Sulphotransferases acting on mucin-type oligosaccharides

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Abstract

This review summarizes the occurrence, properties and role in mucin O-glycosylation pathways of the various members of glycoprotein sulphotransferase families. Although a number of sulphotransferases have been cloned that act on mucin-type substrates in vitro, it is still difficult to determine exactly which enzymes are responsible for mucin sulphation in vivo. Sulphotransferases play a critical role in determining the chemical, physical and biological properties of mucins. Several of these enzymes have been shown to differ in expression and activity in cancer and inflammation.

Introduction

Mucins are high-molecular-mass glycoproteins found on membranes and in mucus gels that protect the underlying cells. The protein cores of mucins have characteristic tandem repeat regions rich in Ser/Thr residues that are extensively O-glycosylated, but they can also have N-glycans. N- and O-glycans of mucins produced in the intestine, stomach, lungs and many other tissues can have sulphate esters. These sulphated glycans are involved in protection of the epithelium, in cell adhesion and in bacterial adhesion. The sulphate esters may mask antigenic or lectin binding sites. They may also regulate the biosynthesis, half-life and biological roles of glycoproteins, and may control lymphocyte homing and inflammation. Families of mucin sulphotransferases (STs) with conserved ST motifs exist. Two families of ST enzymes transfer sulphate from 3′-phosphoadenosine 5′-phosphosulphate (PAPS) to either the 3-position of Gal (Galβ1–3Gal) or the 6-position of GlcNAc (GlcNAcβ1–6ST) of mucins respectively. Other sulphated epitopes on mucins and mucin-like molecules may be synthesized by additional STs, such as those synthesizing Galβ1–3GalNAc β1–3Galβ1–4GlcNAc units that are found on N-glycans of glycoprotein hormones. These sialyl Lewis x structures (6-sulpho-Lewis x and 6′-sulpho-Lewis x respectively). These sialyl Lewis x determinants are attached mainly to O-glycan core 2 [17,18].

Sulphated mucin oligosaccharide structures

At least eight different O-glycan core structures occur in secreted mucins [2,3], where core structures 1–4 and 6 serve as potential substrates for STs [2,4–7]. Sulphation is most frequently found on Gal and GlcNAc residues of N-acetyl-lactosamine sequences, specifically at the 3-position of Gal and the 6-position of GlcNAc of O-glycan cores and their elongated structures. For example, rat gastric and salivary mucins bear linear core 1 (Galβ1–3GalNAc β1–3)GalNAc β1–6(Galβ1–3)GalNAc-structures with 6-sulphated GlcNAc [10–12]. In pig gastric mucin, 6-SO4-GlcNAc seems to be the sole sulphated and acidic carbohydrate [13]. In pig zona pellucida glycoproteins, which contain both O- and N-glycans, O-linked sugars have 6-sulphate linked to GlcNAc both on core 3 (GlcNAcβ1–3GlcNAc-)- and on repeating N-acetyl-lactosamine disaccharides [5]. Respiratory mucins from patients with cystic fibrosis (CF) contain many complex O-glycans, with both 3-SO4-Gal and 6-SO4-GlcNAc esters [14–16]. Rat bone sialoprotein [7] has sulphated GlcNAc residues in the disialylated O-glycan core 2 and the core 6 (GlcNAc β1–6GlcNAc-)-structure.

Glycosylation-dependent cell adhesion molecule-1 (GLYCAM-1), an endothelial mucin-like ligand for L-selectin, carries 6-sulphated GlcNAc and 6-sulphated Gal on sialyl Lewis x structures (6-sulpho-Lewis x and 6′-sulpho-Lewis x respectively). These sialyl Lewis x determinants are attached mainly to O-glycan core 2 [17,18]. 4-SO4-GalNAc β1–4GlcNAc units that are found on N-glycans of glycoprotein hormones are also found on mucin O-glycans, e.g. on those with a core 2 structure [19].

Sulphate may be added to mannosic residues, to the core GlcNAc, or to GlcNAc, Gal or GalNAc on the antennae of N-glycan chains [3,20]. For example, N-glycans from thyroglobulin contain Galβ1–4GlcNAc sequences with

Key words: biosynthesis of glycoproteins, inflammation, O-glycan, mucin, sulphotransferase.

Abbreviations used: CF, cystic fibrosis; OTG, cystic fibrosis transmembrane conductance regulator; KS, keratan sulphate; PAPS, 3′-phosphoadenosine 5′-phosphosulphate; ST, sulphotransferase; TGN, trans-Golgi network; TNF, tumour necrosis factor α.

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sulphate attached to the 3-position of Gal residues as well as to the 6-position of internal GlcNAc residues [21,22].

KS in cartilage or bone glycoproteins and proteoglycans can be O-linked to protein and may contain Gal and GlcNAc residues, both of which are sulphated at the 6-position [23,24]. Proteoglycans secreted by human mammary epithelial cells were also shown to possess O-glycans with sulphated GlcNAc of core 2 structures [25]. In contrast with bone KS, corneal KS chains are longer and can be N-linked to asparagine with 6-sulphated Gal and GlcNAc repeating units [26,27]. The human endometrial mucin MUC1, which has both adhesive and anti-adhesive properties, carries KS that appears to be important for the regulation of embryo implantation [28].

**Role of sulphation**

Sulphated glycans add negative charges to mucins, which influence their chemical and physical properties, hydration and metal ion binding [1]. Sulphated structures are ligands for bacteria, but the sulphate group also masks underlying antigens [26] and protects mucin from degradation by bacterial glycosidases. Sulphation has a distinct role in regulating the biosynthesis of glycan chains. Sulphated glycan structures are involved in cell adhesion, growth factor presentation, cell signalling, development, leucocyte homing, and adhesion and inflammation [29].

Cell surface and secreted mucins, e.g. from ovarian cystadenoma [30] or human bronchial mucins [14,15], may carry sulphated Lewis antigens. Sulphated L-selectin ligands on GLYCAM-1, CD34 and other cell surface glycoproteins expressed on high endothelial venules have been shown to facilitate tethering and initial attachment of leucocytes to the endothelium via selectins [29,31,32]. Counter-receptors of L-selectin on high endothelial venules are sialyl α2–3Galβ1–4(Fuc α1–3)(6-sulpho)GlcNAc β- or sialyl α2–3 (6-sulpho)Gal β1–4 [32]. In addition, Tyr-O-sulphation is required for high-affinity binding to the P-selectin ligand [33].

Sulphation of glycoprotein hormones plays an important role in the metabolism of the hormones [34]. For example, GalNAc 4-sulphate in lutropin is essential in maintaining an effective half-life in the blood. Unsulphated forms of the hormone with terminal Gal residues are rapidly taken up by liver receptors, while sialylated forms last too long in the blood.

Bacteria not only adhere to mucins but can also cause erosion of mucin oligosaccharides. Sulphate groups partly protect mucins from the action of bacterial glycosidases. Helicobacter pylori, bacteria that cause chronic gastritis and gastric ulcers, adhere to the human gastric cancer cell line KATO III, and the adhesion can be inhibited by sulphated gastric mucins [35]. H. pylori also bind to sulphated salivary mucins [36], where 3-sulphated Gal and 3-sulphated Lewis a oligosaccharides bind better than sialylated Lewis a and b. Pseudomonas aeruginosa bacteria that have adhesins that bind to sulphated Lewis x (3′-sulpho-Lewis x) and 3′-sialo-6-sulpho-Lewis x [37]. Carbohydrate sulphation reduces the growth of P. aeruginosa. Therefore sulphated mucins are thought to provide host protection, and to reduce the nutrient pool and thereby the growth of bacteria. This may be an important mechanism for CF patients, who suffer from infections of P. aeruginosa and have increased sulphation in their respiratory mucins [38].

**Classes of STs**

Mammalian STs can be cytosolic or Golgi-membrane-bound [1]. Cytosolic STs act on alcohol groups of hydroxysteroids, drugs, phenols, oestrogens and environmental chemicals, and on amino groups of drugs. These reactions are involved in hormone homoeostasis, detoxification, and activation of xenobiotics. Membrane-bound STs act on hydroxy groups of sugars or on the nitrogen group of amino sugars [39]. These Golgi STs have been localized to post-endoplasmic reticulum compartments, the trans-Golgi and trans-Golgi network (TGN), and are involved in the synthesis of proteoglycans, glycosaminoglycans, glycolipids and glycoproteins [40]. Subcellular fractionation has shown that STs acting on GlcNAc are localized in the medial/trans-Golgi, and STs acting on Gal are in the Golgi and TGN. STs acting on Tyr are also found on membranes and have been characterized in a number of tissues, including gastric mucosa [41].

Cytosolic and membrane STs have little sequence similarity. Analysis of the gene sequences and crystallographic data for STs shows that conserved motifs in the N-terminal region and a central sequence are involved in binding of the 5′- and 3′-phosphate groups respectively of PAPS [32,42–45]. All membrane-bound STs have the type II membrane protein domain structure, similar to that of Golgi-bound glycosyltransferases, with a cytosolic N-terminus, a transmembrane domain and a catalytic domain at the C-terminus, directed towards the Golgi lumen. The human GlcNAc deacetylase/ST involved in the synthesis of heparan sulphate expressed with a FLAG epitope in mouse LTA cells and localized to the TGN. Expression of a truncated enzyme suggested that the N-terminal region was sufficient for Golgi localization [46]. The crystal structure of this enzyme shows that Glu, Lys and possibly Thr residues are involved in PAPS binding [47]. In the GlcUA3ST enzyme, which synthesizes the 3-SO4-GlcUA linkage in the HNK-1 epitope (HNK-1ST), Arg-189 and Ser-197 hydrogen-bond with the 3′-phosphate of PAPS. Asp-190 and Pro-191 are in the core of the 3′-phosphate binding site, while Lys-128 is in the 5′-phosphate binding site, although it may also bind the acceptor substrate [48].

STs involved in proteoglycan synthesis transfer sulphate to the 6-position of internal GalNAc units of chondroitin and to the 6-position of non-reducing terminal GalNAc 4-sulphate of chondroitin sulphate [49], or to hydroxy groups of sugar units in heparan sulphate [50]. The ST involved in sulphation of the amino group of heparan sulphate has an additional N-deacetylase activity that converts GlcNAc residues into glucosamine before N-sulphation [51]. There is
Table 1 | Mucin STs

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Acceptor substrate</th>
<th>Tissue</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlcNAc6ST-1</td>
<td>GlcNAc, core 2</td>
<td>Ubiquitous</td>
<td>[72-74]</td>
</tr>
<tr>
<td></td>
<td>GlcNAcβ1–2/6Man</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Sialyl-Lewis x</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not core 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GlcNAc6ST-2</td>
<td>GlcNAc, core 2, 3</td>
<td>Mucinous adenocarcinoma</td>
<td>[43,75,76,95]</td>
</tr>
<tr>
<td></td>
<td>Sialyl-Lewis x</td>
<td>High endothelial venules</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GlcNAcβ1–2/6Man</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GlcNAc6ST-3 (hIGlcNAc6ST)</td>
<td>GlcNAc, core 2</td>
<td>Human intestine</td>
<td>[76,94]</td>
</tr>
<tr>
<td></td>
<td>Not core 3, not KS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GlcNAc6ST-4</td>
<td>GlcNAcβ-R</td>
<td>Ubiquitous</td>
<td>[81,82]</td>
</tr>
<tr>
<td>GlcNAc6ST-5 (hCGlcNAc6ST)</td>
<td>GlcNAc</td>
<td>Cornea, human brain</td>
<td>[77,78]</td>
</tr>
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<td>Galβ3ST-2</td>
<td>Type 1, 2 chains, core 1</td>
<td>Lung</td>
<td>[69]</td>
</tr>
<tr>
<td>Galβ3ST-3</td>
<td>Galβ1–4GalNAc-on</td>
<td>Brain, kidney, thyroid</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>N-glycans, core 2</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Galβ4(6-SO4-)GlcNAc</td>
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<tr>
<td></td>
<td>Not core 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galβ3ST-4</td>
<td>Core 1, core 2</td>
<td>Ubiquitous</td>
<td>[4]</td>
</tr>
</tbody>
</table>

also a 2-ST acting on iduronic acid (IdUA) residues [52] and a
3-ST acting on glucosamine [53]. STs involved in the synthesis
of KS chains attached to N- and O-glycans add sulphate
to the 6-position of Gal and the 6-position of GlcNAc
[27,54]. Members of another family of STs, GalNAc4ST-1 and
-2, synthesize the 4-SO4-GalNAc linkage in glycoprotein
hormones [47,55]. Other STs add sulphate to the 3-position of
Gal and the 6-position of GlcNAc, the linkages found mainly
in mucins. The ST that synthesizes the 6-SO4-Gal linkages of
sialyl-Lewis x-related sugars [56] is expressed specifically in
lymph nodes and the spleen [57].

All of these STs are members of families of enzymes,
with some enzymes being expressed ubiquitously and some occurring in a cell-type-specific manner. The specificity of
the enzymes may be similar within a family, but usually
varies between families. It is thus the combination of the
cell-type-specific expression and the distinct acceptor substrate
specificity that determines the degree of sulphation of
glycoproteins and proteoglycans. It is difficult to separate STs
acting on mucins from those acting on other glycoconjugates
because of the overlap in ST acceptor specificities (Table 1).
Proteoglycans, mucins and other glycoproteins are all assemled in the Golgi in particular cells and presumably are
exposed to an assembly line consisting of the same enzymes.
Thus the initial glycosylation reactions, directed by the amino
acid sequences of the acceptor substrates, will form a basis
on to which oligosaccharide structures grow and become
acceptor substrates for specific ST reactions.

Mucin STs

Cell types committed to the production of mucins express
not only mucin STs, but also STs involved in the synthesis of
other glycoconjugates. The relative activities of STs acting on
specific glycan structures will determine the extent of mucin
sulphation, as well as the amounts of the different sulphated
structures in a mucin. From structural work of mucins and
the specificities of cloned STs, it can be deduced that members
of the GlcNAc6ST and Gal3ST families are the main mucin
STs. The enzymes can be assayed using low-molecular-mass
substrates with mucin-type structures. Separation of the
enzyme products depends on the substrate structures, and
has been achieved by TLC and paper chromatography, paper
electrophoresis, HPLC and anion exchange chromatography.
Lectin chromatography can also be successful when the added
sulphate masks the lectin binding site.

Gal3STs

Rat colon has a very high activity of a Gal3ST acting
on core 1 substrates, i.e. Galβ1–3GalNAc-R. This activity
has been characterized in rat colon homogenate [58]. Core
1 Gal3ST activity is ubiquitous [58–65], e.g. in rat and human colon tissue and cells, breast cancer cells, human
lymphoid cells, bovine synoviocytes, and several other tissues
(I. Brockhausen, unpublished work), but not in COS-1
[4], CHO or BHK [64] cells. Accordingly, the mRNA of core
1 Gal3ST has been shown to be expressed ubiquitously [4].
The optimal pH for the rat colon enzyme is 6.3 [58], similar
to that of the recombinant enzyme expressed in COS-1
cells [4]. The metal ion stimulation patterns are similar, but
not identical, between rat and human enzymes. The 3- and 4-
hydroxy groups of Gal and the 2-acetamido group of GalNAc
of the core 1 substrate are essential for activity of the rat
colon Gal3ST [58]. Substitution of GalNAc (of core 1) at the
6-position by GlcNAc, sulphate or a deoxy function supports the activity. However, when Gal (of core 1) is substituted by 3- or 6-sulphate or 3- or 4-deoxy functions, activity is eliminated [58]. It is interesting that very few structures of mucins with a 3-SO4-Gal linkage of cores 1 and 2 have been found, although the activity is abundant.

Chandrasekaran et al. [66,67] identified two distinct GalST activities in human breast, ovarian and endometrial cancer tissues and cells. Group A activity was active on core 1 only, while group B activity was towards Gal-R. There were differences in optimal pH and metal ion requirements between the two activities. The Group A enzyme activity was found in a number of human breast cancer cell lines and calf lymph nodes. An ovarian cancer cell line and a number of colon cancer cell lines showed group B activity. GlcNAcST activities were very low in all of these cell lines, but variable and higher in calf lymph nodes.

Microsomal fractions derived from human airway mucosa were also found to possess a Gal3ST acting on Galβ-methyl and carbohydrate chains with Gal termini. This is consistent with the occurrence of these sulphated structures in human lung mucin [16]. This human airway ST is identical in specificity to Gal3ST-3 [16,68]. To date, four members of the Gal3ST family have been cloned (Table 1), based on sequence similarity to other STs and Gal3ST-1 acting on Galceramide. Gal3ST-2 acts on type 1 and 2 chains and core 1 [69] and synthesizes 3-SO4-Galβ1–3(Fucα1–4)GlcNAc-, while Gal3ST-3 in brain, thyroid and kidney prefers N-acetyl-lactosamine chains and core 2, and can act on Galβ1–4(6-SO4)-GlcNAc-, but not on core 1 [68]. Gal3ST-4 prefers the Galβ1–3GlcNAc- structure found in core 1 and core 2, but also has activity towards Galβ1–3/4GlcNAc- [4].

GlcNAc6STs

Human respiratory mucosa has a GlcNAc6ST activity that can use GlcNAc β-methyl and GlcNAc-terminating mucin oligosaccharides as substrates [54]. A GlcNAc6ST activity was also found to be present in the Golgi fraction of rat stomach; this enzyme acts on rat gastric mucin to form a product characterized as 6-SO4-GlcNAcβ1–3Galβ1–3GalNAc [8]. 6-Sulphated GlcNAc has been found in all regions of the rat stomach [8,70]. GlcNAc6ST activity is also present in human respiratory mucosa [4], rat sublingual gland [71], rat colon and cells derived from the colon [58,60,71].

The genes for a number of GlcNAc6STs have been cloned (Table 1). GlcNAc6ST-1 is involved in the synthesis of the sulpho-sialyl-Lewis x determinant on core 2 O-glycans and N-glycans [72–74], but the enzyme does not act on core 3 (GlcNAcβ1–3GalNAc-). The gene is expressed in many tissues. GlcNAc6ST-2 also synthesizes the selectin ligand sulpho-sialyl-Lewis x in high endothelial venules [43,75], and can act on core 2 and core 3, as well as on N-glycan structures. The gene encoding GlcNAc6ST-2 is expressed in mucinous adenocarcinoma [76]. A human GlcNAc6ST-3 (hGlcNAc6ST) is expressed mainly in human intestines. It can act on terminal GlcNAc residues of N-acetyl-lactosamine chains, core 2 and GlcNAc-Man, but not on core 3 or KS [76]. A closely related enzyme is expressed in corneal tissue, GlcNAc6ST-5 (CGlcNAc6ST). The corneal enzyme synthesizes corneal KS [77] and is also present in human brain [78]. A mouse IGlcNAc6ST has combined activities of the two enzymes, GlcNAc6ST-3 and -5, and can synthesize KS, as well as 6-SO4-GlcNAc linkages in glycoproteins [79,80]. GlcNAc6ST-4 is expressed in many different organs and can utilize N-acetyl-lactosamine chains as substrates [81,82].

Sulphation pathways of O-glycans

The pathways in the biosynthesis of complex sulphated sugar chains can be derived from the specificities of STs and glycosyltransferases, and the occurrence of glycan structures. Sulphation may block pathways and turn off branching and elongation reactions. Oligosaccharides with terminal 6-sulpho-GlcNAc are substrates for β4-Gal-transferase from rat colon and bronchial mucosa. β4-Gal-transferase can act on 6-sulphated, but not 3- or 4-sulphated, GlcNAc [58], and GlcNAc6ST adds sulphate only to terminal GlcNAc residues [9,76,80] (Scheme 1). Thus extension of sulphated poly(N-acetyl-lactosamine) chains occurs after 6-sulphation of GlcNAc. In the assembly of the L-selectin ligand 6-sulpho-sialyl-Lewis x (Scheme 1), GlcNAc is first 6-sulphated, then a Galβ1–4 residue is added [83]. Subsequently, α2–3 sialic acid is added to Gal and α1–3Fuc to GlcNAc. While the α3-sialyltransferase tolerates the sulphate group on the 6-position of GlcNAc [84], it does not act on 6-sulphated Gal residues. Although the sequence of reactions has not been established, the 6′-sulpho-sialyl-Lewis x determinants are probably assembled by first α3-sialylating and α3-fucosylating the N-acetyl-lactosamine structures, and this is followed by 6-sulphation of the Gal residues (Scheme 1). Especially in respiratory mucins, the Gal moiety of N-acetyl-lactosamines can also be 3-sulphated, and this may be followed by the addition of an α3-Fuc residue to GlcNAc to form 3′-sulpho-Lewis x [85].

Core 1 Gal3ST recognizes the Gal1–3GlcNAc unit of cores 1 and 2, which therefore can both become sulphated. However, once core 1 is 3-sulphated, core 2 cannot be formed [58]. Thus sulphated core 2 structures are synthesized after the core 2 branch has been synthesized. Sulphation of this core residue of core 1 also stops elongation and sialylation (Scheme 1).

Sulphated N-glycans present in recombinant human tissue plasminogen activator contain sialic acid and sulphate attached to the same Gal residue [NeuAcα3(6SO4)Gal]. Since sialic acid cannot be transferred to sulphated Gal, it appears that sulphation is a later event than sialylation and occurs in late Golgi compartments [87]. Since a number of glycosyltransferases can act on sulphated substrates, e.g. β4-Gal-transferase [58] and α2–α3/4-Fuc-transferases [86,88,89], sulphation is not necessarily the last step of biosynthesis.

KS is synthesised by β4 Gal-transferase-1 and β3GlcNAc-transferase-2, which are ubiquitous, and of all the members
Scheme 1 | Sulphation pathways of mucin oligosaccharides

A

B

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of these glycosyltransferase families have the greatest KS synthesizing ability [80]. β3GlcNAc-transferase cannot add GlcNAc to sulphated Gal [80]. KS Gal6ST can add internal 6-sulphate on to Gal; thus, in the assembly pathway of KS, Gal can be 6-sulphated during or after completion of the KS backbone.

Mucin sulphation in disease

Human intestinal mucosa is rich in STs and sulphomucins, with selective secretion of a more highly sulphated mucin population in the rectum. The sulphate content of mucins is decreased in colon cancer as well as in ulcerative colitis [90–92]. Specific sulphatases degrading mucin oligosaccharides have been isolated from faecal extracts and from specific types of bacteria which cleave 6-sulphated sugar linkages. The hyperactivity of these enzymes may explain the undersulphation of mucins in pathological conditions such as ulcerative colitis [93].

A significant loss of mucin sulphation was also seen in colorectal adenoma cells during their progression to cancer. This decrease in mucin sulphation corresponded to decreased core 1 Gal3ST and GlcNAc6ST activities [60]. Lower core 1 Gal3ST activity was also found in colon cancer tissue [58,59]. In non-mucinous carcinoma, the expression of Gal3ST-2 was found to be down-regulated [94]. Breast cancer cells also have lower Gal3ST activity compared with normal mammary cells [61]. However, breast cancer tissues had high activity of the enzyme [67]. Since sulphation reactions depend on the presence of appropriate substrate, alterations in the biosynthesis of mucin cores and their modifications in cancer [3,59] play a pivotal role in controlling the amount of mucin sulphation.

GlcNAc6STs in human colonic mucosa have been named according to their substrate specificities: SulT-a was shown to act on GlcNAcβ1–6(Galβ1–3)GlcNAc (core 2) and GlcNAcβ1–2Man, whereas SulT-b has a broader specificity and also acts on core 3 and GlcNAcβ1–3Galβ1–4Glc. SulT-a activity was lower in mucinous adenoma than in adjacent mucosa. SulT-b was expressed in mucinous adenocarcinoma and adenocarcinoma with a mucinous component [95,96]. The gene encoding GlcNAc6ST-2 was found to be expressed in a number of mucinous adenocarcinomas, while there was a low expression level in normal and non-mucinous adenocarcinoma [94,95]. GlcNAc6ST-3, which corresponds to SulT-a in its substrate specificity, was expressed at higher levels in normal mucosa compared with adenocarcinoma.

The gene mutated in CF, i.e. that encoding the CF transmembrane conductance regulator (CFTR), may be connected to sulphation by regulating the ability to transfer sulphate into the cytoplasm, the site of PAPS synthesis. CFTR gene transfer into pancreatic carcinoma cells as well as epithelial cells derived from CF patients could correct a sulphate transport deficiency [97]. Mucins associated with CF have been shown to be hypersulphated, e.g. in intestines [98], tracheobronchial secretions and nasal epithelial cells from CF patients [99,100]. A 2-fold higher level of sulphation was observed in airway xenografts from CF patients compared with non-CF controls [101]. Respiratory mucins from CF patients have sulpho-Lewis x and sulpho-sialyl-Lewis x determinants [15]. The synthesis of these epitopes was significantly stimulated in bronchial mucosal explants after incubation with the inflammatory cytokine tumour necrosis factor α (TNFα). Thus Gal3ST activity towards Galβ-methyl and GlcNAc6ST activity towards GlcNAcβ-methyl substrate were increased [102]. In human myeloid cells, TNFα stimulated 6-sulphation of GlcNAc attached to the cell adhesion glycoprotein CD44 [29]. Mainly N-glycans, but also O-glycans, were affected, while the sulphation of chondroitin sulphate was decreased. In contrast, treatment of cultured bovine articular synoviocytes with TNFα caused a decrease in core 1 Gal3ST activity [65]. This suggests a cell type- and ST-specific regulation of sulphation, and indicates that inflammatory stimulation can alter the ratio of specific sulphated glycan structures produced in a cell.

CFTR(−/−) knockout mice die of intestinal obstruction several days after weaning unless kept on a liquid diet. There is accelerated mucin secretion, and colonic crypt lumina are deep and dilated, and filled with mucin. These mice apparently have abnormal degrees of mucin sulphation, based on the different acidity of secreted and cellular mucins [103]. ST activities in these mice varied during the development of the disease [64]. A tissue survey showed that colon tissue of CFTR(−/−) mice was rich in STs acting on core 1. Ileum, stomach mucosa, duodenal tissues, kidney and lung tissues had a lower activity of core 1 ST. The enzyme was not detected in the colon of CF mice that were very ill and died of obstructive disease, and the activity in the ileum was very low, while the lung showed normal levels. Intestinal obstruction particularly affected STs in the colon and ileum, and core 1 Gal3ST activity showed an increase in a later stage of the disease, but then declined in the final stage. This suggested that mice with late-stage obstructive disease have lost the ability to produce sulphated mucin oligosaccharides.

In another animal model of CF, rats treated intraperitoneally with reserpine have an increased production of sulphated mucins [104]. Sulphomucin prominent in the distal one-third of small intestine spread to the middle and proximal third. Incorporation studies with 35S showed that sulphate was incorporated into O-glycans.

Conclusions

In conclusion, the class of mucin STs may include STs normally acting on other glycoproteins or proteoglycans. The combined activities of glycosyltransferases synthesizing the basic and specific substrate structures for ST reactions, and the individual STs with different specificities, assemble sulphated mucin oligosaccharides. The synthesis of sulphated glycans is altered in inflammatory disease and cancer, through different expression and activities of individual glycosyltransferases and STs, leading to a new sulpho–mucin phenotype that is expected to be functionally distinct. After
identification of the genes and activities of STs, future work should focus on these functional roles.

The support of The Arthritis Society, the Canadian Cystic Fibrosis Foundation, Materials and Manufacturing Ontario and the National Research Council of Canada is gratefully acknowledged.

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