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Redox regulation of human estrogen sulfotransferase (hSULT1E1)☆

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ABSTRACT

Sulfotransferases (SULTs) are enzymes that catalyze the sulfation of hydroxyl-containing compounds. Sulfation regulates hormone activities and detoxifies xenobiotics. Human estrogen sulfotransferase (hSULT1E1) catalyzes the sulfation of estrogens and regulates estrogen bioactivities. Oxidative regulation provides a biological mechanism for regulating enzyme activities *in vivo*. The oxidative regulation of human SULTs has not been reported. In this study, we used amino acid modification, manipulation of intracellular redox state, and site-directed mutagenesis to study the redox regulation of human SULTs and specifically the mechanism of hSULT1E1 inhibitory regulation by oxidized glutathione (GSSG). Of the four major human SULTs, hSULT1A1, hSULT1A3, and hSULT2A1 do not undergo redox regulation; hSULT1E1, on the other hand, can be redox regulated. GSSG inactivated hSULT1E1 activity in an efficient, time- and concentration-dependant manner. The co-enzyme adenosine 3′-phosphate 5′-phosphosulfate protected hSULT1E1 from GSSG-associated inactivation. A reduced glutathione (GSH) inducer (*N*-acetyl cysteine) significantly increased while a GSH depletor (buthionine sulfoxamine) significantly decreased hSULT1E1 activity, but both failed to affect the amount of hSULT1E1 protein in human hepatocyte carcinoma Hep G2 cells. Crystal structure suggested that no Cys residues exist near the active sites of hSULT1A1, hSULT1A3, and hSULT2A1, but Cys residues do exist within the active site of hSULT1E1. Site-directed mutagenesis demonstrated that Cys83 is critical for the redox regulation of hSULT1E1. This first report on the redox regulation of human SULTs suggests that the redox regulation of hSULT1E1 may interrupt the regulation and function of estrogens under various physiological and pathological conditions.

1. Introduction

Sulfotransferases (SULTs) are enzymes that catalyze the sulfation (sulfonation) of hydroxyl-containing compounds [1,2]. The universal sulfuryl group donor (co-substrate) for SULT-catalyzed sulfation is adenosine 3′-phosphate 5′-phosphosulfate (PAPS). The reaction products are a sulfated product and adenosine 3′,5′-diphosphate (PAP). One of the

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Abbreviations: SULT, sulfotransferase; SULT1E1, estrogen sulfotransferase; NAC, *N*-acetyl cysteine; BSO, buthionine sulfoxamine; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; E_2, 17β-estradiol; ER, estrogen receptor; ROS, reactive oxygen species; RNS, reactive nitrogen species

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main biological functions of SULTs is the regulation of various hormones [3]. Many of the endogenous hormones are substrates for different SULT isoforms [4]. Sulfation usually leads to the inactivation of hormones, as the sulfated forms of hormones are usually unable to bind to receptors [5]. Sulfated hormones could also be used for transport or storage of bioactive hormones [3].

SULTs also catalyze the sulfation of many structurally diverse drugs, carcinogens, and other xenobiotics. The substrate specificities of many SULTs are very broad [6]. Most hydroxyl groups in phenols, alcohols, and N-substituted hydroxylamines function as substrates for given cytosolic SULT isoforms. Sulfation of drugs and xenobiotics is primarily associated with detoxification by which a relatively hydrophobic xenobiotic is biotransformed into a more water-soluble sulfuric ester, which in turn is readily excreted. However, there are numerous important exceptions wherein the formation of chemically reactive sulfuric esters is an essential step in metabolic pathways leading to toxic or carcinogenic bioactivation [6]. Detoxification or bioactivation depends on the chemical properties of the sulfated product.

Human SULT1E1 (hSULT1E1) catalyzes the sulfation of estrone and estradiol with extremely high efficiency. Sulfation is believed to be involved in the inactivation of estrogens in target tissues [7]. Sulfation of active 17β-estradiol (E_2) forms inactive estradiol sulfate, which can be reactivated following desulfation by estrogen sulfatase [8].

The cytoplasm is a highly reducing environment (containing millimolar levels of GSH) in which protein cysteine residues are maintained primarily in their thiol state [9]. Redox modification of Cys residues of an enzyme provides a mechanism for regulating enzyme activity [10]. Proteins can be S-glutathionylated [11] or S-nitrosylated [12], especially during oxidative stress. Oxidative stress is involved in the pathogenesis of various degenerative diseases, including cancer [13]. Oxidative stress may be involved in breast cancer, and high levels of GSH is associated with a favorable outcome and good prognosis, whereas, low levels of GSH is associated with more aggressive or more advanced disease [14].

Many factors, including clinical oxygen treatment, chemical (toxicants) stress, physical stress, aging, virus infection, and different pathological conditions, can cause oxidative stress. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) can modify thiol bonds that affect a protein's function [15]. Oxidative stress is a well-known cause of changes in GSSG/GSH ratios and levels *in vivo* [10]. S-Glutathionylation regulates the activity of various enzymes [16,17], although no data has been reported regarding *in vivo* SULT regulation. Only *in vitro* redox regulation using *E. coli*-expressed rat aryl sulfotransferase IV (AST-IV or rSULT1A1) has been reported [18]. Our recent work [19,20] demonstrated that hyperoxia, physical stress, and chemical (parathion) stress regulate rSULT1A1 enzyme activity *in vivo*. Our *in vitro* redox regulation mechanism studies suggest that rSULT1A1 oxidative regulation occurs through Cys thiol modification. Oxidative stress that occurs in various pathological conditions may significantly alter SULT enzymatic activity, leading to changes in hormone regulation and xenobiotic drug detoxification/metabolism. Thus, it is important to understand the oxidative regulation of human SULTs and its mechanisms.

To the best of our knowledge, oxidative regulation of human SULTs has not been reported. In the present investigation, we used human liver cytosol and purified recombinant human SULTs to study the effect of GSSG on human SULT activity. We applied either a GSH inducer (*N*-acetyl cysteine [NAC]) or a GSH depletor (buthionine sulfoxamine [BSO]) to human hepatocyte carcinoma (Hep G2 cells) to test how varying intracellular GSH levels affect hSULT1E1 enzymatic activity. These results, as well as site-directed mutagenesis studies we report here, clearly reveal that hSULT1E1 activity can be regulated by redox modification of its cysteine residues.

2. Methods and materials

2.1. Materials

Ampicillin,17β-estradiol (E_2), 3′-phosphoadenosine 5′-phosphosulfate (PAPS), dithiothreitol, buthionine sulfoxamine (BSO), *N*-acetylcysteine (NAC), dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), and oxidized glutathione (GSSG) were purchased from Sigma. SDS-polyacrylamide gel electrophoresis reagents were purchased 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 membranes (Immobilon-P; Millipore Corporation, Bedford, MA) were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Protein assay reagent was purchased from Bio-Rad. All other reagents and chemicals were of the highest analytical grade available.

2.2. hSULT1E1 enzyme activity assay

hSULT1E1 activities in human liver cytosol, Hep G2 cell cytosol, bacterial cytosol (50 μg protein from each) and in purified enzyme (3 μg protein) were determined by the radioactive assay method [21–24]. [^{3}H]E_2 (0.9 Ci/mmol; 0.15 μM final concentration) was used as substrate for the reaction. For all assays, 20 μM PAPS was used in a total of 250 μl reaction mixture containing 50 mM Tris buffer (pH 6.2). After a 30-min incubation in a shaking water bath (37 °C), the reaction was stopped by adding 250 μl of 0.25 M Tris (pH 8.7). Extraction was performed twice by adding 0.5 ml of water-saturated chloroform each time. After the final extraction, 100 μl of aqueous phase was used for scintillation counting. The data collected from the enzymatic assay from each protein source were the average of results obtained from three independent experiments.

2.3. hSULT1E1 inactivation by GSSG

Samples containing either human liver cytosol (final concentration, 1.0 mg/ml) or purified hSULT1E1 (final concentration, 0.1 mg/ml) were incubated in Tris buffer (pH 6.2) at room temperature with various concentrations of GSSG for different durations, as indicated in the figures. Aliquots (50 μl) of the mixture were used to determine E_2 sulfation (hSULT1E1) activity as described above. The proper control experiments were performed by adding an equal volume of water.