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CHARACTERIZATION OF A TYROSYLPROTEIN SULFOTRANSFERASE IN HUMAN LIVER

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Abstract—Sulfation of tyrosyl residue(s) has been found to be a post-translational modification that precedes the secretion of many biologically active proteins or peptides. In the present paper, we report on the characterization of human liver tyrosylprotein sulfotransferase (TPST), the enzyme responsible for sulfation of tyrosine in proteins. Using poly Glu,Ala,Tyr (6:3:1; EAY) as the model substrate, human liver TPST was recovered in the microsomal fraction after differential centrifugation. This enzyme displayed a pH optimum of 6.4 and was stimulated approximately 2.5-fold in the presence of 0.5% non-ionic detergents, such as Lubrol-PX and Triton X-100. The divalent cation Mn^{2+} was required for enzymatic activity and produced maximal activation at 30 mM, whereas other divalent cations, including Mg^{2+} and Co^{2+}, failed to enhance sulfoconjugation at this concentration. Using the optimized assay condition, the apparent K_m for EAY was found to be approximately 1.5 μM, with significant substrate inhibition at EAY concentrations above 2 μM. The 16 amino acid peptide of the C-terminus of C4 possessed an apparent K_m of approximately 2.1 μM. Using EAY as a substrate, TPST activity was measured in liver samples from ten organ donors to detect the variability of this enzyme among human subjects. The activity in the male group (1.065 ± 0.074 pmol/min/mg) was significantly ($P < 0.005$) higher than that of the female group (0.662 ± 0.158 pmol/min/mg), suggesting that TPST activity may be regulated, in part, by sex hormones.

Sulfation of the tyrosyl residue is now known to be a widespread post-translational modification of many secreted proteins and peptides, including the C4 component of complement [1], α_2-antiplasmin [2] and cholecystokinin (CCK†) [3]. For several sulfated peptides, sulfation has been shown to be required for optimal biological activity [4, 5], yet the functional consequence of this process for the majority of the sulfated proteins or peptides that have been identified remains unknown. The enzyme responsible for this modification, tyrosylprotein sulfotransferase (TPST), has been identified and characterized in various species and tissues including rat PC-12 cells, bovine adrenal medulla, rat brain and rat liver [6–9]. These studies demonstrated that TPST is a membrane-bound enzyme most likely associated with the Golgi apparatus. Using either the acidic polymer, poly Glu,Ala,Tyr (6:3:1; EAY), or Boc-cholecystokinin-8 as the substrate, these reports further demonstrated that TPSTs identified in the different tissues share certain biochemical properties including an acidic pH optima and the requirement for the presence of a detergent and divalent cations for optimal activity. However, evidence is also provided in these articles suggesting that differences exist between these enzymes including the pH optima and the requirement for specific divalent cations.

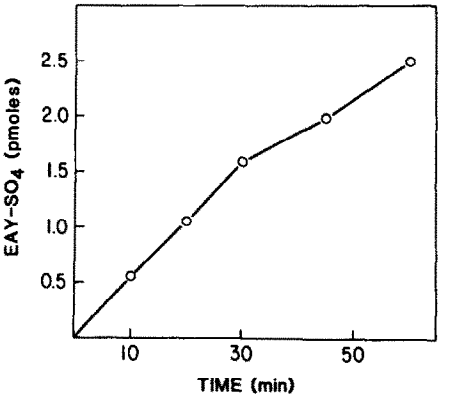

Fig. 1. Reaction of EAY sulfation as a function of time. Reactions were performed as described (Materials and Methods) with each assay containing 0.24 mg microsomal protein from autopsy liver. The reaction product was quantitated at the indicated time points. Each data point is the average of triplicate results.

In a previous study from our laboratory, it was shown that liver from several animals including humans contains a membrane-bound TPST which is capable of sulfating EAY [9]. The properties of the human TPST were not characterized further, although this would be of considerable interest since prior studies have demonstrated significant differences in the biochemical properties between the soluble sulfotransferases from human and animal tissues [10]. Therefore, it is necessary to determine

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† Abbreviations: CCK, cholecystokinin; Boc, *tert*-butoxycarbonyl; C_4, fourth component of complement; EAY, poly Glu,Ala,Tyr (6:3:1); MES, 2-[*N*-morpholino]ethanesulfonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; TFA, trifluoroacetic acid; TPST, tyrosylprotein sulfotransferase; and DDT, dithiothreitol.

whether similar differences exist for the membrane-bound sulfotransferases as well. In this study, we report the initial characterization of human liver TPST and demonstrate that this enzyme shares many biochemical properties with its counterpart from other species.

MATERIALS AND METHODS

Materials. The polymer, Glu,Ala,Tyr (6:3:1; average mol. wt 47.000), buffers, detergents, and unlabeled PAPS were purchased from the Sigma Chemical Co., St. Louis, MO. Glycerol, Na_2SO_4, NH_4HCO_3 and trichloroacetic acid were obtained from J. T. Baker, Phillipsburg, NJ, and ultrapure sucrose from Schwarz/Mann, ICN, Cleveland, OH. Bicinchoninic acid protein assay reagents were purchased from Pierce, Rockford, IL, and [^{35}S]PAPS (1.5 to 2.0 Ci/mmol) from New England Nuclear, Boston, MA. C4-P16‡, the 16 amino acid carboxy terminus of the C4 component of complement containing the tyrosyl residues previously shown to be sulfated *in vivo*, was synthesized and provided by Dr. G. Hortin, Department of Pediatrics, Washington University, St. Louis, MO. Sep Pak C_{18} cartridges were obtained from Waters Associates, Milford, MA. All other chemicals used were the purest available from commercial sources.

Tissue preparation. Human liver samples used in characterization of TPST were obtained at autopsy within 4 hr after death. Liver specimens were also obtained from organ donors with no apparent liver diseases and were stored at −80°. These specimens were provided by Dr. C. Falany, Department of Pharmacology, University of Rochester, Rochester, NY.

Microsomes of human liver were prepared by differential centrifugation. Briefly, each liver sample was weighed, minced, and mixed with 4 vol. of homogenization buffer consisting of 0.25 M sucrose, 10 mM Tris buffer, pH 7.0, and 10 mM EDTA. The mixture was initially homogenized with a Waring Blender at low speed for 6–8 bursts of 5 sec each. When preparing smaller specimens from organ donors, a Brinkmann PT 10/35 Polytron was used to initially homogenize the tissue at setting 4 for 10 sec. The following methods were used subsequently for each liver preparation. The tissue was homogenized further using a standard Teflon/glass motorized homogenizer at 2000 rpm. The homogenate was then filtered through three layers of gauze and the filtrate was centrifuged at 1000 *g* for 5 min. The resulting pellet was resuspended using the same volume of buffer as above and again centrifuged at 1000 *g* for 5 min. The combined supernatant solution was then centrifuged at 10,000 *g* for 10 min. The resulting pellet was rehomogenized and recentrifuged under the same conditions, and the combined supernatant solution was then centrifuged at 146,000 *g* for 60 min to obtain the microsomal pellet, which was resuspended in a buffer consisting of 20% glycerol, 10 mM MES buffer, pH 6.6, and 1 mM dithiothreitol (DTT). All the above procedures were performed at 4°. The pooled microsomal preparation was immediately stored at −80° until assayed for TPST activity. No significant loss of enzyme activity was found for at least 3 weeks under this storage condition or with the frozen intact liver samples also stored under these conditions.

Polymer sulfation assay. The assays were performed essentially as described by Rens-Domiano and Roth [9]. The assay mixture contained, in a final volume of 100 μL, 40 mM MES buffer, pH 6.4, 30 mM $MnCl_2$, 0.5% Lubrol-PX, 50 mM NaF, 1 mM 5′-AMP and 3′-AMP, 2 μM [^{35}S]PAPS, 2 μM poly Glu,Ala,Tyr (EAY), and an appropriate amount of microsomal protein in 30 μL of resuspension buffer. The reactions initiated by the addition of enzyme were incubated at 30° for 30 min or an appropriate period of time as indicated. The assays were terminated by transfer of 70-μL aliquots onto 2.5 × 2 cm squares of Whatman 3MM filter paper, which were immersed immediately into a washing solution containing 10% trichloroacetic acid and 10 mM Na_2SO_4. After adequate washing, the filter papers were then dried and the radioactivity was measured by liquid scintillation spectrometry. Reactions performed in the absence of EAY were used to determine the blank values.

Sulfation of C4-P16. Reaction conditions were similar to those described above for EAY. Reactions were terminated by addition of 400 μL of ice-cold 75 mM EDTA, pH 6.7, to the assay. To separate the sulfated C4-P16 from PAPS, 450-μL aliquots of the assay mixture were applied to Sep Pak C_{18} cartridges which were prewashed with 5 mL of 0.05% trifluoroacetic acid (TFA) in methanol followed by 5 ml of 0.05% TFA in water. PAPS was eluted from the columns with 20 mL of 0.05% TFA in water, and the sulfated products were eluted with 10 mL of 0.05% TFA in methanol. Reactions performed in the absence of C4-P16 represented the blanks for sulfation of endogenous substrates. The eluted [^{35}S]-sulfation products were quantitated by liquid scintillation spectrometry. Studies in our laboratory have shown that the recovery and separation of the PAPS and sulfated products from the C_{18}-columns were essentially complete using this method (Rens-Domiano S and Roth JA, unpublished observations).

RESULTS

The subcellular distribution of TPST activity from autopsied human liver, using differential centrifugation, is indicated in Table 1. In the experiment illustrated, approximately 55% of the activity was recovered in the microsomal fraction with approximately a 4-fold increase in specific activity, as compared to the 1000 *g* supernatant solution. In contrast, the 10,000 *g* mitochondrial pellet contained less than 30% of the initial activity and possessed a specific activity less than half that observed in the microsomal fraction.

PAPS, the sulfate donor, has been shown to be degraded rapidly by a variety of tissue preparations including rat and human tissues, which results in a decrease in the linearity of the TPST reaction *in vitro* [9, 11, 12]. Accordingly, to minimize PAPS catabolism, NaF, 5′-AMP and 3′-AMP were included

‡ C4-P16, EANEDYEDYEYDELPA.

Table 1. Subcellular distribution of TPST activity

Fraction	Volume (mL)	Protein (mg/mL)	Specific activity (pmol/min/mg)	Recovery (%)	Fold-purification
1000 *g* Supernatant	240	11.94	0.054	100	1
10,000 *g* Pellet	70	7.8	0.082	28.9	1.52
Microsomal pellet	50	7.98	0.215	55.5	3.98
Soluble fraction	420	4.37	ND*		

TPST activity in each fraction was assayed by incubation of an appropriate aliquot of each fraction (0.1 to 0.2 mg) protein in 100 μL of reaction mixture using the standard assay conditions described in Materials and Methods.

* Not detected.

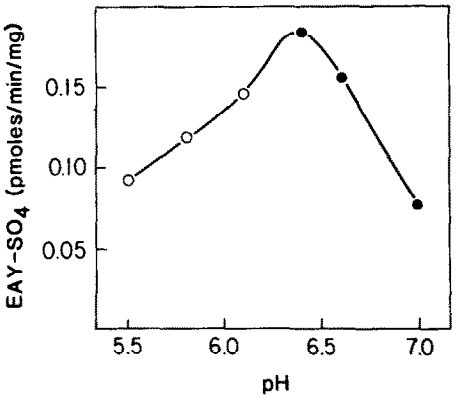

Fig. 2. Effect of pH on TPST activity. This experiment was performed as described (Materials and Methods). Each assay contained, in a final volume of 100 μL, 30 mM $MnCl_2$, 0.5% Lubrol-PX, 50 mM NaF, 1 mM 5'-AMP, 1 mM 3'-AMP, 2 μM [^{35}S]PAPS, 2 μM EAY, 0.24 mg protein with a 40 mM concentration of either MES (—○—) or HEPES (—●—) buffer at the pH indicated. Data shown here are the result of a typical experiment performed in triplicate. This experiment was repeated four times with similar results and no significant differences between using MES or HEPES at the same pH.

Table 2. Effect of detergents on EAY sulfation

Detergent	Specific activity (pmol/min/mg)	Percentage over control
None (control)	0.091	100
Lubrol-PX	0.255	280
Triton X-100	0.234	257
Tween-20	0.167	184
Digitonin	0.200	220
Octyl-glucoside	0.163	179

The effect of detergents was determined by assaying TPST activity in the presence of 0.5% of the detergent indicated.

routinely in our assays. As illustrated in Fig. 1, the sulfation of EAY catalyzed by the microsomal fraction was linear with time for up to 30 min with 0.24 mg of protein in the assay. Therefore, all subsequent experiments were performed in the presence of 0.12 to 0.24 mg of protein. In the absence of NaF, 5'-AMP and 3'-AMP, the product formed at 30 min of the reaction was about 80% less than that in the presence of these known PAPS degradation inhibitors. Although not shown, addition of any one of these compounds alone did not increase the TPST activity significantly, which implied the existence of multiple pathways of PAPS degradation.

Prior studies have demonstrated that TPST from other species required an acidic pH and the presence of a non-ionic detergent for optimal activity [7–9]. As shown in Fig. 2, human liver TPST similarly possessed an acidic pH optimum of approximately 6.4. Although not shown, the activity of TPST was similar in the presence of either of the two buffers employed, HEPES or MES, at pH 6.4. In regard to detergents, all detergents examined showed various stimulatory effects on TPST activity at a fixed concentration of 0.5% (Table 2). Of the detergents tested, Lubrol-PX and Triton X-100 appeared to be the most effective, stimulating the enzyme activity by greater than 2.5-fold. Based on these results, the effect of various Lubrol-PX concentrations on enzyme activity was examined. The data reported in Fig. 3 demonstrated that Lubrol-PX began to enhance activity at 0.05% and reached a maximum effect at 0.1%. Higher concentrations were slightly less stimulatory (Fig. 3). Since the stimulatory effect produced by Lubrol-PX was relatively constant between 0.25 and 2.0%, we chose to include 0.5% Lubrol-PX in all assays with human liver TPST.

TPST isolated from various species has also been shown to be activated by divalent cations including Mn^{2+}, Mg^{2+} and Co^{2+} [7–9]. In the present study, we measured the abilities of various divalent cations to stimulate TPST activity from human liver and found that 30 mM $MnCl_2$ maximally increased TPST activity (Fig. 4). Although not shown, chlorides of other divalent cations at 30 mM, including Mg^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, failed to promote a stimulatory effect on TPST activity.

The apparent K_m value for EAY sulfation was also

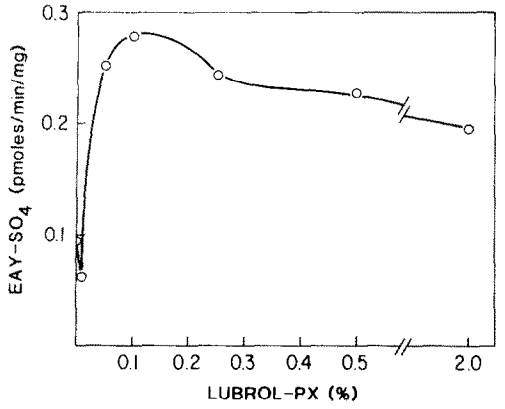

Fig. 3. Effect of Lubrol-PX on TPST activity. Data show the average of results obtained from four experiments each performed in triplicate, as described (Materials and Methods), with various concentrations of Lubrol-PX as indicated.

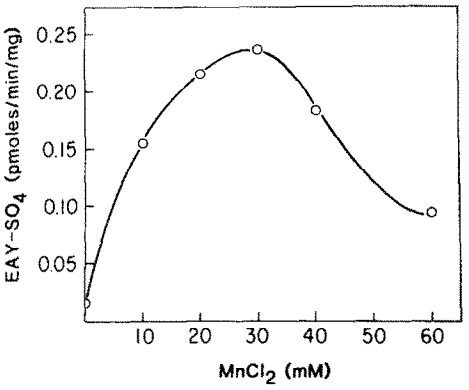

Fig. 4. Effect of Mn^{2+} on TPST activity. Data shown here are the average of results obtained from two experiments each performed in triplicate, as described (Materials and Methods), with various concentrations of $MnCl_2$ as indicated.

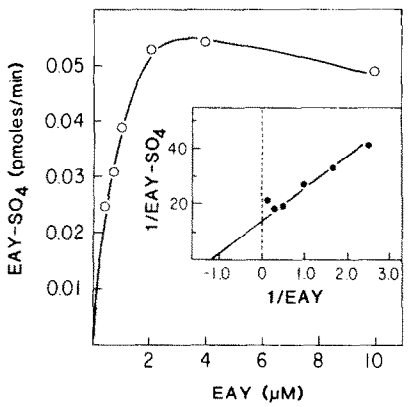

Fig. 5. Effect of EAY concentration on sulfation by TPST. Data shown are obtained from an experiment performed in triplicate using 0.24 mg microsomes from an autopsied sample and various concentrations of EAY. Other reaction conditions were as described (Methods and Materials). Inset: Lineweaver–Burk plot of the data. The K_m value derived from linear regression of the four data points where there was no significant substrate inhibition was 0.7 μM. Correlation coefficient = 0.993, $P < 0.01$. The average K_m value obtained from three experiments was 1.5 μM.

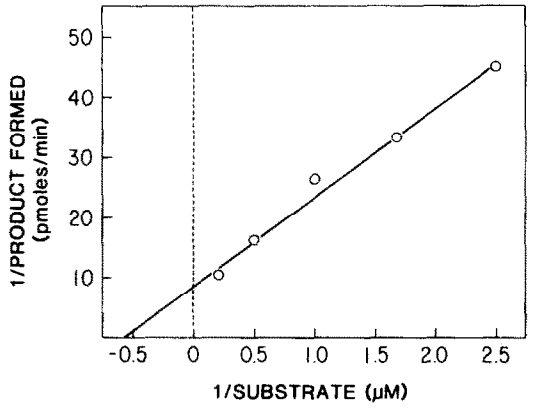

Fig. 6. Lineweaver–Burk plot of C4-P16 sulfation. Data shown were obtained from an experiment done in triplicate using 0.24 mg protein from an autopsied sample and various concentrations of C4-P16. Other reaction conditions were as described (Methods and Materials). K_m = 1.7 μM, correlation coefficient = 0.992, $P < 0.001$. This experiment was repeated twice with an average K_m of 2.1 μM.

determined as illustrated in Fig. 5. The average apparent K_m value from three independent experiments was approximately 1.5 μM, as determined from Lineweaver–Burk analysis. In all experiments, EAY concentrations above 2 μM resulted in substrate inhibition.

Since the C4 component of complement is known to be sulfated, C4-P16, a synthetic 16 amino acid peptide of the carboxy terminus of C4 containing three sulfatable tyrosyl residues [13, 14], was also examined to determine whether it can be sulfated by human liver TPST. As illustrated by the data in Fig. 6, C4-P16 was readily sulfated by human liver TPST and possessed an apparent K_m value of approximately 2.1 μM under the assay condition employed. When C4-P16 was used over a broad range of concentrations, the Lineweaver–Burk plot showed a downward biphasic curvature. The higher K_m value observed was estimated to be approximately 12 μM. We assume that this activity is caused by the presence of the soluble phenol sulfotransferase (P-PST) nonspecifically bound to the microsomes, since prior studies have demonstrated that the soluble phenol sulfotransferase is capable of sulfating tyrosine groups of a number of peptides *in vitro*, such as angiotensin, enkephalin and oxytocin [10]. It has also been observed that Boc-CCK was sulfated by a soluble phenol sulfotransferase from rat liver (Rens-Domiano S and Roth JA, unpublished observation), rat brain [15] and human liver [16]. In addition, preliminary studies in our laboratory with purified human P-PST confirmed that this enzyme is capable of sulfating C4-P16. However, we do not exclude the

Table 3. Distribution of TPST activity in human livers

Sex	Age	Specimen	Specific activity (pmol/min/mg)
Male*	34	HL-4	0.969
	56	HL-5	1.107
	41	HL-7	1.047
	35	HL-10	1.136
Female*	6	HL-1	0.718
	56	HL-2	0.367†
	55	HL-3	0.742
	14	HL-6	0.662
	43	HL-8	0.653
	66	HL-9	0.858

Methods used in isolating microsomes and assaying activities were as described in Materials and Methods. Results shown are the averages of three experiments, each performed in triplicate. Different protein concentrations were used in each case to secure the linearity of the reaction.

* Mean TPST activities ± SD of males and females were 1.065 ± 0.074 and 0.662 ± 0.158 pmol/min/mg respectively; $P < 0.005$.

† Sample HL-2 was directly homogenized with a Teflon/glass homogenizer after mincing, without using a polytron.

possibility that the biphasic Lineweaver–Burk plot may have resulted from multiple enzyme forms of human liver TPST that sulfate C4-P16 with different affinities.

It was also of interest to determine the biological variability of TPST in the human population. To address this issue, we measured TPST activity using EAY as substrate in liver samples obtained from ten organ donors. As shown in Table 3, the relative specific TPST activity determined in the microsomes from these livers ranged from 0.367 to 1.136 pmol/min/mg. This 3-fold difference in enzyme activity did not correlate with age of the subjects, but did correlate statistically with their sex. The activity in the male group (1.065 ± 0.074 pmol/min/mg) was significantly higher than that of the female group (0.662 ± 0.158), with $P < 0.005$ using Student's *t*-test.

DISCUSSION

The data presented in this paper demonstrate the existence of a membrane-bound tyrosylprotein sulfotransferase in human liver. Since previous studies on the TPST from bovine adrenal medulla and rat liver have indicated that this enzyme is associated with the Golgi apparatus, we initially attempted to isolate this membrane fraction from human liver using a discontinuous sucrose gradient as previously described [9]. Although the relative specific activity obtained in the Golgi fraction was 2.5-fold enriched over the crude homogenate, this method was not utilized for this study because of the low yield (11–15% of the initial activity). Using differential centrifugation as described in this paper, the majority of TPST activity was consistently recovered in the microsomal fraction (55–70%) with a 3- to 5-fold increase in specific activity over the 1000 *g* supernatant fraction. These results are consistent with the enzyme being located in the Golgi apparatus although they do not exclude the possibility that this enzyme may also be present in other subcellular fractions. The EAY sulfating activity is not present in the cytosolic fraction, further indicating that TPST is distinct from the soluble phenol sulfotransferase. Prior studies established that a cytosolic phenol sulfotransferase from rat and human liver are capable of sulfating small peptides such as angiotensin, enkephalin [10] and Boc-CCK, but not larger protein such as the synthetic polymer EAY (Rens-Domiano S and Roth JA, unpublished observation). Two forms of soluble phenol sulfotransferase have been purified and characterized from human liver [17] and platelet [18]. In contrast to the membrane-bound TPST, these enzymes have a pH optimum of approximately pH 7.4 and are not stimulated by divalent cations such as Mn^{2+}.

An acidic pH optimum has been a common property of TPST from all tissues thus far investigated. Using EAY as a substrate, we found that the pH optimum for TPST from human liver was 6.4, which is very similar to the previously reported pH optima of 6.5 and 6.7 for TPST from bovine adrenal medulla and rat liver, respectively [7, 9], yet considerably higher than the pH optimum of 5.8 for TPST from rat brain reported by Vargas *et al.* [8]. As will be noted later, it is possible that the lower pH value obtained in rat brain may have resulted from the fact that the substrate used was Boc-CCK instead of EAY.

The activation of tyrosine sulfation by divalent cations also has been reported with TPST isolated from different tissues. Mn^{2+} was shown to produce maximal stimulation at 25 mM using the enzyme from rat liver [9], while it was also shown to be effective at concentrations of 2 mM in rat brain [8] and 5 mM in bovine adrenal medulla [7]. In addition, Mg^{2+} was found to be stimulatory when using enzyme isolated from bovine adrenal medulla, whereas Co^{2+} was partially effective at stimulating TPST activity isolated from rat liver. In the present study, Mn^{2+} was found to enhance significantly human liver TPST activity with the maximal effect obtained at approximately 30 mM. Other divalent cations including Mg^{2+} and Co^{2+} at this concentration failed to produce a stimulatory effect.

Since TPST is a membrane-bound enzyme, it is also important to examine the effects of detergents on TPST activity. As shown in Table 2, all the non-ionic detergents stimulated TPST activity, with Lubrol-PX and Triton X-100 being the most effective at the concentration tested. These results are consistent with previous studies [7–9] with TPST from rat and bovine tissues, indicating that a non-ionic detergent is required for optimal activity.

EAY has been reported to produce substrate inhibition at concentrations above 10 μM using the enzymes from rat brain [15] and bovine adrenal medulla [7]. In the present studies, EAY also produced substrate inhibition at concentrations above 2 μM. The apparent K_m value of EAY using the microsomes from autopsied samples was approximately 1.5 μM. A similar apparent K_m value for EAY has been reported by Lee and Huttner [7] using TPST from bovine adrenal medulla.

The results from our study demonstrate that TPST from human liver exhibits certain biochemical properties in common with those from other species, although minor differences are also observed. The observed differences in the properties of TPST among species and tissues may reflect the existence of diverse enzyme forms, differences in the microenvironment of the enzyme, or differences in assay conditions or substrate employed to measure enzyme activity. In regard to the latter point, studies in our laboratory have revealed that the pH optimum for rat liver TPST sulfation of Boc-CCK was lower than that for EAY even though our data strongly suggest that both substrates are esterified by the same form of TPST (Rens-Domiano S and Roth JA, unpublished observation). These data indicate that the substrate employed to measure TPST activity may affect the apparent optimal assay conditions. In this latter case, the degree of ionization and/or solubility of EAY and Boc-CCK within the Golgi membranes may likely have affected TPST activity *in vitro*.

The similarities in biochemical properties of TPST from different species revealed in the present study suggest that protein and peptide sulfation is a well conserved process and, therefore, implies that it may be an essential post-translational modification required for the functioning of certain peptides and proteins. Consistent with this is the observation by Friederich *et al.* [19] demonstrating that mouse fibroblasts transfected with the genomic DNA for yolk protein 2 of *Drosophila melanogaster* secreted the intact yolk protein in a sulfated form apparently identical to that isolated from the *Drosophila*. Thus, mouse fibroblasts contain a functionally similar enzymatic system for protein sulfation as that of *Drosophila*, which suggests that the sulfation process is highly conserved during evolution. The results of our study support this hypothesis.

To help understand whether TPST activity is regulated *in vivo* and whether it possibly functions to control the production of the biologically active tyrosine sulfated proteins and peptides, it is first necessary to determine the variability of the activity of this enzyme in the human population and the factors which potentially can influence its activity *in vivo*. Accordingly, TPST activities were determined in ten liver samples from organ donors with no apparent liver disorders with the exception of HL-6, which was obtained from an individual chronically exposed to phenobarbital for treatment of a seizure disorder. There was about a 3-fold variation in TPST activity in these livers, and statistical analysis revealed a significant difference between the male and female activities. These observations are the first to implicate that sex hormones may regulate the expression of TPST activity, although the physiological role of this regulation remains to be established. Interestingly, Andersen *et al.* [20] measured the serum concentration of sulfated and non-sulfated gastrin in the fasting state of healthy subjects and observed considerable variability among individuals with no correlation to sex or age. However, gastrin is synthesized and sulfated in antrum and small intestine; therefore, it is possible that TPST in these sites is regulated differently than that in the liver. In addition, availability of the sulfate donor PAPS may also play a role in regulating the formation of sulfation products. Regulation of enzymes associated with Golgi apparatus by steroid hormones, including sex hormone, has been reported although the mechanism involved and its implication are still unclear. Haffar *et al.* [21] reported that glucocorticoids were capable of regulating the activity of a post-translational processing enzyme in the Golgi apparatus, thus leading to alteration of the expression of a viral protein on the cell membrane. More recently, Dahiya *et al.* [22] reported that administration of testosterone to rats resulted in an altered glycosphingolipid composition of the small intestinal mucosa. The testosterone treatment also led to an increase in glycotransferase activities in the intestinal mucosa which could account, at least in part, for the change in glycosphingolipid composition [22]. The sex difference in human TPST activity presently observed herein, therefore, may be the result of a direct regulation by sex hormones or secondary to the change in microenvironment of the enzyme induced by sex hormones.

In summary, we have identified a membrane-bound tyrosylprotein sulfotransferase from human liver, which is capable of sulfating a variety of tyrosine containing peptides. These studies also reveal that the biochemical properties of human liver TPST are similar to those in rat liver and other species. The sex-related distribution of TPST activity in human liver suggests that TPST activity may be regulated by sex hormone, although the functional role of this regulatory process is unknown. Further study is required for better understanding of this enzyme and its functional roles in humans.

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REFERENCES

1. Karp DR, Post-translational modification of the fourth component of complement. *J Biol Chem* **258**: 12745–12748, 1983.
2. Hortin G, Fok KF, Toren PC and Strauss AW, Sulfation of a tyrosine residue in the plasmin-binding domain of α_2-antiplasmin. *J Biol Chem* **262**: 3082–3085, 1987.
3. Mutt V and Jorpes E, Hormonal polypeptides of the upper intestine. *Biochem J* **125**: 51P–58P, 1971.
4. Jensen SL, Holst JJ, Nielsen OV and Rehfeld JF, Effect of sulfation of CCK-8 on its stimulation of the endocrine and exocrine secretion from the isolated perfused porcine pancreas. *Digestion* **22**: 305–309, 1981.
5. Hortin GL, Farries TC, Graham JP and Atkinson JP, Sulfation of tyrosine residues increases activity of the fourth component of complement. *Proc Natl Acad Sci USA* **86**: 1338–1342, 1989.
6. Lee RWH and Huttner WB, Tyrosine-*O*-sulfated proteins of PC12 pheochromocytoma cells and their sulfation by a tyrosylprotein sulfotransferase. *J Biol Chem* **258**: 11326–11334, 1983.
7. Lee RWH and Huttner WB, $(Glu^{62},Ala^{30},Tyr^{8})_n$ serves as high-affinity substrate for tyrosylprotein sulfotransferase: A Golgi enzyme. *Proc Natl Acad Sci USA* **82**: 6143–6147, 1985.
8. Vargas F, Frerot M, Tuong MDT and Schwartz JC, Characterization of a tyrosine sulfotransferase in rat

brain using cholecystokinin derivatives as acceptors. *Biochemistry* **24**: 5938–5943, 1985.

9. Rens-Domiano S and Roth JA, Characterization of tyrosylprotein sulfotransferase from rat liver and other tissues. *J Biol Chem* **264**: 899–905, 1989.
10. Roth JA, Phenol sulfotransferase. In: *Neuromethods* (Eds. Boulton AA, Baker GB and Yu PH), Vol. 5, pp. 575–604. Humana Press, Clifton, 1986.
11. Miller RR and Waechter CJ, Enzymatic sulfation of a large molecular weight glycoprotein associated with pig brain membranes. *Arch Biochem Biophys* **198**: 31–41, 1979.
12. Whittmore RM and Roth JA, Effect of phosphatase inhibition on *in vitro* dopamine sulfation and 3′-phosphoadenosine-5′-phosphosulfate catabolism in humon brain. *Biochem Pharmacol* **34**: 3853–3856, 1985.
13. Hortin G, Sims H and Strauss AW, Identification of the site of sulfation of the fourth component of human complement. *J Biol Chem* **261**: 1786–1793, 1986.
14. Hortin G, Folz R, Gordon JI and Strauss AW, Characterization of sites of tyrosine sulfation in proteins and criteria for predicting their occurrence. *Biochem Biophys Res Commun* **141**: 326–333, 1986.
15. Vargas F and Schwartz JC, Apparent identity of cerebral tyrosylsulfotransferase activities using a cholecystokinin derivative or an acidic amino acid polymer as substrate. *FEBS Lett* **211**: 234–238, 1987.
16. Young WF Jr, Human liver tyrosyl sulfotransferase (TST). *Pharmacologist* **30**: A12, 1988.
17. Falany CN, Vazquez ME, Heroux JA and Roth JA, Purification and characterization of human liver phenol-sulfating phenol sulfotransferase. *Arch Biochem Biophys* **278**: 312–318, 1990.
18. Heroux JA and Roth JA, Physical characterization of a monoamine-sulfating form of phenol sulfotransferase from human platelets. *Mol Pharmacol* **34**: 194–199, 1988.
19. Friederich E, Baeuerle P, Garoff H, Hovemann B and Huttner WB, Expression, tyrosine sulfation, and secretion of yolk protein 2 of *Drosophila melanogaster* in mouse fibroblasts. *J Biol Chem* **263**: 14930–14938, 1988.
20. Andersen BN, Magistris LD and Rehfeld JF, Radioimmunochemical quantitation of sulfated and non-sulfated gastrins in serum. *Clin Chim Acta* **127**: 29–39, 1983.
21. Haffar OK, Aponte GW, Bravo DA, John NJ, Hess RT and Firestone GL, Glucocorticoid-regulated localization of cell surface glycoproteins in rat hepatoma cells is mediated within the Golgi complex. *J Cell Biol* **106**: 1463–1474, 1988.
22. Dahiya R, Ahlawat RS and Sharma A, The glycosphingolipid composition and glycosyltransferase activities of the small intestinal mucosa of testosterone-treated rats. *Biochem Cell Biol* **67**: 42–47, 1989.