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The Biological Roles of Steroid Sulfonation

Paul Anthony Dawson

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1. Introduction

Although its role in mammalian physiology is currently underappreciated, sulfate is an obligate nutrient for numerous cellular and metabolic processes in human growth and development [1]. The diet provides approximately one third of sulfate requirements in adults [2], although sulfate intake can vary greatly (1.5-16mmol/day) and is dependent on the source of drinking water (negligible to >500 mg/L) and types of food [3-5]. Brassica vegetables and commercial breads have a high sulfate content (>8.0umol/g) whereas low sulfate levels (<0.5umol/g) are found in some foods, including fresh onions, apples and oranges [5]. Once consumed, sulfate is absorbed through the intestinal epithelium into the blood, where it is maintained at approximately 0.3mM, making sulfate the fourth most abundant anion in human circulation [6, 7]. Blood sulfate levels are maintained by the kidneys, which filter sulfate in the glomerulus and then reabsorb the majority of sulfate back into circulation [8]. The process of sulfate reabsorption occurs in the proximal tubule of the kidney, and is mediated by two sulfate transporter proteins, SLC13A1 (aka NaS1, Sodium sulfate transporter 1) and SLC26A1 (aka SAT1, Sulfate anion transporter 1) [9]. The NaS1 protein is expressed on the apical membrane of epithelial cells in the proximal tubule where it mediates the first step of sulfate reabsorption [10], and SAT1 mediates the second step across the basolateral membrane [11] (Figure 1A). Mice lacking the NaS1 or SAT1 genes have sulfate wasting into the urine which leads to low blood sulfate levels (hyposulfataemia) [12, 13]. Humans with loss of function mutations (R12X and N174S) in the NaS1 gene also exhibit renal sulfate wasting and hyposulfataemia [14]. This depletion of sulfate from circulation reduces sulfate availability to cells throughout the body and leads to a reduced intracellular sulfate conjugation (sulfonation) capacity, as shown in the NaS1 and SAT1 null mice [12, 13, 15].

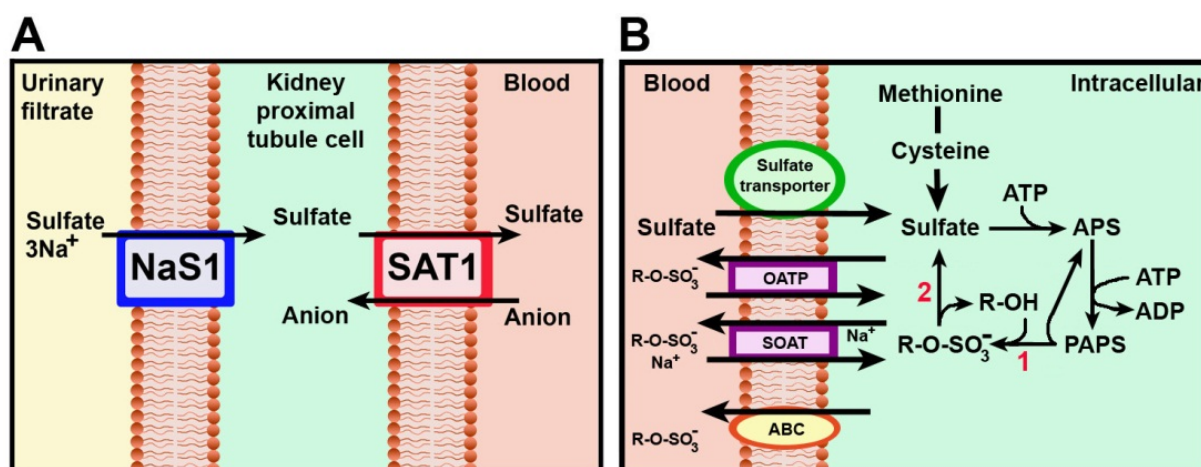


Figure 1. Sulfate levels need to be maintained for sulfonation reactions to function effectively. (A) In the kidneys, filtered sulfate is reabsorbed through epithelial cells in the proximal tubule via NaS1 on the apical membrane and then by SAT1 on the basolateral membrane. (B) Intracellular sulfate is obtained from extracellular sources via sulfate transporters, and is derived from the metabolism of methionine and cysteine. Sulfate and ATP are converted to the universal sulfonate donor, PAPS. Both (1) sulfonation and (2) de-sulfonation reactions are active within intracellular metabolism. Sulfonated molecules are transported across the plasma membrane of cells via ATP binding cassette (ABC) proteins, sodium-dependent organic anion transporter (SOAT) and organic anion transporter polypeptides (OATPs), where they provide a circulating reservoir for cellular uptake and intracellular de-sulfonation. R-O-SO₃⁻ represents sulfonated substrates, including steroids.

Intracellular sulfate is derived from the uptake of sulfate across the plasma membrane via sulfate transporters, and from the intracellular metabolism of sulfur-containing amino acids and thiols, as well as the removal of sulfate from substrates via sulfatases (Figure 1B). Certain cell types in adults, including chondrocytes, endothelial cells and hepatocytes have a high requirement for intracellular sulfonation, and are more reliant on transport of extracellular sulfate into the cell [16, 17]. In addition, the placenta and developing fetus are reliant on sulfate from the maternal circulation because placental and fetal cells have a relatively low capacity to form sulfate from methionine and cysteine [1, 18, 19]. Sulfonation reactions in all organisms require the conversion of sulfate to the universal sulfonate (SO₃⁻) donor, 3'-phosphoadenosine 5'-phosphosulfate (PAPS) [20]. The generation of PAPS is mediated by the bifunctional enzyme, PAPS synthetase, which sulfurylates ATP to form adenosine 5'-phosphosulfate (APS) followed by phosphorylation to form PAPS [21] (Figure 1B). The sulfonate group from PAPS is then transferred to the target substrate via sulfotransferase enzymes, which can be grouped into two classes: (i) membrane-bound in the golgi where they sulfonate glycosaminoglycans, proteins, peptides and lipids; and (ii) cytosolic sulfotransferases which sulfonate neurotransmitters, bile acids, xenobiotics and steroids [22].

Early studies described the presence of steroid sulfates in biological samples, including urine [23]. Biochemists had also described the chemical incorporation of sulfate (SO₄²⁻) into steroids [24], a process which we refer to as sulfation and not to be confused with the metabolic process of sulfonation which is mediated by sulfotransferases with PAPS as the sulfonate (SO₃²⁻) donor [20]. In 1955, De Meio and Lewycka provided initial evidence that DHEA could be enzymatically conjugated with sulfate using rat liver extracts [25]. These findings

were supported by subsequent studies showing that rabbit liver extracts could mediate sulfate conjugation of 14 steroids, including testosterone and deoxycorticosterone [26]. The landmark report of sulfate activation to PAPS [27] and the subsequent identification of sulfotransferases [22, 27], has led to our current understanding of sulfonation, and the physiological importance of this process in modulating the biological activity of steroids [28, 29]. Over the past decade, interest in steroid sulfonation and links to mammalian pathophysiology has expanded (Figure 2).

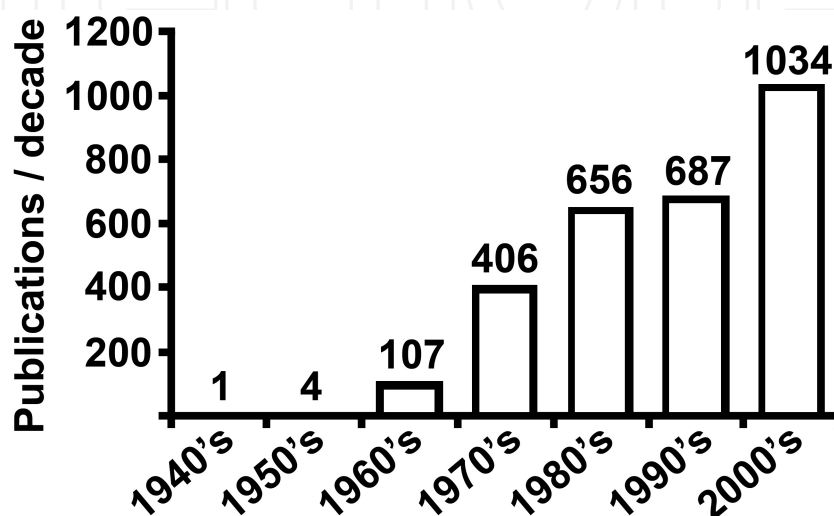


Figure 2. The number of articles published in the field of steroid sulfonation. Articles were identified in PubMed using the key words ((sulfonation or sulfation or sulfotransferase or sulfatase) and steroid). The increasing number of articles in recent years reflects the current interest in steroid sulfonation.

2. Steroid sulfotransferases

To date, five gene families of mammalian sulfotransferases (SULT1, SULT2, SULT3, SULT4 and SULT5) have been identified [22]. In humans, five subfamily members of SULT1 and SULT2 have been linked to steroid sulfonation (Table 1) with some overlap in the specificity of substrates (Figure 3).

Human SULT1A1 was initially cloned from liver where it mediates the sulfonation of numerous phenolic compounds [30]. SULT1A1 has since been found in several extrahepatic tissues, such as platelets which have been widely used for biochemical phenotyping of six SULT1A1 alloenzymes, each with a different enzyme activity due to the presence of amino acid variants: SULT1A1*1, SULT1A1*2 (R213H), SULT1A1*3 (M223V), SULT1A1*4 (R37G), SULT1A1*V (A147T + E181G + R213H), and SULT1A1*VI (P90L + V243A) [22, 31-33]. SULT1A1 exhibits high specific activity towards 17β -estradiol, 17β -estrone, DHEA and 2-methoxyestradiol at relatively high nonphysiological concentrations (i.e. micromolar) *in vitro* [33], suggesting that SULT1A1 may not play a major physiological role in steroid sulfonation.

SULT	Tissue expression	Steroid substrates	References
SULT1A1	liver, adrenal, bladder, platelets, bone,	17 β -estradiol,	[31,34]
	brain, eye, intestine, kidney, lung,	17 β -estrone, DHEA,	
	lymph, ovary, breast, spleen, pancreas,	2-methoxyestradiol	
	thyroid, testis, stomach, placenta,		
	salivary gland, prostate, uterus		
SULT1E1	adrenal, liver, heart, kidney, lung, eye,	17 β -estradiol,	[22,35]
	muscle, pharynx, larynx, placenta,	17 β -estrone, 17 β -estriol	
	trachea, endometrium, stomach, brain	DHEA, pregnenolone	
SULT2A1	adrenal, liver, small intestine, muscle,	DHEA, pregnenolone	[28,32,36]
	brain, breast, placenta, stomach	androgens, 17 β -estradiol	
SULT2B1a	ovary, lung, kidney, colon, skin, prostate	pregnenolone, DHEA	[37,38]
SULT2B1b	placenta, skin, prostate, colon	cholesterol, DHEA,	[39,40]
	lung, kidney, colon, stomach, spleen,	pregnenolone,	
	small intestine, thymus thyroid, liver	oxysterols	
	breast, platelets, ovary, brain		

Table 1. Human Sulfotransferases (SULTs), tissue expression and steroid specificity.

SULT1E1, also referred to as estrogen sulfotransferase, shows high affinity for 17 β -estradiol, 17 β -estrone and 17 β -estriol, at physiological (nanomolar) concentrations, to form estrogen-3-sulfates (Figure 3). This enzyme also sulfonates DHEA and pregnenolone, as well as numerous synthetic estrogens, including diethylstilbestrol [33]. Human SULT1E1 is expressed in several tissues, with high levels detected in the liver and adrenal glands [22, 33]. Endometrial SULT1E1 levels are influenced by the stage of pregnancy and by the menstrual cycle [41, 42]. This most likely reflects the up-regulation of SULT1E1 gene expression by progesterone [43].

Originally named DHEA sulfotransferase [44], SULT2A1 is strongly expressed in the fetal adrenal gland (zona reticularis), as well as the adult liver, adrenal gland and duodenum, where it plays a major role in sulfonating DHEA [22, 33]. SULT2A1 also sulfonates other hydroxysteroids including pregnenolone, as well as 17 β -estradiol and testosterone to form estradiol-17-sulfate and testosterone-17-sulfate, respectively [33].

The SULT2B1a and SULT2B1b proteins are encoded by the same gene but differ in the amino acid sequences at their amino-terminal ends, as a result of an alternative exon 1 [45]. SULT2B1a preferentially sulfonates pregnenolone [37, 38], whereas SULT2B1b plays a major role in cholesterol sulfonation, particularly in the skin [39, 40].

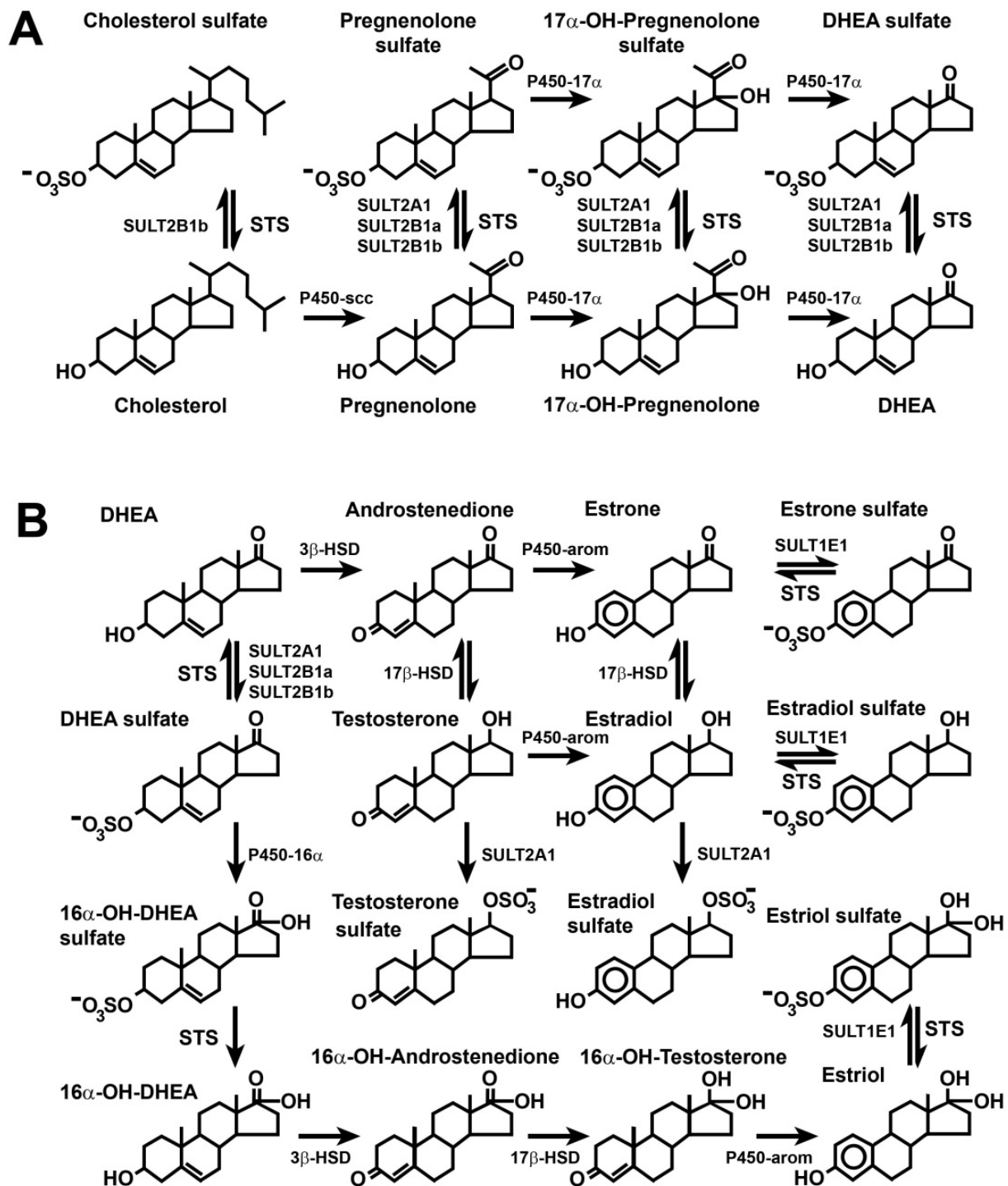


Figure 3. Steroid sulfonation and de-conjugation pathways play an important role in steroid metabolism, as well as regulating steroid half-life and activity. (A) Steroid sulfatase (STS) converts cholesterol sulfate to cholesterol, which is then transported into mitochondria for conversion to pregnenolone, and downstream adrenal products including DHEA and DHEA sulfate. (B) DHEA sulfate serves as the precursor molecule for synthesis of the non-adrenal steroid hormones, including testosterone and estrogens. In most cases, sulfonation decreases the biological activity of steroids by preventing binding to steroid receptors.

3. Steroid sulfatase

In humans, 17 sulfatases have been identified [46], of which steroid sulfatase (aka STS or aryl sulfatase C) mediates the hydrolysis of alkyl (e.g. DHEA-S, pregnenolone sulfate, deoxycorticosterone sulfate and cholesterol sulfate) and aryl (e.g. estrone sulfate, estradiol sulfate and estriol sulfate) steroid sulfates [47-49] (Figure 3). STS is a membrane-bound enzyme (EC 3.1.6.2), which has been detected in the rough endoplasmic reticulum, Golgi cisternae, trans-Golgi and plasma membrane, as well as in the coated pits, endosomes and multivesicular endosomes [50]. The STS gene is located on the short arm of the X chromosome, within a region (Xp22.3) that partially escapes X chromosome inactivation [51]. As a consequence, STS enzymatic activity in XX females is higher (by ≈ 1.6 -fold) when compared to XY males. The human Y-chromosome contains an STS pseudogene that is most likely a gene duplication of STS on the X-chromosome, but it lacks the 5'-regulatory DNA sequences necessary for gene expression and hence does not express a functional STS protein [52]. STS is expressed in numerous fetal tissues, including brain, adrenal gland, small and large intestine, liver, thyroid, thymus, lung, heart and kidney [50]. In adults, STS expression is most abundant in testis, uterus, prostate, thyroid, lung, liver and skin. In addition, STS is strongly expressed in the placenta where it is responsible for desulfonating DHEA sulfate, which is derived from maternal circulation and the fetal adrenal glands. Placental STS also deconjugates 16α -hydroxy-DHEA sulfate (Figure 3), which is produced in the fetal liver [53]. Thus, STS plays an important step in the pathway for generating estriol, which is the most abundant estrogen during human pregnancy.

Deficiency of STS leads to X-linked ichthyosis (XLI, OMIM 308100), which affects approximately 1 in 6000 males [54, 55]. Most cases (≈ 80 -90%) are caused by complete deletion of the STS gene, whereas small deletions or point mutations account for the remainder of cases [56]. Loss of STS leads to an accumulation (up to 20-fold increase) of its substrate, cholesterol sulfate, in plasma and red cell membranes, as well as the epidermis [57]. Excess cholesterol sulfate in the skin, delays desquamation which leads to hyperkeratosis that appears as large, polygonal, dark brown scales on the skin. Extracutaneous manifestations of this disorder include corneal opacity, cryptorchidism, epileptic seizures and reactive psychological disorders. Some isolated cases of X-linked ichthyosis have presented with pyloric hypertrophy, acute lymphoblastic lymphoma and congenital defect of the abdominal wall [57], however, the link between these clinical conditions and STS is not clear. The clinical manifestations of X-linked ichthyosis in STS deficient patients present after birth, indicating that loss of STS activity may not be essential for fetal development.

To function effectively, all sulfatases including STS, need to be post-translationally modified by the formylglycine generating enzyme (FGE), which is encoded by the sulfatase modifying factor 1 (SUMF1) gene [58]. Mutations in the SUMF1 gene, leads to multiple sulfatase deficiency (MSD, OMIM 272200), which is characterised by congenital growth retardation, skeletal abnormalities, neurological defects and early mortality. Similar phenotypes have been observed in Sumf1 knockout mice [59], confirming that other genes do not compensate

for loss of SUMF1. These findings highlight the importance of maintaining the required balance of sulfonated substrates, including steroid sulfates, in mammalian physiology.

4. Physiological roles of sulfonated steroids

Steroid sulfates were initially thought to be end products of metabolism, with the sulfate merely increasing the water solubility of the steroid and enhancing its excretion into the urine [60]. More recent studies have revealed steroid sulfates to be important precursors for the formation of biologically active steroids, or to have physiological roles that are distinct from non-sulfonated steroids [28, 32].

The physicochemical properties of steroids is markedly changed when conjugated to sulfate. In most cases, sulfonation decreases the biological activity of steroids by preventing their binding to steroid receptors [28]. For example, whilst estrogens bind to their genomic estrogen receptors, estrogen sulfates do not bind. This finding is supported by the over-expression of SULT1E1 in cultured human breast carcinoma-derived cells, as well as uterine endometrial Ishikawa cells, which abolish estrogen-stimulated cell proliferation [61, 62]. Conversely, increased expression of STS which increases unconjugated (active) steroid levels, leads to enhanced estrogen-stimulated cell proliferation [63]. Sulfate also contributes to the modulation of cholesterol function. In addition to serving as a substrate for adrenal and ovarian steroidogenesis, cholesterol sulfate has been linked to several biological processes, including: regulation of cholesterol synthesis; plasmin and thrombin activities; sperm capacitation; and activation of protein kinase C [50].

Sulfonation in the brain modulates the nongenomic actions of neurosteroids on GABA_A, N-methyl-D-aspartate, glutaminergic and σ -opioid receptors, usually in opposing ways [64]. For example, pregnenolone sulfate is a picrotoxin-like antagonist, whereas unconjugated pregnenolone is a barbiturate-like agonist. In addition, DHEA sulfate stimulates acetylcholine release from the hippocampus but unconjugated DHEA does not. These findings may be relevant to the association of pregnenolone sulfate and DHEA sulfate with enhanced cognitive function in animals [64]. Furthermore, reduced circulating DHEA sulfate and pregnenolone sulfate levels have been linked with decreased cognitive function in humans. Studies have also reported reduced circulating DHEA sulfate levels in patients with Alzheimer's disease and multi-infarct dementia [65, 66]. These findings, together with the detection of SULT1A1, SULT1E1, SULT2A1, SULT2B1 and STS in the fetal and adult brain, suggests that sulfonation and deconjugation of neurosteroids contributes to neurodevelopment and maintenance of brain function. Of great interest is the detection of SULT4A1 in brain [67], however, its substrate and physiological role is yet unknown.

Steroid sulfates avidly bind to serum proteins, particularly albumin as well as corticosteroid binding globulin (aka CBG, transcortin) and sex hormone binding globulin (aka SHBG, androgen-binding protein, testosterone-binding β -globulin) [28, 68-71]. Binding of steroid sulfates to serum proteins slows their urinary clearance by approximately 2 orders of magnitude, when compared to unconjugated steroids [72]. Accordingly, circulating steroid

sulfate levels are higher when compared to their non-sulfonated forms. For example, the ratio of estrogen sulfate to estrogen is approximately several-fold [28, 32]. The high level of albumin-bound steroid sulfates in circulation is proposed to provide a pool of inactive steroids which can be taken up by peripheral target tissues, where deconjugation via STS generates active steroids. Animal studies have provided evidence linking reduced sulfonation capacity with decreased plasma steroid sulfate levels and increased urinary steroid secretion [73]. The NaS1 knockout mouse, which exhibits hyposulfataemia and reduced sulfonation capacity [12, 15], has decreased (by $\approx 40\text{-}50\%$) circulating levels of DHEA, DHEA-S and corticosterone, whereas urinary levels of corticosterone and DHEA were increased (up to 40%) [73]. This study implied that the reduced sulfonation of steroids, led to the observed increased urinary steroid secretion which lowered circulating steroid levels. This proposal is supported by an earlier study, which reported reduced circulating DHEA-S levels in mice with low *Sult2a1* and sulfate donor 3'-phosphoadenosine 5'-phosphosulfate synthase 2 (*PAPSS2*) mRNA levels [74]. These findings highlight the functional consequences of steroid sulfonation in maintaining a circulating reservoir of steroids that can be drawn upon by target cells in the body.

Sulfonated steroids are moved through the plasma membrane of cells by several different transporter proteins, including the sodium-dependent organic anion transporter (aka SOAT) [75], the sodium-independent organic anion transporting polypeptides (aka OATPs) [76] and the ATP binding cassette (ABC) proteins [77] (Figure 1). ABC transporters are ubiquitously expressed and are mostly considered responsible for the efflux of steroid substrates, whereas SOAT and OATPs mediate tissue-specific bi-directional transport of steroid sulfates across the plasma membrane of cells. Initially identified in rat adrenal glands, SOAT has since been detected in human adrenal glands, as well as numerous additional tissues in rodents, including kidney, lung, mammary gland, liver, uterus, brain and testis. SOAT shares homology with the apical sodium-dependent bile acid transporter (aka ASBT, SLC10A2) but is not a bile acid transporter. Rather, SOAT transports steroid sulfates, including estrone-3-sulfate, pregnenolone sulfate and DHEA sulfate. Four families of OATP (OATP1, OATP2, OATP3 and OATP4) have been shown to transport DHEA sulfate and estrone-3-sulfate. The OATP1 genes are expressed throughout the body, with highest expression levels for sub-family members: OATP1A2 in the brain, liver, lung, kidney and testis; OATP1B1 and OATP1B3 specifically expressed in the liver; and OATP1C1 in the brain and testis. The OATP2 sub-family member OATP2B1 is expressed in numerous tissues, including liver, syncytiotrophoblasts of the placenta, mammary gland, heart, skeletal muscle and endothelial cells of the blood-brain barrier. The OATP3A1_v1 transporter is expressed in the germ cells of the testis, as well as in the choroid plexus and frontal cortex. Two OATP4 sub-family members have been identified in the following tissues: OATP4A1 in heart, lung, liver, skeletal muscle, kidney, pancreas and syncytiotrophoblasts in the placenta; whereas OATP4C1 is localised to the basolateral membranes of renal proximal tubules. Whilst certain sulfonated steroids (i.e. DHEA sulfate and estrone-3-sulfate) have been used to test the substrate specificity of the above ABC, SOAT and OATPs, further studies are required to investigate all known naturally occurring (as well as synthetic) steroid sulfate substrates.

Together, these studies demonstrate that sulfate plays important but unappreciated roles in modulating circulating steroid levels and cellular efflux and uptake of steroids, as well as biotransforming the biological activity of steroids.

4.1. Steroid sulfates in pregnancy

Sulfonation of cholesterol in maternal and placental tissues provides an essential precursor for the synthesis of steroid sulfates, including DHEA sulfate. Whilst the steroid biosynthetic pathway is limited in the fetus, DHEA sulfate is produced in the fetal adrenal gland (zona reticularis) and then circulated to the placenta where it provides the major supply of DHEA sulfate ($\approx 90\%$) for production of estrone, estradiol and other fetal steroids [1]. DHEA sulfate is also converted to 16α -hydroxy DHEA sulfate in the fetal liver, via 16α -hydroxylase, and subsequently converted to estriol ($>60\text{mg/day}$ during the third trimester of human gestation) in the placenta (Figure 3). Whilst decreased levels of estriol in maternal circulation have been used as a marker for Down and trisomy 18 syndromes, pregnancy loss, as well as gross neural tube defects such as anencephaly [78], the role of perturbed DHEA and estriol sulfonation in modifying maternal estriol levels and possibly human fetal development, awaits further investigation.

Steroid sulfates are the major form of steroids supplied to fetal tissues. For example, placental estradiol-3-sulfate is taken up by the fetal brain where it is de-sulfonated by STS to estradiol (Figure 4), which acts as a potent stimulator of fetal adrenocorticotropin (ACTH) secretion and hypothalamus-pituitary-adrenal (HPA) axis [79]. Accordingly, the ratio of sulfonated (inactive) to unconjugated (active) steroids plays an important role in many of the steroid-responsive molecular events that regulate placental and fetal growth and development [28]. This is relevant to the mid-gestational fetal loss and placental thrombosis that was observed in mice lacking the *Sult1e1* estrogen sulfotransferase [80]. *Sult1e1* is highly expressed in the placenta where it is essential for generating estrone sulfate, estradiol-3-sulfate and estriol sulfate (Figure 3). In addition, *Sult1e1* is abundantly expressed in the testis. Male *Sult1e1* knockout mice develop Leydig cell hypertrophy/hyperplasia, seminiferous tubule damage, reduced sperm motility and sire smaller litters when compared to age-matched control males. These studies highlight the importance of estrogen sulfonation in maintaining mammalian pregnancy and normal male reproductive function.

A sufficient supply of intracellular sulfate needs to be maintained for sulfonation reactions to function effectively [60, 81]. During human and rodent pregnancy, maternal circulating sulfate levels increase approximately 2-fold, with levels peaking in the second and third trimesters [82-85]. This increase is associated with elevated kidney *NaS1* and *Sat1* gene expression [83, 86] and renal sulfate reabsorption [87] in the pregnant mother (Figure 4). The increased circulating sulfate level in pregnant humans (from ≈ 0.26 to 0.59 mM) [82, 88, 89] and mice (from ≈ 1.0 to 2.3 mM) [83] enhances sulfate availability to the placenta and fetus, and is remarkable since most circulating ions usually decrease slightly due to haemodilution [90]. Since the placenta and fetus have a relatively low capacity to generate sulfate from methionine and cysteine [18, 19], most of the sulfate in these tissues must come from the maternal circulation (Figure 4). This is consistent with fetal hyposulfatemia and negligible amniotic fluid sulfate levels in fetuses from pregnant

hyposulfataemic NaS1 null mice [83]. Of great interest is the reduced fecundity of female NaS1 null mice [12], as a result of fetal death in late gestation (from embryonic day 12.5) [83] which is a similar gestational age when fetal death occurs in the Sult1e1 null mice [80]. These studies highlight the importance of maintaining a sufficient supply of sulfate to placental and fetal cells in mammalian gestation.

Recently, the relative abundance and cellular expression of all known placental sulfate transporters was described [91]. That study identified Slc13a4 (aka NaS2, Sodium sulfate transporter-2) to be the most abundant placental sulfate transporter, which was localised to the syncytiotrophoblasts of mouse placenta, where it is proposed to supply sulfate into the placenta from maternal circulation. The role of placental NaS2, as well as kidney NaS1 (Figure 4), in modulating placental endocrine function awaits further investigation.

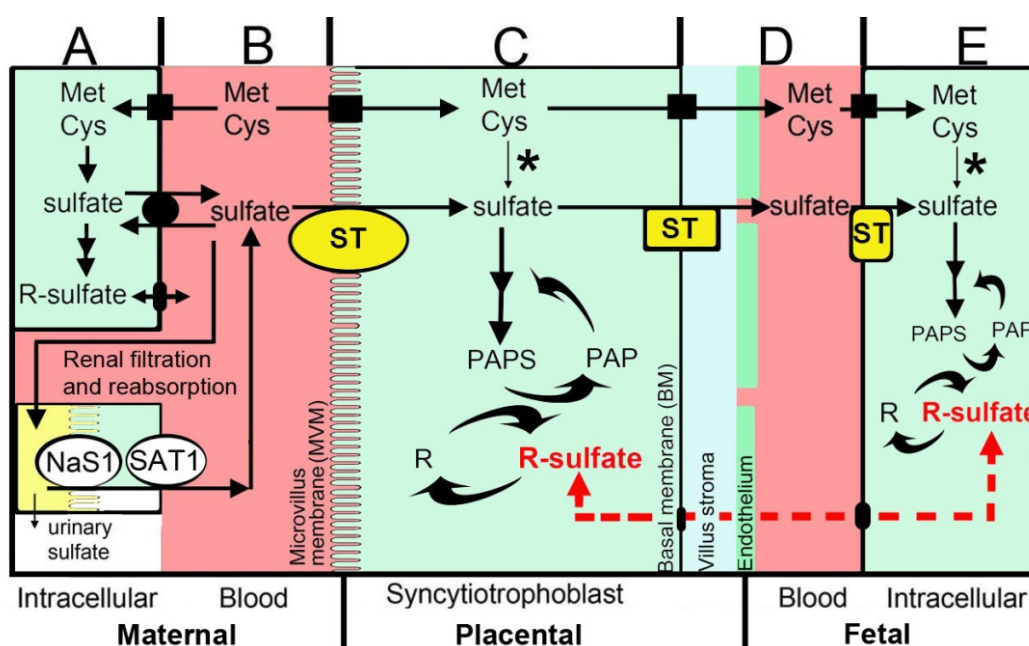


Figure 4. Sulfate supply from mother to placental and fetal tissues is essential for sulfonation reactions to function effectively. R-sulfate represents sulfonated substrates, including steroids such as estrogens and DHEA. During pregnancy: (A) Increased kidney NaS1 and SAT1 expression from early gestation (in mice from E4.5) enhances renal sulfate reabsorption, which leads to (B) ≈ 2 -fold increased maternal blood sulfate levels. (C) NaS2 expression in syncytiotrophoblasts mediates sulfate transport (ST) for generation of the universal sulfate donor PAPS (3'-phosphoadenosine 5'-phosphosulfate). Both sulfonation and de-sulfonation are active within placental and fetal metabolism. (D) Sulfate is moved through the villus stroma and inter-endothelial clefts into fetal circulation. (E) Fetal intracellular sulfate levels are maintained by sulfate transporters (ST). *Negligible sulfate is derived from Methionine (Met) and Cysteine (Cys) in fetal and placental cells.

4.2. Role of steroid sulfotransferases and sulfatase in cancer

Over the past decade, interest in steroid sulfonation/de-sulfonation and cancer has expanded following our knowledge of sulfonated (inactive) and unconjugated (active) steroids and the requirement for unconjugated steroids (particularly 17 β -estradiol) for maintaining growth of some carcinoma cells [28, 32]. STS is upregulated in many hormone-dependent

neoplasms, including breast, endometrial, ovarian and prostate cancers [50, 64]. Increased STS causes the conversion of: (i) estrone sulfate to estrone which is then reduced to estradiol; and (ii) estradiol-3-sulfate to estradiol (Figure 3). Excess estradiol then binds to the estrogen receptor and causes cell proliferation [92]. In addition, upregulation of STS causes the conversion of DHEA sulfate to DHEA, which is then further metabolised to the active androgens: androstenediol, testosterone and dihydrotestosterone that bind the androgen receptor, leading to cell proliferation. STS is detected in most cases of: (i) malignant prostate cancer tissue (85%) but not in the non-neoplastic peripheral tissues; breast tumours (90%) and may be a predictor of recurrence of breast cancer in ER positive tumours; and (iii) ovarian cancer (97%) with low STS activity linked to increased survival time [92]. The link between STS, unconjugated estradiol, active androgens and tumour cell proliferation has led to the development of STS inhibitors [92]. Clinical trials with sulfatase inhibitors in patients with estrogen- and androgen-driven malignancies are in progress and we await outcomes.

The potential roles of steroid sulfotransferases in the induction and maintenance of hormone-dependent cancers has also gained attention. SULT1E1 activity is more abundant in normal breast cells lines when compared to cancer cells lines. In cultured carcinoma cells, transfection of SULT1E1 led to effective reductions in estrogen-mediated cell proliferation [61, 62]. This can be relevant to polymorphisms in the SULT1E1 gene which have been associated with increased risk of breast cancer and reduced disease free survival [93]. Additional studies have linked human sulfotransferase polymorphisms with numerous neoplasias, including endometrial, breast, prostate, lung, mouth, gastric, colorectal and bladder cancers [73, 93-95]. In addition, reduced SULT2A1 expression has been found in hepatocellular carcinoma cells (HCC), and the lowest level of SULT2A1 expression correlates with a higher grade and stage of HCC [96].

Animal studies have demonstrated the link between reduced sulfonation capacity and increased tumour cell growth [97]. The hyposulfataemic NaS1 null mouse, which has reduced circulating DHEA sulfate levels [73], was injected with tumour cells (TC-1) derived from lung epithelium. After 14 days, tumour weights from the NaS1 null mice were increased \approx 12-fold when compared to the control mice with normal sulfonation capacity [97]. The tumours grown in NaS1 null mice also showed an increased abundance of vessels, indicating that reduced sulfate supply exacerbates angiogenesis in tumour cell growth. That study highlighted the importance of blood sulfate levels as a possible modulator of tumour growth.

4.3. Role of PAPS synthetase in steroid homeostasis

In addition to the requirement for intracellular sulfate levels, steroid sulfonation requires a sufficient supply of the PAPS sulfonate donor [20]. Generation of PAPS is the rate limiting step for all sulfonation reactions. PAPS is synthesized in two steps: (1) sulfurylation of ATP to form adenosine 5'-phosphosulfate (APS); (2) phosphorylation of APS to form PAPS. Both sulfurylation (EC 2.7.7.4) and kinase (EC 2.7.1.25) activities are mediated by the bifunctional enzyme, PAPS synthetase [21]. Two PAPS synthetase enzymes (PAPSS1 and PAPSS2) have been identified in rodents and humans [98-100]. PAPSS2 is the more abundant isoenzyme in

tissues from adults such as the liver and adrenal glands that have a high sulfonation capacity, and its catalytic activity is approximately 10- to 15-fold higher when compared to PAPSS1 [32]. A number of gene variants have been found in the PAPSS1 gene [101] but their effect on sulfonation capacity is not yet known. Since PAPSS1 is the predominant PAPS synthetase in the developing central nervous system and bone marrow, its loss is proposed to be embryologically lethal [32]. Mutations in the PAPSS2 gene have been linked to developmental dwarfism disorders, including spondyloepimetaphyseal dysplasia in humans, and brachymorphism in mice [98].

In one case, inactivating mutations in the PAPSS2 gene were linked to premature pubarche, hyperandrogenic anovulation and increased androgen levels in a young female patient [102]. Her endocrine profile showed androstenedione and testosterone levels at 2-fold above the upper limit of normal ranges, a DHEA level at the upper limit of the normal range, and DHEA sulfate level at one order of magnitude below the normal range. The clinical presentations of this patient were proposed to be a consequence of reduced DHEA sulfonation, which led to increased circulating levels of unconjugated DHEA that were converted to androgens. A more recent study showed a trend ($P=0.06$) for a lower ratio of circulating DHEA sulfate to DHEA, in a cohort ($n=33$) of children with premature adrenarche, and harbouring a polymorphism (rs182420) in the SULT2A1 gene [103]. SULT2A1 genetic variants have also been associated with reduced DHEA sulfate and inherited adrenal androgen excess in some women with polycystic ovary syndrome [104]. These findings identify a link between PAPSS2, SULT2A1, reduced sulfonation of DHEA and androgen excess disorders.

In addition, studies have linked reduced gene expression of both PAPSS2 and Sult2A1 to reduced circulating DHEA sulfate levels in mice with lipopolysaccharide-induced acute-phase response [74]. These findings suggest that perturbed steroid sulfonation may be a mechanism for decreased DHEA sulfate levels found in patients with infection, inflammation and trauma that induces metabolic changes in the liver as part of the acute-phase response.

In summary, there is growing body of evidence that disruption of the steroid sulfonation pathway, via sulfate/PAPS supply and sulfotransferase activity, leads to perturbed endocrine homeostasis and associated clinical manifestations.

5. Conclusion

Sufficient intracellular sulfate levels and its sulfonate donor PAPS, as well as sulfotransferases (see Table 1), are required for maintaining steroid sulfonation capacity. Furthermore, sulfatases are needed to generate unconjugated active steroids. Together, sulfate transporters, PAPS synthetases, sulfotransferases and sulfatases are essential for maintaining a balance of steroid sulfates and unconjugated steroids, which play different biological roles in humans and animals. Whilst the field of steroid sulfonation is largely unappreciated, its significance is being realised with experimental findings from animal models of reduced sulfate supply (i.e. NaS1 null mouse) and loss of sulfotransferase activity (i.e. Sult1e1 null mouse), as well as links to certain cancers and increased androgen levels.

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Author details

Paul Anthony Dawson

Address all correspondence to: paul.dawson@mmri.mater.org.au

Mater Medical Research Institute, South Brisbane, Queensland, Australia

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