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A nano switch mechanism for the redox-responsive sulfotransferase

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ABSTRACT

Cellular redox signaling is important in diverse physiological and pathological processes. The activity of rat phenol sulfotransferase (*r*SULT1A1), which is important for the metabolism of hormone and drug, is subjected to redox regulation. Two cysteines, Cys232 and Cys66, nanometer away from each other and from the enzyme active site were proposed to form disulfide bond to regulate the activity of *r*SULT1A1. A nano switch, composed of a flexible loop from amino acid residues 59–70, explained how this long distance interaction between two cysteines can be achieved. The enzyme properties were investigated through site-directed muatagnesis, circular dichroism, enzyme kinetics and homologous modeling of the *r*SULT1A1 structures. We proposed that the formation of disulfide bond between Cys232 and Cys66 induced conformational changes of sulfotransferase, then in turn affected its nucleotide binding and enzyme activity. This discovery was extended to understand the possible redox regulation of other sulfotransferases from different organisms. The redox switch can be created in other redox-insensitive sulfotransferases, such as human phenol sulfotransferase (*h*SULT1A1) and human alcohol sulfotransferase (*h*SULT2A1), to produce mutant enzymes with redox regulation capacity. This study strongly suggested that redox regulation of drug and hormone metabolism can be significantly varied even though the sequence and structure of SULT1A1 of human and rat have a high degree of homology.

1. Introduction

Cellular redox signaling contributes to the control of cell development, differentiation, growth, death and adaptation, and has been implicated in diverse physiological and pathological processes [1]. The non-equilibrium nature of biological systems necessitates temporal or spatial suppression or enhancement of specific biochemical pathways, either gene regulation or protein modification, according to cellular metabolism or exogenous signals [2]. 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 [3]. Oxidative stress is a well-known cause of changes in GSSG/GSH ratios and levels *in vivo* [4].

Previous study indicated that stress and oxidative regulation of rat phenol sulfotransferase (*r*SULT1A1) occurs at the level of protein modification, but not at gene expression [5,6]. *In vitro* studies confirmed that a highly conserved Cys66 is important for the regulation of *r*SULT1A1 catalytic activity [7,8]. Redox status and nucleotide binding have been shown to regulate catalytic activity of *r*SULT1A1 and are shown to be responsible for the complete interconversion of two enzyme forms [9]. It is intriguing to observe that cytosolic SULTs can be regulated either at gene level or at protein level through redox treatments [7,8]. What is the distinction between these two types of enzyme and if the redox regulation can be created in all cytosolic SULT are the subjects of this research.

Sulfonation (Fig. 1) catalyzed by cytosolic sulfotransferase in biological system is a popular and important biotransformation that involves in detoxication of a broad range of endobiotics and xenobiotics and activation and deactivation of hormones and carcinogens [10,11]. Macromolecular substrates such as proteins and polysaccharides are metabolized by membrane-bound SULTs [12]. Recent studies have implicated the SULTs in a number of disease states including various forms of cancer [13], entry of the herpes virus [14], enterovirus [15,16] or HIV [17,18], and chronic inflammation [19]. They are novel therapeutic targets to discover new drugs [20].

Our approach to study this phenomenon is to first understand the mechanism for SULT redox regulation according to the sequence, structure and catalytic function of SULTs. It has been proposed that two distanced cysteines, Cys232 and Cys66, in this enzyme may be responsible for the redox regulation of its catalytic

Abbreviations: Bis–tris propane, 1,3-bis[tris(hydroxymethyl) methylamino] propane; CD, circular dichroism; DEAE, diethylaminoethyl; GSSG, glutathione (oxidized form); GST, glutathione S-transferase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SULT, sulfotransferase; TCEP, tris(2-carboxyethyl) phosphine.[2]

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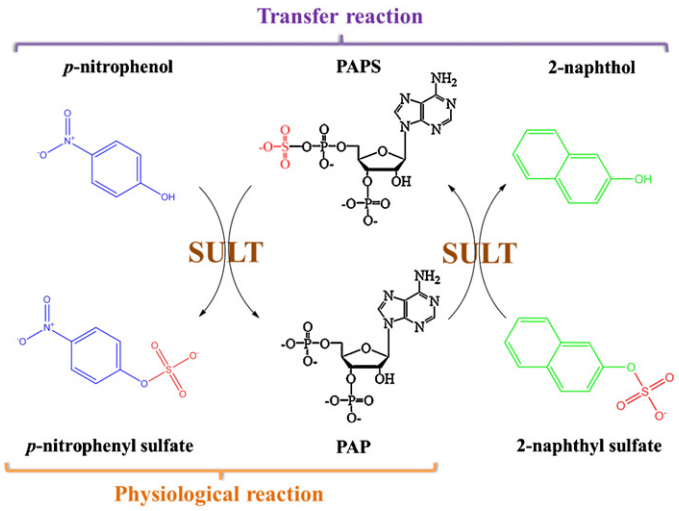

Fig. 1. Reactions catalyzed by SULT1A1.

activity [8]. Neither cysteine is near the enzyme active site. How the formation of potential disulfide bond can affect the *r*SULT1A1 activity is unclear. Furthermore, the involvement of the three other cysteines in *r*SULT1A1 needs to be explained in a redox environment. Sequence and structural analyses of SULTs indicated that the flexible loop (amino acid residue 59–70) and Trp49 of *r*SULT1A1 may closely relate to redox regulation of its activity. How these amino acids coordinate to function as a redox switch is to be explained. Finally, it is important to understand the difference between rat and human SULT1A1 because rat is frequently used as animal model to study human diseases. To understand the structural and mechanistic basis for redox regulation of cytosolic SULTs would be a remarkable progress to elucidate the role in the metabolism of carcinogens, drugs, neurotransmitters and xenobiotics, and may assist in chemical risk assessment and the design of more effective therapeutics.

2. Materials and methods

2.1. Reagents

Bis–tris propane, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), dithiothreitol, (ethylenedinitrilo) tetraacetic acid (EDTA), glutathione reduced form (GSH), glutathione oxidized form (GSSG), 4-methylumbelliferone (MU), 4-methylumbelliferyl sulfate (MUS), 2-naphthol, N^6-(6-aminohexyl)adenosine 3′,5′-diphosphate, 3′-phosphoadenosine 5′-phosphate (PAP), PAP agarose, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), *p*-nitrophenol (*p*NP), *p*-nitrophenyl sulfate (*p*NPS) were purchased from Sigma (USA). Tris(2-carboxyethyl) phosphine (TCEP) was obtained from Pierce (USA). Glycerol, potassium phosphate and sucrose were obtained from J.T. Baker (USA). DEAE Sepharose fast flow, glutathione S-transferase sepharose fast flow, and HiTrap™ desalting column was obtained from GE Healthcare (USA). All other chemicals were obtained commercially at the highest purity possible.

2.2. Computational analysis of protein sequence and structure

SULT sequences were obtained from National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). SULT structures used in this report were solved by X-ray crystal diffraction and obtained from Protein Data Bank (PDB, http://www.rcsb.org/pdb/). The molecular modeling of *r*SULTT1A1 structure was done at SWISS-MODEL (http://www.expasy.org/swissmod/SWISS-MODEL.html). The first approach mode was run [21] and hSULT1A1 was used as template. Amino acid sequences of SULTs were aligned using ClustalW program at European Bioinformatics Institute (EBI, http://www.ebi.ac.uk/Tools/clustalw/index.html), and their protein structures were compared and aligned using the Combinatorial Extension (CE) method (http://cl.sdsc.edu/ce.html).

2.3. Site-directed mutagenesis of cDNA encoding SULTs

Site-directed mutagenesis was performed with *PfuTurbo*® DNA polymerase using QuickChange (Stratagene, La Jolla, CA). All primers for mutagenesis were purchased from Mission Biotech Co., Ltd. (Taiwan). The cDNA of *r*SULT1A1 and human SULT incorporated in the pET-3c and pGEX-2TK expression vector were used as templates in conjunction with specific mutagenic primers, respectively. Mutated cDNA sequences were confirmed using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA) following the standard protocol. Six mutant clones and their primers for mutagenesis were prepared: *r*SULT1A1 mutant C66S (forward primer, 5′-G CTA GAG AAG **AGT** GGC CGC GCC CCC-3; reverse primer, 5′-G GGC GCG GCC **ACT** CTT CTC TAG CTT GCC-3′), *r*SULT1A1 mutant C232S (forward primer, 5′-G AAA GAG AAC **AGC** ATG ACT AAC TAC AC-3′; reverse primer, 5′-A GTT AGT CAT **GCT** GTT CTC TTT CAT TTT C-3′), *h*SULT1A1 mutant P236C (forward primer, 5′-G AAG AAG AAC **TGC** ATG ACC AAC TAC ACC-3′; reverse primer, 5′-A GTT GGT CAT **GCA** GTT CTT CTT CAT CTC-3′), *h*SULT2A1 mutant I66C (forward primer, 5′-T GCC AAG TGG **TGC** CAA TCT GTG CCC ATC-3′; reverse primer, 5′-G CAC AGA TTG **GCA** CCA CTT GGC ATC CCC-3), *h*SULT2A1 mutant K227C (forward primer, 5′-G AAA GAA AAC **TGC** ATG TCC AAT TAT TCC C-3′; reverse primer, 5′-A ATT GGA CAT **GCA** GTT TTC TTT CAT GCT C-3′), and *h*SULT2A1 mutant I66C/K227C. Bold and underlined nucleotides indicated the designated positions for site-directed mutagenesis.

2.4. Expression and purification of SULT recombinant enzymes

Recombinant wild type and mutant SULT clones were transformed into *Escherichia coli* BL21 (DE3) and over-expressed. Detail methods were described previously [22–24]. SULTs were extracted by sonication, and purified by DEAE Sepharose chromatography and GST gene fusion system (GE Healthcare, USA). The two forms of SULT1A1 were separated by PAP-agarose chromatography as described previously [9]. All enzymes used in this study were purified and the purity was at least 95% homogeneity according to SDS-PAGE.

2.5. Preparation of reduced and oxidized SULTs

SULTs were continuously reduced, oxidized and reduced again to prepare enzymes in different redox stages and to demonstrate that these enzymes were reversible under redox environments. Purified SULTs were passed through HiTrap™ desalting column with exchange buffer (1 mM EDTA, 10% glycerol, and 125 mM sucrose in 100 mM bis–tris propane at pH 7.0) to remove possible interfering reagents. SULTs (1 ml, 20 μM dimmer) were then incubated in 1 mM TCEP for 60 min at 25 °C to give the reduced SULTs. Excess amount of the reducing agent was removed by desalting chromatography. The oxidized SULTs were prepared by incubating the reduced SULTs (1 ml, 10 μM dimmer) with GSSG (1 mM) at 25 °C for 60 min. The excess amount of GSSG was removed through HiTrap™ desalting column. The oxidized SULTs

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