Tyrosine sulfation: a modulator of extracellular protein–protein interactions

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Tyrosine sulfation is a post-translational modification of many secreted and membrane-bound proteins. Its biological roles have been unclear. Recent work has implicated tyrosine sulfate as a determinant of protein–protein interactions involved in leukocyte adhesion, hemostasis and chemokine signaling.

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The study of living systems has been transformed by an explosion of genetic information. It is clear, however, that the structure of a mature protein is not dependent solely upon its gene. Post-translational modifications, such as glycosylation, proteolysis, phosphorylation, acylation, adenylation, farnesylation, ubiquitination, and sulfation, abound in living systems. These modifications affect a wide range of protein behaviors and characteristics, including enzyme activity, protein lifespan and protein–protein interactions.

Protein tyrosine sulfation is emerging as a widespread post-translational modification in multicellular eukaryotes. To our knowledge, protein tyrosine sulfation was first observed over four decades ago, in a peptide derived from fibrinogen [1]. In the mid-1980s, a series of tyrosine-sulfated proteins was discovered, which includes diverse molecules such as α-choriogonadotrophin and heparin cofactor II (a list of human proteins known to be tyrosine sulfated is shown in Table 1) [2]. Many of these tyrosine-sulfated proteins participate in protein–protein interactions that seem to be driven, at least in part, by recognition of the sulfate group itself. In the past few years, tyrosine sulfation has been identified as a key modulator of protein–protein interactions that mediate inflammatory leukocyte adhesion. The recent discovery of tyrosine-sulfated chemokine receptors suggests an even broader role in the inflammatory response.

Biological roles of tyrosine sulfation in the vasculature

At sites of inflammation, leukocytes roll on, adhere to, and transmigrate between the endothelial cells lining blood vessel walls (Figure 1a). Rolling is the requisite initial adhesion event in leukocyte recruitment to sites of inflammation. It is mediated in some sites by the interaction of P-selectin on activated endothelial cells with the P-selectin glycoprotein ligand (PSGL)-1 on cognate leukocytes [3]. PSGL-1 was identified at the molecular level in 1993, using expression cloning [4]. Glycosylation of PSGL-1 was known to be a major determinant of P-selectin binding, but was not sufficient to explain the binding avidities observed *in vivo*.

Further investigations revealed that tyrosine sulfation near the amino terminus of PSGL-1 plays an essential role in P-selectin binding (shown schematically in Figure 1b). Three papers published within one month of each other in 1995 provided the first evidence of this phenomenon [5–7]. Progressive deletions of the PSGL-1 amino terminus by Pouyani and Seed [5] revealed that a 20-residue peptide was required for high-affinity binding. Furthermore, mutagenesis within this sequence showed that the three tyrosine residues carried sulfate and were required for binding to P-selectin. Similar results were obtained by Sako *et al.* [6]. In a third report, Wilkins *et al.* [7] found that treating PSGL-1 with bacterial arylsulfatase reduced P-selectin binding activity. More recent work determined that proteolytic cleavage of the amino-terminal ten residues from mature PSGL-1 eliminated P-selectin binding [8]. Notably, this information was used to design a simple glycosulfopeptide that binds P-selectin with high affinity and inhibits its interaction with neutrophils [9].

Tyrosine sulfation is also involved in hemostasis. When blood vessels are injured, the subendothelial matrix is exposed to blood flow. The body responds by forming a thrombus, or platelet aggregate, at the site of injury. The complete mechanism of platelet attachment is an area of active research, but von Willebrand factor (vWF) and the platelet membrane protein GP Ibα are known to be involved in the early steps of adhesion [10,11]. vWF is a plasma protein that bridges subendothelial collagen and platelet GP Ibα, thereby mediating the initial interactions between platelets and the subendothelium. Platelet binding to vWF is partially dependent upon the sulfation of three tyrosine residues within GP Ibα [12]. Proteolytic removal of the tyrosine-sulfated segment reduced binding potency by an order of magnitude [13,14].

Tyrosine sulfation of chemokine receptors

Last year, the importance of tyrosine sulfation suddenly increased once again, with the first report of a tyrosine-sulfated chemokine receptor. Chemokines are small, secreted proteins that are involved in leukocyte trafficking, angiogenesis, angiostasis, battling viral infections, and the host immune response to cancer [15]. These proteins exert their

Table 1

Human proteins known to be tyrosine sulfated.

Protein	Sulfation site(s), if known	Reference
Amyloid precursor		[36]
α-2-Antiplasmin	PPMEEDYPQFGSP	[37]
CCR5	H_2N-MDYQVSSPIYDINYYTSEPCQ	[16]
Cholecystokinin	RISDRDYMGWMDF	[38]
α-Choriogonadotropin	CHCSTCYYHKS-COOH	[39]
Coagulation factor V		[40]
Coagulation factor VIII	NEEAEDYDDDLTD	[41]
	DKNTGDYYEDSYEDS	
	DQEEIDYDDTISV	
	KEDFDIYDEDEN	
Complement C4	MEANEDYEDYEYDELPAK	[42]
Dermatan sulfateproteogylcan		[43]
α-Fetoprotein		[44]
Fibrin		[45]
Fibronectin		[44]
Gastrin	EEEEEAYGWMDF	[46]
Glycoprotein Ib-α	EGDTDLYDYYPEEDTE	[12]
Heparin cofactor II	GEEDDDYLDLEKIFSEDDDYIDIVDS	[47]
PSGL-1	H_2N-QATEYEYLDYDFLPET	[5]
Procollagen type II		[48]
S-protein		[49]

effects through G-protein-coupled receptors on target cells. Farzan *et al.* [16] showed that the chemokine receptors CCR5 and CXCR4 are tyrosine sulfated. CCR5 carries four tyrosine residues in the amino-terminal region, all of which show some degree of sulfation in cell culture. Incubation of CCR5-expressing Cf2Th cells with sodium chlorate, a global inhibitor of sulfation, decreased binding of MIP-1α and MIP-1β—two natural chemokine ligands for CCR5 [16]. Sulfotyrosine 14 of CCR5 seems to play a particularly important role in binding to the chemokines MIP-1α and RANTES (M. Farzan, personal communication).

In addition to binding native chemokines, CCR5 also serves as a co-receptor for HIV gp120, working together with CD4 to mediate attachment of the virus and its subsequent invasion [17,18]. Mutation of the four sulfotyrosine residues in CCR5 to phenylalanine inhibits HIV infection by 50–75% in cultured cells, depending on the HIV isolate tested [16]. This information suggests that inhibitors of tyrosine sulfation might serve as anti-HIV therapeutics, as long as disruption of the modification is not globally toxic. Alternatively, peptides that mimic the tyrosine-sulfated sequence of CCR5 might compete for binding to HIV gp120 and block viral adhesion.

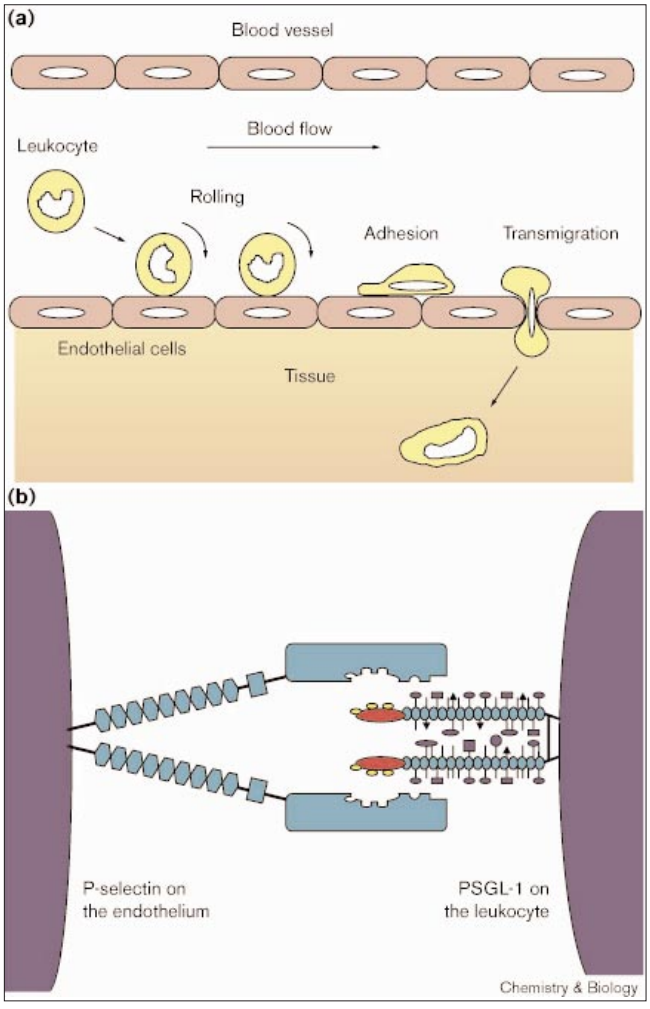
Prediction of tyrosine sulfation

The prevalence of tyrosine sulfation across the proteome is still unknown. The enzymes that generate the modification, tyrosyl protein sulfotransferases (TPSTs, described below), reside in the Golgi compartments and therefore have access to secreted and membrane-bound proteins. One might hope to find a consensus sequence for tyrosine sulfation amongst these proteins that would be of predictive value. Examination of the local sequences at known sites of tyrosine sulfation reveals a preponderance of acidic residues, but no defined consensus is evident (Table 1). Still, the common features of tyrosine sulfation sites have been identified in numerous chemokine receptors, as well as other seven-transmembrane segment (7TMS) receptors. The sequences listed in Table 2 have been shown to acquire tyrosine sulfate when expressed as fusions with a carrier protein. These modifications, if they exist on the native 7TMS receptors, may contribute to ligand binding affinity and/or specificity.

Tyrosyl protein sulfotransferases

A major advance in the field came with the molecular cloning of two tyrosyl protein sulfotransferases, TPST-1 and TPST-2, from human and mouse in 1998 [19–21]. Both are membrane-bound, N-glycosylated Golgi enzymes with a lumenal catalytic domain, a single-span transmembrane domain, and a short cytosolic tail. The human TPSTs share 67% sequence identity. *Caenorhabditis elegans* was found to have a related protein, called TPST-A, that is 54% identical to human TPST-1 [20]. A second *C. elegans* gene, whose function is unknown, has 39% identity and 62% similarity to TPST-A. Northern analysis indicates that both human TPSTs are expressed in many tissues. It has not been determined, however, whether the two TPSTs are co-expressed in the same cells. It is possible

Figure 1



Tyrosine sulfation plays an important role in the immune response. **(a)** Leukocytes roll upon, adhere to and transmigrate between endothelial cells at sites of inflammation. P-selectin and its ligand, PSGL-1, are often required for this process. **(b)** PSGL-1 is a mucin-like glycoprotein that appears to be an extended rod shape *in vivo* [50]. The extreme amino terminus of PSGL-1 carries three tyrosine sulfation sites, shown in yellow. These sulfate esters, and specific glycans on PSGL-1, are key binding determinants for P-selectin. See Table 1 for the sequence of the sulfation site.

Table 2

Some 7TMS receptors with possible sulfation sites [16].

Receptor	Presumed tyrosine sulfation site
CCR2b	VTTFFDYDYGAPCHK
CCR3	TFGTTSYYDDVGLLCE
CCR8	H_2N-MDYTLDLSVTTVTDYYYPDIFSSPC
d6	AENSSFYYYDYLDEVAF
CXCR3	ENFSSSYDYGENESD
CXCR4	H_2N-MEGISIYTSDNYTEEMGSGDYDSMKEP
str133	H_2N-MAEHDYHEDYGFSSFN
dez	H_2N-MRMEDEDYNTSISYGDEYPDYLDSIVV
gpr1	TLFEEFENYSYDLDYYSLESDL
gpr15	EETSVYLDYYYATSPNS
apj	GGDFDNYYGADNQSECEYTDW
c5aR	H_2N-MNSFNYTTPDYGHYDD

that additional TPSTs have not yet been discovered. The existence of more than one TPST might explain the diversity of sequences that are tyrosine sulfated; each enzyme might have a different substrate specificity and act upon a different subset of target proteins.

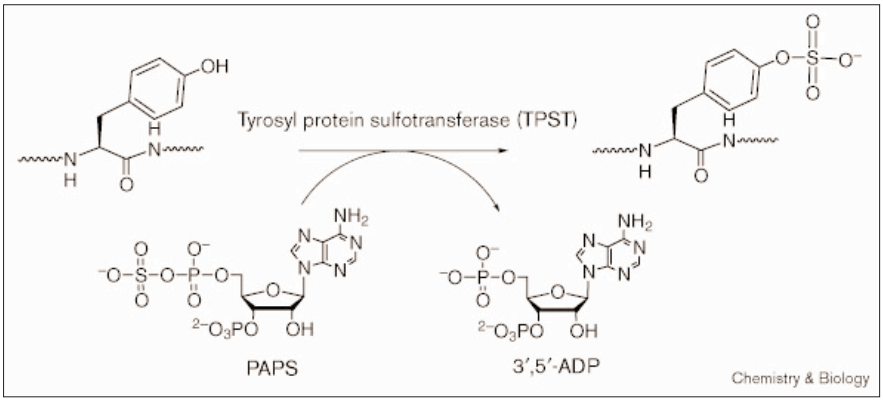
Like all sulfotransferases, the TPSTs use 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as a sulfate donor (Figure 2). Mammals, yeast and *Escherichia coli* can all produce PAPS through a similar pathway ([22] and references therein). ATP and inorganic sulfate are first converted to adenosine 5′-phosphosulfate (APS) by the action of ATP sulfurylase. APS kinase then phosphorylates APS to produce PAPS. In mammals, the sulfurylase activity and the kinase activity both reside within the same polypeptide chain [23]. Finally, PAPS is imported into the Golgi compartments by a specific transporter [24,25]. The existence of PAPS in an organism or cell does not mandate the presence of tyrosine sulfation. To our knowledge, there are no instances of tyrosine sulfation in yeast or prokaryotes.

Structural aspects of sulfotransferases

Although no structural data exists for the TPSTs, insights can be drawn from sequence comparisons with other sulfotransferases of known structure. Two cytosolic sulfotransferases have been crystallized: estrogen sulfotransferase (EST) [26] and the catecholamine sulfotransferase SULT1A3 [27,28]. The structure of the sulfotransferase domain of heparan sulfate *N*-deacetylase/*N*-sulfotransferase 1, a Golgi-localized enzyme, has also been reported [29]. These three structures all revealed a PAPS binding motif—the 'PSB loop'—which has the sequence K(S/T)GTT. This motif is found in a wide range of sulfotransferases [30], and it is also found in the TPSTs. In a co-crystal structure with mouse EST, 3′,5′-diphosphoadenosine (3′5′-ADP) interacts with the PSB loop through hydrogen bonding with amide protons of the peptide backbone and sidechain atoms from lysine and threonine residues [26]. The TPSTs might have similar interactions; future mutagenesis and crystallographic studies will be required to address this.

Structural and mechanistic studies of cytosolic and carbohydrate sulfotransferases suggest that these enzymes share some features with kinases. The fold of EST most resembles that of the uridylate, adenylate and guanylate kinases [26]. Co-crystal structures of EST bound to vanadate indicate that the active site is designed for an in-line attack on sulfate by a hydroxyl group of the substrate estradiol [31].

Figure 2



The reaction catalyzed by TPSTs.

The catalytic domain of heparan sulfate reveals a similar design [29]. The reactions catalyzed by kinases are also thought to proceed via an in-line direct transfer mechanism [32]. The relationship of TPSTs to kinases with respect to structure and mechanism should be addressed in crystallographic and mechanistic studies.

Regulation of tyrosine sulfation

How dynamic is tyrosine sulfation? Many signaling pathways are known to depend upon the phosphorylation state of various tyrosine residues. Living systems produce both kinases and phosphatases to adjust phosphorylation rapidly in response to signals. So far as we know, there is no comparable dynamic regulation of tyrosine sulfation. The TPSTs appear to be constitutively active, and the location of tyrosine sulfated proteins — secreted or exposed on the extracellular face of the plasma membrane — might preclude the rapid removal of sulfate from a particular target protein. Both prokaryotic and eukaryotic arylsulfatases exist, but we are not aware of any whose primary physiological role is the removal of sulfate from tyrosine. The human arylsulfatases A and B are, however, known to accept a range of artificial sulfated substrates that carry a phenolic ring [33]. These sulfatases reside in the lysosome and might participate in degradation of a broad spectrum of tyrosine-sulfated proteins [34]. In contrast, arylsulfatase E is found in the Golgi compartment [35]. A possible regulatory role for this enzyme has not yet been addressed.

Future directions

The functional significance of tyrosine sulfation, as revealed by studies of PSGL-1 and chemokine receptors, underscores the importance of the many still-unanswered questions. One of the most fundamental of these questions is: which proteins normally carry tyrosine sulfate *in vivo*? The identification of the full complement of tyrosine-sulfated proteins would allow a better understanding of the roles tyrosine sulfate plays in living systems. At present there is no simple way to accomplish this. We suspect that generating an anti-tyrosine sulfate antibody using conventional methods would be difficult because tyrosine sulfate is found on numerous secreted and membrane-bound proteins. The purpose of multiple TPSTs is also unclear. This situation could benefit greatly from the tools of chemical biology. For example, cell-permeable small-molecule inhibitors that are specific for each TPST could be used to deconvolute their substrates. Coupling TPST inhibition with functional assays of protein–protein binding could uncover hitherto unknown roles for tyrosine sulfation.

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