Crystal structures of SULT1A2 and SULT1A1*3: Insights into the substrate inhibition and the role of Tyr149 in SULT1A2

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1. Introduction

In mammals, the cytosolic sulfotransferases (SULTs) play important roles in the regulation of the levels and activities of neurotransmitters and steroid/thyroid hormones as well as in the inactivation and elimination of xenobiotics [1–3]. These enzymes catalyze the transfer of a sulfonate group from the “active sulfate”, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to nucleophilic sites of their substrates. The resulting sulfuric esters usually have increased water-solubility and can be readily excreted. Thus, sulfonation as catalyzed by the SULTs is often associated with inactivation and removal of metabolites and waste compounds. In some cases, however, the sulfuric esters derived from benzylic and allylic alcohols or aromatic hydroxylamines may be chemically reactive toward nucleophilic sites on DNA, RNA, and proteins, inducing toxic effects or mutations thereby leading to carcinogenic response [4–6].

It is now known that all SULTs from mammals constitute a gene superfamily and, based on amino acid sequence homology, distinct gene families have been further categorized [7]. Two major gene families among them are the phenol SULT family (designated SULT1) and hydroxysteroid SULT family (designated SULT2). For humans, the SULT1 family presently includes SULT1A1, 1A2, 1A3, 1B1, 1C1, 1C2, and 1E1. Although each of these SULTs has distinct substrate preference, they may exhibit somewhat broad and overlapping substrate specificity to span the diversity of chemicals requiring sulfonation. For example, SULT1A1 and SULT1A2 preferentially catalyze the sulfonation of neutral phenolic compounds such as p-nitrophenol (pNP), but they can also use dopamine, the classical substrate of SULT1A3, as substrate albeit with lower affinity [8,9]. Both SULT1A1 and SULT1A2, which share 49% amino acid identity with SULT1E1, also are capable of sulfonating estrogens and their derivatives, catecholestrogens (CEs) [10]. In addition, a wide range of (more) hydrophobic molecules, including (pro)carcinogens, 1-hydroxymethylpyrene (1-HMP), 2-hydroxylaminophenolpyridine (OH-APP), and N-hydroxy-2-acetylaminofluorene (OH-AAF) [11], and endogenous compounds, e.g., 3,3’-diiodothyronine (T2), can be the substrates of SULT1A2 and SULT1A1 [12,13]. Recent pharmacogenetic studies have revealed the genetic polymorphisms of SULT1A1 and SULT1A2 [14,15]. Both SULT1A1 and SULT1A2 genes include a series of allelic variant forms encoding allozymes that show individual differences in enzyme activity as well as other biochemical and physical properties. For example,

** Abbreviations: SULT, cytosolic sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; pNP, p-nitrophenol.
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The cytosolic sulfotransferases (SULTs) in vertebrates catalyze the sulfonation of endogenous thyroid/steroid hormones and catecholamine neurotransmitters, as well as a variety of xenobiotics, using 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as the sulfonate donor. In this study, we determined the structures of SULT1A2 and an allozyme of SULT1A1, SULT1A1*3, bound with 3′-phosphoadenosine 5′-phosphate (PAP), at 2.4 and 2.3 Å resolution, respectively. The conformational differences between the two structures revealed a plastic substrate-binding pocket with two channels and a switch-like substrate selectivity residue Phe247, providing clearly a structural basis for the substrate inhibition. In SULT1A2, Tyr149 extends approximately 2.1 Å further to the inside of the substrate-binding pocket, compared with the corresponding His149 residue in SULT1A1*3. Site-directed mutagenesis study showed that, compared with the wild-type SULT1A2, mutant Tyr149Phe SULT1A2 exhibited a 40 times higher V_max and two times lower K_m with p-nitrophenol as substrate. These latter data imply a significant role of Tyr149 in the catalytic mechanism of SULT1A2.

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SULT1A1*2, a SULT1A1 allelic variant, having a His residue at position 213, is associated consistently with the inherited traits of lower sulfating activity and thermal stability than variants having an Arg residue at that position [14]. Moreover, SULT1A1*2 activates certain procarcinogens with a much lower activity than those allelic variants having an Arg residue at position 213 [6,16]. Interestingly, SULT1A2, which shares 96% amino acid identity with SULT1A1, unexpectedly showed much stronger activities in the bioactivation of OH-AAF and OH-APP compared with SULT1A1 [11].

To elucidate the structural basis of their catalytic mechanisms and substrate specificity, an increasing number of SULT crystal structures have been determined. Some examples include the mouse SULT1E1 complexed with PAP/17b-estradiol (E2) (Protein Data Bank (PDB) Code 1AQU) [17], human SULT1A1 complexed with pNP (1LS6) [18], human SULT1E1 complexed with PAPS (1HY3) [19], human SULT1A3 (1CJM) [20,21], human SULT2A3 (1EHF) [22], and SULT2B1 complexed with dehydroepiandrosterone (1Q22) or pregnenolone (1Q20) [23]. All of these structures contain a common PAPS-binding region and a variable substrate-binding region. Among them, only the structure of a SULT1A1 allozyme, SULT1A1*2, was determined in the presence of two xenobiotic substrates and provided the first clue for substrate inhibition and binding. As mentioned above, however, SULT1A1*2 is a SULT1A1 variant having lower stability and decreased activity for procarcinogens.

The genetic polymorphisms of members of the SULT1A subfamily represent a good experimental model for unraveling the critical features that determine the substrate preference and enzymatic activity of SULTs. Here, we report the first crystal structure of SULT1A2 and the crystal structure of a SULT1A1 allozyme, SULT1A1*3. These two structures revealed apparent conformational changes around the substrate-binding pocket. Site-directed mutagenesis study was performed to verify the importance of residue Tyr149 which in SULT1A2, extends approximate 2.1 Ä further to the inside of the substrate-binding pocket, compared with the corresponding residue in SULT1A1*3.

2. Experimental procedures

2.1. Materials

pNP, aprotinin, thrombin, Trizma base, and reagents used for crystallization and data collection were obtained from Sigma Chemical Company. BL21 Escherichia coli cells were from Stratagene. pGEX-2TK glutathione-S-transferase gene fusion vector and glutathione Sepharose 4B were products of Amersham Biosciences. pGEX-2TK harboring SULT1A2 or SULT1A1*3 cDNA was prepared as previously described [24]. All other chemicals were of the highest grade commercially available.

2.2. Expression, purification, and crystallization

Human SULT1A2 or SULT1A1*3 cDNA was constructed into pGEX-2TK vectors and expressed in BL21 cells as previously described [24]. The proteins were purified by affinity chromatography on a glutathione–Sepharose 4B column based on a previously described procedure [25]. Human SULT1A2 Tyr149Phe mutant was generated using the QuickChange Site-directed Mutagenesis Kit and purified as above.

Crystallization of SULT1A2 and SULT1A1*3 was carried out using the hanging drop vapor diffusion method and the sitting drop vapor diffusion method, respectively. Initial conditions were identified from Hampton Research commercial screens and then optimized by incremental scanning around the initial conditions.

SULT1A2 crystals were grown at 18 °C after mixing 2 µl of 10 mg/ml purified protein with 2 µl of reservoir solution (0.1 M Hepes, pH 7.0, 18% polyethylene glycol 8000, 0.3 M Ca(Ac)2) on the coverslip. This was equilibrated over 500 µl of the reservoir solution and monoclinic crystals appeared after 4–5 days. In the crystallization of SULT1A1*3, 10 mg/ml purified protein was preincubated with 4 mM PAP and 0.08% (w/v) BAM at 4 °C for 20 min. Thereafter, 4 µl of reservoir solution (0.1 M MES, pH 6.0, 14.75% polyethylene glycol 4000, 20 mM Ca(Ac)2) was mixed with 4 µl of protein solution on the Micro-bridge, placed inside the well containing 500 µl reservoir solution and incubated at 7 °C. Orthorhombic crystals (0.2 × 0.2 × 0.05 mm) appeared after about 2 weeks.

2.3. Data collection and refinement

X-ray diffraction data of SULT1A2 were measured on a Rigaku R-AXIS IV° system at the Institute of Biophysics, CAS (Beijing, China). The crystal was immersed in cryoprotectant (20% polyethylene glycol 8000, 0.1 M Hepes, pH 7.0, 15% MPD, and 0.3 M Ca(Ac)2) 3–5 min prior to flash-cooling in liquid nitrogen. The SULT1A1*3 data set was collected on a MAR-DTB area plate system at BSRF (Beijing, China) beamline 3W1A using the cryoprotectant of 0.1 M MES, pH 6.0, 15% PG-4000, 10 mM Ca(Ac)2, and 15% MPD. For data measurement, the pre-frozen crystals were transferred into a stream of nitrogen gas cooled to 100 K. Diffraction data of SULT1A2 and SULT1A1*3 extended to 2.4 Å and 2.3 Å, respectively, and were integrated, merged, and scaled using the DENZO/SCALEPACK program package [26].

The structure of SULT1A2 was solved by using the program Molrep [27] using SULT1A1*2 (96% sequence identity, PDB Code 1LS6) as the search model. The structure of SULT1A1*3 was solved by using the program AmoRe [28] using mouse estrogen SULT (SULT1E1) (PDB Code 1AQU) as the search model. Subsequently, SULT1A2 and SULT1A1*3 structures were refined to 2.4 Å and 2.3 Å resolution, respectively, in CNS software package [29] and iterative manual model building were carried out with the program O [30]. All statistics for data collection, model building, and refinement were summarized in Table 1. Structure drawings were generated using the programs Molscript [32], Raster3D [33], and Pymol [34].

2.4. Enzymatic assay

Procedures for the determination of the sulfating activity of wild-type and mutated SULT1A2 were the same as previously described [24].

3. Results and discussion

3.1. Overall structure of SULT1A2 and SULT1A*3

The basic organization of the crystal structures of SULT1A1*3 and SULT1A2 with PAP but without acceptors are for all intents and purposes identical (Fig. 1). Therefore, the discussion will focus on the SULT1A2 structure and the differences between SULT1A2 and SULT1A1*3. The overall structure of SULT1A2 is that of the classical SULT fold consisting of α/β motif comprised of a central 5-stranded parallel β-sheet surrounded by helices on either side. Interestingly, PAP was bound to the protein prior to the crystallization in the binding mode as described previously for the interaction of PAP with SULT1A1*2 [18]. Furthermore, there were on calcium cation and one acetate anion at the acceptor binding site of SULT1A2, which is probably because of the presence of high concentrations of calcium acetate in the crystallization condition (Fig. 4).
3.2. Substrate-binding pocket

The substrate-binding sites of the acceptor-free SULT1A1*3 and ion-bound SULT1A2 are more like a V-shaped pocket, which was described as an L-shaped pocket in the structure of SULT1A1*2 [18]. In SULT1A2, Phe247 and Ile89 occupy the opening of the V-shaped pocket so that the two ‘wings’ of ‘V’ represent two substrate-binding channels with an angle about 65° and the bottom of ‘V’ is the catalytic cavity (Fig. 2). The channel I is between Phe247 and Val148, and the channel II is composed of Phe76, Met77, Ile89, and Pro90. Phe24, Phe81, and Phe84 constitute the common hydrophobic base of the pocket, while Phe142, Met248, and Phe255 form the top of the cavity (not shown in Fig. 2 for clarity). Although the wings, the base and the top of the substrate-binding pocket incorporate predominantly hydrophobic aromatic and aliphatic residues, the bottom and the side of the catalytic cavity are hydrophilic. The proposed catalytic residue Lys48 and His108 lie at the bottom of the cavity. The hydroxyl headers of Tyr240 and Tyr149 extend to the cavity from both sides, which constitute the hydrophilic side wall of the substrate-binding pocket. It is worthy of mention that the residues constituting the substrate-binding pocket in SULT1A2 are the same as those in SULT1A1*3 except that, in SULT1A2, Tyr149 (substituting His149 in SULT1A1*3) extends approximate 2.1 Å further to the inside of the substrate-binding pocket (Fig. 3).

Significant conformational changes are also observed in two conserved regions, residues 82–92 (loop 82–92) and residues Phe247 and Val148, where calcium and acetate ions bind in the active site of SULT1A2. Compared with acceptor-free SULT1A1*3, the aromatic ring of Phe247 rotates by about 90° to the inside of the substrate-binding pocket and the side chain of Val148 turns up in SULT1A2. Thus, the distance between the Phe247 and Val148 in SULT1A2 is decreased to 4.2 Å, which is much shorter than that (6.4 Å) in SULT1A1*3 (Fig. 2). As in the structure of SULT1A1*2 bound with two pNP molecules [18], the changed Phe247 and Val148 in SULT1A2 represent a substrate-inhibition conformation, resulting in a closed channel I and a narrowed channel II upon binding with calcium and acetate ions (Fig. 2A). Moreover, loop 82–92 in SULT1A2 tilts up about 18° on the pivot of Cα in residue Lys85, revealing a plastic architecture around the substrate-binding pocket. Site-directed mutagenesis reveals that Phe247 of SULT1A1, which interacts with both p-nitrophenol (pNP) molecules in the active site, is important for substrate inhibition [18,35]. The switch-like conformation transition of Phe247 and Loop 82–92 in SULT1A2 and SULT1A1*3 structures suggests that the opening form of Phe247 and Loop 82–92 allows the enzyme to bind two pNP molecules or some larger substrate molecules. At the time of substrate inhibition, the channel II may be firstly occupied by one substrate, and then residue Phe247 will change its conformation and close the channel I to prevent the other substrate from binding to the catalytic site.

3.3. Sulfonation activity of mutant Tyr149Phe SULT1A2 toward a prototype substrate, p-nitrophenol

As SULT1A1, SULT1A2 uses the same residues to constitute the catalytic sites except for one substitution at the position of 149.
Compared with His149 in SULT1A1*3, Tyr149 in SULT1A2 extends approximately 2.1 Å further to the inside of the substrate-binding pocket. To verify the role of Tyr149 in SULT1A2, site-directed mutagenesis was performed to generate a Tyr149Phe mutant. As shown in Table 2, compared with wild-type SULT1A2, the Tyr149Phe mutant displayed a $K_m$ that is 40 times higher and a $V_{max}$ that is two times lower, with $p$-nitrophenol as substrate. The catalytic efficiency, as reflected by $V_{max}/K_m$, therefore is nearly two orders of magnitude lower for the Tyr149Phe mutant in comparison with the wild-type SULT1A2, indicating a significant role of Tyr149 in the catalytic mechanism of SULT1A2.

In SULT1A2 and $p$-nitrophenol are highlighted by blue dashed lines, and the electrostatic interaction between His149 in SULT1A1s is shown by red dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

**Fig. 3.** Structure comparison of residue at position 149 of SULT1A2 (blue), SULT1A1*2 (red) and SULT1A1*3 (yellow). $p$-Nitrophenol molecules (magenta) from the crystal structure of SULT1A1*2 were superposed onto the substrate-binding pocket of SULT1A2 based on the structure comparison. The putative interactions between Tyr149 in SULT1A2 and $p$-nitrophenol are highlighted by blue dashed lines, and the electrostatic interaction between His149 in SULT1A1s is shown by red dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Divalent cation binding

The SULT1A2 crystal was grown in the presence of calcium acetate at a high concentration (0.3 M). Interestingly, the active
binding site was found to be bound with one calcium cation and one acetate anion (Fig. 4). Cation–π interaction of Ca2+ with the aromatic ring of Tyr240 is “observed” here by geometry [38]. In the structure of SULT1A2, the aromatic ring of Tyr240 interacts with Ca2+ at a distance of 4.3 Å and an acute angle of 44°. The acetate anion forms ionic-bond interaction (2.6 Å) with the calcium cation in the opposite position of the aromatic ring of Tyr240.

Although extensive studies have been carried on the variations in levels of enzyme activity as well as other biochemical and physical properties of human cytosolic SULTs, not much is known about how these enzymes are regulated. Recent studies have revealed that divalent metal cations can exert dramatic inhibitory/stimulatory effects on various human cytosolic SULTs [39,40]. The IC50 or EC50 values determined for different divalent cations were, however, mostly above their normal physiological concentration ranges, implying the human cytosolic SULTs in general may not be easily influenced by these metal cations in vivo. During crystallization, the growth conditions of SULT1A2 and SULT1A1*3 crystals were very similar, except the concentration of calcium acetate. The crystal of SULT1A1*3 was grown in 20 mM Ca(Ac)2, whereas the crystal of SULT1A2 was grown in a much higher concentration (0.3 M) of Ca(Ac)2. Due to the high concentration of calcium, a cation–π interaction was observed in the crystal structure of SULT1A2 but not in that of SULT1A1*3. Cation–π interactions with three aromatic amino acids, Phe, Tyr, and Trp, are known to contribute to the stability of protein or protein–ligand complex. For example, the side chains of aromatic amino acid residues have been found to coordinate with Ca2+, a large alkali metal ion, in crystal structures of rhodanase [41], glutamine synthetase [42], and methylamine dehydrogenase [43]. In a recently reported crystal structure of lysozyme (PDB Code 1LPI), a Na+ cation was observed to interact with a solvent-exposed Trp side chain [44].

![Fig. 4. Cation–π interactions of the aromatic ring of Tyr240 with Ca2+. Cyan, 2Fo – Fc annealed omit map contoured at 1.5σ; red, a Fo – Fc; fourier difference map (4.0σ) calculated without Ca2+ and acetate anion; green, a Fo – Fc; fourier difference map (4.0σ) calculated without Ca2+; blue, a Fo – Fc; fourier difference map (3.5σ) calculated without acetate anion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)](Image 76x98 to 260x276)

strong structural evidence that Mg2+ interacts directly with the π face of the bases in DNA and RNA [38]. Similarly, it can be speculated that the cation–π interaction of calcium with Tyr240 may stabilize the substrate-binding pocket. Thus, the crystal structure of SULT1A2 bound with Ca2+ adopts the substrate-binding conformation as the structure of SULT1A1*2 bound with two pNP molecules. The cation–π interaction in the structure of SULT1A2 therefore suggests that divalent cations are able to modify the conformation of the substrate-binding pocket, which is a potentially prevalent mode of divalent metal cations in their regulatory effects on cytosolic SULTs.

In summary, the crystal structures of SULT1A2 and SULT1A1*3 revealed a plastic substrate-binding pocket and a switch-like substrate selectivity residue, providing a reasonable mechanism of the substrate inhibition. The binding of calcium cation to SULT1A2 suggested a possible divalent cation binding site in their inhibitory effects on the sulfonating activity of various human SULTs. Furthermore, Site-directed mutagenesis study indicated clearly a significant role of Tyr149 in the catalytic mechanism of SULT1A2. These findings suggest that the genetic polymorphism of SULT1A2 and SULT1A1 may affect the individual susceptibility towards procarcinogens, in particular certain aromatic amines and amides.

### 3.5. Protein Data Bank atomic coordinates

Coordinates and structure-factor amplitudes have been deposited with RCSB Protein Data Bank (PDB) with entrance codes of 1Z28 and 1Z29 for SULT1A1*3 and SULT1A2, respectively.

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