Biochemistry

 Volume 41, Number 44 November 5, 2002

Current Topics

Carbohydrate Sulfotransferases of the GalNAc/Gal/GlcNAc6ST Family†

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Received August 2, 2002

Sulfation of small molecules, proteins, and sugars is an essential modification in organisms ranging from bacteria and plants to mammals. Sulfation is implicated in a wide variety of cellular signaling events and in the modulation of receptor−ligand binding in normal processes and disease states including chronic inflammation, cancer cell metastasis, cartilage formation, hormone regulation, and solubilization of toxic xenobiotics (*1*−*5*). Sulfated glycoproteins and proteoglycans mediate cell−cell adhesion (*6*), cell proliferation by the activation of growth factors (*7*), cell differentiation (*8*−*11*), viral and bacterial invasion (*12*−*17*), blood clotting (*18*−*20*), and activation of chemokines and cytokines (*21, 22*). More specifically, the sulfation of sugar residues on glycoproteins modulates the binding of leukocytes to endothelial cells at sites of chronic inflammation (*6, 23*−*28*), the migration of neural cells (*29*−*31*), the outgrowth of neurons and astrocytes (*32, 33*), and the circulatory half-life of glycoprotein hormones (*34*−*36*).

The sulfotransferases (STs) are the enzymes responsible for the transfer of a sulfuryl group from the substrate 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to the alcohol (or amino) group of a sugar (*37*), a small molecule (*1, 2, 38*−*40*), or a tyrosyl residue in a protein (*41*) (Figure 1). The sulfotransferases can be divided into two main classes: the cytosolic and the Golgi-resident enzymes. A substantial amount of work has been published characterizing the structures and functions of the cytosolic sulfotransferases, including crystallography (*42*−*45*), mutagenesis (*46*−*51*), photoaffinity labeling (*52*−*54*), and mechanistic studies (*2, 38, 39, 55*−*57*). Several reviews have been written on the involvement of the cytosolic sulfotransferases in xenobiotic inactivation and hormone regulation (*1, 2, 38, 40, 58*). The structures and functions of the cytosolic sulfotransferases have also been reviewed (*59*−*61*). By contrast, very little mechanistic or structural information exists for the Golgi-resident carbohydrate sulfotransferases and tyrosylprotein sulfotransferases (TPSTs). This is due in part to their membrane-associated state and the lack of highly productive expression systems. Furthermore, the regulation of sulfation is poorly understood, and the identification and prediction of endogenous substrates of the Golgi enzymes remains difficult.

The study of carbohydrate sulfation began with the identification of keratan sulfate sulfotransferase activity in the chick cornea in 1978 (*62*). The first mammalian carbohydrate sulfotransferase activity, a heparan sulfate glucosamine *N*-sulfotransferase, was characterized in 1979 (*63*), and the enzyme responsible was cloned in 1994 (*64*). Since then, 31 carbohydrate STs have been described. The Golgi-resident STs are all type II single-span transmembrane proteins that are composed of a short N-terminal cytosolic tail and a C-terminal catalytic domain within the Golgi lumen. In addition, there are now two TPSTs known in humans [(*65*) and reviewed in (*41*)]. Sulfation of tyrosine residues has been shown to provide a critical recognition element for HIV-1 infection of human cells (*66*) and for mediating the interaction between leukocytes presenting

† This work was supported by a grant from the National Institutes of Health (GM59901).

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10.1021/bi020507h CCC: $22.00
Published on Web 10/09/2002

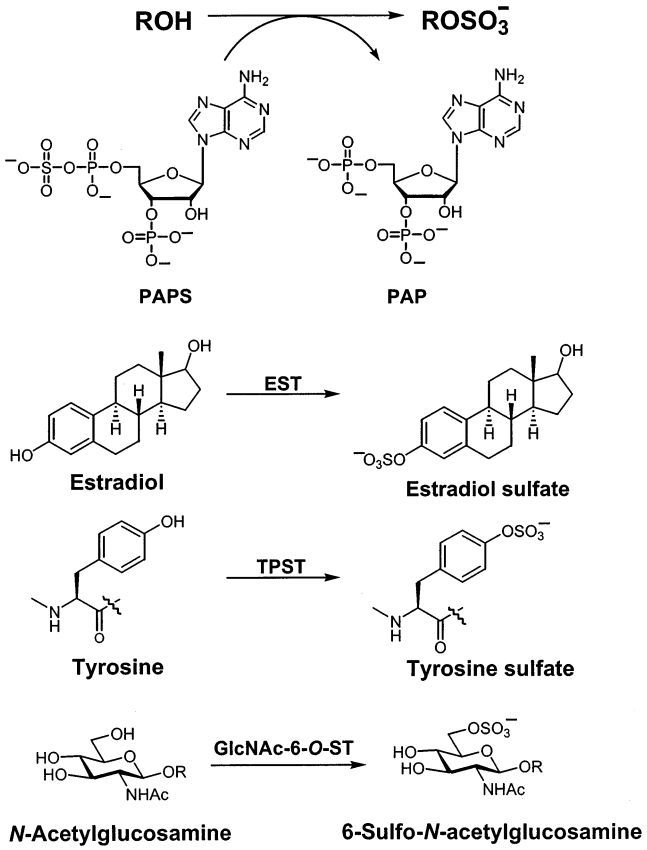

FIGURE 1: Examples of cytosolic and Golgi-resident sulfotransferase reactions. Sulfotansferases catalyze the transfer of a sulfuryl group to an acceptor molecule using the activated sulfate donor PAPS as a substrate. Sulfotransferases are known to sulfate hormones such as estradiol, the tyrosine residues of proteins, and the carbohydrate residues of glycoproteins, proteoglycans, and glycolipids.

P-selectin glycoprotein ligand-1 (PSGL-1) and P-selectin at the surface of endothelial cells (*67*).

This review focuses on a subtype of STs that acts on the 6-position of GalNAc, Gal, or GlcNAc residues, termed the "GST" family (*68*). We first discuss the roles of carbohydrate sulfotransferases in the inflammatory response and in the biosynthesis of glycosaminoglycans (GAGs). A more detailed overview of GAG biosynthesis and associated STs can be found in a recent review (*69*). The cloning, tissue distribution, and substrate specificity of the seven cloned human GSTs are summarized, and the protein sequences of the cytosolic ST and GST sequences are compared to identify sites of potential structural similarity between the two subclasses.

The Role of Sulfated L-Selectin Ligands in Leukocyte Homing and the Inflammatory Response. Interest in carbohydrate sulfation has increased considerably since the demonstration of a key role in leukocyte homing both in the normal immune system and in chronically inflammed tissues. Immune surveillance requires the continuous trafficking of lymphocytes from the bloodstream through peripheral lymph nodes. This process occurs via high endothelial venules (HEVs), specialized regions of the microvasculature designed to recruit lymphocytes under flow (*70*). There are three stages of leukocyte extravasation into the lymph node. First, leukocytes tether to the surface of HEV and then roll along the endothelium. They become activated by specific chemokines, then firmly adhere to the venules. Finally, the cells migrate across the endothelial barrier into the lymph node tissue. L-Selectin is a receptor expressed on all classes of circulating leukocytes that mediates the initial tethering and rolling interaction on endothelium in peripheral lymph node HEVs (*6, 71*−*74*). L-Selectin requires glycoprotein ligands on the HEV that present the sulfated carbohydrate epitope 6-sulfo sialyl Lewis x (sLe^x) (*75*−*77*). This unusual epitope is a posttranslational modification of various mucin proteins that include the secreted GlyCAM-1 in the mouse and cell-surface-bound CD34, sgp200, podocalyxin, and MAdCAM-1 in human tissue (*6, 71*). The sulfate ester installed at the 6-position of GlcNAc increases the binding affinity to L-selectin above a threshold so that lymphocytes can be captured from the blood under conditions of shear stress (*78, 79*). HEC-GlcNAc6ST (GST-3) is the sulfotransferase implicated in the installation of the sulfate ester at the 6-position of GlcNAc as its expression is limited to peripheral lymph node high-endothelial cells (HEC) (*80, 81*). The biological roles of GST-3 have been studied in a mouse model in which the *GST-3* gene was disrupted (*82*). The loss of GST-3 activity was directly responsible for the loss of recombinant L-selectin binding to the luminal aspects of HEV, the elimination of lymphocyte binding to HEV glycoproteins in vitro, and the marked reduction of in vivo homing of lymphocytes to peripheral lymph nodes in vivo (*82*).

Blood vessels in chronically inflamed tissues adopt an HEV-like structure, and, likewise, leukocytes are recruited to these tissues in response to pro-inflammatory cytokines such as IL-4 and TNFα (*70, 83*−*86*). Modulation of sulfation of L-selectin ligands may therefore control leukocyte recruitment to chronically inflamed tissues. Thus, the responsible STs are potential therapeutic targets for small-molecule therapies for a host of chronic inflammatory diseases such as diabetes, rheumatoid arthritis, inflammatory bowel disease, transplant rejection, psoriasis, and asthma (*87*−*90*).

The Cloning and Substrate Specificity of the GalNAc/Gal/GlcNAc-6-O-sulfotransferase Family. There are currently seven members of the Gal/GalNAc/GlcNAc-6-*O*-sulfotransferase (GST) family: GST-0, -1, -2, -3, -4α, -4*β*, and -5. Their substrate preferences, chromosomal location, tissue distribution, protein length, and value of K_M(PAPS) are summarized in Table 1. Some appear to play a role in GAG biosynthesis whereas others sulfate sugars within *O*-linked or *N*-linked glycoproteins. Mouse homologues are known for all of the aforementioned human carbohydrate sulfotransferases, except that only one GST-4 isozyme exists in mouse, and human GST chromosome localization is syntenic to the mouse (*37*). Within their catalytic domains, the GSTs share more than 40% similarity and are roughly 30% identical at the amino acid level. The GSTs can be divided into two subfamilies, the GalNAc/Gal-6-*O*-sulfotransferases and the GlcNAc-6-*O*-sulfotransferases, with approximately 50% amino acid sequence identity in the subfamily (*68*). Interestingly, single-nucleotide polymorphism (SNP) profiling in a Japanese population showed no SNPs within the exons of GST-0, -1, -2, -3, -4α, -4*β* or -5, suggesting that members of this gene family are under selective pressure to conserve the sequence in these regions (*91, 92*).

The GalNAc/Gal-6-O-sulfotransferases. (A) GST-0. Chondroitin 6-sulfotransferase (C6ST or GST-0) catalyzes the transfer of a sulfate group to the 6-position of GalNAc within chondroitin sulfate, a member of the GAG family. The

Table 1: Seven Cloned Human Golgi-Resident GalNAc/Gal/GlcNAc-6-*O*-sulfotransferases (GSTs)[a]

name	nomenclature in literature	previous abbreviations	ref	accession no.	sulfated substrate	tissue expression	chromosomal location	protein length	K_M (PAPS) (μM)
GST-0	chondroitin 6-*O*-sulfotransferase	C6ST	99	AB012192	Gal,GalNAc	broad	10q21.3	479	ND
		CH6ST-1	103		GluAβ1,4-GalNAc				
GST-1	keratan sulfate 6-*O*-sulfotransferase	KSGal6ST	106	AB003791	Galβ1,4-GlcAc	broad	11p11.1-11.2	411	0.58
	chondroitin 6-*O*-sulfotransferase	C6ST	108	U65637					
	carbohydrate sulfotransferase-1	CHST-1	109	AF090137					
GST-2	*N*-acetylglucosamine 6-*O*-sulfotransferase	GlcNAc6ST-1	110	AB014680	terminal GlcNAc	broad	7q31	530	1.2
	carbohydrate sulfotransferase-2	CHST-2	109	AF83066			3q24		
GST-3	HEC-restricted *N*-acetylglucosamine 6-*O*-sulfotransferase	HEC-GlcNAc6ST							
		GlcNAc6ST-2	81	AF131235	terminal GlcNAc	HEC	16q23.1-23.2	386	5.9
GST-4α	intestinal *N*-acetylglucosamine 6-*O*-sulfotransferase	I-GlcNAc6ST							
		GlcNAc6ST-3	111	AF176838	terminal GlcNAc	intestine	16q23.1-23.2	390	14
GST-4β	corneal *N*-acetylglucosamine 6-*O*-sulfotransferase	C-GlcNAc6ST	102	AF219990	terminal GlcNAc	cornea	16q23.1-23.2	396	ND
		GlcNAc6ST-5	116	AF280086					
GST-5	chondroitin 6-*O*-sulfotransferase-2	C6ST-2	112	AB037187	(GluAβ1,4GalNAc)	broad	Xp11	486	ND
			114	AF280089	terminal GlcNAc				
		GlcNAc6ST-4	113	AB014680	GlcNAcβ1,6-ManOMe				

[a] ND = not determined. The nomenclature in the GST literature can be confusing. For example, GST-3 is also known as GlcNAc6ST-2. Abbreviations used by others are listed in the table for each of the GSTs.

enzyme can also sulfate Gal residues of keratan sulfate (*93*), another GAG, and the Gal residues in sialyl *N*-acetyllactosamine (sialyl LacNAc) oligosaccharides (*94*). GST-0 was cloned by Fukuta et al. from a human fetal brain cDNA library based on homology to a previously discovered chick C6ST (*95*). The chick and human enzymes are 74% identical at the amino acid level. Two in-frame ATG transcription start sites were found at the N-terminus of the protein, and the first ATG site encodes for a 479 residue protein containing a 15 residue hydrophobic putative transmembrane domain. Six potential *N*-linked glycosylation sites are conserved between the chick and human enzymes (*93*). Tsutsumi et al. independently cloned GST-0 from a human placental cDNA library. The enzyme was seven times more active at transferring sulfate to the 6-position of GalNAc in $(GlcA\beta1 \rightarrow 3GalNAc\beta1 \rightarrow 4GlcA)_n$ structures than with substrates bearing another sulfate group at the 4-position of GalNAc (*96*). Furthermore, GST-0 did not transfer a sulfate group to IdoAα1 $\rightarrow$ 3GalNAc (*96*).

(B) GST-1. Several groups have reported the cloning of KSGal6ST (KSST or GST-1) (*97*−*99*) and determined it to be composed of 411 residues with 4 potential *N*-linked glycosylation sites (*97*). A hydropathy plot predicted the presence of a 14 amino acid transmembrane domain found between amino acids 7 and 20 (*97*). Mazany et al. identified two potential tyrosine kinase phosphorylation sites as well as an RGD motif. The N-terminus contained a signal sequence that could allow for secretion from cells. The RGD sequence in secreted GST-1 could aid in integrin binding; however, this signal sequence is most likely a Golgi-retention sequence as the majority of the enzyme appears to be intracellular. GST-1 is expressed in a wide range of tissues (*97*−*99*). The enzyme catalyzes the transfer of a sulfate group to the 6-position of Gal in the repeating disaccharide polymer Galβ1 $\rightarrow$ 4GlcNAc(6-SO_3^-) of keratan sulfate. GST-1 can sulfate the 6-position of galactose residues within synthetic sialyl LacNAc oligosaccharides as efficiently as in keratan sulfate (*100*). This ST has a preference for sulfating keratan sulfate (Galβ1 $\rightarrow$ 4GlcNAc(6-SO_3^-), but will also transfer sulfate to the unsulfated polymer (Galβ1 $\rightarrow$ 4GlcNAc) (*97*). The sulfotransferase activity on sialyl LacNAc structures was much higher than the corresponding desialylated substrate, and only internal Gal residues were sulfated by GST-1 (*100*). Interestingly, Mazany et al. described GST-1 as a chondroitin sulfate ST as well as a keratan sulfate ST, while Fukuta et al. identified GST-1 as only a keratan sulfate ST.

The GlcNAc-6-O-sulfotransferases. (A) GST-2. CHST2 (GST-2) was identified as a 530 residue sulfotransferase from a HUVEC cDNA library. GST-2 has a C-terminal domain that is 43% and 45% identical at the amino acid level to human and chick C6ST, or GST-0, respectively. In contrast to GST-0 and GST-1, GST-2 catalyzes the sulfation of the 6-position of GlcNAc within keratan sulfate-like structures on *N*-linked glycans and within mucin-associated glycans that can ultimately serve as L-selectin ligands. GST-2 will sulfate the GlcNAc residues at terminal, nonreducing ends of oligosaccharide chains (*81, 101*). Uchimura et al. found that the human cDNA encodes for two isoforms with short and long cytosolic tails (*101*). The two in-frame ATG start sites are 41 bp apart. It is postulated that perhaps the short form encodes for a Golgi-resident version of the enzyme while the long-tail version may be expressed at the cell

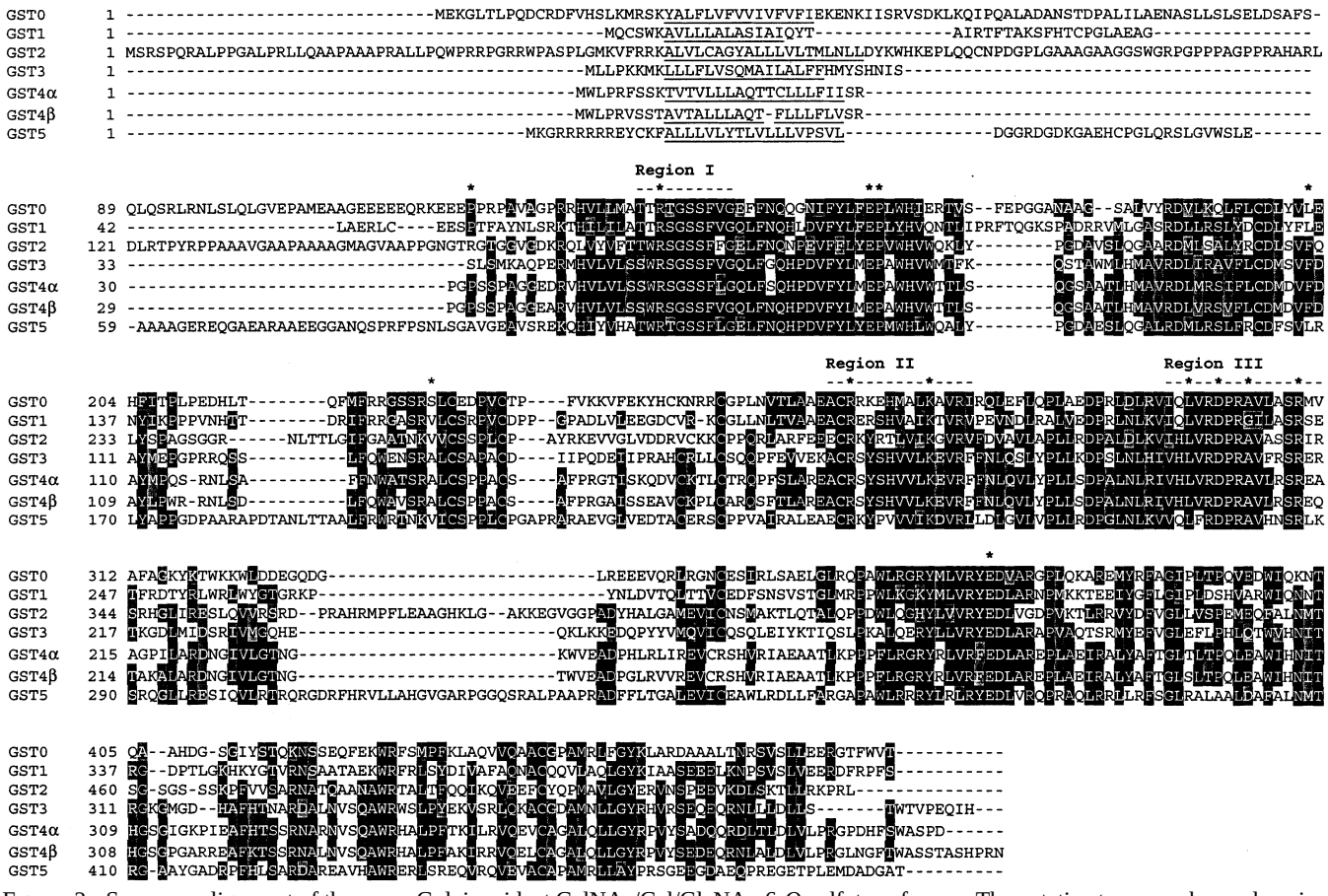

FIGURE 2: Sequence alignment of the seven Golgi-resident GalNAc/Gal/GlcNAc-6-*O*-sulfotransferases. The putative transmembrane domains are underlined. Region I corresponds to the 5′-phosphosulfate PAPS binding sequence, Region II corresponds to the putative substrate binding site, and Region III is the 3′-phosphate PAPS binding site. Asterisks above the sequence alignment indicate the point mutations found in GST-4β that correlate to patients with MCD. Black and gray shading indicate that > 50% of the residues at that position are identical or similar, respectively.

surface as is the case for a β1 → 4-galactosyltransferase (*101*). GST-2 transcripts are observed in most human tissues. The carbohydrate structures synthesized by GST-2 may be involved in the differentiation and behavior of blood cells, their progenitor cells, and in neurons of the central nervous system (*101*).

(B) GST-3. HEC-GlcNAc6ST (GST-3) was cloned from a high-endothelial cell cDNA library. The cDNA is 1158 nt in length and encodes for a 386 residue protein with 3 potential *N*-linked glycosylation sites (*81*). GST-3 is 73% identical to mouse GST-3 (LSST) (*80*), and is 31% identical to chick GST-0, and 32% identical to human GST-1 at the amino acid level. GST-3 is also known as HEC-GlcNAc6-ST (*81*) for its highly restricted expression in peripheral lymph node HEC. The mouse homologue is named LSST (*80*) for its ability to synthesize 6-sulfo sLe^x, the L-selectin binding epitope. Flow cytometry experiments indicate that the transfection of both GST-1 and GST-3 into cells greatly enhances binding of an L-selectin/IgM chimera compared to either sulfotransferase alone (*81*). It is possible that GST-3 is also induced at sites of chronic inflammation where HEV-like vessels develop. Indeed, Fukuda and co-workers observed GST-3 expression in the mouse hyperplastic thymus, where leukocytes are being continuously recruited (*80*). Thus, GST-3 is of particular interest as an antiinflammatory target. The importance of GST-3 for biosynthesis of HEV-associated L-selectin ligands is understood by the dramatic phenotype of the GST-3 knockout mouse, mentioned earlier.

*(C) GST-4*α. I-GlcNAc6ST (GST-4α) is a GlcNAc-6-*O*-sulfotransferase that is expressed in both the small and large intestines where many mucin glycoproteins and GAG chains are found (*102*). The sulfation events catalyzed by GST-4α result in the formation of sulfated GAG chains within proteoglycans and in the sulfation of *O*-linked chains within sialomucins of the gut lymphatic tissue; however, GST-4α does not appear to generate L-selectin ligands. GST-4α was first identified by screening an expressed sequence tag (EST) database using GST-1 and GST-3 as queries (*102*). GST-4α is 1173 bp with no introns detected within the coding region, as is the case with all the GSTs. There were two possible ATG initiation sites within the gene, with the second ATG favored according to Kozac's rules (*102*). The second start position results in a translated protein sequence of 390 amino acids with 55% identity to GST-3 and 35.8% identity to GST-1 (*102*). GST-4α is a transmembrane protein with a 9 residue N-terminal cytoplasmic tail, an 18 residue transmembrane domain, and a C-terminal catalytic domain with three potential sites of *N*-linked glycosylation (*102*).

(D) GST-4β. C-GlcNAc6ST (GST-4β) is the GlcNAc-6-*O*-sulfotransferase that is located 50 kb downstream from GST-4α as a tandem repeat of genes on human chromosome 16q23.1-23.2. GST-4α and -β are 85.6% identical at the amino acid level. While GST-4α is expressed intestinally, GST-4β is expressed in the brain and cornea (*103*). Null mutations of GST-4β are associated with human macular corneal dystrophy (MCD) and result in the undersulfation

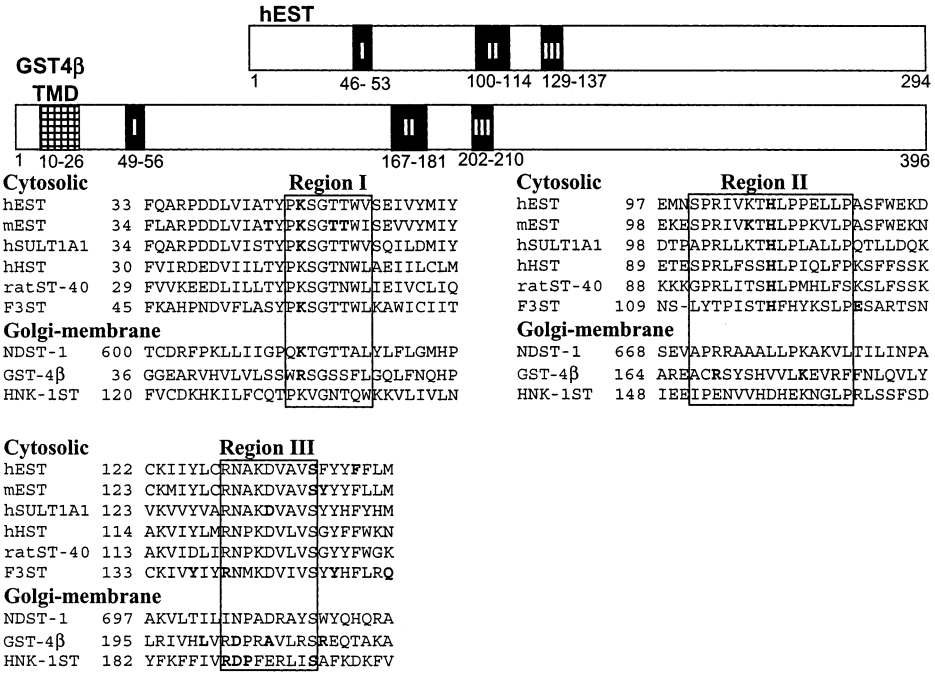

FIGURE 3: Sequence alignment of several cytosolic and Golgi-resident carbohydrate sulfotransferases. A schematic view of the region distribution in hEST and GST-4β is shown above the sequence alignments. Residues in boldface indicate amino acids that have been mutated and found to effect enzymatic activity. hEST, human estrogen sulfotransferase; GST-4β, human GlcNAc-6-*O*-sulfotransferase-4β (corneal); mEST, mouse estrogen ST; hSULT1A1, human phenol ST; hHST, human hydroxysteroid ST; F3ST, flavonol 3-ST; NDST-1, the sulfotransferase domain of human *N*-deacetylase/*N*-sulfotransferase-1; HNK-1ST, human natural killer cell ST.

of keratan sulfate in the cornea, leading to corneal opacity and eventual blindness (*104*−*109*). Several gene rearrangements and missense mutations result in both type I and type II MCD (*104*). Type I MCD patients have point mutations within the catalytic domain of the enzyme that result in the lack of keratan sulfate in the cornea and serum (*104, 107, 108*). Type II MCD patients have mutations in the 5′ promoter region of their gene, which result in an absence of keratan sulfate from the cornea only (*104, 107, 108*).

The genes encoding GST-3 and GST-4α/β are clustered on chromosome 16, and a number of human patients with a variety of developmental and morphological abnormalities were described as having deletions in this region on a single chromosome (*68, 110, 111*). The tissue-specific expression of GST-3 and GST-4α/β, and the incidence of humans with morphological consequences from mutations that map to the same chromosomal location, implicates these GSTs in development processes.

(E) GST-5. C6ST-2 (GST-5) has been cloned by three groups; however, it was first reported by Kitagawa et al. and identified as a GalNAc-6-*O*-sulfotransferase of chondroitin polymers (GlcAβ1 → 3GalNAc)$_n$ (*112*). GST-5 was found from a BLAST analysis of an EST database using GST-1 as a probe (*112*). This search resulted in the discovery of an ORF encoding a 486 residue protein with three potential sites of *N*-linked glycosylation and with 24% amino acid sequence identity to GST-1, with the highest homology occurring at the C-terminal catalytic region (*112*). GST-5 is 41%, 26%, and 31% identical at the amino acid level to GST-2, -3, and -4α, respectively (*113*). GST-5 is unique among the GlcNAc-6-*O*-sulfotransferases in that it has a cluster of six arginine residues within its 13 residue cytoplasmic tail. Like several of the other GSTs discussed, transcripts of GST-5 are detected in a variety of human tissues (*112, 114*).

The Substrate Binding Regions of the Carbohydrate Sulfotransferases. The carbohydrate sulfotransferases have two regions of homology with the cytosolic sulfotransferases, the 5′-PSB and 3′-phosphate (3′-PB) binding regions (*115*). Figure 2 shows a sequence alignment of the GSTs, while Figure 3 shows a sequence alignment of sulfotransferases from several subfamilies. Two regions, labeled I and III in Figures 2 and 3, form a 5′-PSB and a 3′-PB site, respectively, while Region II is defined as the putative carbohydrate substrate binding site. The cytosolic sulfotransferases contain a fourth region called the P-loop, a feature commonly found in nucleotide binding proteins (*116*), that is essential for PAPS binding. This region, however, has no correlate in the carbohydrate sulfotransferases. Residues implicated in maintaining catalytic function and structural integrity of the carbohydrate sulfotransferases have been identified by sequence alignment with the cytosolic sulfotransferases for which crystal structures are available. Many of these residues are conserved among the carbohydrate sulfotransferases, and some of these conserved residues are mutated in GST-4β from patients suffering from MCD type I, indicating their importance in enzymatic function.

X-ray crystal structures exist for mouse and human estrogen sulfotransferases (mEST and hEST) (*42, 45*), human hydroxysteroid sulfotransferase (hHST, hSULT2A3) (*117*), human catecholamine/dopamine sulfotransferase (hSULT1A3) (*43, 118*), and the sulfotransferase domain of the Golgi-resident human heparan sulfate *N*-deacetylase/*N*-sulfotransferase-1 (NDST-1) (*44, 60, 61*). This latter enzyme sulfates the amino group of glucosamine during the biosynthesis of the GAG heparan sulfate. mEST has been cocrystallized with estradiol and 3′-phosphoadenosine 5′-phosphate (PAP) as well as with a putative transition-state analogue, vanadate/estradiol complex (*42, 119*). The kinetics of sulfate transfer

Table 2: List of All Point Mutations Made in Various Cytosolic Sulfotransferases, the Sulfotransferase Domain of NDST-1, and HNK-1ST That Had an Effect on the Catalytic Activity of the Enzyme[a]

mutation	enzyme	ref	wt % activity	K_M(rel) [PAPS]	K_M(rel) [substrate]	k_{cat}(rel) V_{max}(rel)	substrate
			Region I: 5′-PSB				
T45V	mEST	119	ND/ND	1.2	1.2/ND	0.18/ND	estradiol/DHEA
K48M	mEST	119	ND/ND	NA	NA	NA	estradiol/DHEA
K48M	hSULT1A1	123		NA/NA	NA/NA		dopamine/pNP
K48M	hSULT1A3	123			NA/NA	*0.06/NA*	dopamine/pNP
K59A	F3ST	48	0.3	ND	ND	ND	quercetin
K128A	HNK-1	134	1				HNK-1 glycan
K614A	NDST-1	135	NA	NA	NA	NA	K5 polymer
K48M	hSULT1A1	123	NA/NA	NA/NA			dopamine/pNP
K48M	hSULT1A3	123	NA/NA			0.06/NA	dopamine/pNP
T51V	mEST	119	ND/ND	7.7	1.6/ND	0.19/ND	estradiol/DHEA
T52V	mEST	119	ND/ND	2.3	3.7/ND	0.05/ND	estradiol/DHEA
			Region II: SBS				
K106A	mEST	119	ND/ND	2.5	6.8/ND	0.01/ND	estradiol/DHEA
H108K-GST	mEST	119	ND/ND	ND	3.1/ND	0.008/ND	estradiol/DHEA
H107A	hEST	136	NE	NE	ND	NE	estradiol/DHEA
H108A	hSULT1A1	123			NA/NA	*NA/NA*	dopamine/pNP
H108A	hSULT1A3	123			1.3/NA	*NA/NA*	dopamine/pNP
H98A	rat	ST-40	163	ND/ND			andosterone/DHEA
H118A	F3ST	49	0.08	ND	ND	ND	quercetin
E126K	F3ST	49	53	0.68	1.6	0.64	quercetin
			Region III: 3′-PB				
K134G	F3ST	49	24	1.2	0.82	0.19	quercetin
Y137A	F3ST	49	0.8	ND	ND	ND	quercetin
R140S	F3ST	49	0.1	ND	ND	ND	quercetin
R189A	HNK-1	134	1				HNK-1 glycan
D190A	HNK-1	134	<1				HNK-1 glycan
P191A	HNK-1	134	<1				HNK-1 glycan
S197A	HNK-1	134	<1				HNK-1 glycan
S137A	hEST	134	4				
Y150A	F3ST	49	12	4.9	3.5	0.29	quercetin
F142A	mEST	119	55/NA	ND	50/ND	0.5/ND	estradiol/DHEA
I146A	mEST	119	72/NA	ND	ND/ND	ND/ND	estradiol/DHEA
S148A	mEST	119	19.5/7.7	ND	ND/ND	ND/ND	estradiol/DHEA
Y149F	mEST	119	60.2/131	ND	ND/ND	ND/ND	estradiol/DHEA
Q175H	F3ST	49	62	1	1.56	0.95	quercetin
H186A	F3ST	49	2.7	1.3	1.49	0.04	quercetin
C751A	NDST-1	57	104	ND	0.89	0.96	K5 polymer
Y240F	mEST	123	122	3.2	1.3	0.93	estradiol
E246A	hSULT1A1	55	2.7	2.7	0.68	*0.55/0.76*	2-naphthol

[a] Residues are categorized according to the equivalent residue mutated in the sequence based on alignment. In column 7, numbers in italics are V_{max} values, whereas all other values are k_{cat} values. In entries where two values are specified, these correspond to two different substrates as shown in the far right column. Abbreviations: mEST, mouse estrogen ST; hSULT1A1, human phenol ST; hSULT1A3, human monoamine ST; F3ST, flavonol 3-ST; HNK-1ST, human natural killer cell ST; NDST-1, sulfotransferase domain of human heparan sulfate *N*-deacetylase/*N*-sulfotransferase; H108K-GST, a glutathione *S*-transferase fusion with a mutant MEST; ND, not determinable; NE, not expressed; NA, not available.

for a variety of substrates have been examined, and a number of mutants have been made to probe specific residues involved in enzyme stability, substrate specificity, and catalysis (*46*, *48*−*51*, *119*−*124*). A list of the mutants for both cytosolic and carbohydrate sulfotransferases that had the most deleterious effect on enzymatic activity is provided in Table 2. The structure of the vanadate/estradiol complex supports the proposed direct in-line transfer mechanism of sulfate from PAPS to the acceptor estradiol (*119*). The order of substrate binding to the enzyme has been determined to occur in either an ordered bi-bi (*125*−*128*) or a random bi-bi process for aryl sulfotransferase-IV (*129*, *130*). Kinetic studies of a PLN GlcNAc6ST activity are also consistent with either random or ordered binding, and a direct in-line transfer mechanism (*131*, *132*).

Region I: The PSB-loop or 5′-Phosphate PAP Binding Region. The PSB-loop resides at the N-terminal region of the sulfotransferases sequence and interacts with the 5′-phosphosulfate of PAPS (*116*). The crystal structure of mEST reveals that the 5′-phosphosulfate binding region is composed of the sequence $T_{45}YPK_{48}SGTTW_{53}$ where Trp53 is in a parallel stacking arrangement with the adenine ring of bound PAP (*45*). Lys48 of mEST corresponds to Arg50 in GST-4β and Arg174 in GST-2 when the GSTs are aligned with mEST. The sequence $K_{48}SGTT_{52}$ in mEST corresponds to the sequence $R_{50}SGSS_{54}$ (GST-4β sequence), in the GSTs (*48*). K48 also correlates with K128 of human natural killer cell-1 ST (HNK-1ST), another carbohydrate ST, and with K614 in human NDST-1. Lys to Arg/Ala mutations in HNK-1ST and NDST-1 result in a loss of catalytic activity (*133*, *134*). The crystal structure of mEST shows that Lys48 is directly coordinated to an oxygen of the 5′-phosphate of bound PAP. The arrangement of side-chains around PAPS in mEST is depicted in Figure 4. An arginine residue replaces Lys48 in the GST 5′-PSB sequence (*45*). Patients suffering from MCD type II have an R50C mutation in the gene encoding GST-4β within the 5′-PSB region, and this arginine residue is conserved among all seven GSTs (*104*, *135*).

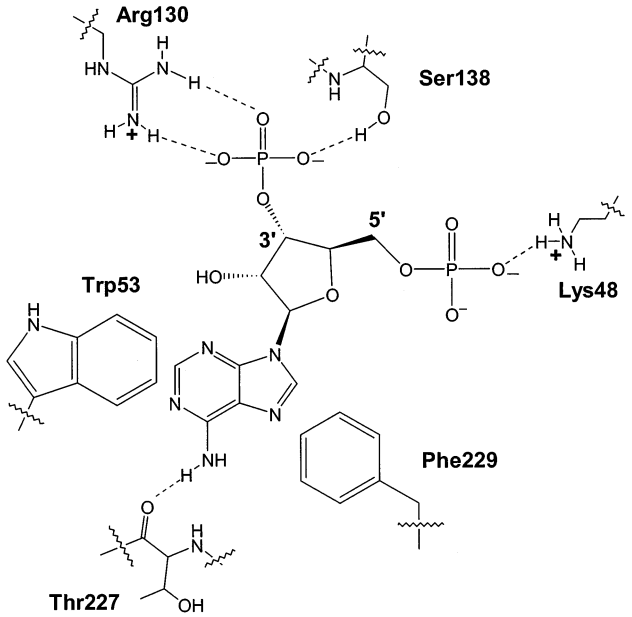

FIGURE 4: mEST residues involved in 5′-phosphate and 3′-phosphate stabilization and binding of the adenine ring. Lys48 hydrogen-bonds with the 5′-phosphate, while the guanidino group of Arg130 and the hydroxyl group of Ser138 interact with the 3′-phosphate of PAP. Trp53 is involved in a parallel π-stacking interaction, and Phe229 is orientated perpendicular to the adenine ring.

Region III: The 3′-Phosphate PAP Binding Region. It is known from crystallographic studies of mEST that Arg130 and Ser138 directly interact with the 3′-phosphate group of bound PAP (*45*) (Figure 4). The cocrystal structure of human EST with bound PAPS indicates that S137 (S138 in mEST) interacts with K47 (K48 in mEST) when PAPS is bound and with an oxygen atom of the 3′-phosphate when PAP is bound. This interaction indicates a molecular level of control over enzyme activity as S137 only releases K47 when K47 is needed to stabilize the end product (*42*). Additionally, Trp53 and Phe229 are in a parallel ring-stacking arrangement with the aromatic adenine ring of PAP (*45*). Site-directed mutagenesis studies have shown that Arg140 and Arg277 of flavonol 3-ST (F3ST), which correspond to Arg130 and Arg257 in mEST, are important for F3ST activity (*136*). Thr227 interacts with the adenosine of PAPS, while Arg257, Lys258, and Gly259 of mEST are all within hydrogen-bonding distance to the oxygen atoms of the 3′-phosphate group of PAP (*45*). Gly259 is the first Gly residue of the P-loop, GxxGxxK, consensus motif at the C-terminal region of all of the cytosolic STs, a feature lacking in the GSTs. Residues Asp134 and Glu263 in human phenol sulfotansferase (hSULT1A1) are conserved among the cytosolic sulfotransferases and were determined to be essential for catalysis by mutating these two acidic residues to alanines (*55*). The mutation D134A results in a complete loss in activity of hSULT1A1, while the D263A mutant had an 80% decrease in activity when compared to wild-type enzyme (*55*). R130, K197, R257, and K258 are all within 5 Å of D134 and D263 based on sequence alignment and mapping of hSULT1A1 onto the mEST structure (*55*). The basic residues surrounding the two crucial acidic residues are predicted to be important in maintaining the PAPS binding site structure which is essential for catalytic activity (*55*). MCD type I patients have mutations D203E and R211W in the putative 3′-phosphate PAPS binding region of GST-4β (*104*). The spacing between the conserved basic residues in the cytosolic sulfotransferases, $R_{130}xxK_{133}xxxxS_{138}$, is conserved among all GSTs with the sequence $RD_{203}PRAVxxSR_{211}$ (*135*). HNK-1ST, which also acts on a carbohydrate substrate, has a similar motif, as shown in the sequence alignment in Figure 3 (*30*, *137*). The only mutagenesis of a Golgi-resident carbohydrate sulfotransferase in the 3′-PB region has been performed on HNK-1ST (*134*). In this study, the mutations R189A, D190A, P191A, and S197A were made and were shown to abolish nearly all catalytic activity (*134*). These residues make up the RDP motif that is conserved in all of the GSTs. Ser197 resides within the second turn of a predicted α-helix and terminates the 3′-PB region, as is indicated by the mapping of this region of the HNK-1ST sequence onto the crystal structures of NDST-1 and mEST (*134*).

Region II: The Substrate Binding Region. Sandwiched between the 5′-PSB (Region I) and the 3′-PB (Region III) sites in the primary sequence is the substrate binding site, Region II. The cytosolic sulfotransferases typically recognize hydrophobic steroids and phenolic/catecholamine-type small molecules, while the Golgi STs typically recognize the hydrophilic sugars of carbohydrates or tyrosyl peptides. Thus, it is not surprising that no homology exists between the Golgi and cytosolic enzymes in this region.

Patients suffering from MCD type I have mutations R166P and K174R in a region of high homology nestled between the putative 5′-PSB and 3′-PB binding sites of corneal GST-4β (*104*) (Figure 2). These arginine and lysine residues are highly conserved in all seven GSTs, perhaps indicating the carbohydrate binding pocket of the GSTs. The mutation data from patients underscore the potential importance of this region, and may define a functionally conserved motif in the GSTs, as these charged residues may be involved in general acid or electrostatic catalysis during the transfer of sulfate from PAPS to the sugar acceptor molecule. MCD type I patients have an additional mutation, E274K, in their GST-4β sequence that decreases sulfotransferase activity. However, this region of the GST-4β has no recognizable consensus in the cytosolic STs (*104*).

The Carbohydrate Specificities of the GSTs. The substrate specificity for the GSTs is generally defined as the 6-position of GlcNAc, GalNAc, or Gal. However, each enzyme appears to have some preference for certain contexts in which that residue occurs. GST-1, -2, -3, -4α, -4β, and -5 have all been expressed as soluble, secreted enzymes, and their activities with synthetic substrates have been extensively analyzed (*100*, *113*, *138*−*140*). All the GlcNAc-6-*O*-sulfotransferases (GST-2−5) prefer GlcNAc in a terminal position. Thus, they must participate in an intermediate stage of oligosaccharide biosynthesis, before glycan extension. Both GST-2 and GST-3 require the 2-*N*-acetyl group and the equatorial 4-hydroxyl group on the target GlcNAc residue, and while both GSTs tolerated a variety of reducing terminal pyranoses, some distinct preferences were observed (*141*). GST-2 and GST-3 can sulfate a variety of terminal GlcNAc Core 2, β1 → 2Man and β1 → 6Man substrates (*139*, *140*), while GST-4α seems to prefer GlcNAc in the context of the Core 2 trisaccharide (*139*). Interestingly, GST-5 has the highest activity toward Man-linked GlcNAc acceptors followed by Core 2 and finally LacNAc oligomers (*113*). These substrate-

profiling results indicate that GST-5 may preferentially sulfate *N*-linked glycans. Knowing which elements of the GlcNAc $\beta(1 \rightarrow 6)$GalNAc are crucial for enzymatic recognition and function could aid in inhibitor design.

CONCLUSIONS

In summary, we have reviewed the GST subfamily of Golgi-resident carbohydrate sulfotransferases that catalyze the transfer of sulfate to the 6-position of GalNAc/Gal/GlcNAc residues during the biosynthesis of unique carbohydrate structures, such as 6-sulfo sLe^x. As discussed, the dysregulation of carbohydrate sulfation can lead to serious disease states. Much work remains in defining the substrate specificities, catalytic mechanisms, and biological roles of these enzymes. Furthermore, the regulation of carbohydrate sulfotransferase expression remains to be elucidated. It has been proposed that the expression of GST-4α and β may be controlled by the Zn-dependent transcription factor Sp1 as there is a triplet repeat of Sp1 binding sites in their 5′ UTR (*68*). Localization within the Golgi stacks also dictates a level of regulation, as only certain substrates are available within specific cisternae. Antibodies raised toward this protein family will facilitate histological and immuno-localization studies. At the biochemical level, the development of productive expression systems of active enzyme will enable in vitro studies to define the substrate specificities and catalytic mechanisms of the sulfotransferases through the mutagenesis of key residues. A robust expression system may also lead to the solution of the crystal structures of GST family members. Gene knockouts will allow the study of the phenotypic consequences of the lack of sulfation enzyme in the cell line or animal under scrutiny, and to this end, several mouse GST knockouts already exist. The discovery of selective small molecule inhibitors may allow the creation of "chemical knock-outs" of enzyme activity in vivo to identify the physiological role of these proteins in wild-type animals, and these inhibitors may serve as leads toward novel therapeutics.

ACKNOWLEDGMENT

We thank Drs. Penelope Drake, Jennifer Kohler, and Spencer Williams, as well as Dawn Verdugo, Brian Cook, and Dr. Stefan Hemmerich for helpful comments. Research in the authors' laboratory was supported by a grant from the NIH (GM59907).

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BI020507H