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Differential Enzymatic Characteristics and Tissue-Specific Expression of Human TPST-1 and TPST-2

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Protein tyrosine sulfation is emerging as a widespread post-translational modification in multicellular eukaryotes. The responsible enzyme, named tyrosylprotein sulfotransferase (TPST), catalyzes the sulfate transfer from 3′-phosphoadenosine 5′-phosphosulfate to tyrosine residues of proteins. Two distinct TPSTs, designated TPST-1 and TPST-2, had previously been identified. In the present study, we cloned human TPST-1 and TPST-2 expressed and characterized the recombinant enzymes using peptide substrates. These enzymes displayed distinct acidic pH optima and stimulatory effects of Mn^{2+}. Additionally, the activity of TPST-2, but not TPST-1, was stimulated in the presence of Mg^{2+}. Compared with TPST-2, TPST-1 displayed considerably lower K_m and V_{max} for the majority of the tested peptide substrates, implying their differential substrate specificity. Quantitative real-time PCR analysis showed that although the two TPSTs were co-expressed in all 20 human tissues examined, the levels of expression of TPST-1 and TPST-2 varied significantly among different tissues. These latter findings may imply distinct physiological functions of TPST-1 and TPST-2.

Key words: enzyme, post translational modification, protein, sulfation, tyrosine-sulfated protein, tyrosylprotein sulfotransferase.

Post-translational protein modification by tyrosine sulfation, first discovered in bovine fibrinogen (*1*), is now known to have a widespread occurrence among proteins of multicellular eukaryotic organisms (*2*). It has been implicated in the alteration of biological activity of proteins (*3–5*), proteolytic processing of bioactive peptides (*6*), change in half-life of proteins in circulation (*7*), intracellular transport of secretory proteins (*8*), and modulation of extracellular protein-protein interaction including inflammatory leukocyte adhesion (*9–12*). The recent discovery of the tyrosine sulfation of chemokine receptors (*13*) suggests an even broader role in the inflammatory response.

Tyrosylprotein sulfotransferases (TPSTs) are the enzymes that catalyze the transfer of a sulfonate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS), the sulfate donor, to tyrosine residues of target proteins (*14*). TPST activity, following its first detection in rat pheochromocytoma (PC-12) cells (*14*), has been demonstrated to be present in numerous cell types and tissues (*15–22*). Comparison of the characteristics reported for TPSTs from various mammalian tissues revealed some common properties including acidic pH optimum, requirement for Mn^{2+} as a cofactor, and the utilization of PAPS as the sulfate donor (*15–22*). Biochemical evidence indicates that the enzyme is an integral membrane protein, located in the *trans*-Golgi network, with a luminally oriented active site (*15*, *23*).

Possibly due to the membrane-bound nature of TPST as well as itsinstability upon solubilization, it took several years for the successful purification of the protein (*24*), which required some 140,000-fold enrichment from the crude homogenate of bovine adrenal medulla as the source of the enzyme.

A major advance in the field came in 1998 with the molecular cloning of two TPSTs, designated TPST-1 and TPST-2, from human and mouse (*25–27*). Sequence analysis revealed that both TPST-1 and TPST-2 display Type II transmembrane topology with a short N-terminal cytoplasmic domain, a single 17-residue transmembrane domain, and a luminal catalytic domain. TPST-1 and TPST-2 are similar size (370–377 amino acid residues), and each has two putative *N*-glycosylation sites and six conserved luminal cysteine residues. Human TPST-1 and TPST-2 share 67% amino acid sequence identity. The human *TPST-1* and *TPST-2* genes have been localized to, respectively, 7q11.21 and 22q12.1 (*26–28*). Northern blot analysis indicated that both TPSTs are expressed in all human tissues examined (*25–27*). Interestingly, a recent study revealed that shear stress–dependent down-regulation of TPST-1 involves tyrosine kinase, while up-regulation of TPST-2 is mediated by a protein kinase C–dependent pathway (*29*). The existence of two TPSTs might explain the diversity of sequences that are tyrosine sulfated (*13*, *26*); and the two enzymes might have differential substrate specificity and act upon different subsets of target proteins. Recent gene-knockout studies revealed that mice deficient in TPST-1 had reduced body mass and increased postimplantation fetal death (*30*), whereas deficiency in TPST-2 lead to infertility in male mice (*31*). These findings indicated distinct physiological functions of TPST-1 and TPST-2. It is unclear, however, whether the distinct physiological involvement of the two TPSTs is due to their differential substrate specificity and/or tissue-specific expression.

Although considerable progress has been made in recent years in the study of TPST, some fundamental issues

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Table 1. **Oligonucleotide primers used for cloning of TPST-1 and TPST-2 cDNAs and for quantitative real-time PCR.**

I. For cloning of TPST-1 and TPST-2 cDNAs:		
TPST-1	Sense	5′-CCGCTCGAGATGGTTGGAAAGCTGAAGCAG-3′
	Antisense	5′-CGCGGATCCCGCTCCACTTGCTCAGTCTGTGG-3′
TPST-2	Sense	5′-CCGCTCGAGATGCGCCTGTCGGTGCGGAGG-3′
	Antisense	5′-CGCGGATCCCGCGAGCTTCCTAAGTGGGAGG-3′
II. For quantitative real-time PCR:		
β-actin	Sense	5′-CTGGAACGGTGAAGGTGACA-3′
	Antisense	5′-AAGGGACTTCCTGTAACAATGCA-3′
TPST-1	Sense	5′-ATGTGGTCACGGTCAAGTAAAGAG-3′
	Antisense	5′-GCTCCCCATGCTTAACGATAAT-3′
TPST-2	Sense	5′-TCAGCTCGGCTATGACCCTTA-3′
	Antisense	5′-GCTGGTGTTTTATAGTCCCCTTTC-3′

concerning their substrate specificity, tissue-specific expression, regulation, and physiological involvement remain poorly understood. Moreover, the majority of previous studies examining biochemical characteristics of the TPST enzymes in numerous cell types and tissues made no distinction between TPST-1 and TPST-2. As a first step toward clarifying these issues, we report here the expression of human TPST-1 and TPST-2 in 293T human embryonic kidney cells. The enzymatic properties of the expressed human TPST-1 and TPST-2 were analyzed using a panel of peptides encompassing tyrosine sulfation sites of known tyrosine-sulfated proteins (*3*, *9–13*, *32–36*). The pH optimum, divalent cation requirement, substrate specificity, as well as kinetic constants (K_m and V_{max}) of the two enzymes were determined. Moreover, by employing quantitative real-time PCR, the expression of TPST-1 and TPST-2 in different human tissues was investigated.

MATERIALS AND METHODS

Materials—*N*-2-Hydroxylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), 2-(*N*-morpholino) ethanesulfonic acid (Mes), tris (hydroxymethyl) aminomethane (Tris), dithiothreitol (DTT), and Dulbecco's modified Eagle's medium (DMEM) were products of Sigma Chemical Company. Z-Glu-Tyr was obtained from Peptide Institute. ReverTra Ace and KOD -plus- were obtained from TOYOBO. LA *Taq* DNA polymerase and SYBR® Premix Ex *Taq* (Perfect Real Time) were purchased from Takara Bio. Human Total RNA Master Panel II was from Clontech. pcDNA3, Lipofectamine Plus reagent were products of Invitrogen. pGEX-4T-1 prokaryotic GST fusion vector and Glutathione Sepharose 4B were from Amersham Biosciences. T4 DNA ligase and all restriction endonucleases were from New England Biolabs. Oligonucleotide primers were synthesized by NIPPON EGT and Hokkaido System Science. 293T SV40–transformed human embryonic kidney cells (ATCC CRL11268) was from American Type Culture Collection. Protease inhibitor cocktail tablets were obtained from Roche. Sep-Pak Plus C18 cartridges were from Waters. [^{35}S]PAPS (45 Ci/mmol) was synthesized from ATP and [^{35}S]sulfate using recombinant human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase as described previously (*37*). Cellulose thin-layer chromatography (TLC) plates were products of Merck. All other reagents were of the highest grades commercially available.

Molecular Cloning of Human TPST-1 and TPST-2—One microgram of human liver total RNA was reverse-transcribed using ReverTra Ace and oligo $(dT)_{20}$-primer. With the first-strand cDNA synthesized as the template, the open reading frames of human TPST-1 and TPST-2 were amplified by PCR using LA *Taq* DNA polymerase and oligonucleotides shown in Table 1 as primers. Amplifications conditions were 30 cycles of 30 s at 94°C, 30 s at 60°C, and 90 s at 72°C, followed by a final step of 10 min at 72°C. At the end of the PCR, the reaction mixtures were applied onto a 0.7% agarose gel, separated by electrophoresis, and visualized by ethidium bromide staining. The PCR product bands detected were excised from the gel, and the cDNAs therein were isolated by spin filtration. Purified TPST-1 and TPST-2 cDNAs were individually cloned into pcDNA3, an eukaryotic expression vector, yielding pcDNA3/TPST-1 and pcDNA3/TPST-2.

Transient Expression of TPST-1 and TPST-2 in 293T Human Embryonic Kidney Cells—293T cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 37°C and under 5% CO_2/95% air. 293T cells, grown in 100 mm dishes, were individually transfected with 8 μg of pcDNA3 vector only (as a control) or pcDNA3/TPST-1 or pcDNA3/TPST-2 using LipofectAMINE and Plus reagent-mediated procedure. Transfection was for 24 h at 37°C. Afterwards, the transfected cells were incubated at 37°C in DMEM containing 10% fetal bovine serum. At the end of a 48-h incubation, the cells in individual dishes were rinsed twice with phosphate-buffered saline, scrapped off, and homogenized in 1 ml of ice-cold 50 mM HEPES-NaOH (pH 7.0) containing 250 mM sucrose, 1 mM EDTA, protease inhibitor mixture, 1 mM PMSF and 1 mM DTT using a polytron type homogenizer. The homogenates thus prepared were subjected to centrifugation at 100,000 × g for 1 h. Pelletted membrane fractions were lysed in an ice-cold 20 mM HEPES-NaOH (pH 7.0) buffer containing 1% Triton X-100, 5% glycerol, protease inhibitor mixture, and 1 mM DTT. The lysates were subjected to a brief centrifugation and the supernatants collected were aliquoted and stored at –80°C prior to being used for the enzymatic characterization.

Preparation of Peptide Substrates for Enzymatic Assay—A number of peptides corresponding to regions encompassing tyrosine sulfation sites in P-selectin glycoprotein ligand-1 (PSGL-1), chemokine receptors (CCR2, CCR3, CCR5, CCR8), gastrointestinal hormone

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Table 2. **Amino acid sequences of the peptide substrates and their coding sense and antisense oligonucleotides used for cloning and expression.**

Name	Amino acid sequence*	Sense and antisense oligonucleotides
PSGL-1	ATE**Y**E**Y**LD**Y**DFL	5′-GATCCgccaccgaatatgagtacctagattatgatttcctgG-3′
		5′-AATTCcaggaaatcataatctaggtactcatattcggtggcG-3′
CCR5	PI**Y**DIN**YY**TSE	5′-GATCCccaatctatgacatcaattattatacatcggagtagG-3′
		5′-AATTCctactccgatgtataataattgatgtcatagattggG-3′
CCR2	TFFD**Y**D**Y**GAP	5′-GATCCaccttttttgattatgattacggtgctccctagG-3′
		5′-AATTCctagggagcaccgtaatcataatcaaaaaaggtG-3′
CCR3	GTTS**YY**DDVG	5′-GATCCggtaccacatcctactatgatgacgtgggctagG-3′
		5′-AATTCctagcccacgtcatcatagtaggatgtggtaccG-3′
CCR8	TVTD**YYY**PDI	5′-GATCCacagtgaccgactactactaccctgatatctagG-3′
		5′-AATTCctagatatcagggtagtagtagtcggtcactgtG-3′
CCK	SDRD**Y**MGWMD	5′-GATCCgaccgggactacatgggctggatggatttttagG-3′
		5′-AATTCctaaaaatccatccagcccatgtagtcccggtcG-3′
C4	ED**Y**ED**Y**E**Y**DE	5′-GATCCgaggactatgaggactatgagtacgatgagtagG-3′
		5′-AATTCctactcatcgtactcatagtcctcatagtcctcG-3′

*The sulfatable tyrosine residues are in bold-type.

[cholecystokinin (CCK)] and complement C4 (C4) were prepared using the pGEX-4T-1 prokaryotic expression system. Table 2 shows the amino acid sequences and the corresponding coding nucleotide sequences of the peptides prepared through bacterial expression. Briefly, complementary sense and antisense oligonucleotides coding for each peptide were hybridized and subcloned into the *Bam*HI/*Eco*RI site of pGEX-4T-1 prokaryotic expression vector and the vector construct was transformed into *E. coli* BL21 cells. Procedure for the induction of expression and subsequent affinity fractionation and purification of the peptide expressed was as previously described (*38*).

For the generation of cDNAs encoding mutated PSGL-1 peptides (Fig. 1), sense and antisense mutagenic oligonucleotides with codon(s) (TAT or TAC) encoding tyrosine changed to codon(s) (TTT or TTC) encoding phenylalanine were synthesized and used for the preparation of mutated PSGL-1 peptides based on the above-mentioned procedure.

Enzymatic Assay—The standard assay mixture (20 μl final volume) consisted of 50 mM MES-NaOH (pH 6.5 for TPST-1 and pH 6.0 for TPST-2), 50 mM NaF, 20 mM (for TPST-1) or 25 mM (for TPST-2) $MnCl_2$, 0.2% Triton X-100, 20 μM peptide substrate and 0.5 μM [^{35}S]PAPS (45 Ci/mmol). The reaction was started by the addition of 16 μg of the enzyme preparation, allowed to proceed for 30°C for 15 min, and terminated by heating at 100°C for 3 min. The precipitates formed were cleared by centrifugation, and the supernatants collected were analyzed for [^{35}S]sulfated peptide using a previously developed thin-layer chromatography (TLC) procedure, with *n*-butanol/pyridine/formic acid/water (5:4:1:3; by volume) as the solvent system. Afterwards, the plates were air-dried and analyzed using a Fluoro Image Analyzer (FLA-3000G).

Quantitative Real-Time PCR Analysis—For use as templates in real-time PCR, first-strand cDNAs were reverse-transcribed from total RNAs isolated from twenty human tissues (Human Total RNA Master Panel II; BD Clontech) using random hexamers according to the manufacturer's instruction (TOYOBO). Oligonucleotide primers (Table 1) for quantitative real-time PCR were designed using Primer Express software (Applied Biosystems). PCR amplification was performed using the ABI PRISM 7000® Sequence Detection System (Applied Biosystems). Reaction conditions were 10 s at 95°C for initial denaturation, followed by 50 cycles of 5 s at 95°C, 31 s at 60°C. The expression values obtained were normalized against those for the control human β-actin (*39*).

PSGL-1 wt	A T E **Y** E **Y** L D **Y** D F L
PSGL-1 1Y	A T E **Y** E **F** L D **F** D F L
PSGL-1 2Y	A T E **F** E **Y** L D **F** D F L
PSGL-1 3Y	A T E **F** E **F** L D **Y** D F L
PSGL-1 1, 2Y	A T E **Y** E **Y** L D **F** D F L
PSGL-1 1, 3Y	A T E **Y** E **F** L D **Y** D F L
PSGL-1 2, 3Y	A T E **F** E **Y** L D **Y** D F L
PSGL-1 F	A T E **F** E **F** L D **F** D F L

Fig. 1. **Amino acid sequences of wild-type (wt) and mutated PSGL-1 N-terminal peptides.** The three tyrosine residues and mutated phenylalanine residues are in bold-type.

RESULTS AND DISCUSSION

A good number of studies using lysates of cultured cells or homogenates of tissues or organs have been carried out to characterize the enzymatic properties of TPST (*14–22*). These previous studies, however, made no distinction between the two forms of TPSTs, TPST-1 and TPST-2, identified in mammalian animals such as human and mouse (*25–27*). The problem was further complicated by the possibility of differential expression of TPST-1 and TPST-2 in different cell types, tissues, and organs. The current study aimed to clarify these issues by expressing individually TPST-1 and TPST-2 for unambiguous characterization of their enzymatic properties and analyzing the level of expression of the two TPSTs.

Expression of Recombinant Human TPST-1 and TPST-2 in 293T Cells—pcDNA3 harboring the full-length cDNA encoding human TPST-1 or TPST-2 was transfected into 293T cells for the expression of the recombinant enzyme. Lysates of transfected cells, as well as untransfected cells and cells transfected with pcDNA3 vector alone, were assayed for TPST activity using the wild-type PSGL-1 peptide as substrate. As shown in Table 3, lysates of untransfected cells and cells transfected with pcDNA3 vector alone showed approximately the same level of TPST activity. This basal TPST activity was due to the

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Table 3. **Expression of recombinant human TPST-1 and TPST-2 in 293T cells.**

	Specific activity (pmol/min/mg)*	Relative activity (-fold)
None	0.288 ± 0.029	1.0
pcDNA3	0.325 ± 0.004	1.1
TPST-1-pcDNA3	3.129 ± 0.135	10.9
TPST-2-pcDNA3	3.195 ± 0.157	11.1

*Values shown are means ± SD derived from three determinations.

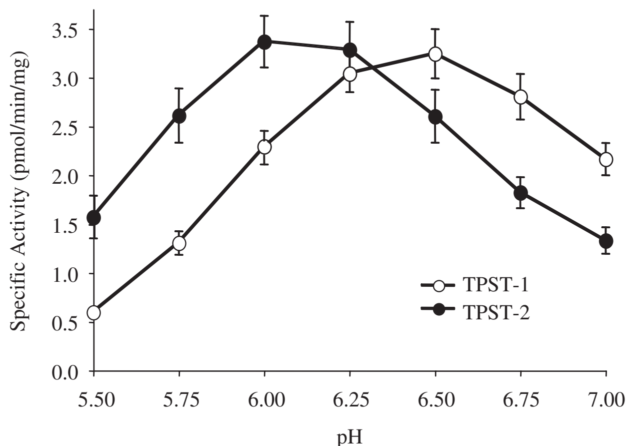

Fig. 2. **pH-dependence of the sulfating activity of human TPST-1 and TPST-2 with the wild-type PSGL-1 N-terminal peptide as substrate.** The enzymatic assays were carried out under standard conditions, as described in "MATERIALS AND METHODS," using different buffer systems. The data represent calculated mean values derived from three experiments. The open circles correspond to the activities detected using TPST-1, and the solid circles correspond to the activities detected using TPST-2.

endogenous TPST of 293T cells. In contrast, there was a 10-fold increase in TPST activity in lysates of 293T cells transfected with pcDNA3 harboring cDNA encoding TPST-1 or TPST-2, indicating clearly the production of functionally active recombinant TPST-1 and TPST-2.

Enzymatic Properties of Recombinant Human TPST-1 and TPST-2—Recombinant TPST-1 and TPST-2 expressed in 293T cells were analyzed with regard to their pH-dependency, divalent metal cation requirement, substrate specificity, and kinetic parameters.

Effects of pH and Divalent Metal Cations—We first examined the pH-dependency of the two recombinant enzymes using wild-type PSGL-1 peptide as substrate. As shown in Fig. 2, TPST-1 and TPST-2 displayed pH optima at, respectively, 6.5 and 6.0. Previous studies had revealed that TPST-1 and TPST-2 are Golgi enzymes with their catalytic domain located in the Golgi lumen, which has an acidic environment (*15*, *23*). The acidic pH optima determined for the two TPSTs, therefore, are compatible with the acidic environment in Golgi lumen (*40*). Previous studies using Golgi membrane lysates had revealed stimulatory effects of Mn^{2+} on the activity of TPST (*41*). We were interested in examining whether Mn^{2+} exerted differential stimulatory effects on the activity of TPST-1 and TPST-2. In a concentration-dependence experiment (Fig. 3A), it was found that TPST-1 was stimulated maximally (by approximately 17 times) in the presence of 20 mM

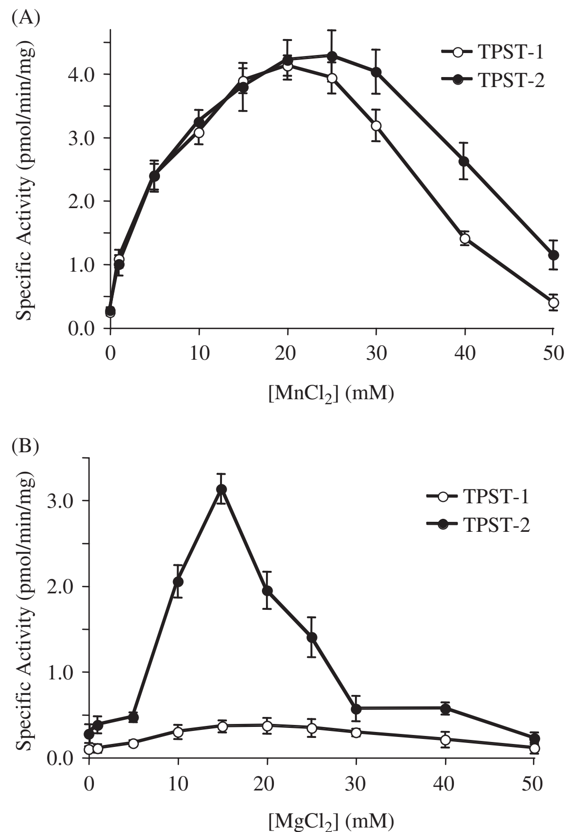

Fig. 3. **Manganese- and magnesium-dependence of the sulfating activity of human TPST-1 and TPST-2 with the wild-type PSGL-1 N-terminal peptide as substrate.** The enzymatic assays were carried out in the presence of different concentrations of $MnCl_2$ (A) or $MgCl_2$ (B) under standard conditions as described in "MATERIALS AND METHODS." The data represent calculated mean values derived from three experiments. The open circles correspond to the activities detected using TPST-1, and the solid circles correspond to the activities detected using TPST-2.

$MnCl_2$, whereas TPST-2 displayed optimal activity (16-fold of the activity that determined in the absence of $MnCl_2$) in the presence of 25 mM $MnCl_2$. When the effects of Mg^{2+} were similarly examined (Fig. 3B), it was found that the activity of TPST-1 was not much affected in the presence of $MgCl_2$ over a concentration range up to 50 mM. In contrast, the activity of TPST-2 was stimulated, with optimal stimulation (by approximately 11 times) observed in the presence of 15 mM $MgCl_2$. Interestingly, 20 mM $CaCl_2$ inhibited completely the sulfating activity of both TPSTs (data not shown). It is worthwhile pointing out that this is the first time that TPST-1 and TPST-2, individually expressed in 293T cells, were shown to be differentially stimulated by Mn^{2+} and Mg^{2+}. Previous studies employed mostly Golgi-enriched membrane fractions that contained both TPST-1 and TPST-2, and therefore were not able to reveal differential stimulatory effects of Mn^{2+} and Mg^{2+} on these two enzymes. Whether the stimulatory/inhibitory effects of these divalent metal cations are indeed physiologically relevant, however, remains to be clarified.

Substrate Specificity and Kinetic Parameters—Previous studies indicated that a key feature of the tyrosine sulfation sites in known tyrosine-sulfated proteins is the

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presence of acidic residues on both the N-terminal side and C-terminal sides of sulfatable tyrosine residue (*42*, *43*). To probe further the importance of neighboring acidic amino acid residues in tyrosine sulfation, we decided to use the PSGL-1 N-terminal peptide as a model. As shown in Fig. 4, wild-type PSGL-1 peptide contains three tyrosine residues and all three of them meet the criteria for the requirement of neighboring acidic amino acid residues. We were interested in finding out whether the three tyrosine residues of PSGL-1 peptide are equally or differentially sulfated by the two TPSTs (Fig. 4). A panel of mutated PSGL-1 peptides with one, two, or all three of the tyrosine residues being replaced by phenylalanine were prepared and used as substrates. As shown in Fig. 4, both TPST-1 and TPST-2 appeared to be most active toward wild-type peptides and the mutated peptides that retained the C-terminal tyrosine residue. In contrast, mutated peptides lacking the C-terminal tyrosine residue served as very poorly substrates for either TPST. These results indicated the specificity of the TPSTs for the C-terminal tyrosine residue in wild-type PSGL-1 peptide. Kinetic constants for the sulfation of wild-type and those mutated PSGL-1 peptides that contain the C-terminal tyrosine residue, as well as peptides encompassing tyrosine sulfation sites of chemokine receptors (CCR2, CCR3, CCR5, CCR8), cholecystokinin (CCK) and complement C4 (see Table 4), were determined. As shown in Table 4, compared with TPST-2, TPST-1 displayed lower K_m and lower V_{max} for the majority of the tested peptide substrates. It should be pointed out that, in general, both TPST-1 and TPST-2 exhibited relatively high K_m values for the peptides tested. Whether the two enzymes in fact have higher affinity for their parent proteins is an interesting issue for future investigation. Nevertheless, the data compiled in Table 4 do provide additional support for differential substrate specificity of TPST-1 and TPST-2.

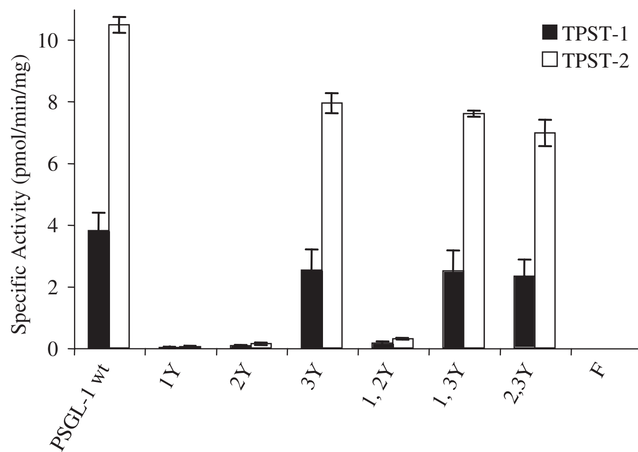

Fig. 4. **Substrate specificity of human TPST-1 and TPST-2 with the wild-type and mutated PSGL-1 N-terminal peptide as substrates.** The enzymatic assays were carried out under standard conditions as described in "MATERIALS AND METHODS," by using 40 μM peptide substrate. The data represent calculated mean values derived from three experiments. The solid bars correspond to the activities detected using TPST-1, and the open bars correspond to the activities detected using TPST-2.

Tissue-Specific Expression of Human TPST-1 and TPST-2—We were interested in the expression patterns of TPST-1, TPST-2 in different human tissues/organs. Quantitative real-time PCR was employed to investigate the tissue distribution of TPST-1 and TPST-2 transcripts in 20 human tissues (Fig. 5). Data obtained were normalized against those for the control human β-actin. Both TPST-1 and TPST-2 were expressed in all tissue samples examined, albeit at different levels. TPST-1 mRNA appeared to be expressed at higher levels in neuronal tissues such as brain cerebellum (12.8-fold higher compared with TPST-2 mRNA), fetal brain (4.9-fold), adult brain (2.8-fold), and spinal cord (2.9-fold), and in reproductive organs, such as testis (2.7-fold) and uterus (2.5-fold), and prostate (1.9-fold). TPST-2 was more strongly expressed in liver (2.3-fold compared with TPST-1 mRNA), salivary gland (2.1-fold), thymus, heart, and placenta (1.4-fold). Collectively, these data showed clearly the differential expression of TPST-1 and TPST-2 in different tissues. The differential expression of TPST-1 and TPST-2 may imply that the two enzymes act upon different subsets of proteins in different cell types, tissues, and organs.

To summarize, we have cloned and expressed human TPST-1 and TPST-2 and characterized the recombinant enzymes. The two TPSTs exhibited distinct enzymatic properties, including pH optimum, stimulation by Mn^{2+} or Mg^{2+}, and substrate specificity. Quantitative real-time PCR analysis showed that while the two TPSTs were both expressed in all 20 human tissues examined, they differed in the level of expression in different tissues. Further studies concerning the ontogeny, regulation, and

Table 4. **Kinetic constants of TPST-1 and TPST-2 with different peptides as substrates.**

Substrate	TPST-1			TPST-2		
	K_m (μM)	V_{max} (pmol/min/mg)	V_{max}/K_m	K_m(μM)	V_{max}(pmol/min/mg)	V_{max}/K_m
PSGL-1 wt	9.67	3.95	0.409	26.89	71.43	2.657
3Y	17.52	2.48	0.142	22.01	14.86	0.675
1, 3Y	19.75	2.41	0.122	79.95	14.01	0.175
2, 3Y	87.41	4.10	0.047	115.82	15.08	0.130
C4	6.83	6.31	0.923	11.23	58.48	5.206
CCR2	47.56	3.66	0.077	25.54	29.76	1.166
CCR3	1,418.65	0.486	0.0003	3,603.67	5.65	0.0016
CCR5	1,345.88	0.90	0.0007	4,137.83	1.47	0.0004
CCR8	1,384.88	4.04	0.0029	1,197.40	13.28	0.0011
CCK	825.69	1.83	0.0022	1,647.81	21.93	0.013
Z-Glu-Tyr	1,297.31	0.997	0.0008	1,222.92	2.29	0.0019

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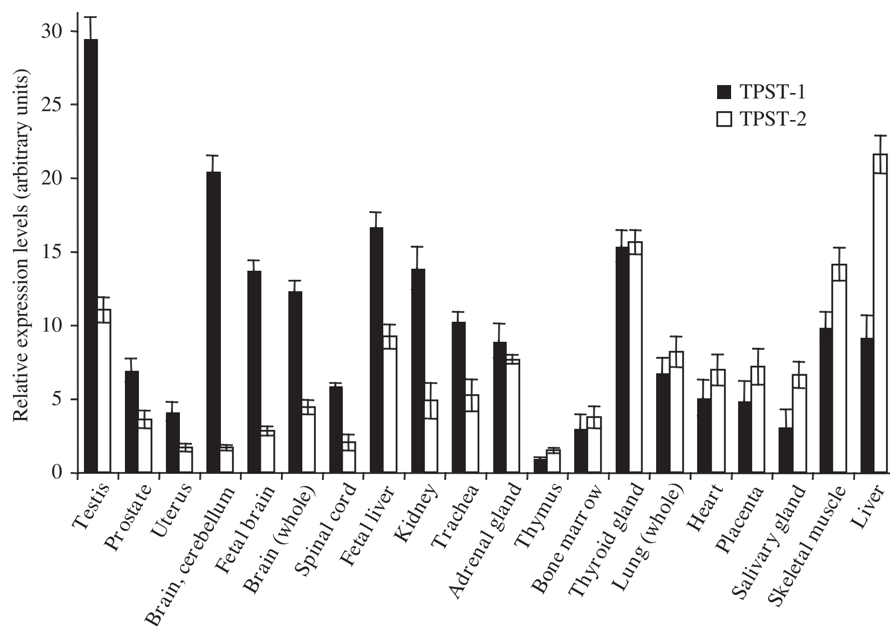

Fig. 5. **Comparison of the expression level of TPST-1 and TPST-2 in 20 human tissues.** Human TPST-1 and TPST-2 mRNA levels were quantified in arbitrary units, normalized against β-actin signal, as described in "MATERIALS AND METHODS." The data represent calculated mean values derived from three experiments. The solid bars correspond to the levels detected for TPST-1, and the open bars correspond to the levels detected for TPST-2.

physiological involvement of TPST-1 and TPST-2 are currently underway.

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