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ScienceDirect
The International Journal of Biochemistry & Cell Biology 39 (2007) 685–689
IJBCB
www.elsevier.com/locate/biocel

Molecules in focus

Human cytosolic sulfotransferase SULT1A1

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Received 4 September 2006; received in revised form 21 September 2006; accepted 3 October 2006
Available online 7 October 2006

Abstract

Sulfonation is an important conjugation reaction required for a range of biological processes including phase II metabolism, whereby sulfo-conjugation renders a compound more hydrophilic to aid its excretion. The major enzyme responsible for xenobiotic sulfonation is the widely expressed cytosolic sulfotransferase SULT1A1. The SULT1A1 crystal structure has provided insights into this enzyme's substrate specificity and catalytic function, including its role in the sulfonation of endogenous substrates such as oestrogens. Contrary to its metabolic role, SULT1A1 can also bioactivate compounds; it is known to sulfonate pro-carcinogens such as hydroxymethyl polycyclic aromatic hydrocarbons leading to highly reactive intermediates capable of forming DNA adducts, potentially resulting in mutagenesis. Given the role of SULT1A1 in these diverse functions and the discovery of allelic variants with differing catalytic activities, this enzyme has been the focus of numerous polymorphic studies investigating the link between inter-individual SULT1A1 variance and the etiology of a variety of cancers.

Keywords: Human cytosolic sulfotransferase; SULT1A1; Phase II metabolism; Bioactivation; PAPS; *p*-Nitrophenol

1. Introduction

The conjugation to a sulfonate moiety (SO_3^{-1}), donated by the cofactor 3′-phosphoadenosine-5′-phosphosulfate (PAPS), is one of the major phase II metabolic reactions resulting in increased hydrophilicity of xenobiotic compounds, facilitating their excretion from the body (Hempel et al., 2005). More recently sulfo-conjugation or sulfonation has been shown to play an important role in the modulation of endogenous compounds ranging from steroids to polysaccharide chains, as well as bioactivation of a number of xenobiotics (Hempel et al., 2005; Strott, 2002). Sulfo-conjugation of small molecular weight compounds is carried out by cytosolic sulfotransferases. These are grouped by sequence identity into six families. Members of the SULT1 sulfotransferase family are primarily involved in xenobiotic metabolism and steroid sulfonation. This family is further divided into five subfamilies and their members display distinct, as well as overlapping, substrate preferences. A solitary SULT1A member has been characterised in species such as rat, mouse, cow, pig, dog, guinea pig, platypus and monkey (Hempel et al., 2005). In humans three closely related SULT1A members have been isolated (SULT1A1, SULT1A2 and SULT1A3), which share >90% sequence identity (Hempel et al., 2005).

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doi:10.1016/j.biocel.2006.10.002

Human SULT1A1 exhibits a broad substrate range with specificity for small phenolic compounds. The SULT1A1 cDNA was first isolated from a liver cDNA library and its 295 amino acid, 32 kDa protein characterised by its high affinity for the model substrate *p*-nitrophenol ($K_m \sim 1$ μM), sensitivity to inhibition by 2,6-dichloro-4-nitrophenol (DCNP) and its thermostability at 45 °C (Veronese, Burgess, Zhu, & McManus, 1994; Wilborn et al., 1993). It was therefore originally referred to as aryl/phenol or thermostable sulfotransferase (P-PST or TS-PST). The SULT1A1 gene is located on chromosome 16p11.2-12.1, in close proximity to its related isoform SULT1A2 (Hempel et al., 2005).

2. Structure

The structure of human SULT1A1 (Gamage et al., 2003) comprises a single α/β domain that forms a central five stranded parallel β-sheet surrounded on either side with α-helices to form a fold that is characteristic of all known cytosolic SULT structures (Fig. 1a). The β-strands contribute the residues that form the PAPS-binding site as well as the critical catalytic residues. Interestingly, the SULT structural fold is similar to that of nucleotide kinases such as uridylate kinase suggesting an evolutionary relationship between sulfonation and phosphorylation (Kakuta, Petrotchenko, Pedersen, & Negishi, 1998).

Residues 45-TYPKSGT-51 of SULT1A1 provide the major binding interactions for the 5′-phosphate group of the sulfate donor PAPS and this region has been termed the phosphosulfate binding (PSB) loop (Kakuta et al., 1998). The PSB loop is important for orienting the cofactor for in-line sulfuryl transfer to the acceptor substrate (Kakuta et al., 1998; Pedersen, Petrotchenko, Shevtsov, & Negishi, 2002). The 3′-phosphate of PAPS interacts with residues 257–259 located at the beginning of a highly conserved GxxGxxK motif, and residues Arg130 and Ser138. The positioning of the adenine ring of PAPS is determined by the side chains of aromatic residues Trp53, Thr227 and Phe229. The structural features of the PAPS-binding site are well conserved among sulfotransferases.

Like most cytosolic sulfotransferases, the crystal structure of SULT1A1 has a covered hydrophobic substrate binding site, where PAP and two molecules of the model xenobiotic substrate *p*-nitrophenol are bound in a very hydrophobic L-shaped binding pocket (Gamage et al., 2003). Human SULT1A1 sulfonates a wide range of compounds such as small flat aromatics (*p*-nitrophenol, dopamine), L-shaped aromatics (3,3′-diiodothyronine; *N*-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, *N*-OH-PhIP) and fused ring compounds (17β-oestradiol; Hempel et al., 2005). SULT1A1 has a "plastic" binding site, which can adopt varying architectures to interact with these very different compounds. Recent modeling studies with the N-hydroxylated metabolites of the model carcinogen 2-acetylaminofluorene (2-AAF) and the major food-derived mutagen PhIP show that the hydroxyl groups of these compounds are within hydrogen bonding distance of the donor sulfonate group of PAPS and of the catalytic residue His108 (Fig. 1b and c; Gamage et al., 2006). In the SULT1A1:PAPS:*N*-OH-2-AAF model the ligand forms an unfavourable interaction with the side chain of Phe81, the gate residue at the active site, suggesting that a conformational change is required to accommodate the ligand. This may explain the lower activation of *N*-hydroxy-2-acetylaminofluorene (*N*-OH-2-AAF) by SULT1A1 compared with the allozyme SULT1A2 (Glatt et al., 2001; Meinl, Meerman, & Glatt, 2002). *N*-OH-PhIP exhibits favourable stacking interactions between its benzyl group and the side chains of residues Phe76 and Phe84 with no steric clashes between the ligand and SULT1A1 residues (Fig. 1c). This favourable accommodation of PhIP in the SULT1A1 active site could explain the high metabolic activation of PhIP by SULT1A1 (Gamage et al., 2006). These preliminary modeling studies of N-hydroxy metabolites to human SULT1A1 indicate the possibility of using in silico modeling of substrate binding to human sulfotransferases as predictive tools for chemical risk assessment in humans, as alternatives to interpretations of animal data.

The sulfonation reaction mechanism has been shown to take place via an S_N2 in-line displacement, similar to that of phosphoryl transfer in uridylate kinase (Kakuta et al., 1998; Pedersen et al., 2002). In the active site, the highly conserved residue His108 is directly co-ordinated to the 3-phenolic group of the substrate and acts as the catalytic base in the sulfuryl transfer. His108 is thought to deprotonate the acceptor 3′OH of the substrate, converting it to a strong nucleophile that attacks the sulfur of PAPS. This leads to build up of negative charge at the bridging oxygen and forces Lys48 to donate its proton to the bridging oxygen resulting in sulfonate dissociation (Kakuta et al., 1998; Pedersen et al., 2002). The conserved residue Ser138 appears to be required to prevent PAPS hydrolysis in the absence of the substrate (Pedersen et al., 2002).

The discovery of substrate inhibition of sulfotransferases at high substrate concentrations has challenged the classical Michaelis–Menten kinetics of these enzymes. Based on kinetic analysis, substrate inhibition at high concentrations appears to be due to impeded