Review

Human sulfatases: A structural perspective to catalysis

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Abstract. The sulfatase family of enzymes catalyzes hydrolysis of sulfate ester bonds of a wide variety of substrates. Seventeen genes have been identified in this class of sulfatases, many of which are associated with genetic disorders leading to reduction or loss of function of the corresponding enzymes. Amino acid sequence homology suggests that the enzymes have similar overall folds, mechanisms of action, and bivalent metal ion-binding sites. A catalytic cysteine residue, strictly conserved in prokaryotic and eukaryotic sulfatases, is post-translationally modified into a formylglycine. Hydroxylation of the formylglycine residue by a water molecule forming the activated hydroxylformylglycine (a formylglycine hydrate or a gem-diol) is a necessary step for the enzyme's sulfatase activity. Crystal structures of three human sulfatases, arylsulfatases A and B (ARSA and ARSB), and estrone/dehydroyeipandrosterone sulfatase or steroid sulfatase (STS), also known as arylsulfatase C, have been determined. While ARSA and ARSB are water-soluble enzymes, STS has a hydrophobic domain and is an integral membrane protein of the endoplasmic reticulum. In this article, we compare and contrast sulfatase structures and revisit the proposed catalytic mechanism in light of available structural and functional data. Examination of the STS active site reveals substrate-specific interactions previously identified as the estrogen-recognition motif. Because of the proximity of the catalytic cleft of STS to the membrane surface, the lipid bilayer has a critical role in the constitution of the active site, unlike other sulfatases.

Keywords. Sulfatase, steroid sulfatase, aryl sulfatase, three-dimensional structure, crystal structure, catalysis, membrane protein, estrone sulfate.

Introduction

The sulfatase family of enzymes catalyzes the hydrolysis of sulfate ester bonds of a wide variety of substrates ranging from sulfated proteoglycans to conjugated steroids and sulfate esters of small aromatics. Seventeen human sulfatase proteins and their genes have been identified [1, 2]. Several of them are associated with genetic disorders leading to reduction or loss of function of corresponding enzymes. The sequence homology among the members of the sulfatase family ranges between 20% and 60%. This is reflected in similarity of the tertiary structures as well as of the active site architectures. The catalytically active residues, mostly from the N-terminal half of the polypeptide chain, are highly conserved, indicating a common catalytic mechanism shared by the members of the family. One particular active site amino acid that is strictly conserved in prokaryotic and eukaryotic sulfatases is a cysteine, which is post-translationally modified into a formylglycine (FG) [3]. Hydroxylation of FG by a water molecule forming the activated hydroxylformylglycine (HFG; an FG
hydrate or a gem-diol) is a necessary step for the enzyme’s sulfatase activity [3, 4].

Human sulfatases are active at either acidic or neutral pH, based on their subcellular localization. The lysosomal sulfatases have pH optima in the acidic range, while those localized in the endoplasmic reticulum (ER), Golgi apparatus and cell surface are most active at neutral pH. Human arylsulfatases, such as arylsulfatases A (ARSA) and B (ARSB), are lysosomal and represent soluble forms of the enzyme. Mutations in genes of these lysosomal enzymes lead to diseases such as metachromatic leukodystrophy and mucopolysaccharidosis [5]. Estrone (E1)/dehydro-piandrosterone (DHEA) sulfatase (steroid sulfatase; STS), also known as arylsulfatase C, is a microsomal enzyme and is an integral membrane protein of the ER [6, 7]. It is most active at or near neutral pH and can be solubilized only in the presence of detergents [7, 8]. STS is expressed in several tissues including human placenta, skin fibroblasts, breasts, and fallopian tubes [6, 7, 9–16]. Mutations in the STS gene and inactive enzyme have also been associated with X-linked ichthyosis, a disease related to scaling of the skin [17–19]. Localization of STS in the smooth and rough ER was demonstrated by immunohistochemical labeling [9]. Arylsulfatases D, E, F, G, H, J and K are reported to be localized in ER or Golgi compartments [1, 2]. Iduronate 2-sulfatase, sulfamidase, galactose 6-sulfatase, N-acetyl galactosamine-4-sulfatase, and glucosamine sulfatase, other members of the human sulfatase family, are localized in lysosomes. Mutations in these genes, causing deficiency of one or more of these enzymes necessary for normal cell metabolism, are commonly referred to as lysosomal storage disorders, known collectively as mucopolysaccharidosis, such as hunter, sanfilippo and morquio syndromes [2]. Two new sulfatases, recently reported, Hsulf1 and Hsulf2 are heparin sulfate endosulfatases that release sulfate groups on the C6 position of GlcNAc from an internal subdomain in intact heparin and are localized on the cell surface [2, 20]. The cause of multiple sulfatase deficiency (MSD) has been attributed to mutations in the gene encoding the enzyme known as FG generating enzyme that activates all human sulfatases by catalyzing the conversion of catalytic cysteine to FG [21–23].

Crystal structures of three members of the human sulfatase family, human placental STS [8], ARSA [24], ARSB [25] and one homologous bacterial arylsulfatase from Pseudomonas aeruginosa (PAS) [26] have been determined. The structure of the native, full-length human placental STS provides the first direct evidence of membrane integration of these enzymes, suggesting roles for the lipid bilayer, and possibly for the ER membrane, in catalysis [8]. The overall three-dimensional structures of all three soluble sulfatases exhibit a high degree of homology for the domain of STS that scaffolds the catalytic residues. Furthermore, the spatial arrangement of amino acids responsible for hydrolysis of sulfate esters is virtually identical in all four sulfatases, demonstrating the high degree of similarity of their catalytic mechanism. Nonetheless, subtle differences in the sequences of the substrate-binding cleft result in differences in the active site architecture that account for the variation in substrate specificity. Having a membrane-spanning domain bordering the lipid bilayer and partially contributing to the architecture of the active site makes STS uniquely different from the other three known structures of the sulfatase family. In this article, we describe the commonality of the sulfatase structures in relation to their function with special emphasis on STS, and probe issues related to catalytic mechanism and substrate specificity from examination and analysis of their molecular structures.

**STS in hormonal breast and prostate cancers**

In addition to adrenal sources, hydrolysis of conjugated steroids catalyzed by STS is an alternative source of sex-steroid precursors for the local biosynthesis of active estrogens and androgens. STS catalyzes the hydrolysis of E1-sulfate to unconjugated E1, which is subsequently reduced to 17β-estradiol (E2) by 17β-hydroxysteroid dehydrogenase 1 (17HSD1) (Fig. 1). Androstenedione (A) to E1 and testosterone (T) to E2 aromatization steps are catalyzed by aromatase (P450arom). However, local biosynthesis of E2 from E1-sulfate has been proposed to be the major cause of high levels of active estrogens in the breast for post-menopausal women [27, 28]. The presence of STS in breast carcinomas and STS-dependent proliferation of breast cancer cells have been demonstrated [27, 29]. STS immunoreactivity was detected in 84 out of 113 breast carcinoma cases and was significantly associated with their mRNA levels as well as enzymatic activities [30]. Immunoreactivity was also found to correlate with tumor size and increased risk of recurrence. Higher mRNA levels were associated with poor prognosis in patients with estrogen receptor-positive breast cancer [31]. Additionally, STS has also been found in LNCaP prostate cancer cells [32]. STS hydrolyzes DHEA-sulfate to DHEA, which is then converted to dihydrotestosterone (DHT), the most potent agonist of the androgen receptor, by the actions of 3HSD, 17HSD3, and 5α-reductases 1 and 2 (5R1/5R2) (Fig. 1). Recently published transcriptional data showing increased expres-