Strategies for drug discovery by targeting sulfation pathways

Stefan Hemmerich, Dawn Verdugo and Virginia L. Rath

Posttranslational modifications of proteins such as phosphorylation have been recognized as pivotal modulators of biological activity in healthy and diseased tissues. Sulfation is a key posttranslational modification the role of which in physiology and pathology is only now becoming appreciated. Whereas phosphorylation is central to intracellular signal transduction, sulfation modulates cell–cell and cell–matrix communication. Sulfation involves a class of enzymes known as sulfotransferases, which transfer sulfate from the ATP-like sulfate donor 3′phosphoadenosine-5′phosphosulate to glycoproteins, glycolipids or metabolites. This review focuses on Golgi-localized sulfotransferases, their molecular biology and biochemistry, and strategies towards discovery of sulfotransferase inhibitors that could have potential as therapeutics in inflammation, cancer and infectious diseases.

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▼ One of the surprising results of the recent mapping and sequencing of the human genome has been the number of genes, ~25,000–35,000 rather than the >100,000 predicted by earlier estimates [1,2]. Therefore, at least in terms of number of genes, humans do not appear to be significantly more complex than invertebrates such as the worm *Caenorhabditis elegans* (25,000 genes) [3] or the fly *Drosophila melanogaster* (15,000 genes) [4]. Indeed, we are only about fivefold more gene-rich than the yeast *Saccharomyces cerevisiae* (6,000 genes) [5]. At the time, Venter and colleagues [2] speculated that the unexpectedly low number of genes found in mammals might be compensated for by combinatorial diversity generated through posttranslational modification of proteins. Posttranslational modifications include glycosylation [6], methylation (of histones) [7], acylation (myristoylation [8], prenylation [9]), and phosphorylation [10]. These modifications are pivotal to protein structure and function and play fundamental roles in health and disease. Phosphorylation in particular has become a prime target for drug discovery [11] and several inhibitors of phosphorylation, including Gleevec (imatinib mesylate), an inhibitor of the ABL kinase, and Iressa (gefitinib), an inhibitor of EGF kinase, are now approved drugs for various cancer indications.

Sulfation is another, so far under-exploited, posttranslational modification of proteins and glycoproteins. Sulfation involves attachment of a sulfate ester to a hydroxyl or, less frequently, an amino moiety on a biomolecule and is catalyzed by a class of enzymes known as sulfotransferases (STs). The reverse process, desulfation, is the removal of a sulfate from a biomolecule that is catalyzed by another class of enzymes known as sulfatases. The sulfated biomolecule can be a glycoprotein, a proteoglycan, a glycolipid, or a small molecule metabolite. This review will focus on sulfation of glycoconjugates as sulfation of metabolites and tyrosines has been reviewed extensively elsewhere [12,13].

Sulfation pathways

Although sulfation is mechanistically analogous to phosphorylation [14], its biological consequences are not in intracellular signal transduction but rather in extracellular cell–cell and cell–matrix interactions. Thus, sulfated glycoproteins and proteoglycans are usually targeted to the cell surface or secreted into the extracellular medium, where they support cell adhesion or presentation and modulation of growth and differentiation factors, chemokines and other molecules involved in intercellular communications.

Whereas sulfation exerts its biological function outside the cell, the sulfate cycle in a mammalian cell (Figure 1) commences with uptake of inorganic sulfate from the extracellular medium into the cytosol, mediated through a variety of sulfate transporters [15],

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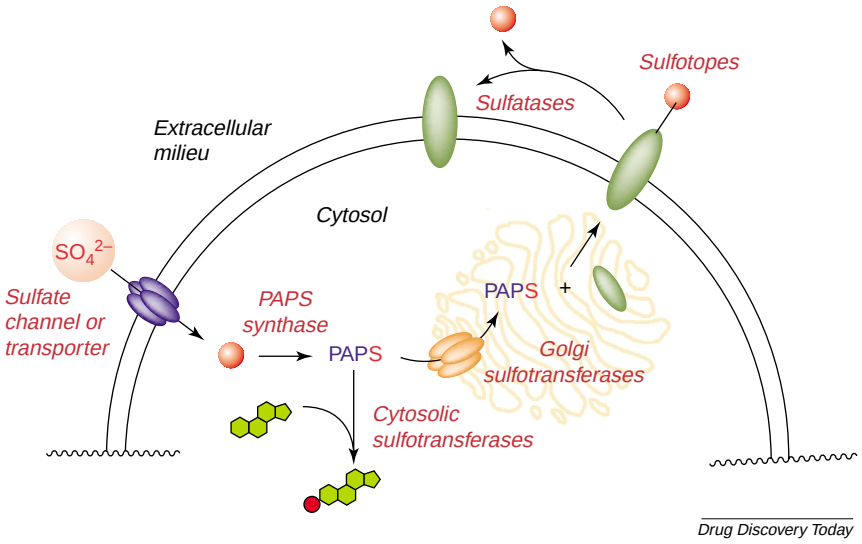

Figure 1. The sulfation cycle. Cells take up inorganic sulfate from the outside through sulfate channels or transporters. In the cytosol, the enzyme PAPS synthase catalyzes the formation of PAPS from ATP and SO_4^{2-}. PAPS is the universal sulfate donor for all STs and is either utilized by cytosolic STs for sulfation of small metabolites, or it is translocated into the Golgi compartment through a PAPS transporter, recently described as the human homologue of the *Drosophila melanogaster* slalom protein. Golgi-localized STs then transfer sulfate from PAPS onto nascent glycoprotein or glycolipid substrates as they pass through the Golgi on their way to presentation on the plasma membrane or secretion. The resulting sulfotopes often constitute specific binding epitopes for adhesion molecules or soluble factors involved in intercellular communication. In some cases, membrane-associated or extracellular sulfatases further modulate the biological function of these sulfotopes by removal of a subset of the critical sulfate residues.

some of which have remarkably restricted expression patterns. In the cytosol, one sulfate is then reacted with one molecule of ATP to form a sulfo-nucleotide known as 3′-phosphoadenosine 5′-phosphosulfate (PAPS). This is an energy-consuming two-step process catalyzed by the bifunctional enzyme PAPS synthase [16], the universal sulfate donor for all STs [17]. Although the STs involved in sulfation of metabolites reside in the cytosol [12], the enzym es-involved in sulfation of glycoconjugates and the subject of this review are Golgi-resident type-2 transmembrane proteins that extend their catalytic domains into the Golgi lumen. To serve as a substrate for Golgi-localized STs, PAPS is transported into this compartment through a specific transporter known in human as PAPST1, an ortholog of the *D. melanogaster* slalom (sll) protein [18]. In the Golgi compartment, resident STs transfer the sulfate from PAPS onto an appropriate acceptor on the nascent glycoconjugates that are being shuttled to the plasma membrane or to the extracellular medium. The sulfated epitopes (sulfotopes) presented by the mature glycoconjugate often constitute specific binding sites for adhesion molecules or soluble factors involved in cell–cell and cell–matrix communication. These sulfotopes can be desulfated by membrane-associated or extracellular endosulfatases, such as the recently discovered mammalian heparin endosulfatases SULF-1 and SULF-2 [19]. The activity of sulfated proteoglycans is also modulated through internalization into lysosomes, where they are processively degraded by specific exosulfatases and exoglycosidases [20].

Analogous to the kinases, which are key enzymes in the regulation of signal transduction, the Golgi-localized STs are involved in the regulation of sulfate-dependent cellular interactions. Their central role in critical cell-adhesion events in multiple diseases qualifies them as targets for drug discovery. The fact that STs are enzymes and, as such, potentially inhibitable by small molecules, makes them better tractable drug targets than the protein–protein and protein–carbohydrate interactions involved in communication between cells [21]. In this review we discuss Golgi-localized STs and their role in biosynthesis of physiologically and pathologically important sulfotopes. We further summarize current approaches to screen for inhibitors of these enzymes. In a subsequent review in a later issue of this journal we consider current ST inhibitors and explore how the knowledge of the structural biology of this class of enzymes might facilitate discovery of the next generation of ST antagonists.

The human sulfotransferase proteome

Biological sulfation was first discovered by E. Baumann in 1876 in sulfated metabolites in urine [22]. In the 1930s, sulfation of glycoconjugates (heparin, chondroitin sulfate and dermatan sulfate) was described by Karl Meyer and colleagues. Tyrosine sulfation was discovered in 1954 in fibrinogen [23] and enzymes capable of sulfating proteoglycans were first identified biochemically in the 1960s. The first cDNA encoding a Golgi-associated ST, heparin deacetylase N-sulfotransferase 1, was cloned in 1992 [24].

STs share a conserved amino acid sequence motif that binds the universal sulfate donor PAPS [25,26]. The presence of this conserved motif has been used to identify genes encoding novel carbohydrate STs [26]. Since the completion of the human genome sequencing project, most, if not all, of the human ST genes have been identified, and many

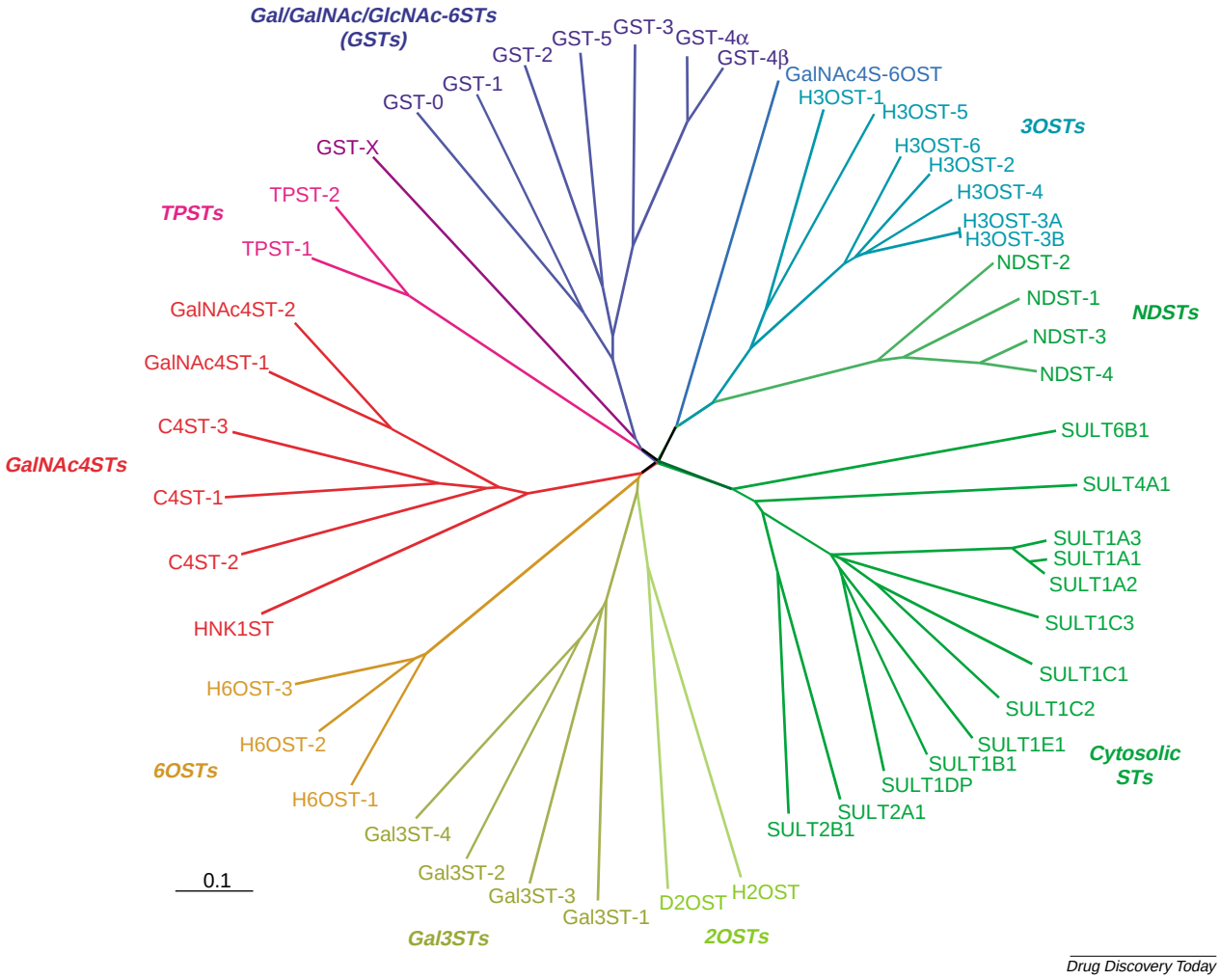

Figure 2. The human ST proteome (sulfome). The abbreviations for the different enzymes and enzyme families refer to Table 1. The catalytic domains encoded by the known human ST genes were aligned to each other using the clustalx algorithm [60]. The alignment was then entered into the TreeView program (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) to generate the above phylogenetic tree. The alignment of Golgi STs was performed using only the predicted catalytic domains: starting between 7 and 14 residues N-terminal of the characteristic P-loop motif known to interact with the 5′phosphate of PAPS [25], and ending at the C-terminus of the enzyme (tabulated in Table 1). The scale bar corresponds to 0.1 change per nucleotide.

have now been expressed as recombinant proteins and characterized. There are at least 50 genes known to encode, with splice-variants, a somewhat larger number of enzymes. These enzymes can be organized into families characterized by sequence homology and functional properties.

The 50 human ST genes illustrated in Figure 2 and tabulated in Table 1 include 13 cytosolic STs and 37 Golgi-associated STs. Within the Golgi-ST class, there are two protein tyrosine STs; the remainder adds sulfate to carbohydrates within glycoconjugates (proteoglycans, glycoproteins and glycolipids).

Of those STs that act on glycoconjugates, the two largest families are the heparin STs (NDSTs, 2OSTs, 3OSTs and 6OSTs) and the Gal/GalNAc/GlcNAc 6-O-STs (GSTs). In addition there are galactose 3-O-STs, which catalyze 3-O-sulfation at galactose in glycolipids (sulfatides) [27] and glycoproteins [28,29], as well as GalNAc 4-O-STs, which add sulfate to a variety of substrates, including: chondroitin sulfates A and E [30], the HNK-1 epitope [31] and the N-glycans elaborated on pituitary hormones [32]. Finally, there is a 6-O-sulfotransferase specific for GalNAc-4-sulfate moieties (GalNAc4S6ST) that is important in biosynthesis of chondroitin sulfate E and dermatan sulfate [33]. This enzyme does not belong to the GST family and cannot be assigned clearly to one of the other families presented in Figure 2 and Table 1. It was originally discovered as a protein coexpressed with B-rag and speculated to play an important role in B-cell development [34].

Table 1. The sulfotransferase proteome in human

Family	Enzyme	Gene	Accession number	Chromosome locus	ST-domain* (residue nos.)
Cytosolic STs	Phenol ST	SULT1A1	NP_001046	16p11.2	EPS#
		SULT1A2	NP_001045	16p11.2	EPS#
	Catecholamine ST	SULT1A3	NP_003157	16p12.2	EPS#
	Thyroid hormone ST	SULT1B1	NP_055280	4q13.3	EPS#
	Cytosolic ST family 1C	SULT1C1	NP_001047	2q12.3	EPS#
		SULT1C2	NP_006579	2q12.3	EPS#
		SULT1C3	DAA01771	2q12.3	EPS#
	Cytosolic ST 1D pseudogene	SULT1DP	NG_002642	4q13.3	EPS#
	Estrogen ST	SULT1E1	NP_005411	4q13.3	EPS#
	Dehydroepiandrosterone ST	SULT2A1	NP_003158	19q13.33	EPS#
	3 β-hydroxysteroid ST	SULT2B1	NP_004596	19q13.33	EPS#
	Cytosolic ST 4A	SULT4A1	NP_055166	22q13.31	EPS#
	Cytosolic ST 6B	SULT6B1	DAA01772	2p22.2	EPS#
TPSTs	Protein tyrosine ST-1	TPST-1	NP_003587	7q11.21	62–377
	Protein tyrosine ST-2	TPST-2	NP_003586	22q12.1	66–356
Gal/GalNAc/GlcNAc 6STs	Chondroitin 6-O-ST 1	GST-0	NP_004264	10q22.1	131–479
	Keratan sulfate galactose 6–O–ST	GST-1	NP_003645	11p11.2	59–411
	N-acetylglucosamine 6-O-ST	GST-2	NP_004258	3q24	163–530
	L-selectin ligand ST	GST-3	NP_005760	16q22.2	41–386
	Intestinal GlcNAc 6-O-ST	GST-4α	NP_036258	16q22.1	40–390
	Corneal GlcNAc 6-O-ST	GST-4β	NP_067628	16q22.1	39–395
	Chondroitin 6-O-ST 2	GST-5	NP_063939	Xp11.3	100–486
	NCAG1 (similar to ST)	GST-X	NP_115536	18q22.1	861–1222
2OSTs	Dermatan 2-O-ST	D2OST	NP_005706	6q25.1	105–406
	Heparin 2-O-ST	H2OST	NP_036394	1p22.3	49–307
3OSTs	Heparin 3-O-ST	H3OST-1	NP_005105	4p15.33	110–367
		H3OST-2	NP_006034	16p12.2	148–406
		H3OST-3A	NP_006033	17p12	133–399
		H3OST-3B	NP_006032	17p12	208–471
		H3OST-4	XP_056254	16p12.1	86–346
		H3OST-5	AAN37737	6q21	86–346
		H3OST-6	AAK61299	16p13.3	55–311
6OSTs	Heparin 6-O-ST	H6OST-1	NP_004798	2q21.1	79–410
		H6OST-2	NP_671703	Xq26.2	73–459
		H6OST-3	NP_703157	13q32.1	139–471
NDSTs	Heparin deacetylase N-ST	NDST-1	NP_001534	5q33.1	599–882
		NDST-2	NP_003626	10q22.2	598–884
		NDST-3	NP_004775	4q26	590–873
		NDST-4	NP_072091	4q26	589–872
Gal3STs	Galactosylceramide (sulfatide) ST	Gal3ST-1	NP_004852	22q12.2	72–423
	Glycoprotein β-Gal 3-O-ST	Gal3ST-2	NP_071417	2q37.3	48–398
	β-Galactose-3-O-ST 3	Gal3ST-3	NP_149025	11q13.2	59–431
	Galβ1–3GalNAc 3′-O-ST	Gal3ST-4	NP_078913	7q22.1	63–486
GalNAc4STs	HNK-1 ST	HNK1ST	NP_004845	2q11.2	79–256
	Chondroitin 4-ST	C4ST-1	NP_060883	12q23.3	76–352
		C4ST-2	NP_061111	7p22.3	119–414
		C4ST-3	NP_690849	3q21.3	61–341
	GalNAc 4-O-ST	GalNAc4ST1	NP_071912	19q13.11	151–424
		GalNAc4ST2	NP_113610	18q11.2	168–438
GalNAc4S6ST	GalNAc 4-sulfate 6-O-ST	GalNAc4S6ST	NP_055678	10q26.13	251–561

*used for alignment to generate Figure 2. #EPS: entire peptide sequence

Role of sulfotransferases in health and disease

Sulfotransferases act at critical steps in the biosynthesis of sulfotopes that constitute recognition motifs for adhesion molecules, chemokines, growth factors and their receptors, and pathogens. Inhibition of the responsible ST(s) poses an opportunity to modulate the downstream sulfotope and thus potentially ameliorate diseases that involve sulfation-dependent binding events. Here we focus on the function of heparin STs and Gal/GalNAc/GlcNAc-6-O-STs in generation of specific sulfotopes with suspected roles in cancer, viral infection and inflammatory diseases.

Heparin sulfotransferases

Heparan sulfate (HS) is a glucosaminoglycan (GAG) comprising a polymer of the disaccharide unit, GlcNAcα1→4GlcAβ1→4. It is elaborated from core proteins such as syndecan and perlecan, forming HS proteoglycans that are expressed on almost all types of cell. Following polymerization by the EXT1–EXT2 polymerase complex, HS undergoes a series of modification reactions catalyzed by at least four families of heparin STs and one epimerase [6]. Sulfate is first added to the nitrogen in GlcNAc, followed by 2-O-sulfation of the iduronic acid residues resulting from epimerization of GlcA. These modifications are followed by 6-O-sulfation and, less frequently, 3-O-sulfation of GlcNAc. Sulfation occurs in clusters along the HS chains, with regions devoid of sulfate separating the modified tracts. The related GAG heparin is produced exclusively by mast cells and differs from HS by having more extensive sulfation and uronic acid epimerization.

The specific arrangements of sulfated residues in heparin and HS gives rise to specific binding sites that are recognized by HS-binding factors and receptors. The best known example of such a binding site is the antithrombin binding site in heparin, which depends on specific 3-O-sulfation and 6-O-sulfation within a pentasaccharide [35]. Upon binding of heparin, antithrombin very efficiently neutralizes most of the enzymes generated during activation of the blood-clotting cascade, especially thrombin, factor IXa and factor Xa. Heparin, and low-molecular weight heparin fragments are used extensively in prevention and treatment of thrombosis.

Heparin 3-O-ST 1 (H3OST-1) has been shown to catalyze the rate-limiting step in the synthesis of anticoagulant heparin, however, blood clotting is not impaired in H3OST1-deficient mice [36]. It remains to be seen whether this result is due to redundancy with other 3OST isozymes (at least seven total), or because heparin is not involved in physiological hemostasis. HS sulfation is also important in viral infection, specifically in docking and cell entry of Hepatitis C virus [37] and Herpes simplex virus [38]. In the latter case, a 3-O-sulfation catalyzed by H3OST-3 was shown to be critical for viral entry. Therefore, specific heparin ST inhibitors could be antiviral drugs that might act synergistically with current therapies.

More generally, HS proteoglycans bind specific growth factors in a sulfation-dependent fashion [39]. In some cases, binding of the factor to HS and presentation of the HS-bound factor to its receptor is necessary for a productive biological effect. For example, fibroblast growth factors (FGFs) signal through their receptors only when bound and presented by HS. Binding of basic FGF to HS requires critical 6-O-sulfation [40], which is mediated by heparin 6-O-STs (at least three isozymes are known in mouse [41] and human, cf. Table 1). In other cases, binding of the factor by HS sequesters it in a biologically inactive form, from which it could potentially be released by upstream regulators such as heparanases or sulfatases. HS-dependent sequestration of growth and differentiation factors has been shown to occur in the Wnt pathway during development, as well as in the vascular endothelial growth factor (VEGF) pathway. In the quail embryo, Emerson and colleagues have shown that a novel sulfatase known as QSULF mobilizes HS-bound Wnt, which then triggers several developmental processes [42]. VEGF is also known to bind to a sulfated HS-epitope with a demonstrated involvement of glucosamine 6-O-sulfate [43].

Furthermore, in transgenic mouse models of cancer angiogenesis, VEGF is not synthesized *de novo*, but released from extracellular matrix HS [44]. Rosen and colleagues recently cloned and characterized human and mouse homologues of QSULF (SULF-1 and SULF-2) and defined them as extracellular glucosamine 6-O-endosulfatases that are active on HS [19]. These investigators showed both of these enzymes specifically hydrolyse sulfate from the 6-position of GlcNAc in a trisulfated IdoA2S-GlcNS(6S) disaccharide unit within heparin. Treatment of heparin with recombinant human SULF-2 released VEGF from heparin *in vitro* (K. Uchimura and S.D. Rosen, pers. commun.). Therefore, specific GlcN 6-O-sulfations in HS appear to be crucial for sequestration of important growth and differentiation factors and a potential mechanism for modulating these associations is through the SULFs. As the aforementioned three human heparin 6-O-STs could show subtle differences in their specificities for sugar motifs and their respective sulfation patterns [41], one or the other of these enzymes might modulate VEGF retention directly. Specific inhibitors of the enzyme(s) responsible could be beneficial in angiogenic therapy for conditions such as stable angina, where new cardiac blood vessel formation is desirable.

A third subfamily of heparin STs catalyzes deacetylation and N-sulfation of GlcNAc in HS. N-sulfation precedes all

other steps in HS sulfation [6], therefore, these enzymes are less selective drug targets than the more downstream heparin STs, such as the 6OSTs and 3OSTs. In fact, NDST-1-deficient mice show an embryonic lethal phenotype [45] and cultured embryonic fibroblasts from these mice lack >60% HS N-sulfation. However, the remaining three NDST isozymes appear to be more restricted in tissue expression and/or function. Specifically, NDST-2, although ubiquitously expressed, appears to be required in mast cell heparin biosynthesis only, because otherwise normal NDST-2-deficient mice have reduced numbers of abnormal mast cells and these lack heparin in their granules [46]. These mast cells, however, are still able to secrete histamine upon Fc receptor ligation. The striking discovery that mast cell-deficient mice are protected from experimental arthritis [47] raises the possibility of a role for mast cell heparin in the disease process and suggests that specific inhibitors of NDST-2 could be potential novel anti-inflammatory drugs.

In summary, heparin and HS STs are crucially involved in biosynthesis of biologically important HS epitopes that function in blood homeostasis, cell proliferation and differentiation, angiogenesis and viral infection. Inhibitors of these enzymes could be developed into novel small-molecule drugs that target inflammation, cancer and infection.

Gal/GalNAc/GlcNAc-6-O-sulfotransferases

The second largest family of Golgi-associated STs are the Gal/GalNAc/GlcNAc 6-O-STs [26,48]. These fall into two subfamilies. The galactose 6-O-STs, which include GST-0 and GST-1, catalyze 6-O-sulfation at galactose and, in the case of GST-0, GalNAc in chondroitin sulfate, keratan sulfate and (at least in transfected cells) mucin-type glycoproteins. The GlcNAc-6-O-STs include GST-2, GST-3, GST-4α, GST-4β and GST-5. They catalyze 6-O-sulfation of GlcNAc in keratan sulfate and/or N-linked and O-linked glycoproteins. GST-5 was also reported to sulfate GalNAc in chondroitin sulfate. Whereas GST-2 and GST-5 are widely expressed, expression of GST-3 and the two GST-4 isozymes is highly restricted. GST-X is a large, chimeric protein with an N-terminal domain that is highly homologous to a lung carcinoma antigen known as SART-2. The C-terminal ST domain of GST-X is the most divergent of the family and its *in vivo* substrate(s) is unknown. Using immunofluorescence, GST-X has been localized to the Golgi (C. de Graffenried and C. R. Bertozzi, pers. commun.) and its gene is localized to human chromosome 18q, a region that has been implicated in the etiology of bipolar disorder [49].

Within the GST family, GST-3 (also known as HEC-GlcNAc6ST, GlcNAc6ST-2, LSST or CHST4) and to a lesser degree GST-2 (also known as GlcNAc6ST-1 or CHST-2) are the best validated for their potential role in human disease, specifically inflammation. GST-3 was discovered in an effort to identify and characterize the enzyme catalyzing the critical sulfation modification in L-selectin ligands [50,51]. L-selectin is a lectin-like cell adhesion molecule on leukocytes that mediates the initial adhesion step in lymphocyte recirculation to peripheral lymphoid organs. It binds to a specific set of glycosylated endothelial mucins known as peripheral node vascular addressin (PNAd). These are expressed constitutively on high-walled endothelial venules (HEV) within the lymph-node vasculature. Endothelial L-selectin ligands are also expressed *de novo* at sites of chronic inflammation, including rheumatoid synovium, asthmatic lung and in allografts undergoing chronic rejection [52]. Rather than recognizing a protein ligand, L-selectin binds to sialyl-Lewis x type complex O-glycans that are 6-O-sulfated on GlcNAc within the sialyl Lewis x capping groups (sulfoadhesin) [53]. Lack of the GlcNAc 6-O-sulfate in sulfoadhesin precludes high-affinity binding. Direct selectin antagonism with small molecules has met with little success, so the discovery of this critical sulfation allows modulation of selectin-mediated cell adhesion indirectly by inhibition of the relevant ST [21]. This is attractive as enzymes are generally easier to inhibit than macromolecular interactions.

Three lines of evidence validate GST-3 as the enzyme primarily responsible for sulfoadhesin biosynthesis: (1) GST-3 message and protein are expressed only in HEV of lymph nodes and sites of aberrant lymphocyte recruitment, such as the hyperplastic thymus in the AKR mouse [50,51]; (2) cell transfection with GST-3 cDNA promotes expression of L-selectin ligands on the cell surface [51]; and (3) a GST-3-deficient mouse expresses no luminal L-selectin ligands on high endothelial venules and exhibits 50% reduced lymphocyte homing to peripheral lymph nodes [54]. However, GST-3 is not the only enzyme responsible for L-selectin biosynthesis, as in GST-3-knockout animals, sulfated L-selectin ligands are maintained on the abluminal surface of lymph node HEV. Fukuda and colleagues recently reported a mouse in which the gene encoding GST-2 had been deleted along with the GST-3 gene. This mouse was shown to be lacking luminal and abluminal L-selectin ligands and to have 70% reduced lymphocyte homing to peripheral lymph nodes [55].

Based on its role in selectin-mediated cell adhesion and cell migration discussed above, selective inhibitors of GST-3 and dual inhibitors of GST-3 and GST-2 could have potential as anti-inflammatory drugs. The discovery of inhibitors of this novel class of enzymes requires ST assays adaptable to HTS. This aspect of inhibitor discovery will

be reviewed in the following chapter with a special focus on the GST family of carbohydrate STs.

Recent advances in high-throughput ST assay development

Until very recently, the assays used to measure ST activity *in vitro* relied almost exclusively upon the use of radioactive sulfur, in the form of [^{35}S]-PAPS for transfer to corresponding substrates. These assays included the separation of [^{35}S]-labeled products via thin layer/paper chromatography, or capture of hydrophobic substrates onto reversed-phase cartridges [56,57]. Although these approaches were suitable for low- to medium-throughput kinetic analyses of a handful of enzymes, they often depend upon expensive substrates, large quantities of enzymes, and suffered from being labor-intensive. Similarly, their suitability for large screening projects was hindered by the concomitant production of radioactive waste, necessitating expensive disposal and containment.

In 1999, a continuous assay for measuring ST activity was developed by Wong and co-workers [58]. This assay eliminated the need for radioactivity, and relied upon the unique reversibility of β-aryl ST IV (β-AST-IV), a cytosolic ST from rat, under certain reaction conditions. β-AST-IV, in the presence of concentrated *p*-nitrophenyl sulfate, catalyzes the reverse physiological transfer of a sulfuryl group from *p*-nitrophenyl sulfate to PAP, forming PAPS and *p*-nitrophenolate anion. In the presence of another ST of interest, β-AST-IV converts the PAP formed in the ST reaction back into PAPS for further reaction cycles, while producing a stoichiometric amount of *p*-nitrophenolate. This byproduct can be monitored by UV absorbance as an indirect measure of ST activity. Unfortunately, although ideal for kinetic studies, large quantities of enzyme are exhausted by this assay, and STs with substrate K_Ms in the nanomolar range (such as estrogen ST) do not produce enough PAP to generate a detectable amount of *p*-nitrophenylate signal via UV absorbance. In addition, the presence in the assay of β-AST-IV along with the ST of interest complicates ST inhibitor screening.

A recent 96-well 'dot-blot' capture assay for screening carbohydrate STs has addressed some of the technical difficulties inherent to earlier methods [59]. This assay features polymers of carbohydrates as the substrate molecules, which under optimized conditions can be captured onto either ionic, or hydrophobic membranes depending on the nature of the carbohydrate substrate. Although still relying upon monitoring [^{35}S]-labeled products, this assay obviates the need for secondary capture reagents, and excessive washing or elution steps as the product can be quantitated on membranes directly via phosphorimaging.

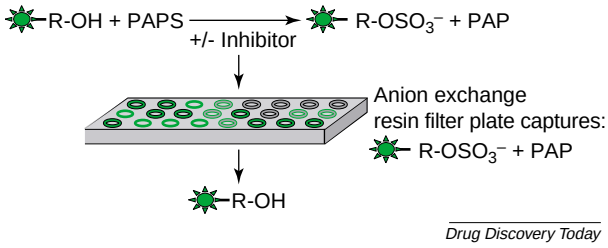

Figure 3. A 96/384-well ST-activity assay with fluorescent readout. The ST to be assayed is incubated with PAPS and an appropriate oligosaccharide substrate derivatized at its reducing end through a short spacer with a fluorophore. Quenched reactions are applied to either 96- or 384-well cationic capture plates. A simple elution removes any unreacted substrate from the membrane, while the now negatively charged, sulfated (and fluorescent) products are retained and quantitated in a fluorescent plate reader.

We have optimized the 'dot-blot' capture assay to enable GST activity measurements by fluorescence instead of radioactivity. In this fluorescence-based GST assay (Figure 3), an appropriate oligosaccharide substrate is synthetically modified at the aglycone with a fluorophore. Such modification of their respective substrates does not impair the enzymatic activities of the GSTs. To measure GST activity, quenched reactions are applied to either 96- or 384-well cationic capture plates. A simple elution removes any unreacted substrate from the membrane, while the negatively charged, sulfated (and fluorescent) products are captured and quantitated via fluorescence. In theory, any ST can be screened by this robust method, provided that the ST tolerates the inclusion of a small fluorophore tethered to an unreactive portion of the substrate.

Conclusions

Sulfotransferases are enzymes that catalyze biological sulfation processes shown to modulate fundamental biological processes in cell–cell and cell–matrix communications. Sulfotransferases are functionally and mechanistically analogous to kinases; however, the ST proteome in human is about one order of magnitude smaller than the human kinase proteome. As drug targets, sulfotransferases offer the advantage of reducing technically challenging approaches, such as direct antagonism of cell adhesion and growth factor interactions, to enzyme inhibition with its inherently superior chemical tractability. As the human sulfotransferase family becomes better defined and screening assays are developed, sulfotransferase inhibitor discovery could soon yield novel drugs for treatment of cancer, inflammation and infection, which will improve and compliment current therapies.

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