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Tyrosine Sulfation

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The O-sulfation of tyrosine residues of membrane and secretory proteins that transit through the secretory pathway of eukaryotic cells is a ubiquitous posttranslational modification conserved in all multicellular organisms. Tyrosine sulfation is catalyzed by tyrosylprotein sulfotransferase (TPST) isoenzymes, which are integral membrane proteins of the trans-Golgi network. Tyrosine sulfation has been shown to be important for protein–protein interactions occurring in diverse biological processes, ranging from the receptor binding of regulatory peptides to the interaction of viral envelope proteins with the cell surface.

Tyrosine-Sulfated Proteins

Occurrence

Sulfation is one of the most abundant posttranslational modifications of tyrosine, since up to 1% of the tyrosine residues of total protein in an organism can be sulfated. Tyrosine is the only amino acid residue in proteins known to undergo sulfation. Following the first description of a sulfated-tyrosine residue in a peptide derived from fibrinogen by Bettelheim in 1954, it has been known since 1982 that tyrosine-sulfated proteins occur in all animals, from lower invertebrates up to humans. Tyrosine-sulfated proteins also exist in the plant kingdom, for example, in the green alga volvox, one of the earliest truly multicellular organisms, or in higher plants such as rice. While occurring throughout metazoan evolution, tyrosine-sulfated proteins appear to be absent in unicellular eukaryotes and prokaryotes, implicating this post translational modification in some aspect of multicellularity.

In a given animal, tyrosine-sulfated proteins have been observed in all tissues examined. Each tissue appears to contain a characteristic set of tyrosine-sulfated proteins, suggesting that proteins with tissue-specific expression are major targets for tyrosine sulfation. In cell culture, tyrosine sulfation of proteins has been detected in all primary cultures and cell lines investigated, including various secretory cells, epithelial cells, fibroblasts, neuronal cells, and cells of the immune system.

Tyrosine-sulfated proteins can be identified by various methods, including labeling using radioactive sulfate followed by tyrosine sulfate analysis of a given protein. In line with the intracellular localization of tyrosylprotein sulfotransferase (TPST) in the trans-Golgi network, all known tyrosine-sulfated proteins are either secretory or plasma membrane proteins. Reviews with comprehensive lists of tyrosine-sulfated proteins have been published, and several of these proteins have been shown to play important biological roles.

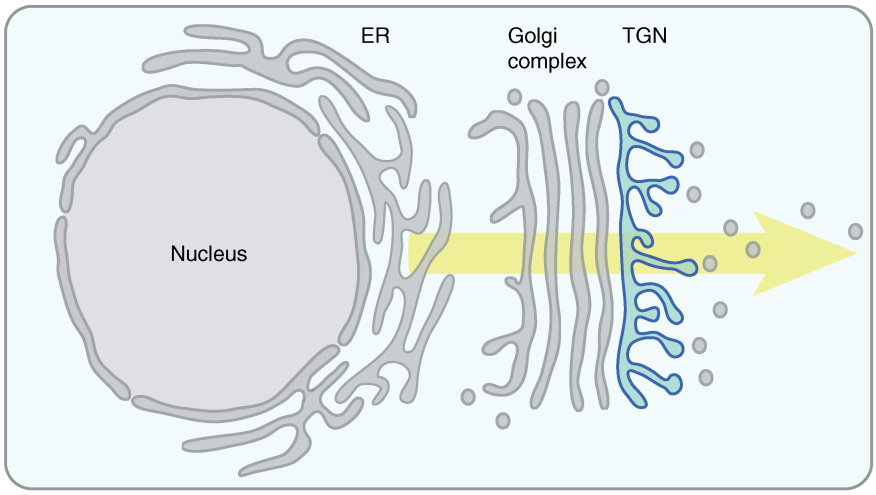
Structural Determinants of Tyrosine Sulfation

The recognition, by TPST, of the tyrosine residue to be sulfated in a secretory protein or the extracellular domain of a membrane protein requires the presence of certain structural features. These have been deduced from the comparison of identified tyrosine sulfation sites and the *in vitro* tyrosine sulfation of synthetic peptides. Although no strict consensus sequence for tyrosine sulfation exists, all sequences exhibit the presence of acidic amino acid residues in the vicinity, i.e., positions -5 (N-terminal) to $+5$ (C-terminal), of the sulfated tyrosine residue. A particularly critical position appears to be amino-terminal (-1) to the tyrosine. Turn-inducing amino acids (P, G) are also frequently present. The few examples of tyrosine sulfation sites lacking proline and glycine are located near the N- or C-terminus of the protein and/or contain several of the three other amino acid residues (D, S, N) with significant turn-conformational potential. Finally, another characteristic feature common to most identified tyrosine sulfation sites is the absence of cysteine residues or potential N-glycosylation sites (NXS or NXT) in the vicinity (positions -7 to $+7$). In either case, the presence of a disulfide-bridge or N-linked oligosaccharides is likely to prevent sulfation of nearby tyrosine residues due to steric hindrance.

Tyrosylprotein Sulfotransferase (EC 2.8.2.20)

The Tyrosine Sulfation Reaction

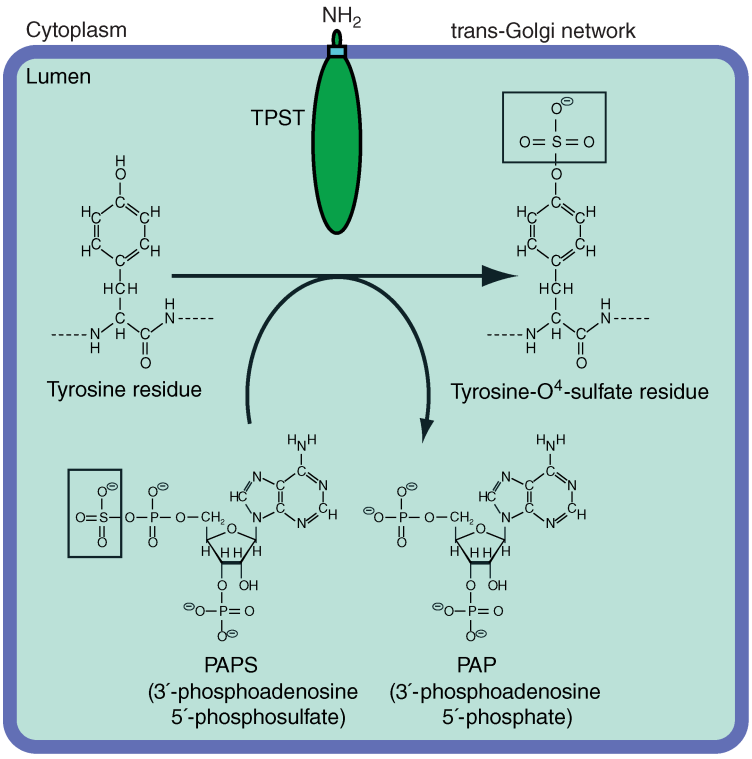
The sulfate transfer reaction to tyrosine residues is catalyzed by TPST, first described in 1983, and used



A

Tyrosine sulfation–dependent extracellular protein–protein interactions
Secretory protein and plasma membrane protein
Secretory protein and secretory protein
Plasma membrane protein and plasma membrane protein

C



B

F0005 **FIGURE 1** (A) Tyrosine sulfation occurs in the trans-Golgi network (TGN). (B) TPST, a type II transmembrane protein, catalyzes the transfer of the sulfate group (boxes) from the co-substrate PAPS to tyrosine residues of secretory proteins and ectodomains of membrane proteins passing through the lumen of the trans-Golgi network. (C) The three principal types of tyrosine sulfation-dependent protein–protein interactions.

as a sulfate donor, the cosubstrate 3′-phosphoadenosine 5′-phosphosulfate (PAPS) (Figure 1B). The transfer is thought to occur in an ordered reaction mechanism that involves the following sequential steps: (1) cosubstrate PAPS binding; (2) substrate binding; (3) sulfate transfer; (4) tyrosine-sulfated product release and, (5) PAP release. TPST activity can be detected by incubation of appropriate membrane preparations with [^{35}S]PAPS, using either endogenous or exogenous protein substrate. Biologically, tyrosine sulfation appears to be an irreversible event *in vivo* due to the lack of a sulfatase capable of catalyzing the desulfation of tyrosine-sulfated proteins under physiological conditions.

S0030 Properties of TPST

P0035 TPST is an integral membrane protein residing in the trans-Golgi network (Figure 1A). The TPST protein can be identified in membrane preparations by MSC (modification after substrate *c*ross-linking) labeling, which is based on the cross-linking of a substrate peptide to TPST followed by intramolecular [^{35}S]sulfate transfer from the cosubstrate PAPS. Various nonionic detergents can be used to solubilize TPST from membranes.

P0040 TPST purified from bovine adrenal medulla is a 50–54 kDa sialoglycoprotein with an apparent S-value of 6. Its pH optimum is between 6.0 and 6.5, which is in line with the slightly acidic pH of the trans-Golgi network. The catalytic activity of the enzyme, towards endogenously as well as exogenously added proteins, is stimulated by divalent cations (Mg^{2+}, Mn^{2+}), and fluoride ions are required for maximal activity. The K_m for the cosubstrate PAPS is 1.4 μM. Like most sulfotransferases, TPST is inhibited by PAP. *In vitro*, the activity of the enzyme is also inhibited by certain lipids such as sphingosine, but the physiological relevance of this observation is unknown. Synthetic inhibitors of TPST (with IC_{50} values of 30–40 μM) have been generated by a combinatorial target-guided ligand assembly technique.

P0045 The K_m for most peptides (8–13 amino acid residues) with a single tyrosine sulfation site is in the range of Q1 10–100 μM. It is likely that similar values hold true for individual tyrosine sulfation sites in proteins. Interestingly, K_m values for synthetic substrates containing multiple tyrosine sulfation sites are considerably lower. Given that several proteins exist with multiple adjacent sulfation sites, e.g., cionin, heparin cofactor, and prepro-cholecystokinin, the increased affinity of TPST for such substrates might be of physiological significance, e.g., by promoting stoichiometric sulfation. A remarkable example of the sequential sulfation of four tyrosine residues within a short stretch of amino acids is the N-terminal domain of the CC-chemokine receptor 5 (CCR5), which is a coreceptor for HIV.

S0035 Molecular Cloning and Membrane Topology of TPST

P0050 Distinct TPST isoenzymes, anticipated from the differential substrate specificities of various TPST preparations, exist in the human and mouse genome. Each contains two TPSTs, TPST-1 (see Swiss-Prot Database, Accession No. 060507) and TPST-2 (see Swiss-Prot Database, Accession No. 060704), which were first characterized at the molecular level in 1998. The human *TPST-1* (7q11) and *TPST-2* (22q12.1) genes encode for 370- and 377-amino acid proteins, respectively, that share an overall 65% identity. The murine *TPST* genes are located on chromosome 5, and the corresponding proteins show ~95% identity to their human counterpart. Both TPST isoenzymes are predicted to have a type II membrane topology, with a very short NH_2-terminal cytoplasmic domain (8 residues) and the bulk of the polypeptide, which is responsible for the catalytic activity, being located in the Golgi lumen (see Figures 1A and 1B). Although overall, TPST isoenzymes display a low degree of amino acid identity to other cytosolic and Golgi-associated sulfotransferases, most of the residues involved in PAPS binding, as deduced from comparison with the crystal structure of estrogen sulfotransferase, are conserved. The luminal domain of either isoenzyme also contains two potential N-glycosylation sites, consistent with presence of N-linked glycans in the TPST protein.

P0055 In agreement with the occurrence of tyrosine sulfation in all metazoan species, cDNAs that predict proteins obviously related to mammalian TPSTs are found in various vertebrates and invertebrates, including fish (GenBank Accession No. BI846282), frog (GenBank Accession No. BG815875), chicken (GenBank Accession Nos. BU142429, BU217098), fly (GenBank Accession No. AY124548), and worm.

TPST-1 Versus TPST-2 S0040

P0060 TPST-1 and TPST-2 transcripts are found in all tissues examined, e.g., brain, heart, skeletal muscle, gut, kidney, liver, lung, and leukocytes. Although ubiquitously expressed, the tissue distribution of TPST-1 and TPST-2 are not identical, recombinant TPST-1 and TPST-2 show similar, but not identical, activities toward certain small peptide substrates, consistent with some functional redundancy as well as a certain degree of differential substrate specificity.

TPST-1- and TPST-2-Deficient Mice S0045

P0065 TPST-1- and TPST-2-deficient mice, generated by targeted disruption of the *Tpst-1* and *Tpst-2* genes, also point to functional redundancy between the two isoenzymes, as either line of knock-out mice is viable (K.L. Moore, personal communication). Although TPST-$1^{-/-}$ animals appear normal, their body weight is reduced about 5% and an increase in postimplantation fetal death is observed, suggesting that unidentified proteins involved in regulation of body weight and reproductive physiology require tyrosine sulfation for optimal function. TPST-2 deficient mice show a transient delay in growth during postnatal development. In addition, the males, but not females, appear to be infertile. Together, these observations are consistent with the idea that TPST-1 and TPST-2 have distinct, but partially overlapping, physiological roles.

Physiological Function and Medical Relevance

P0070 For most tyrosine-sulfated proteins, the physiological function of this posttranslational modification is presently unknown. With regard to the cases in which the biological role of tyrosine sulfation of a particular protein has been elucidated, the common denominator has emerged that tyrosine sulfation promotes extracellular protein–protein interactions. In line with the identification of numerous secretory and plasma membrane proteins that are tyrosine-sulfated, paradigmatic examples exist showing that tyrosine sulfation promotes the interaction between (1) a secretory and a plasma membrane protein, (2) two secretory proteins, or (3) two plasma membrane proteins (Figure 1C).

P0075 The regulatory peptide cholecystokinin is a classical example where tyrosine sulfation of a secretory protein dramatically promotes its interaction with a plasma membrane protein, i.e., its cell surface receptor. Thus, sulfated cholecystokinin is 260 times more potent than its unsulfated form. Of the several examples of tyrosine sulfation promoting the interaction between two secretory proteins, the case of the binding of the tyrosine-sulfated blood coagulation factor VIII to von-Willebrand-factor is particularly intriguing, as it also documents the medical relevance of this posttranslational modification. Humans with a mutation in the critical tyrosine residue of factor VIII that is sulfated and involved in its binding to von-Willebrand-factor are afflicted with hemophilia A.

P0080 An example of tyrosine sulfation promoting the interaction between two plasma membrane proteins is the important role of this posttranslational modification for the high-affinity binding of leukocyte-associated P-selectin glycoprotein ligand (PSGL)-1 to P-selectin on activated endothelial cells. This crucial interaction initiates adhesion of leukocytes to the vascular wall during inflammation. Tyrosine sulfation also occurs in seven-transmembrane-segment chemokine receptors, e.g., CCR5. Under physiological conditions, these plasma membrane proteins play a central role in chemokine signalling pathway through G proteins. Remarkably, human and simian immunodeficiency viruses use CCR5 as a co-receptor, together with CD4, to mediate their attachment to the host cell membrane. Specifically, sulfation of tyrosine residues in the CCR5 N-terminal domain has been shown to be critical for the interaction of this protein with HIV envelope glycoprotein gp120, leading to HIV infection. Thus, the design of tyrosine- sulfated peptide competitors – mimicking HIV gp120-binding sites – could turn out to be the basis for new therapeutic compounds that will block HIV cellular entry. These examples highlight the medical relevance of protein tyrosine sulfation.

Glossary

G0055

G0085 **PAPS** Sulfate donor in the sulfate transfer reaction. PAPS has been known to be the activated form of sulfate and acts as cosubstrate for the sulfation of a wide variety of substances, including proteins.

G0090 **trans-Golgi network** The last station of the Golgi complex. This site is a major branching point of vesicular transport and the origin of two principal pathways of protein secretion: the regulated and constitutive pathways. Q2

Further Reading

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Biography

Dr. Denis Corbeil is a group leader at the University of Dresden. His research interests are in the cell biology of stem cells, with a focus on prominin/CD133. He holds a Ph.D. degree from the University of Montreal and received postdoctoral training in the laboratory of W.B. Huttner, where he participated in the molecular cloning of TPST.

Dr. Wieland B. Huttner is a professor of neurobiology and Director at MPI-CBG in Dresden. His group made seminal contributions on protein tyrosine sulfation, including the identification, characterization, purification, and cloning of TPST. He holds an M.D. degree from the University of Hamburg, received postdoctoral training with Nobel Laureate Paul Greengard at Yale University, and has been pursuing research on neurosecretory vesicle biogenesis and neurogenesis in the mammalian central nervous system.

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