

Roles of Steroid Sulfatase in Brain and Other Tissues

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Summary

Steroid sulfatase (EC 3.1.6.2) is an important enzyme involved in steroid hormone metabolism. It catalyzes the hydrolysis of steroid sulfates into their unconjugated forms. This action rapidly changes their physiological and biochemical properties, especially in brain and neural tissue. As a result, any imbalance in steroid sulfatase activity may remarkably influence physiological levels of active steroid hormones with serious consequences. Despite that the structure of the enzyme has been completely resolved there is still not enough information about the regulation of its expression and action in various tissues. In the past few years research into the enzyme properties and regulations has been strongly driven by the discovery of its putative role in the indirect stimulation of the growth of hormone-dependent tumors of the breast and prostate.

Key words

Steroid sulfatase • Enzyme • Sulfate • Physiological role • Action

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Introduction

Steroid sulfatase (STS) is an almost ubiquitous enzyme of steroid metabolism. It is an important factor influencing many physiological and pathophysiological processes regulated by steroid hormones. A number of them have been revealed recently (Stanway *et al.* 2007, Hazan *et al.* 2005, Weidler *et al.* 2005, Nakamura *et al.* 2006). Below, the main information on its occurrence, molecular biology and actions are reviewed with

emphasis on its physiological and pathophysiological consequences in brain and nervous tissue.

Steroid sulfatase gene and protein

Steroid sulfatase belongs to a superfamily of sixteen different mammalian sulfatases (Obaya 2006). The gene encoding STS is pseudoautosomal in mice, but not in humans, and escapes X inactivation in both species. The functional nucleotide sequence maps to Xp22.3-Xpter (Yen *et al.* 1987, Meroni *et al.* 1996).

The DNA sequence coding for STS on the X-chromosome was cloned, sequenced and well characterized. The 2.4 kbp cDNA encodes a protein of 583 amino acid residues with a short signal peptide of 21-23 amino acids and four potential N-glycosylation motifs N-X-S/T. Probably two of these sites (N47 and N259) are used for N-linked oligosaccharide connection.

STS is synthesized as a membrane-bound 63.5 kDa polypeptide. Newly synthesized polypeptide is processed to a mature 61 kDa form. The decrease in size is due to the processing of the oligosaccharide chains, which are cleavable by endoglucosaminidase as well as mannosidase(s) (Conary *et al.* 1986). Recently, STS was purified and crystallized from the membrane-bound part of the human placenta (Hernandez-Guzman *et al.* 2001) and shortly after, three-dimensional structures of three human sulfatases were released (Ghosh 2005).

Concerning membrane topology, the enzyme has two membrane-spanning antiparallel hydrophobic α -helices with proline 212 serving as a turn point at the cytosolic side of the endoplasmic reticulum membrane. The polar catalytic domain of the enzyme is situated to the lumen side of the lipid bilayer (Hernandez-Guzman *et*

al. 2003). Nevertheless, participation of the lipid bilayer in maintenance of the active site integrity, passage of the substrate and/or product release was still not exactly determined.

Further details concerning the regulation of STS gene expression on transcriptional and translational levels are beyond the scope of this paper. These issues were excellently reviewed in more comprehensive and specialized articles (Nussbaumer and Billich 2005, Reed *et al.* 2004, Richard 2004).

Enzyme activity

Sulfatases are a unique group of enzymes that carry at their catalytic site a post-translational modification, an alpha-formylglycine residue that is essential for enzyme activity. Formylglycine is generated by oxidation of a highly conserved cysteine or, in some prokaryotic sulfatases, serine residue (Dierks *et al.* 1998, Miech *et al.* 1998). In eukaryotes, this oxidation occurs in the endoplasmic reticulum during or shortly after import of the nascent sulfatase polypeptide. The mechanisms that are responsible for the oxidation are probably associated with the protein translocation apparatus. *In vitro* studies for arylsulfatase A revealed short linear motif (CTPSR), starting with the cysteine residue, which acts as a target site for the post-translational modification. Proline and arginine play crucial roles for appropriate modification as well as seven additional residues (AALLTGR) directly following the CTPSR sequence (Dierks *et al.* 1999, Knaust *et al.* 1998). The proposed catalytic mechanism also includes the presence of bivalent Ca^{2+} rather than Mg^{2+} cation at the active site of the enzyme. Therefore, the reaction mechanism, which STS uses for steroid sulfate hydrolysis, closely resembles the mechanism that was proposed for arylsulfatases (Boltes *et al.* 2001).

Recent results show that also N-terminal and C-terminal regions have important contribution to the STS enzyme activity (Sugawara *et al.* 2006).

Subcellular localization

In the above mentioned studies STS was found mainly in the rough endoplasmic reticulum. Furthermore, in cultured human fibroblasts, immunohistochemistry proved that the enzyme exists in Golgi cisternae and in the trans-Golgi, where it is co-distributed with lysosomal enzymes and the mannose 6-phosphate receptor. STS was

also found at the plasma membrane (Kawano *et al.* 1989) and in the coated pits, endosomes and multivesicular endosomes. These structures may be the sites where sulfated estrogen and/or androgen precursors are hydrolyzed. It also co-localizes with lysosomal enzymes and the mannose 6-phosphate receptor here. Despite microsomal STS and lysosomal enzymes share several cellular compartments, in the first studies the polypeptide was surprisingly not detected in lysosomes (Willemsen *et al.* 1988). One year later, however, STS activity was also found in lysosomes (Stein *et al.* 1989). On the other hand, sulfatase activity in these compartments and the appropriate protein was isolated and characterized as N-acetylgalactosamine-4-sulfatase (Bond *et al.* 1997). The stability of sulfatases in subcellular compartments with low pH is possibly due to their membrane topology. It could be related to the high homology of the two luminal domains of STS with the lysosomal sulfatases, arylsulfatase A, and arylsulfatase B. However, the transport between trans-Golgi and lysosomes possibly uses a mannose receptor-independent mechanism. This was proposed as a result of the absence of mannose-6-phosphate in STS carbohydrate moiety (Stein *et al.* 1989).

Occurrence in tissues

Enzymatic activity of STS was demonstrated for the first time in rat liver microsomes (Dodgson *et al.* 1954). Later, this enzyme was found in testis (Payne *et al.* 1969), ovary (Clemens *et al.* 2000), adrenal glands, placenta, prostate, skin, kidney, fetal lung (Hobkirk *et al.* 1982), viscera, endometrium, aorta, bone, peripheral blood leukocytes (Han *et al.* 1987) and brain tissue (Iwamori *et al.* 1976). Now STS is believed to be an almost ubiquitous enzyme. Tissue and organ distribution varies considerably between different mammalian species. Placenta was found to be the richest source of STS. However, the findings differ in dependence on methodological approaches. Immunohistochemistry in combination with reverse transcriptase polymerase chain reaction (RT-PCR) were largely used for STS localization. Biochemically, sulfatase activity was detected in microsome fraction and/or tissue homogenates. These approaches have been used for identification and characterization of STS activity in rat testis (Payne *et al.* 1969), ovarian granulosa cells (Clemens *et al.* 2000), leukocytes (Han *et al.* 1987) and brain tissue (Iwamori *et al.* 1976). More recently, a

biochemical approach was used for determination and characterization of STS activity in Macaca brain regions (Kříž *et al.* 2005)

At first there was confusion among aryl sulfatase C and STS because these enzymes seemed to be different, but recent biochemical and genetic analyses have confirmed that there is only one enzyme (Keinanen *et al.* 1983, Ruoff and Daniel 1991).

Although there was found only one gene for STS, evidence has been found that two different isoforms (microsomal and nuclear) exist in rodents (Nelson *et al.* 1983) and also two isoforms in humans (Chang *et al.* 1990, Munroe and Chang 1987), probably due to different post-translational modifications.

STS and cholesterol supplementation

It is necessary to mention the involvement of STS in steroidogenic acute regulatory protein (StAR) activity regulation. This 30 kDa phosphoprotein which is derived from its larger precursor plays a crucial role in the intramitochondrial transport of cholesterol (Stocco 2001). Cholesterol can be further cleaved to pregnenolone, which serves as a precursor in steroid hormone synthesis and itself also acts as neuroactive steroid. The process occurs in the mitochondrial matrix and is associated with the inner membrane. Experiments on monkey kidney COS-1 cells showed that STS in the presence of StAR and cytochrome P450 (CYP11A1) significantly increased pregnenolone production in the reaction medium. It is a result of both an increase in StAR translation and prolonged StAR half-life (Sugawara and Fujimoto 2004). The importance of STS lies in conversion of cholesterol sulfate to free cholesterol which is a substrate for CYP11A1. Furthermore, cholesterol sulfate has an inhibitory effect on steroidogenesis in rat adrenal mitochondria by inhibiting the cholesterol transport inside the mitochondrion (Xu and Lambeth 1989, Lambeth *et al.* 1987). Therefore, STS prevents inhibitory effect of cholesterol sulfate on cholesterol intramitochondrial transport. Prolonged StAR half-life might also be explained by STS action on cholesterol sulfate. Released intracellular cholesterol binds StAR and may slightly change its conformation and therefore increases protein half-life. The same effect was observed in MLN 64 transfected cells. This gene contains StAR-homology domain and is expressed in placental mitochondria and lysosomes (Bose *et al.* 2000, Zhang *et al.* 2002).

This phenomenon is probably not only limited to steroidogenic tissues but occurs widely throughout the body. For advances in intracellular cholesterol trafficking and the role of StAR see a recent review by Strauss *et al.* (1999).

STS and central nervous system

Steroid hormones, regardless of their origin, exert a wide variety of biological effects on the nervous system. They play an important role in the growth, development, maturation and differentiation of the central and peripheral nerves. Furthermore, it was demonstrated that there is an association between STS activity and behavior, as shown for instance in male mice (Le Roy *et al.* 1999). Evidence has been found that brain tissue was able to synthesize various steroid hormones such as pregnenolone and DHEA from cholesterol *in situ* (Baulieu and Robel 1990). In brain tissue, steroids act mainly in autocrine and/or paracrine ways. It was found that cerebral concentrations of pregnenolone and DHEA neither respond to the administration of adrenocorticotrophic hormone nor undergo circadian variations as do circulating adrenal steroids (Corpechot *et al.* 1981). A new term neurosteroids was suggested for these steroids.

Neurosteroids include steroid hormones or their precursors that are newly synthesized from cholesterol or other early precursors in the nervous system. These steroids could be detected in measurable amounts even if peripheral steroidogenic glands were removed (Robel and Baulieu 1995).

Neurosteroids occur in the nervous system as free unconjugated steroids, sulfate esters or fatty acids esters (Jo *et al.* 1989). The conjugated forms of steroids frequently exceed those of free steroids and differ in their metabolic, behavioral and also psychological effects such as stress, anxiety, cognition and sleep (Baulieu and Robel 1996, Majewska 1992). Besides the action of neurosteroids at the transcriptional level, these steroids may affect the nerve cells in a nongenomic way *via* alteration of neuronal excitability by modulating the activity of several neurotransmitter receptors such as γ -amino butyrate (GABA_A) receptors, N-methyl-D-aspartate (NMDA) receptors, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate receptors, nicotinic, muscarinic and σ -receptors (Klangkalya and Chan 1988, Monnet *et al.* 1995, Valera *et al.* 1992, Wu *et al.* 1991, Shirakawa *et al.* 2005, Wu

and Chen 1997).

Concerning the role of STS, it maintains the balance between sulfated neurosteroids and their unconjugated forms, which in many cases act in opposite ways. The assumption of a steroid binding site at these ligand-gated channels is based on pharmacological studies concerning the strong stereoselectivity and the structure-activity relationship of the action of neuroactive steroids at these neurotransmitter receptors (Lambert *et al.* 1995). In addition, neurosteroids affect these receptors as non-competitive antagonists. It means that steroid-binding sites are neither situated inside the ion channel nor compete with receptor agonists for their binding sites (Dingledine *et al.* 1999). These various sites, located on the outer receptor surface, may be occupied by various steroids resulting in enhancing or decreasing leakage of a particular ion channel. STS maintains the levels of DHEA/DHEAS and pregnenolone/pregnenolone sulfate (PregnS), which are the most common neuromodulators. Furthermore, unconjugated and sulfated steroid molecules act in many cases in opposite ways on the same receptor. This fact further emphasizes the role of STS in regulation of the equilibrium between sulfated and free neuroactive steroids in brain.

Glutamate receptors may have also other outputs: soluble tyrosine kinases activation and/or mitogen-activated protein kinase pathways activation. Furthermore, NMDA receptors in plasma membranes could be coupled to NO synthase (NOS) through the postsynaptic density protein 95 scaffold (Tochio *et al.* 2000). Treatment with DHEA causes NMDA stimulation which leads to a decrease in Ca^{2+} -sensitive NOS activity. Therefore, NO production is inhibited and as a consequence DHEA exerts a neuroprotective effect on cultured rat hippocampal neurons (Kurata *et al.* 2004).

Imbalance in the ratio of unconjugated steroid/steroid sulfate was shown to influence memory and long-term potentiation in the hippocampus (Flood *et al.* 1992, Yoo *et al.* 1996). Experiments on rats suggest that STS inhibition may become an important tool for enhancing neuronal functions, such as memory, mediated by excitatory neurosteroids. This is caused by increased DHEAS rather than DHEA level, which positively influences brain cholinergic function and leads to memory enhancement (Rhodes *et al.* 1997, Li *et al.* 1997).

PregnS and DHEAS were found to display completely different (antagonistic) action on GABA_A receptors. Both also exert complex effects at NMDA

receptors (Zorumski *et al.* 2000). Therefore, these steroids might possess nootropic properties. Indeed, some studies suggested that intracerebroventricular administration of pregnenolone led to better results in various memory tasks in rodents (Flood *et al.* 1992). Furthermore, DHEA was also found to enhance memory retention in mice (Flood *et al.* 1988). These results are likely related to STS levels in used animal models.

It is well known that in humans DHEA serum levels decreases with age (Šulcová *et al.* 1997). This phenomenon was also reported for DHEAS in patients suffering from Alzheimer's disease and multi-infarct dementia (Sunderland *et al.* 1989, Yanase *et al.* 1996). Unfortunately, direct information on DHEA/DHEAS ratio and STS levels is still missing. To date no study is available concerning STS activity and its relationship to dementia disorders. Studies on the determination of neurosteroids in cerebrospinal fluid may be promising (Kim *et al.* 2003).

PregnS and DHEAS were also suggested to be involved in the development of tolerance to ethanol in mice (Barbosa and Morato 2001). Nevertheless, a connection between ethanol tolerance and STS activity was not demonstrated.

DHEA was also found to have important effects in differentiation of glia and neurons during their development (Roberts *et al.* 1987) and in neocortical organization during development suggesting that DHEA may have trophic factor-like activity (Compagnone and Mellon 1998). Neuronal remodeling can also be explained by DHEA interaction with various neurotransmitter systems (Lapchak and Araujo 2001).

Furthermore, it was proposed that neurosteroids could act on nerve cells *via* hypothesized surface receptors coupled with G-proteins or through specific membrane sites using calcium as an intracellular messenger (Orchinik *et al.* 1992, Ramirez and Zheng 1996). Recently, it was showed that $\sigma 1$ receptor-like protein activity was modulated by PregnS. This protein is coupled with Gi/o protein, and this interaction results in enhancement of short-term presynaptic facilitation onto adult hippocampal CA1 neurons (Schiess and Patridge 2005). As was previously mentioned, PregnS levels are among others under the control of STS.

The detailed STS localization in brain tissue has not been exactly determined. Most studies are limited only to major brain regions (Steckelbroeck *et al.* 2004, Kříž *et al.* 2005). Subcellular localization of STS activity in brain tissues has not been clearly identified. However,

it is generally supposed that subcellular STS activity distribution does not differ significantly from the distribution in other cells.

Preliminary results from our laboratory concerning STS activity in several types of human brain tumors showed surprising differences in STS activity in relation to tumor type. Neither age nor sex of studied subjects significantly influenced obtained results.

STS role in physiology and pathophysiology

STS and breast cancer

Much information concerning the role of STS in breast cancer and its treatment have been published (Sasano *et al.* 2006, Stanway *et al.* 2007, Tsunoda *et al.* 2006). Besides 17 β -hydroxysteroid dehydrogenase (17 β -HSD) type I and aromatase, STS belongs to the most important targets for potential endocrine therapy in humans (Suzuki *et al.* 2005). As such, STS has no direct effect on tumor progression and development. However, it maintains the equilibrium between sulfated and unconjugated steroids whose effects in breast cancer development are, in many cases, completely different (Reed *et al.* 2004).

In hormone-dependent human breast cancer, 17 β -estradiol (E2) contributes highly to tumor growth and development. Furthermore, some carcinoma cells actually require estrogens (especially E2) for their growth (Sasano *et al.* 2006). The importance of STS in intratumoral estrogen production lies in its crucial role in the conversion of estrone sulfate (E1S) to estrone (E1) (Pasqualini 2004). E1 could be further metabolized to E2 by the action of 17 β -HSD type I (Poutanen *et al.* 1995). Sulfated estrogens are unable to bind to estrogen receptors and thus they are inactive as hormones (Pasqualini *et al.* 1989). On the other hand, sulfation rapidly changes the polarity of the hydrophobic steroid ring and therefore is widely used for hormone transport. Furthermore, sulfated steroid conjugates act as a reservoir of active hormones and/or their precursors *via* STS action (Reed *et al.* 2004).

In postmenopausal women, a large contribution to estrogen level comes from the aromatization of circulating androgens (Reed *et al.* 1979). In androgen biosynthesis, STS also plays important role in conversion of DHEAS to DHEA, while DHEA serves as precursor for androstenedione and its further aromatization as shown in Figure 1 (Nakata *et al.* 2003, Suzuki *et al.* 2003). Therefore, STS may affect E2 biosynthesis at two

levels – conversion of DHEAS to DHEA and E1S to E1.

The importance of STS in intratumoral estrogen production has prompted the development of new potent STS inhibitors.

STS and dermatopathies

One of the best known diseases that have been ascribed to STS deficiency is X-linked ichthyosis. It is a relatively common genetic disorder, clinically characterized by a generalized desquamation of large, adherent, dark brown scales of the skin. The majority of all cases are caused by a complete deletion of the STS gene from the distal short arm of the X-chromosome (Richard 2004).

Other STS defects are usually caused by one or more point mutations out of seven possible defects (Ghosh 2004). These mutations lead to disruptions in the active site and/or interference with enzyme's membrane-associating motifs that are crucial for the integrity of the catalytic site. All of these mutations are located near the C-terminal region of the STS enzyme (Ghosh 2004, Alperin *et al.* 1997).

On the other hand, locally enhanced STS activity leads to increased DHEA production, which serves as a precursor of active androgens such as 5 α -dihydrotestosterone (DHT) and/or testosterone (Hoffmann 2001). In women, local excess of DHT may cause an androgenic alopecia. In other target tissues (as pubic region and axillary follicles) it may result in hirsutism (Price 2003). Hence, in women with androgenic alopecia STS inhibition can be effective in treating both disorders.

STS action and the immune system

Concerning STS activity and the immune system, maintaining the DHEA/DHEAS ratio further emphasizes the role of STS. Studies on aged mice revealed that *in vitro* DHEA, but not DHEAS, suppressed the release of Th 2 cytokines and therefore enhanced Th 1 response (Daynes *et al.* 1993). Thus, STS present in macrophages (Hennebold and Daynes 1994) may govern the local availability of DHEA and influence the immune response. It is due to the presence of macrophages in lymphoid tissue where Th cell maturation occurs.

Furthermore, the balance of glucocorticoids to DHEA determines the further progression of Th cells to either Th 1 or Th 2 phenotypic line. Cortisol was found to favor the development to Th 2 cells, while DHEA promotes a Th 1 mediated response (Purohit and Reed 2002).

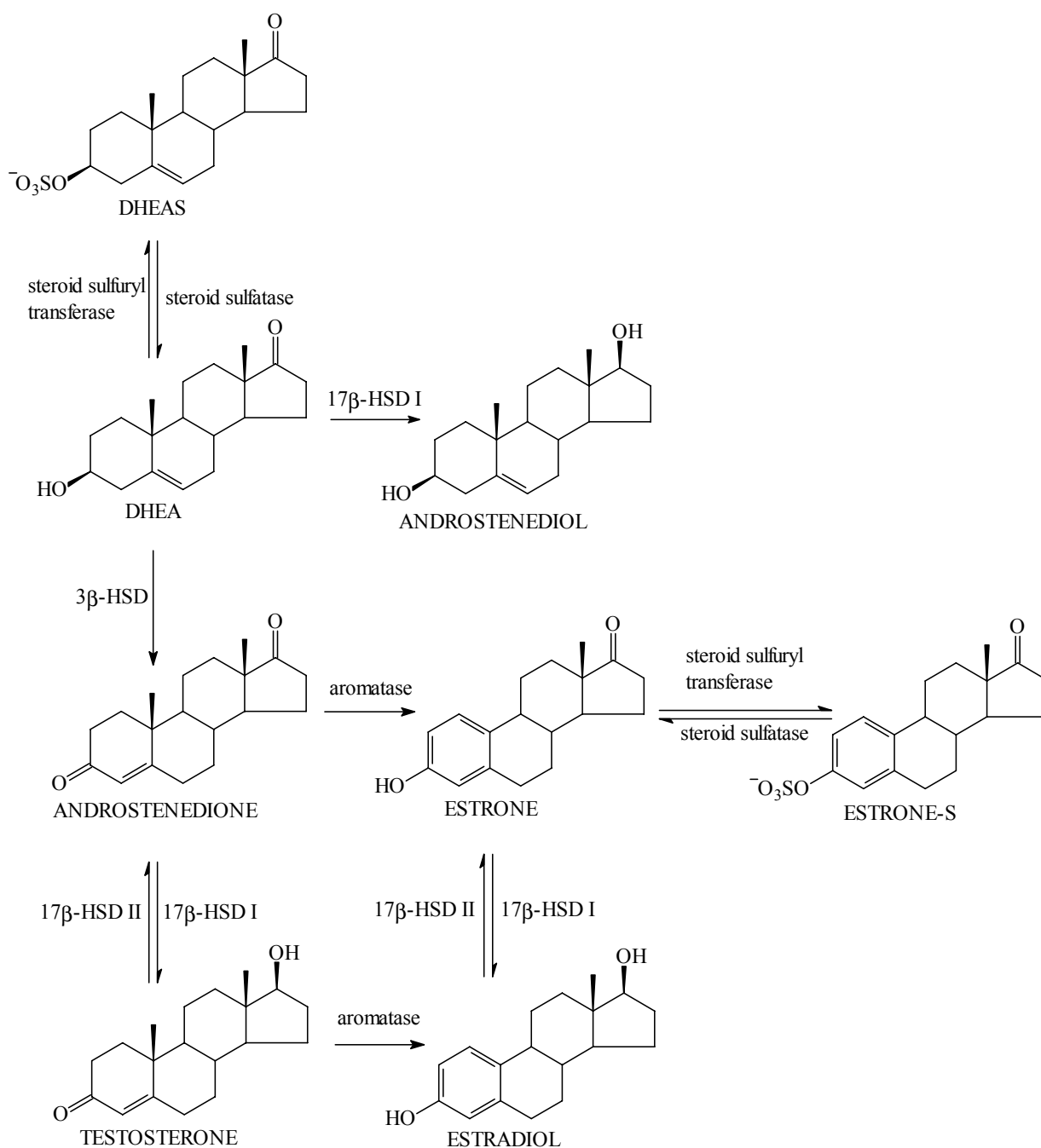


Fig. 1. Steroid hormone metabolism and involved enzymes emphasizing the role of STS in breast cancer tissue. Abbreviations used: 3 β -HSD (3 β -hydroxysteroid dehydrogenase/ $\Delta^{5,4}$ -isomerase), 17 β -HSD I/II (17 β -hydroxysteroid dehydrogenase, type I/II), DHEA (dehydroepiandrosteone), DHEAS (dehydroepiandrosterone sulfate), ESTRONE-S (estrone sulfate).

The enzyme was also found in peripheral blood leukocytes (Han *et al.* 1987), bone (Purohit *et al.* 1992) and thrombocytes (Soliman *et al.* 1993).

Concerning pathological states, many breast and other tumors are infiltrated by lymphocytes and macrophages (Kelly *et al.* 1988). Consequently, it is possible that STS activity of these cells can finally make an important contribution to estrogen synthesis in breast tumors.

Since the STS activity is present in such available tissue, these cells were suggested to provide a relatively simple model for monitoring the efficacy of new STS inhibitors (Purohit *et al.* 1997, Reed *et al.* 1990).

STS action in bone

It was suggested that local formation of estrone could be an important source of estrogens for regulating

bone formation (Janssen *et al.* 1999). Further investigations showed that HOS and MG 63 osteoblast cell lines expressed mRNA for STS and were able to convert both DHEAS and E1S (Fujikawa *et al.* 1997). This pathway involving STS was found to be active not only in cells derived from osteosarcoma but also in osteoblast-like cells cultured from bone fragments. In the latter STS activity was two times higher in comparison with osteosarcoma-derived cells (Muir *et al.* 2004).

The existence of steroid sulfatase in human and rat osteoblast cells suggests that osteoblast cells also possess the capacity to convert circulating sulfo-conjugated steroids to more active androgens and especially estrogens (Janssen *et al.* 1999). This may indicate an important contribution of bone in facilitating the hormonal action.

STS activation and inhibition

Recently explored roles of STS in many pathophysiological processes, especially in breast cancer, prompted efforts for preparation of efficient STS modulators, particularly inhibitors. STS inhibitors may be divided in three basic groups.

Reversible inhibitors: These compounds follow the same strategy: replacement of 3β sulfate moiety in E1S with another one that mimics sulfate group. Attempts to synthesize these derivatives finally led to the preparation of a leading compound. Molecules of such inhibitors compete for STS active site with natural substrates.

Irreversible inhibitors: This group covers the majority of recently reported STS inhibitors and could be further divided to steroidal and non-steroidal irreversible inhibitors. The former group contains estrone-3-O-sulfamate and its derivatives. Despite their efficiency, their use in clinical practice is quite limited because of various side effects. Recently, attention has been focused on non-steroidal derivatives of coumarin and the

exploration of a new leading compound 4-methylcoumarin-7-O-sulfamate (COUMATE) (Purohit *et al.* 1996). Further synthetic efforts led to the preparation of efficient inhibitors derived from COUMATE (Woo *et al.* 2000). Some of these were found suitable for clinical practice and entered clinical trials.

Dual inhibitors: The third direction of STS inhibitor development is focused on compounds with a dual mode of action. These can inhibit both STS and aromatase activities or act as STS inhibitors and antiproliferative or antiangiogenic agents at the same time. According to the mentioned effects they may be especially useful in treatment of hormone-dependent cancers.

A more detailed overview concerning STS inhibitors is beyond the scope of this review. For detailed discussion of the complex molecular structures and mechanisms that govern STS inhibition see other specialized articles (Purohit *et al.* 2003, Nussbaumer and Billich 2005, Reed *et al.* 2004, Nussbaumer and Billich 2004).

It is evident that steroid sulfatase possesses a broad spectrum of actions, often unexpected. This may influence many physiological processes connected with steroid hormones and their genomic as well as non-genomic effects. Development of potent agents, inhibitors or activators of this enzyme, represents one of the tools for effective treatment of various disorders caused by steroid hormone deregulations.

Conflict of Interest

There is no conflict of interest.

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