Carbohydrate sulfotransferases: mediators of extracellular communication

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Sulfated carbohydrates mediate diverse extracellular recognition events in both normal and pathological processes. The sulfotransferases that generate specific carbohydrate 'sulfoforms' have recently been recognized as key modulators of these processes and therefore represent potential therapeutic targets.

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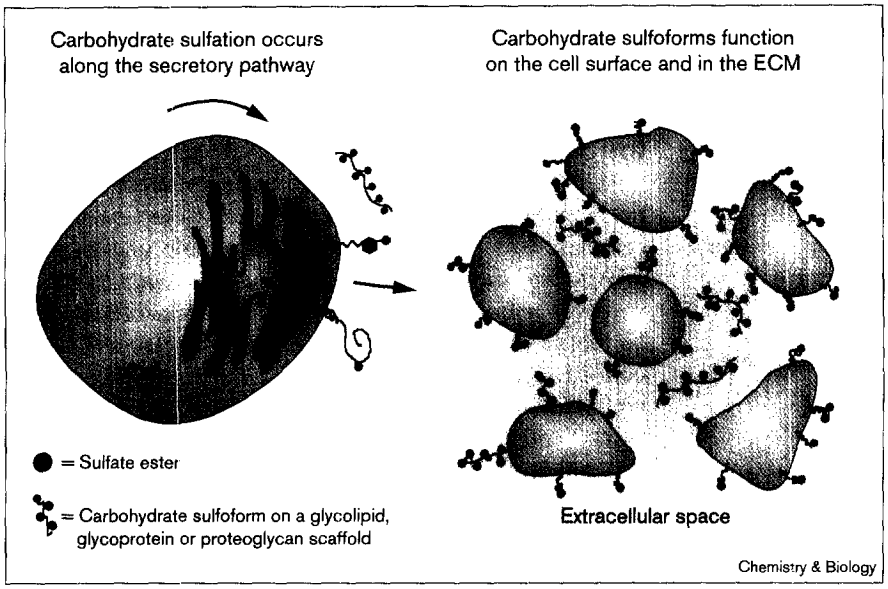
Introduction

The outward world of animal cells is an intensely social and noisy milieu of intercellular chatter. Cells exchange a ceaseless barrage of information, regulating one another's activities to simultaneously maintain collective vitality and suppress anarchy. Despite the potential chaos of extracellular life, an order and solidarity prevails that naturally evokes curiosity about how cells manage such precise and effective communication, particularly when one considers the intrinsic difficulties of interchange through a perilous, cluttered and unbounded space. Communication among cells presents a challenging terrain, often dependent upon passive diffusion of solutes towards their relevant targets, and, conversely, requiring cell-bound receptors to efficiently capture cognate ligands on the fly. Other functions require cell–cell and cell–solute interactions to occur under blood flow and so demand mechanisms adapted to withstand shear stress. How have cells evolved to accommodate the rigors of multicellularity? One necessary response has been the emergence of a versatile extracellular matrix (ECM) that provides both mechanical support and a porous medium for diffusion, as well as specific epitopes necessary for communication. This outer world presents a strikingly different landscape from the viscera of the cell in many ways, but most conspicuous is the preponderant assortment of carbohydrates that populate all corners of extracellular space. Cells present a diverse array of glycosylated lipids and proteins on their surface and in the surrounding expanse. Collectively, these form a dense matrix, called the glycocalyx, that functions as the interface between a cell and the environment in which the cell negotiates its growth, survival and death. Carbohydrates are fundamental to virtually every aspect of extracellular traffic, fulfilling roles from purely structural to mediating the highly specific recognition events that underlie cell–cell communication. Nature has chosen well, as sugars are particularly well suited to mediate such an extraordinary variety of interactions: they are modular, akin to nucleotides and amino acids, but, in addition, sugars can form branched structures with stereospecific linkages, features that confer exponential capacity for structural diversity.

Extracellular sugars are often elaborated with covalent modifications, such as sulfation, acetylation and phosphorylation, that impart further structural variety. Among these modifications the most prevalent is sulfation. Once synthesized along the secretory pathway, carbohydrate sulfate esters are found exclusively in the extracellular milieu, and carbohydrate sulfation could be among the mechanisms chosen by nature to address the challenges of

Figure 1



Sulfated carbohydrates are found only on the cell surface and in the extracellular matrix (ECM) where they mediate intercellular communication. Sulfotransferases are resident Golgi enzymes and modify carbohydrate substrates, such as glycolipids and glycoproteins, along the secretory pathway. Sulfated products are secreted or presented on the cell surface.

multicellular communication (Figure 1). Why so? Sulfate esters have many useful properties. Entirely anionic at physiological pH, they provide an electrostatic component to specific interactions without behaving as a base or nucleophile. In addition, sulfate esters are commonly found in clusters, a very important feature that underlies their versatility and unique function: mooring upon a carbohydrate scaffold allows the spacial arrangement of two or more sulfate anions to contribute to overall ligand structure, an efficient means of generating many unique structures from a few modular features.

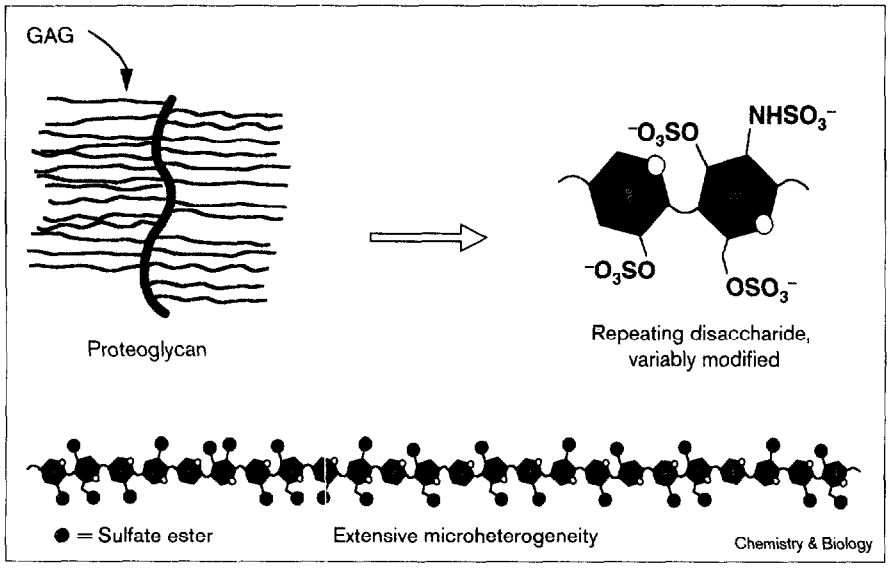
In the past few years, enzymatic placement of sulfate esters onto carbohydrates has been recognized as a mechanism for generating unique ligands with specific receptor-binding activity [1]. Sulfated sugars mediate numerous highly specific molecular-recognition events, and, from a survey of the known sulfate-dependent interactions, two functional themes emerge. The first theme is the combinatorial biosynthesis of numerous carbohydrate sulfoforms within a glycosaminoglycan (GAG) chain that collectively represent a library of unique structures. (We use the term sulfoforms to denote sulfated carbohydrate structures that differ only in their pattern of sulfation.) Combinatorial sulfation is exemplified by heparan sulfate (HS), a GAG that displays extensive microheterogeneity of sulfoforms within one carbohydrate chain, thereby presenting a diverse array of unique motifs, each with the potential of binding a specific receptor (Figure 2 shows schematically the diversity of HS sulfoforms). The broad utility of such a versatile and adaptive scaffold is evident from the great variety of cell-surface interactions known to be mediated by a specific sulfoform of HS, including binding of growth factors, cytokines and pathogens. The second theme is the conversion of a common oligosaccharide into a unique ligand by sulfation (shown schematically in Figure 3). For example, sulfation of a terminal N-acetylgalactosamine (GalNAc) residue on pituitary glycoprotein hormones regulates serum half-life by creating a unique determinant that is selectively recognized by a sulfate-dependent hepatic receptor. These themes underlie a multitude of processes, from angiogenesis and tumor metastasis to leukocyte–endothelium adhesion at sites of inflammation. Accordingly, the carbohydrate sulfotransferases that generate these epitopes have become the focus of intense interest, with the first molecular characterization reported only five years ago. The role of sulfotransferases in modulating both normal and pathogenic processes has brought them to the foreground of research as novel therapeutic targets. Here we summarize the current knowledge of carbohydrate sulfotransferases and the biological functions of carbohydrate sulfoforms, highlighting potential avenues for therapeutic intervention.

Sulfotransferases and the chemistry of sulfate transfer

Carbohydrate sulfotransferases are transmembrane, resident enzymes of the Golgi network that recognize glycans attached to lipids and proteins passing through the secretory pathway (Figure 1 shows the biosynthesis of sulfated biopolymers in the secretory compartments and their delivery to the extracellular milieu). From current knowledge, the carbohydrate sulfotransferases appear distinct both structurally and in their biological function from the cytosolic sulfotransferases, a well-characterized family that recognizes small-molecule substrates such as steroids, flavonoids, neurotransmitters and phenols (reviewed in [2,3]). The roles of cytosolic sulfotransferases are primarily

Figure 2

Proteoglycans are major constituents of the extracellular matrix and are characterized by pendant glycosaminoglycan (GAG) chains. The biosynthesis of GAG chains begins with a repeating disaccharide unit that undergoes extensive modification. The sulfotransferases modify the scaffold in a combinatorial manner to yield highly variable sulfoforms that display tissue-dependent and temporally regulated expression.



metabolic and include the inactivation of xenobiotics, steroid hormones and catecholamines, and the elimination of catabolic end products. In contrast, the carbohydrate sulfotransferases appear to play a fundamental role in extracellular signaling and adhesion by generating unique ligands from a carbohydrate scaffold. In this sense, the carbohydrate enzymes might be more similar in function to the tyrosine sulfotransferases [4–6], also resident in Golgi compartments, that modulate the activity of both secreted and membrane-associated proteins through tyrosine sulfation [7–13].

All eukaryotic sulfotransferases catalyze the transfer of a sulfonyl group from an activated sulfate donor onto a hydroxyl group, or, less commonly, an amino group, of an acceptor molecule (Figure 4). The universal source of activated sulfate for eukaryotic sulfotransferases is 3′-phosphoadenosine-5′-phosphosulfate (PAPS) [14]. Biosynthesis of PAPS occurs in the cytosol by the sequential transformation of ATP by ATP sulfurylase and APS kinase. In mammals, both activities lie within a single, bifunctional cytoplasmic protein called PAPS synthetase; the human enzyme was cloned recently [15]. PAPS traverses the lipid bilayer between the cytosol and the Golgi lumen via a PAPS/PAP translocase (PAP, 3′-phosphoadenosine-5′-phosphate), a transmembrane antiport shuttle that has been purified and characterized [16].

The mechanism of sulfonyl transfer is unknown, although a handful of recent papers have begun to elucidate some of the finer details. An earlier report identified a lysine residue in the PAPS-binding site of a glycolipid sulfotransferase [17]. More recently, a conserved lysine residue has been shown to be essential for sulfotransferase activity of a heparan sulfate N-deacetylase/N-sulfotransferase [18]. Upon sequence alignment, this lysine residue also maps to the active-site lysine required for estrogen sulfotransferase activity. Although currently we have no three-dimensional structure of a carbohydrate sulfotransferase, two recently reported structures of estrogen sulfotransferase and PAP

Figure 3

A common carbohydrate epitope presented on a protein or a lipid scaffold can be converted into a unique ligand for a specific receptor by installation of a sulfate ester.

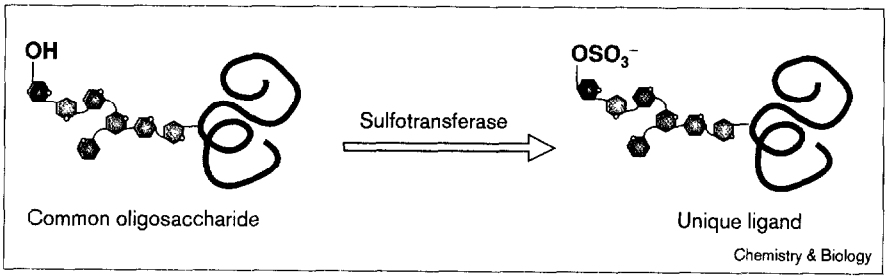
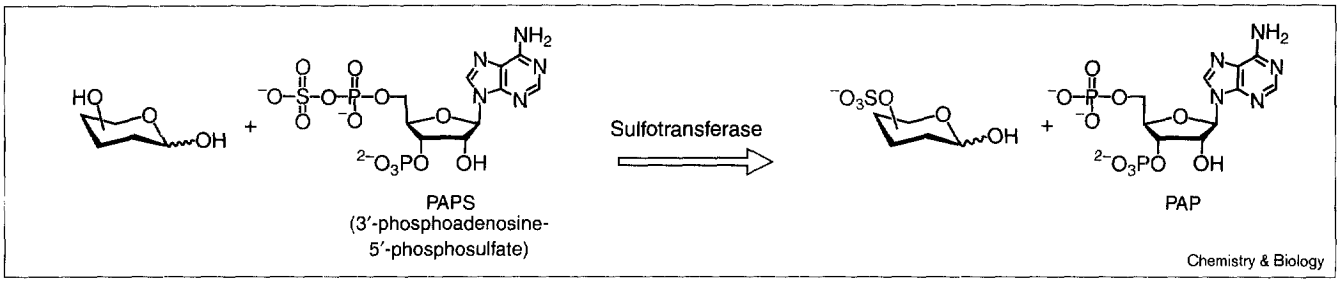


Figure 4



The general carbohydrate sulfotransferase reaction. The universal source of activated sulfate for mammalian sulfotransferases is 3′-phosphoadenosine-5′-phosphosulfate (PAPS).

co-crystallized with estrogen [19] or vanadate [20] provide clues about the active-site architecture. Coupled with mutational analysis of active-site residues, the latter study suggests an in-line sulfonyl transfer mechanism similar to the mechanism of phosphoryl transfer ascribed to many kinases [21]. The carbohydrate sulfotransferases share conserved amino-acid motifs observed in the PAPS binding site of estrogen sulfotransferase, and therefore these studies might be a relevant context in which to consider sulfonyl transfer to carbohydrate substrates.

To date, 17 mammalian carbohydrate sulfotransferases have been cloned, and several more characterized and purified. The structural diversity of known sulfated glycans indicates that many more remain to be discovered. Very modest homology exists between cytosolic, tyrosine and carbohydrate sulfotransferases, evident only at the putative binding site for their common substrate PAPS [22]. Within the carbohydrate sulfotransferase family, initial examination suggests only modest homology among the enzymes that recognize different substrates. Although a few regions within the putative PAPS binding site are conserved among all the carbohydrate sulfotransferases, the remainder of most sulfotransferase sequences do not submit tidily to multiple sequence alignment. Among those enzymes with similar carbohydrate substrates, significant identity (50–95%) is observed between homologs and between isoforms. As the rapid rate of cloning continues, subclasses within the carbohydrate family will probably emerge that clarify the functional significance of disparate domains.

The following discussion of individual carbohydrate sulfotransferases begins with examples of the first paradigm outlined above, in which a complement of enzymes generate a combinatorial array of sulfoforms along a GAG scaffold. This will be followed by an overview of the sulfotransferases that operate on smaller, discrete oligosaccharide substrates, thereby converting them into specific ligands for cognate receptors.

Glycosaminoglycans as scaffolds for combinatorial sulfation

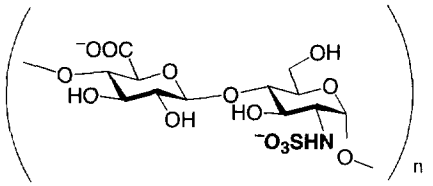
Heparan sulfate sulfotransferases

Proteoglycans pervade the extracellular space and the cell surface in most tissues, and thereby constitute a dominant component of the medium in which cells function and thrive. Proteoglycans comprise extended protein backbones bearing dense arrays of long, heterogeneous GAG chains that are akin to the bristles on a bottle brush, and, in many cases, these chains are sulfated. Table 1 summarizes the sulfated GAGs for which the associated sulfotransferases have been characterized.

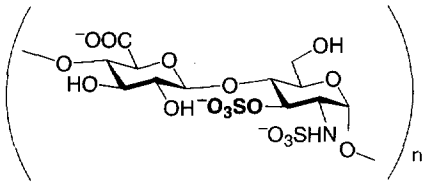
Heparan sulfate (HS) and heparin are common components of a variety of proteoglycans that mediate numerous processes, including cell adhesion [23,24], activation of growth factors [25–27], binding of cytokines and chemokines [28], and infection by bacteria [29,30] and viruses [31–35]. The biosynthesis of HS and heparin involves a multi-enzyme pathway within the secretory compartment that combinatorially modifies the repeating disaccharide $(GluA\beta1\rightarrow4GlcNAc\alpha1\rightarrow4)_n$ (GluA, Glucuronic acid; GlcNAc, N-acetylglucosamine) to yield a variety of sulfoforms within long, highly variable anionic chains. Modifications of HS and heparin include N-deacetylation/N-sulfation of GlcNAc; epimerization of C5 of GluA to give iduronic acid (IdoA); and sulfation of the 3- and 6-hydroxyl groups of glucosamine-N-sulfate, and the 2-hydroxyl group of GluA or IdoA [36]. Although HS and heparin derive from the same repeating core disaccharide and subsequent transformations, they emerge as structurally distinct molecules with different expression levels across tissues, and, in general, heparin exhibits a highly restricted expression and a greater degree of sulfation than the more ubiquitous HS. The presentation of particular sulfoforms of HS and heparin appears to be regulated temporally and spatially, reflecting a finely tuned, tissue-specific expression of the sulfotransferases that correlates with functionality (reviewed in [37]). The capacity of a cell to derive a variety of potential structures through the expression of a few key enzymes

Table 1

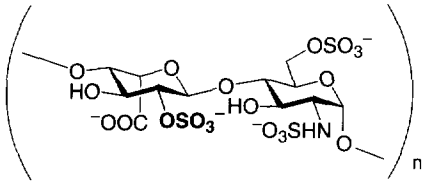
Sulfoforms of glycosaminoglycans (GAGs) and the associated sulfotransferases.



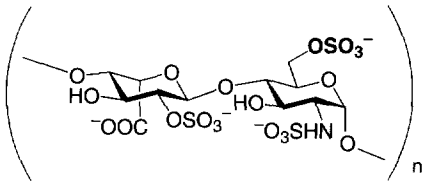
- Heparan sulfate N-deacetylase/N-sulfotransferase
- Human clones: [58,59]
- Initiates modifications of heparin and HS



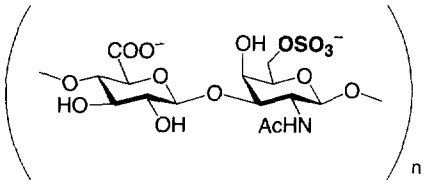
- Heparan sulfate glucosaminyl-3-O-sulfotransferase
- Human and mouse clone: [41]
- Important for antithrombin binding and clotting



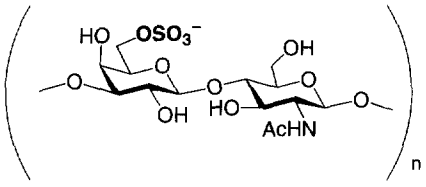
- Heparan sulfate iduronic acid-2-O-sulfotransferase
- CHO cell clone: [63]; gene trap mutant mouse: [43]
- Important for binding of HS to FGF-2 and embryogenesis



- Heparan sulfate glucosaminyl-6-O-sulfotransferase
- Human clone: [61]
- Important for activation of FGF-2 receptor by heparan sulfate-FGF-2 complex



- Chondroitin sulfate GalNAc-6-O-sulfotransferase
- Human clone: [65]
- Involved in development and maintenance of articular cartilage



- Keratan sulfate Gal-6-O-sulfotransferase
- Human clone: [79,80]
- Found in several tissues including cartilage, cornea and brain

represents a powerful and efficient means of modulating the cell-surface chemistry. Accordingly, the orchestration of the sulfotransferases that underlie HS and heparin architecture is an elegant example of combinatorial biochemistry and might provide targets for therapeutic intervention.

The versatility of HS heterogeneity is evident not only from the diversity of proteins that bind a specific HS sulfoform but also by the numerous functional modes of HS interactions. For example, HS can harvest proteins from the ECM, bringing them into the proximity of their cell-surface receptors at a high local concentration [38], or HS can act as a co-receptor, forming an active complex with a protein ligand for receptor binding [26]. HS can inhibit protein activity by binding nonproductively [39]. Furthermore, HS can activate enzymes through allosteric mechanisms [40]. In many

cases, the molecular details of these interactions reveal a recognition requirement for specific HS sulfoforms. For example, high-affinity binding of HS to antithrombin, an inhibitor of the clotting cascade, requires the presence of the rare epitope 3-O-sulfated glucosamine. Consequently, the corresponding glucosaminyl-3-O-sulfotransferase could be an important regulator of blood clotting and a potential therapeutic target (see [41] and references therein).

Another illustration is provided by the presence of iduronic acid-2-O-sulfate within the context of a particular HS pentasaccharide. This specific sulfoform initially binds to fibroblast growth factor-2 (FGF-2) and subsequently forms a ternary complex with the FGF-2 receptor that stimulates endothelial cell growth associated with angiogenesis [26,42]. One strategy for blocking angiogenesis within tumor environments might therefore include disruption of iduronic acid-2-O-sulfotransferase activity. Gene-trap mutation of HS 2-O-sulfotransferase revealed an essential role for this enzyme in mouse embryogenesis [43]. Mice carrying this mutant sulfotransferase exhibited bilateral renal agenesis and defects in eye and skeletal formation, and died during the neonatal period shortly following birth. The sulfoforms generated by HS 2-O-sulfotransferase, therefore, are fundamental to normal development.

In addition, sulfation of heparan sulfate on the 6-position of glucosamine is required for the activation of the FGF receptor by the heparan-sulfate–FGF-2 complex [39] and therefore the corresponding glucosaminyl-6-O-sulfotransferase could be yet another target for anti-angiogenic therapy. On the basis of these studies and others that map the binding of certain FGFs to specific HS sulfoforms, one may speculate that dynamic processes mediated by FGFs could be controlled by the underlying complement of enzymes that dictate HS sulfoform expression.

Numerous other proteins are known to interact with HS or heparin. For example, a recent study investigated the sulfoforms of heparin and HS that bind to the basement membrane glycoprotein laminin-1, an interaction postulated to be involved in tumor–host adhesion during metastasis. The HS fragments with high affinity for laminin-1 were found to be sulfate-rich and comprised six repeating disaccharide units of the structure $((2\text{-}SO_3)IdoA\alpha1\rightarrow4(6\text{-}SO_3)GlcNSO_3\beta1\rightarrow4)$ [44], implicating several enzymes, including the 2- and 6-O-sulfotransferases, acting in concert to promote the pathological interactions. Finally, platelet-derived growth factor-A (PDGF-A) binds the HS motif $((2\text{-}SO_3)IdoA\alpha1\rightarrow4(6\text{-}OSO_3)GlcNSO_3)$, an interaction that could be involved in the regulation of secretion, storage and binding of PDGF-A to its receptor on smooth-muscle cells [45].

It is apparent that dynamic changes in the density or structure of HS sulfoforms accompany several normal and pathological transitions, although the molecular basis for many of these is not yet fully understood. For example, the characteristic presentation of HS sulfoforms during various neural developmental stages correlates to preferential binding of different FGFs [46]. Also, differentiation of colon cells is accompanied by specific alterations in HS sulfoforms, and these changes affect binding of HS to platelet-derived growth factor [47]. Other processes in which HS could engender critical changes include atherosclerosis [48], aging [49] and Alzheimer's disease [50–52]. Also, *Plasmodium falciparum*, the causative agent of malaria, uses both HS [53,54] and chondroitin sulfate [54,55], another GAG, as cell-surface receptors. The interactions underlying these processes probably involve specific HS sulfoforms and could represent opportunities for sulfotransferase-targeted therapy.

Given the generality of HS and heparin in extracellular communication, the associated sulfotransferases represent a significant, albeit largely uncharted, class of modulatory enzymes. Table 1 includes a summary of the sulfotransferases that participate in HS and heparin biosynthesis. The gateway to all modifications is N-deacetylation/N-sulfation as this invariably precedes further enzymatic transformations. A single enzyme, N-deacetylase/N-sulfotransferase (NDNST), catalyzes both reactions [56], and, by virtue of this regulatory role, is a principal determinant of the overall structure and sulfate density of HS and heparin. Several clones have been reported from mouse [57] and human [58,59] that indicate the enzyme has a broad tissue distribution. Given the primary role of this enzyme in regulating HS and heparin architecture, it is possible that other isoforms remain to be discovered. Several clones of the O-sulfotransferase group have been reported. HS glucosaminyl-6-O-sulfotransferase [60] was originally purified from the conditioned media of CHO cells, and the sequence was used later to obtain the human clone from a cDNA library. HS iduronic acid-2-O-sulfotransferase was purified [61] and cloned [62] from CHO cells. Finally, the HS glucosaminyl-3-O-sulfotransferase was purified from a mouse cell line [63] and the sequence of the protein used to clone both the mouse and human genes [41].

Chondroitin GalNAc-6-O-sulfotransferase

Chondroitin sulfate (CS) appears to have a dual role as both a structural component of the ECM and a mediator of signals through specific receptor–ligand interactions. The CS repeating unit is $(GluA\beta1\rightarrow3GalNAc\beta1\rightarrow4)_n$, and sulfation occurs predominantly on the 4- and 6-hydroxyl groups of GalNAc, and less commonly on the 2-hydroxyl group of GluA (Table 1). Comparable with the HS and heparin enzymes, distinct sulfotransferases are thought to operate in concert to generate a particular motif. Although microheterogeneity is not as extensive as on HS, a diverse range of motifs commonly exist within a single strand.

Recently, a human CS GalNAc-6-O-sulfotransferase clone was obtained [64] by homology to the cDNA for a previously identified chick enzyme [65]. This sulfotransferase also recognizes galactose residues of the GAG keratan sulfate (KS), indicating a possible role in both CS and KS biosynthesis. A CS sulfotransferase specific to neural tissue was recently reported that shares 69% homology with the mammalian CS sulfotransferases [66]. The regioselectivity of this enzyme is not known.

CS is a predominant component of cartilage and brain, where it serves in the maintenance of structural integrity. In addition, CS sulfoforms are dynamic and vary across tissues and developmental stages. CS in the brain ECM influences neural cell migration and axonal growth through interactions with other matrix proteins and cell-surface receptors, and therefore differential expression of CS sulfoforms could be important for neuronal development and maintenance (reviewed in [67]). Particular CS sulfoforms might promote neurite outgrowth [68], thereby playing a role in navigating neurons. In addition, developmental regulation of both the sulfation profile of CS chains and the relevant sulfotransferase activities in embryonic chicken brain vary markedly and predictably with development, and these alterations are precisely coordinated [69]. For example, in the latter study the ratio of chondroitin-6-sulfate to chondroitin-4-sulfate and the ratio of their respective sulfotransferase activities were found to progressively decrease with development. These discoveries indicate that developmentally regulated expression of chondroitin sulfotransferases controls stage-specific presentation of CS structures.

The participation of CS sulfoforms in disease is speculated from many examples in the literature. CS is a major component of joint cartilage, and, in the early stages of joint disease, changes have been observed in both the chain length and the pattern of sulfation in animal models and in humans [70–73]. These changes are thought to reflect part of the cellular response by chondrocytes to damage to the articular cartilage matrix. The specificity of the changes shows that the biosynthesis of chondroitin sulfate is under tight cellular control in chondrocytes and suggests that selected patterns of sulfation within chains are expressed to suit different biological functions. The CS 6-O-sulfotransferase appears essential for normal skeletal development and defects in 6-O-sulfation are associated with genetic skeletal deformity [74,75]. Finally, *Plasmodium falciparum* uses certain CS chains, in addition to HS chains, as cell-surface receptors to gain entry into host cells [54,55,76].

Keratan sulfate Gal-6-O-sulfotransferase

KS consists of the repeating disaccharide unit (Galβ1→4GlcNAcβ1→3), which undergoes sulfation of the 6-hydroxyl groups of both Gal and GlcNAc (Table 1). KS is a principle component of cartilage and corneal tissue, and is also found in brain but its precise roles have not been established, although the sulfation pattern varies during development [77] and in some disease states [78]. The human form of a sulfotransferase that generates Gal-6-sulfate on KS has been cloned [79,80]. It appears to be expressed in many tissues and heavily in brain and cornea, and is thought to play a critical role in corneal transparency. Some substrate overlap exists between the KS Gal-6-O-sulfotransferase (KSST) and the CS GalNAc-6-O-sulfotransferase (CSST); for example, KSST accepts chondroitin as a substrate and CSST accepts keratan. (Indeed, the enzyme named the human KSST by Fukuta *et al.* [79] is referred to as a CSST in Mazany *et al.* [80] and in the NCBI gene database. Its activity is sufficiently higher with KS than with CS, however, providing some confidence in its assignment as a KSST.) These enzymes could constitute a family of sulfotransferases characterized by a liberal substrate profile and might participate in the biosynthesis of both KS and CS proteoglycans.

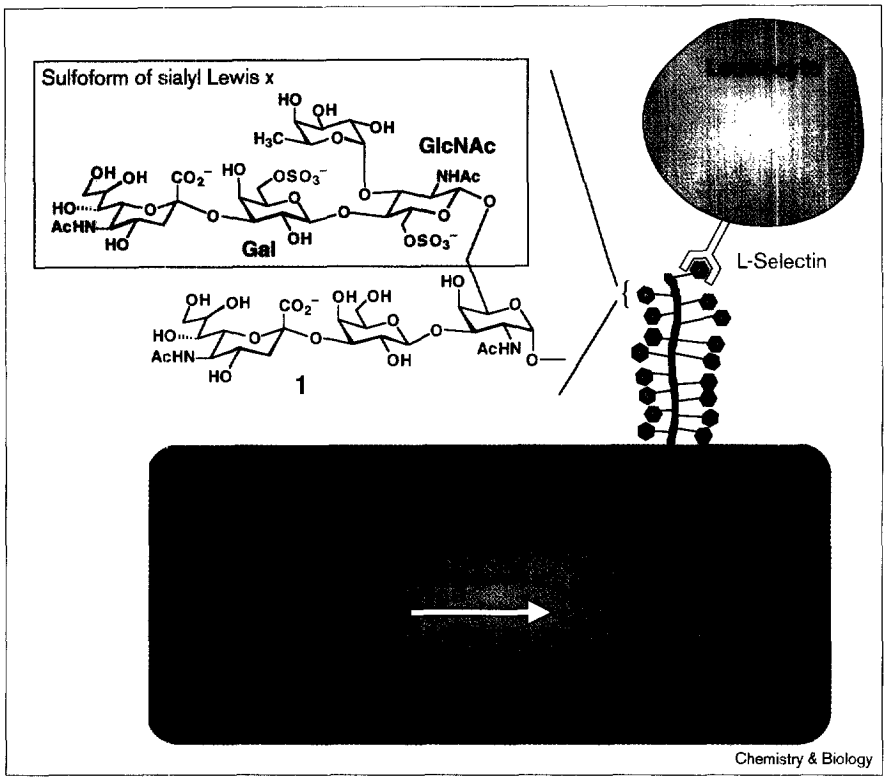
Sulfation converts small glycans into unique ligands

Small, often branched glycans that bear one or two sulfate esters have been identified on a variety of glycolipids and glycoproteins. In several cases, the sulfate ester has been shown to convert the relatively common glycan, which does not have a specific receptor, into a unique ligand for a sulfate-dependent receptor.

GlcNAc-6-O-sulfotransferases and modulation of the inflammatory response

A landmark discovery in the emerging field of sulfotransferase biology was the identification of sulfated oligosaccharides as key modulators of leukocyte–endothelial-cell interactions [81]. L-Selectin, a constitutively expressed receptor on all circulating leukocytes, mediates the initial attachment of blood-borne leukocytes to endothelial cells of the blood vessel walls during the constitutive process of lymphocyte homing to peripheral lymph nodes [82]. In addition, L-selectin contributes to leukocyte adhesion and extravasation at sites of acute and chronic inflammation [83]. The endothelial-derived carbohydrate ligands that mediate lymphocyte adhesion in lymph nodes bear one or both of Gal-6-sulfate and GlcNAc-6-sulfate within sialyl Lewis x-capped oligosaccharides (**1**, Figure 5 and Table 2) [84-87]. These unusual sulfated motifs are thought to be expressed on endothelial cells at sites of chronic inflammation as well [88]. The presence of sulfate esters is strictly required for functional L-selectin binding; although the unsulfated glycan is found on a variety of cells, it does not function as an L-selectin ligand. Enzymatic sulfation during glycan biosynthesis therefore creates a critical recognition motif [89–91], implicating the corresponding sulfotransferases as key modulators of the inflammatory response and as novel targets for anti-inflammatory therapy.

Figure 5



The endothelial ligand for the leukocyte adhesion molecule L-selectin, expressed on circulating leukocytes, is a sulfoform of sialyl Lewis x. Sulfation at the 6-position of Gal and/or GlcNAc converts the underlying glycan to a functional, high-affinity ligand for L-selectin, initiating the extravasation of leukocytes into the surrounding tissue. The corresponding endothelial sulfotransferases might be novel anti-inflammatory targets.

This discovery has prompted several groups to pursue the molecular identification of the endothelial sulfotransferases. A recent report characterized a GlcNAc-6-O-sulfotransferase activity from porcine peripheral lymph nodes that was highly restricted in its expression to endothelial cells within lymphoid tissue, suggesting a role in the biosynthesis of L-selectin ligands [92]. The enzyme required a terminal GlcNAc residue as a component of a synthetic oligosaccharide substrate, and was impeded by a terminal β1→4Gal attached to the GlcNAc residue. A candidate human GlcNAc-6-O-sulfotransferase has now been cloned that shares the same restricted tissue distribution and substrate specificity as the porcine enzyme [93].

A second candidate GlcNAc-6-O-sulfotransferase, cloned from both murine and human sources, was reported recently [94,95]. This enzyme shares some features with the lymph node GlcNAc-6-O-sulfotransferase, such as preference for a terminal GlcNAc residue and expression in lymph node endothelial cells, although it is also expressed in many other tissues, including tumors. This latter enzyme is also capable of generating GlcNAc-6-sulfated sialyl Lewis x when transfected into heterologous cell lines. The potential significance of these GlcNAc-6-O-sulfotransferases in lymphocyte homing and in the inflammatory response, and the regulation of their expression, are subjects of significant current interest. Given the fundamental role of 1 in mediating leukocyte adhesion, and its highly restricted tissue distribution, it is possible that a specific Gal-6-O-sulfotransferase is yet to be discovered.

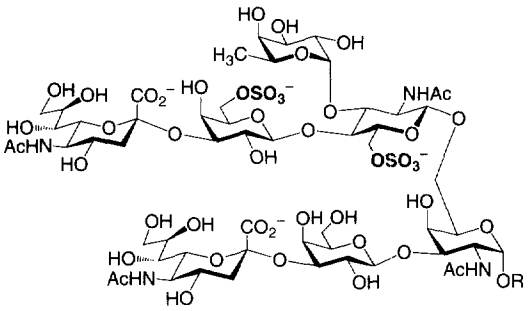
The L-selectin–ligand interaction is not alone as a sulfate-dependent mediator of leukocyte–endothelial cell adhesion. Recently, the leukocyte adhesion molecule CD44 was shown to be sulfated on unidentified carbohydrate chains in response to treatment of the cells with tumor necrosis factor α [96]. Sulfation conferred binding activity to hyaluronic acid, an extracellular matrix component on cognate endothelial cells. In yet a third example, P-selectin, induced on endothelial cells in response to pro-inflammatory signals, interacts with a leukocyte-associated glycoprotein termed PSGL-1 that is sulfated on tyrosine residues. In this case, tyrosine sulfate is critical for high-affinity binding [7,8,97]. The striking similarity of these cell-adhesion events in the vascular compartment with respect to sulfate-dependency leads to an intriguing question: is sulfate a preferred signal for interactions that must occur under conditions of blood flow? The chemical properties of sulfate might contribute to the necessary kinetic and thermodynamic parameters for these events to take place under shear stress [81].

GalNAc-4-O-sulfotransferase and regulation of glycoprotein hormone activity

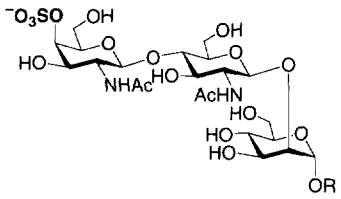
Another unusual sulfated epitope, (4-SO_3)GalNAcβ1→4GlcNAcβ1→2Manα-OR, is found on several glycoprotein

Table 2

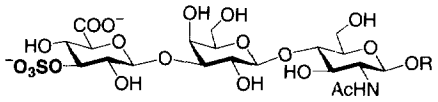
Sulfoforms of small glycans and the associated sulfotransferases.



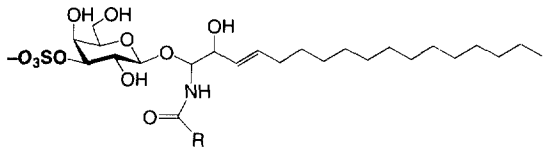
- GlcNAc- and Gal-6-O-sulfotransferases
- Candidate GlcNAc-6-O-sulfotransferase clones: [93-95]
- KS Gal-6-O-sulfotranserases (Table 1) may participate in the biosynthesis
- Epitope found on specialized lymph node endothelial cells and possibly on endothelium in chronically inflamed tissues; this sulfoform is a ligand for the leukocyte adhesion molecule L-selectin



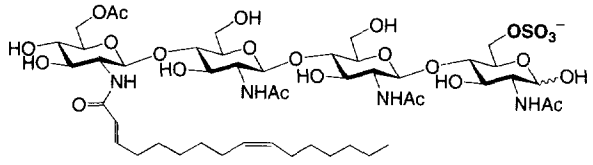
- GalNAc-4-O-sulfotransferase
- Characterization/purification: [102,103]
- Epitope found on pituitary glycoprotein hormones; regulates serum half-life by binding to a hepatic receptor



- Glucuronic acid-3-O-sulfotransferase
- Rat and human clones: [120,121]
- Defining component of the HNK-1 epitope; found on glycoproteins and glycolipids of the nervous system and in the retina; proposed role in neural cell adhesion



- Galactosylceramide-3-O-sulfotransferase
- Human clone: [123]
- Epitope found on myelin, spermatozoa, kidney and small intestine; interacts with ECM proteins, cell adhesion molecules and serum factors



- NodH sulfotransferase
- A component of the *Rhizobium* nodulation gene cluster: [127,128]
- Sulfation of Nod factors confers plant host specificity; epitope binds a specific receptor on plant root hairs

hormones produced by the pituitary gland such as lutropin and thyrotropin (Table 2). The presence of sulfate on the glycan reduces the serum half-life of the hormones, thereby regulating their bioactivity [98]. The effect of sulfation on serum half-life can be accounted for by sulfate-dependent clearance receptors on liver cells that recognize the epitope and remove the hormones from the medium [99–101]. The corresponding GalNAc-4-O-sulfotransferase therefore plays a fundamental role in regulating hormone homeostasis by creating a unique ligand for the clearance receptors. Indeed, this provides an additional level of control over hormone activity beyond simply modulating

the rate of production by the pituitary gland. The enzyme responsible for the modification has not yet been identified at the molecular level, although the protein has been purified from bovine submaxillary gland and extensively characterized [102,103].

Glucuronic acid-3-O-sulfotransferase and the HNK-1 epitope

The sulfated oligosaccharide epitope (3-SO_3)GluAβ1→3Galβ1→4GlcNAc (Table 2) is a highly restricted structure that was first identified on human natural killer (HNK) cells by a specific monoclonal antibody termed HNK-1 [104–107]. Subsequent research has shown this structure to be otherwise confined to the nervous system [108] and eye [109] where it is carried by a variety of cell-surface glycolipids and glycoproteins involved in adhesion and recognition, including the neural cell-adhesion molecule (NCAM), L1, P0, proteoglycans, tenascin and integrins [110,111]. Although the function of this epitope on HNK cells remains unknown, intense interest in the HNK-1 carbohydrate antigen arose from the discovery that IgM from humans with peripheral demyelinating neuropathies recognize this sulfated carbohydrate in a sulfate-dependent manner [106, 112–114]. The role of the HNK-1 epitope in human demyelinating diseases is not fully understood, but several lines of evidence support its participation in pathology [108].

The normal functions of the HNK-1 carbohydrate antigen include an intimate role in the development and maintenance of nervous-system architecture. In particular, the exquisitely precise cell-adhesion and recognition processes that underlie the formation and maintenance of myelin, neurite outgrowth and axonal guidance are mediated, in part, by the HNK-1 epitope [108,111]. HNK-1-bearing molecules have differential expression patterns over time and neural cell type [115–117]. In addition, sulfation occurs at the onset of myelination and regeneration, but ceases after development and does not occur when injured nerves cannot remyelinate [118]. Many of these functions have been shown to be dependent on the presence of the sulfate ester within the epitope. For example, the binding of the HNK-1 carbohydrate epitope to laminin is sulfate dependent [119]. Furthermore, the interactions between HNK-1 and laminin have been shown to mediate neural cell migration and outgrowth, processes that also require the sulfated glucuronic acid moiety [119]. Accordingly, the GluA-3-O-sulfotransferase that generates the epitope plays a central role in the development and maintenance of the nervous system. Two groups reported recently the GluA-3-O-sulfotransferase clone [120,121] paving the way for the essential studies needed to establish the function of the HNK-1 carbohydrate in normal and pathological cell adhesion.

Galactose ceramide 3-O sulfotransferase

The glycolipid (3-SO_3)-galactosylceramide (Table 2) comprises 6% of the lipid in brain, is primarily localized to the myelin sheath, and is also abundant in spermatozoa, kidney and small intestine (reviewed in [122]). Sulfoglycolipids as a class are implicated in many biological functions through their specific interactions with von Willebrand factor, amphoterin, human growth factor, laminin and thrombospondin. The human galactosylceramide-3-O-sulfotransferase has been purified and cloned [123], revealing little homology to any other sulfotransferase, including the GluA-3-O-sulfotransferase. Certain renal-cancer cell lines express abnormally high levels of this enzyme [124], although the significance of this observation has not yet been ascertained.

Rhizobial sulfotransferases and the host specificity of root nodulation factors

Carbohydrate sulfation might be a universal mechanism for cell–cell interchange that extends to unicellular organisms as well. For example, *Rhizobia* secrete specific lipooligosaccharide signals called nodulation or 'Nod' factors that are required for root nodulation and infection of legumes [125]. In response, the host plant will generate nodules, organs in which the bacteria live and convert nitrogen to ammonia. Symbiosis is restricted to particular combinations of legume hosts and bacterial strains, and species specificity of host–plant pairing is derived from the interaction of a bacterium's Nod factor with a specific receptor on host plant root hairs. The nitrogen-fixing bacterium *Rhizobia meliloti* secretes a sulfated lipochitotetraose (Table 2) to initiate symbiosis with the host plant alfalfa [126]. In this case, sulfate on the reducing-terminal GlcNAc residue is the structural determinant that confers host specificity. Without sulfate, the factor elicits symbiosis with vetch rather than alfalfa. The GlcNAc-6-O-sulfotransferase responsible for this modification, the product of the nodH gene, has been fully characterized [127].

Other strains of *Rhizobia* secrete variations of this sulfated lipooligosaccharide that confer specificity to different host species. A related strain, *R. tropici*, expresses a closely related enzyme, sharing 69% identity with *R. meliloti* NodH [128]. The sulfate group is a requirement for infection of the host *Leucaena* plants [129], although *R. tropici* produces a mixture of sulfated and non-sulfated Nod factors, perhaps allowing for symbiosis with a range of host plants. A solitary fucose sulfotransferase has been reported in another strain, *Rhizobium sp.* NGR234. Structural analysis of isolated Nod factors identified both 3- and 4-O-sulfated fucose residues [130], modifications that might modulate host specificity [131,132].

Perhaps it is not a coincidence that bacteria use sulfated carbohydrates for intercellular communication and symbiosis. Indeed, the exclusive role of mammalian carbohydrate sulfoforms in cell–cell communication might be the evolutionary consequence of single-cell organisms signaling for interaction with the more complex world.

Conclusions

How we come to fathom intercellular communication and multicellular function rests, in part, upon our understanding of the sulfotransferases and how they shape and direct extracellular chemistry. In contrast to the growing body of knowledge about the biology of carbohydrate sulfoforms, there is a paucity of chemical information regarding sulfate-dependent recognition events and the catalytic mechanisms of carbohydrate sulfotransferases. There are no three-dimensional structures of carbohydrate sulfotransferases, few mechanistic studies and no inhibitor of any member from this class of enzymes has been reported. The medicinal chemistry of carbohydrate sulfotransferases is therefore a wide-open frontier. More fundamentally, why has nature chosen sulfated oligosaccharides to conduct extracellular information? As the chemical properties of sulfate-dependent binding events are revealed, this and other questions can be answered.

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