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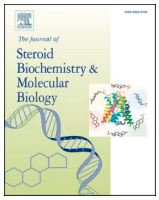

New structural insights provide a different angle on steroid sulfatase action☆

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ABSTRACT

A central part of human sulfation pathways is the spatially and temporally controlled desulfation of biologically highly potent steroid hormones. The responsible enzyme - steroid sulfatase (STS) - is highly expressed in placenta and peripheral tissues, such as fat, colon, and the brain. The shape of this enzyme and its mechanism are probably unique in biochemistry. STS was believed to be a transmembrane protein, spanning the Golgi double-membrane by stem region formed by two extended internal alpha-helices. New crystallographic data however challenge this view. STS now is portraited as a trimeric membrane-associated complex. We discuss the impact of these results on STS function and sulfation pathways in general and we hypothesis that this new STS structural understanding suggests product inhibition to be a regulator of STS enzymatic activity.

1. Introduction

Many steroid hormones require sulfation to increase water solubility and thus expedite circulation in the blood. Examples are the weak androgen dehydroepiandrosterone (DHEA) and the estrogens estrone (E1), estradiol (E2), and estriol (E3); in blood they all are found overtly in their sulfated form [1]. Recently, sulfation was shown to be functionally important even for some vitamin D variants [2]. Within target tissues, sulfated steroids require back-conversion to their free, un-conjugated forms to elicit their biological effects and/or downstream conversion to more potent signaling molecules. Steroid sulfatase (STS) is a key enzyme in the metabolism of human steroids [3]. This enzyme exclusively catalyzes desulfation and therefore is a main enzyme involved in facilitating peripheral steroid action. The STS protein appears to be encoded by a single gene. It receives several post-translational modifications, before operating in most bodily tissues. There seems to be a single STS gene in all vertebrates [4]; in sharp contrast to sulfation pathways that are controlled by numerous sulfotransferase genes (SULTs).

Research on STS expression and activity in physiology and pathology goes back to the 1950's, reviewed in [3,5]. Early studies recognized the importance of STS activity in steroid metabolism in rodent tissues [6] and even in the sea snail *Patella vulgate* [7]. Since then, research on STS and its role in steroid metabolism has shown that STS is involved in many divergent pathologies; most prominently steroid-dependent cancers [1]. STS activity also plays a role in various neurodegenerative conditions, such as Alzheimer's disease and Parkinson's [8], in human mood and behavior [9]. At least in animal models, STS may even be implicated in ageing and lifespan regulation [10].

A complete appreciation of how STS acts has been hampered by our limitations of this enzyme's molecular composition. Structural and functional studies that focus on human steroid sulfatase are scarce [11,12]. This is surprising considering the extended interest this molecular target has received by medicinal chemists over the last two decades [5], who aim to prevent STS from driving hormone-dependent cancers, particularly estrogen-receptor positive breast cancer [13].

So far, the structure of the STS enzyme is thought of as a globular catalytic domain as well as two internal helices that form an extended hydrophobic stem, spanning through a double membrane [11]. This structural view on steroid desulfation, regarded to as a "mushroom" structure, has successfully underpinned many drug-discovery pipelines (see [14] as a recent example).

The established model has left several questions unaddressed about the actual positioning of the protein within the membrane as well as

Abbreviations: STS, steroid sulfatase.

☆ This contribution is dedicated to Cedric Shackleton on the occasion of the Festschrift for his 80th birthday. This publication is part of the accompanying special issue.

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Fig. 1. An unusual membrane domain in the human STS protein. Two internal helices between amino acids 177 and 237 form a stem loop. Noteworthy, this structure does not contain any charged amino acids (with the exception of K183 and R184, depicted in blue). This structural depiction of the stem loop is based on the well-known STS structure PDB: 1P49 [11].

access of the catalytic domain to hydrophobic membrane compartment. Most strikingly, the "stem" of the "mushroom" is void of charged residues at the tip of the loop (Fig. 1), questioning how this structure could anyhow stably anchor electrostatically within double membranes. We here put new structural findings about STS [15] into the context of other recent developments in the field of steroid sulfation pathways.

2. A new structural view on steroid desulfation

A recent crystallographic study challenges our current knowledge about the STS enzyme [15]. This higher-resolution structure of endogenous STS includes several post-translational modifications and portrays this enzyme as a catalytically active trimer (Fig. 2). Amino acids from the stem region, as well as selected side chains from the globular domain, engage in subunit-subunit protein interactions to form a trimeric "bowl" of STS. No longer membrane-spanning, STS now associates with only one of the leaflets of the double-membrane or submerses into it. This configuration is distantly similar to human cyclooxygenases [16] and is much more in line with the amino acid composition of the stem.

Within the trimeric STS complex, the orientation of the catalytic domain is changed from being perpendicular-to-the-membrane to being tilted by some 33 degrees relative to the membrane. This brings the three tryptophan residues Trp550, Trp555 and Trp558 into close proximity of the membrane-cytoplasm interface (these tryptophan residues are schematically shown in Fig. 3). At the same time, the rotational angle of the sulfatase domain is fixed; now all three catalytic centers of the trimer are precisely oriented towards the "inner part of the bowl". Thus, the access channel to its substrates, some membrane-embedded sulfated steroids, may be optimized.

By mapping several crystallization additives, new binding sites for hydrophobic ligands are identified. Two lipid binding sites per trimeric subunit stabilize the complex structure. An additional binding site is noteworthy – this one is close to the catalytic cleft; it may be facilitating substrate binding or even have regulatory function.

The recent study on STS is the crystal structure of an endogenous protein purified from human placenta – with N-terminal proteolytic processing and two post-translational modifications: asparagine residues N47 and N333 carry additional N-acetyl-glucosamine moieties. Finally, there is a specific phosphate coordination site – separate to the actual trimer interface; this may stabilize STS's membrane positioning by binding the heads of specific phospholipids. This could however also result in the formation of higher-order STS complexes, similar to transient interactions recently characterized for some sulfotransferases and other sulfation enzymes [17,18].

3. Regulation of the STS enzyme and substrate inhibition

It is intriguing to speculate about how the new structural details of STS will impact on our understanding of this enzyme's biological regulation. The identification of a secondary ligand site close to STS's catalytic center opens many questions on its action, function, and regulation that were previously unknown. Recent modelling suggests that estrone can be accommodated into this additional binding site [13] and most likely results in a conformational change of STS and/or the STS trimer making the primary ligand site inaccessible to sulfated steroids. Thus, estrone, the product from estrone sulfate desulfation by STS, may ultimately block the enzyme's activity. Other known steroidal substrates of STS or their desulfated products may act similarly [5]; for example DHEAS, the sulfated form of dehydroepiandrosterone.

Interestingly, early research in the 1960′s on sulfation activity in rat testis showed that desulfation of pregnanolone sulfate was inhibited by both pregnanolone and 5-pregnene-3β, 20α-diol [19]. Estrogen derivatives are also known to block STS activity [20], with estrone sulfate inhibiting STS at 7.6 μM in JEG-3 cells. In light of the new STS structure, it is possible that it was desulfation of estrone sulfate by STS leading to increased availability of the reaction product estrone which reduced activity. Furthermore, estradiol at a dose of 10 μM can inhibit STS activity by 53 % in human breast cancer homogenates [21]. Many compounds that inhibit STS activity have estrogen-like chemical structures [5], and it is possible they bind to both STS ligand-binding sites. A recent study has shown that 20 μM and 10 μM estradiol can block STS activity

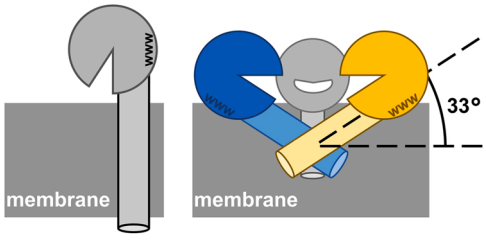

Fig. 3. Representation of the old and new model about STS enzymatic complexes. The membrane loop, the catalytic site and the position of three tryptophan residues is schematically indicated.

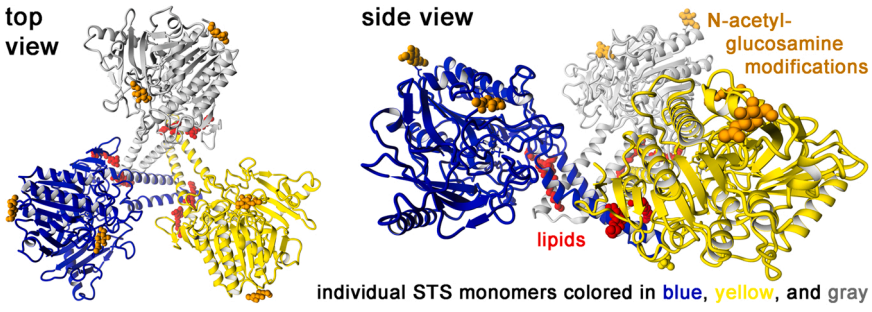

Fig. 2. A new crystal structure of human STS shows this enzyme as a trimer. This visualization in top and side view is based on PDB structure 8EG3 [15]. Lipids are shown in red. Post-translational N-acetyl-glucosamine modifications at asparagine residues N47 and N333 are shown in orange. Within the side view, a phosphate coordination site can be seen in the yellow subunit; the phosphate itself is depicted as space-filling model. See main text for a more detailed description.

in rat liver microsomes, with the 20 μM dose reducing activity by 61.3 % in female livers [22]. Although this is of some interest it should be noted that these estrogen concentrations are not physiologically relevant: Concentrations of estrone and estradiol in the circulation of premenopausal and postmenopausal women rarely rise above 0.5 and 1.5 nM, respectively [23], thus the biological relevance of high dose estrogens inhibiting STS activity seems questionable.

4. Conclusion and open questions

The current structural study looks at the endogenous STS protein. It is N-terminally processed, contains two glycosylated residues and complexes both several lipids as well as some phosphate ions (Fig. 2). The actual substrate spectrum of STS is still not clearly defined. There is the possibility that further conjugations, such as doubly sulfated steroids or mixed conjugated species are excluded from desulfation, thus representing locked metabolites [24,25]. It will be interesting to elucidate the functional significance of all these modifications and binding partners.

If only high dose steroid products can inhibit STS activity what is the biological importance, if any, of the secondary binding site? One possibility is that these secondary binding sites on STS are there to tightly control local steroid synthesis. Many steroids circulate at relatively high concentrations in their sulfated forms. As these steroids can have significant impact on cellular function, and desulfation of them is the first reaction leading to steroid activity, it may make biological sense to have such tight local regulatory control to avoid active steroids swamping cell metabolism and function. However, at the moment, this remains speculation and no experimental evidence exists to support his theory.

Another possibility is that other steroids or as yet unknown factors bind the secondary binding site to alter STS activity. The glucocorticoids cortisol and dexamethasone have been shown to reduce STS activity in the mouse fibroblast cell line NIH-3T3 and in primary mouse fibroblast [26]. However, evidence suggests this is through downregulation of the STS protein rather than through direct enzyme inhibition. These findings are further supported in similar studies showing dexamethasone lowering STS protein expression in human pre-osteoblastic cells MG-63 [27]. Whether other steroids can inhibit STS activity remains to be determined. Please note that a related review was published recently [28].

Data Availability

No data was used for the research described in the article.

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