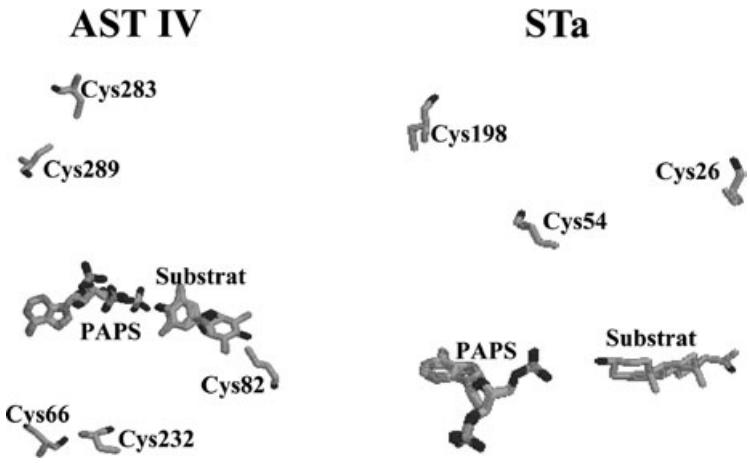

FIGURE 7. Computer modeling structures of AST IV and STa. Swiss-Model was used for the construction of model structures of AST IV and STa based on all known SULT crystal structures. RasMol 2.7 was used to display the structures. Only ligands and Cys residues are shown.

Oxidative regulation of SULTs is isoform specific. STa was not oxidatively regulated either in vivo or in vitro. This agrees with the computer modeling structure of STa (Figure 7).

Unlike lung SULTs, rat liver SULTs are not significantly regulated by oxidative stress. Reports on hyperoxic effects primarily use lung as a model. Liver tissue is not significantly affected under these treatments. The slight but significant increase in AST IV seen in liver after 48 h of >95% oxygen treatment did not correlate with NPSH or GSH/GSSG changes. This increase could be due to gene transcription regulation. The response of lung AST IV activity occurs from 12 to 48 h while liver AST IV occurs from 48 to 72 h. The liver AST IV activity increase correlated well with Western blot results. It is well known that different hormones can regulate SULT expression. Long-term high oxygen exposure may affect hormone levels and lead to an increase in liver AST IV protein expression.

A possible consequence of Cys residue modification is the change in SULT dimer status. SULTs exist in solution as dimers. Modification of Cys by GSSG could change the enzyme from a dimer to monomer. The proposed dimerization motif [52] demonstrated by sequence alignment and site directed mutagenesis studies revealed that there are 10 conserved amino acids near the C-terminus in cytosolic SULTs on the protein's surface. The zipper-like structure of this motif is associated with the homodimer structure of SULT, which is believed to be functionally active (an exception is active mouse estrogen SULT in its monomeric structure form) [52,53]. Cys283 and Cys289 in AST IV are located near the proposed dimerization motif at the C-terminus based on computer modeling structure of this enzyme (data not shown). They may have a role in mono/dimerization of this protein molecule resulting in activity interference. Our gel filtration chromatography results demonstrated that GSH or GSSG treatment of rat liver cytosol did not change AST IV dimer–monomer status. This further supports that Cys chemical modification rather than a change in dimer–monomer status is responsible for AST IV inactivation.

A possible mechanism for oxidative regulation of AST IV is the change of Cys–Cys disulfide bond status. The computer modeling structure (Figure 7) of AST IV suggests that Cys66/Cys232 and Cys283/Cys289 could form disulfide bonds. GSH/GSSG treatment could change the disulfide bond status (could also form GS–Cys disulfide bonds). A few reports on the redox effect on expressed AST IV suggested this mechanism [40–42]. It was suggested that a change in disulfide bond status could alter the protein's PAP binding ability, thus changing its catalytic activity. Our results agree with this possible mechanism.

In conclusion, our results suggest that active site Cys chemical modification can regulate AST IV activity both in vivo and in vitro. This is the first report on in vivo oxidative regulation of a SULT. Understanding how SULTs are regulated through oxidative stress is important clinically. Changes in SULT activities may have a significant health impact occurring through changes in hormone regulation and xenobiotic metabolism/detoxification.

ACKNOWLEDGMENT

The authors deeply appreciate the generous gift of aryl SULT IV (AST IV) and hydroxysteroid SULT a (STa) antibody from Dr Michael W. Duffel of University of Iowa.

REFERENCES

1. Coughtrie MW. Sulfation through the looking glass—Recent advances in sulfotransferase research for the curious. Pharmacogenomics J 2002;2(5):297–308.
2. Duffel MW, Marshal AD, McPhie P, Sharma V, Jakoby WB. Enzymatic aspects of the phenol (aryl) sulfotransferases. Drug Metab Rev 2001;33(3–4):369–395.
3. Klaassen CD, Liu L, Dunn RT II. Regulation of sulfotransferase mRNA expression in male and female rats of various ages. Chem Biol Interact 1998;109(1–3):299–313.
4. Falany CN. Enzymology of human cytosolic sulfotransferases. FASEB J 1997;11(4):206–216.
5. Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, Raftogianis RB. Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. FASEB J 1997;11(1):3–14.
6. Chatterjee B, Song CS, Kim JM, Roy AK. Androgen and estrogen sulfotransferases of the rat liver: Physiological function, molecular cloning, and in vitro expression. Chem Biol Interact 1994;92(1–3):273–279.
7. Dunn RT II, Klaassen CD. Tissue-specific expression of rat sulfotransferase messenger RNAs. Drug Metab Dispos 1998;26(6):598–604.
8. Runge-Morris MA. Sulfotransferase gene expression in rat hepatic and extrahepatic tissues. Chem Biol Interact 1994;92(1–3):67–76.
9. Runge-Morris M. Regulation of sulfotransferase gene expression by glucocorticoid hormones and xenobiotics in primary rat hepatocyte culture. Chem Biol Interact 1998;109(1–3):315–327.
10. Runge-Morris M, Rose K, Falany CN, Kocarek TA. Differential regulation of individual sulfotransferase isoforms by phenobarbital in male rat liver. Drug Metab Dispos 1998;26(8):795–801.
11. Maiti S, and Chen G. Tamoxifen induction of aryl sulfotransferase and hydroxysteroid sulfotransferase in male and female rat liver and intestine. Drug Metab Dispos 2003;31(5):637–644.
12. Maiti S, Chen G. Methotrexate is a novel inducer of rat liver and intestinal sulfotransferases. Arch Biochem Biophys 2003;418(2):161–168.
13. Assem M, Schuetz EG, Leggas M, Sun D, Yasuda K, Reid G, Zelcer N, Adachi M, Strom S, Evans RM, Moore DD, Borst P, Schuetz JD. Interactions between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and Mrp4 knockout mice. J Biol Chem 2004;279(21):22250–22257.
14. Saini SP, Sonoda J, Xu L, Toma D, Uppal H, Mu Y, Ren S, Moore DD, Evans RM, Xie W. A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. Mol Pharmacol 2004;65(2):292–300.
15. Kim MS, Shigenaga J, Moser A, Grunfeld C, Feingold KR. Suppression of DHEA sulfotransferase (Sult2A1) during the acute phase response. Am J Physiol Endocrinol Metab 2004;15:15.
16. Echchgadda I, Song CS, Roy AK, Chatterjee B. Dehydroepiandrosterone sulfotransferase is a target for transcriptional induction by the vitamin D receptor. Mol Pharmacol 2004;65(3):720–729.
17. Fang HL, Shenoy S, Duanmu Z, Kocarek TA, Runge-Morris M. Transactivation of glucocorticoid-inducible rat aryl sulfotransferase (SULT1A1) gene transcription. Drug Metab Dispos 2003;31(11):1378–1381.
18. Sonoda J, Xie W, Rosenfeld JM, Barwick JL, Guzelian PS, Evans RM. Regulation of a xenobiotic sulfonation cascade by nuclear pregnane X receptor (PXR). Proc Natl Acad Sci USA 2002;99(21):13801–13806.
19. Song CS, Echchgadda I, Baek BS, Ahn SC, Oh T, Roy AK, Chatterjee B. Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid × receptor. J Biol Chem 2001;276(45):42549–42556.
20. Paget MS, Buttner MJ. Thiol-based regulatory switches. Annu Rev Genet 2003;37:91–121.
21. Ghezzi P, Romines B, Fratelli M, Eberini I, Gianazza E, Casagrande S, Laragione T, Mengozzi M, Herzenberg LA. Protein glutathionylation: Coupling and uncoupling of glutathione to protein thiol groups in lymphocytes under oxidative stress and HIV infection. Mol Immunol 2002;38(10):773–780.
22. Lind C, Gerdes R, Hamnell Y, Schuppe-Koistinen I, von Lowenhielm HB, Holmgren A, Cotgreave IA. Identification of *S*-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteomic analysis. Arch Biochem Biophys 2002;406(2):229–240.
23. Ji Y, Toader V, Bennett BM. Regulation of microsomal and cytosolic glutathione *S*-transferase activities by *S*-nitrosylation. Biochem Pharmacol 2002;63(8):1397–1404.
24. Narasaraju TA, Jin N, Narendranath CR, Chen Z, Gou D, Liu L. Protein nitration in rat lungs during hyperoxia exposure: A possible role of myeloperoxidase. Am J Physiol Lung Cell Mol Physiol 2003;285(5):L1037–L1045.
25. Gopalakrishna R, Jaken S. Protein kinase C signaling and oxidative stress. Free Radic Biol Med 2000;28(9):1349–1361.
26. Jenkins RR. Exercise, oxidative stress, and antioxidants: A review. Int J Sport Nutr 1993;3(4):356–375.
27. Schwarz KB. Oxidative stress during viral infection: A review. Free Radic Biol Med 1996;21(5):641–649.
28. Spector A. Review: Oxidative stress and disease. J Ocul Pharmacol Ther 2000;16(2):193–201.
29. Maritim AC, Sanders RA, Watkins JB III. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003;17(1):24–38.
30. Poston L, Raijmakers MT. Trophoblast oxidative stress, antioxidants and pregnancy outcome—A review. Placenta 2004;25(Suppl A):S72–S78.
31. Kameyama S, Kondo M, Takeyama K, Nagai A. Air exposure causes oxidative stress in cultured bovine tracheal epithelial cells and produces a change in cellular glutathione systems. Exp Lung Res 2003;29(8):567–583.
32. Cappiello M, Voltarelli M, Giannessi M, Cecconi I, Camici G, Manao G, Del Corso A, Mura U. Glutathione dependent modification of bovine lens aldose reductase. Exp Eye Res 1994;58(4):491–501.
33. Lii CK, Chai YC, Zhao W, Thomas JA, Hendrich S. S-thiolation and irreversible oxidation of sulfhydryls on carbonic anhydrase III during oxidative stress: A method for studying protein modification in intact cells and tissues. Arch Biochem Biophys 1994;308(1):231–239.
34. Cabiscol E, Levine RL. The phosphatase activity of carbonic anhydrase III is reversibly regulated by glutathiolation. Proc Natl Acad Sci USA 1996;93(9):4170–4174.
35. Caselli A, Marzocchini R, Camici G, Manao G, Moneti G, Pieraccini G, Ramponi G. The inactivation mechanism of low molecular weight phosphotyrosine-protein phosphatase by H2O2. J Biol Chem 1998;273(49):32554–32560.

36. Lind C, Gerdes R, Schuppe-Koistinen I, Cotgreave IA. Studies on the mechanism of oxidative modification of human glyceraldehyde-3-phosphate dehydrogenase by glutathione: Catalysis by glutaredoxin. Biochem Biophys Res Commun 1998;247(2):481–486.
37. Reddy S, Jones AD, Cross CE, Wong PS, Van Der Vliet A. Inactivation of creatine kinase by *S*-glutathionylation of the active-site cysteine residue. Biochem J 2000;347(Pt 3):821–827.
38. Eaton P, Fuller W, Shattock MJ. *S*-thiolation of HSP27 regulates its multimeric aggregate size independently of phosphorylation. J Biol Chem 2002;277(24):21189–21196.
39. Weber H, Engelmann S, Becher D, Hecker M. Oxidative stress triggers thiol oxidation in the glyceraldehyde-3-phosphate dehydrogenase of Staphylococcus aureus. Mol Microbiol 2004;52(1):133–140.
40. Marshall AD, Darbyshire JF, Hunter AP, McPhie P, Jakoby WB. Control of activity through oxidative modification at the conserved residue Cys66 of aryl sulfotransferase IV. J Biol Chem 1997;272(14):9153–9160.
41. Marshall AD, Darbyshire JF, McPhie P, Jakoby WB. A review of the effects of manipulation of the cysteine residues of rat aryl sulfotransferase IV. Chem Biol Interact 1998;109(1–3):107–116.
42. Marshall AD, McPhie P, Jakoby WB. Redox control of aryl sulfotransferase specificity. Arch Biochem Biophys 2000;382(1):95–104.
43. Su TM, Yang YS. Mechanism of posttranslational regulation of phenol sulfotransferase: Expression of two enzyme forms through redox modification and nucleotide binding. Biochemistry 2003;42(22):6863–6870.
44. Yang YS, Tsai SW, Lin ES. Effects of 3′-phosphoadenosine 5′-phosphate on the activity and folding of phenol sulfotransferase. Chem Biol Interact 1998;109(1–3):129–135.
45. Chen G, Baron J, Duffel MW. Enzyme- and sex-specific differences in the intralobular localizations and distributions of aryl sulfotransferase IV (tyrosine-ester sulfotransferase) and alcohol (hydroxysteroid) sulfotransferase a in rat liver. Drug Metab Dispos 1995;23(12):1346–1353.
46. Chen G, Battaglia E, Senay C, Falany CN, Radominska-Pandya A. Photoaffinity labeling probe for the substrate binding site of human phenol sulfotransferase (SULT1A1): 7-azido-4-methylcoumarin. Protein Sci 1999; 8(10):2151–2157.
47. Chen G, Rabjohn PA, York JL, Wooldridge C, Zhang D, Falany CN, Radominska-Pandya A. Carboxyl residues in the active site of human phenol sulfotransferase (SULT1A1). Biochemistry 2000;39(51):16000–16007.
48. Chen G, Zhang D, Jing N, Yin S, Falany CN, Radominska-Pandya A. Human gastrointestinal sulfotransferases: Identification and distribution. Toxicol Appl Pharmacol 2003;187(3):186–197.
49. Pacifici GM, Franchi M, Bencini C, Repetti F, Di Lascio N, Muraro GB. Tissue distribution of drug-metabolizing enzymes in humans. Xenobiotica 1988;18(7):849–856.
50. Rahman I, Mulier B, Gilmour PS, Watchorn T, Donaldson K, Jeffery PK, MacNee W. Oxidant-mediated lung epithelial cell tolerance: The role of intracellular glutathione and nuclear factor-kappa B. Biochem Pharmacol 2001;62(6):787–794.
51. Rahman I, Bel A, Mulier B, Donaldson K, MacNee W. Differential regulation of glutathione by oxidants and dexamethasone in alveolar epithelial cells. Am J Physiol 1998;275(1, Pt 1):L80–L86.
52. Petrotchenko EV, Pedersen LC, Borchers CH, Tomer KB, Negishi M. The dimerization motif of cytosolic sulfotransferases. FEBS Lett 2001;490(1–2):39–43.
53. Kiehlbauch CC, Lam YF, Ringer DP. Homodimeric and heterodimeric aryl sulfotransferases catalyze the sulfuric acid esterification of *N*-hydroxy-2-acetylaminofluorene. J Biol Chem 1995;270(32):18941–18947.