Recombinant full-length tissue factor pathway inhibitor fails to bind to the cell surface: implications for catabolism in vitro and in vivo

Guyu Ho, Masaaki Narita, George J. Broze Jr, and Alan L. Schwartz

Tissue factor pathway inhibitor (TFPI) plays a key role in the regulation of tissue factor-initiated blood coagulation secondary to loss of the integrity of the blood vessel wall. TFPI is a naturally occurring Kunitz-type protease inhibitor that inhibits coagulation factor Xa and, in a factor Xa-independent manner, mediates feedback inhibition of the factor VIIa/tissue factor catalytic complex. In vivo full-length TFPI is thought to be primarily bound to the vascular endothelium and the high affinity binding requires an intact carboxy terminus. Here we describe a full-length TFPI molecule, expressed in mouse C127 cells (TFPI<sup>C127</sup>), which exhibits virtually no cellular binding yet contains the intact carboxy terminus. This TFPI (TFPI<sup>C127</sup>) is neither internalized nor degraded via the TFPI endocytic receptor, LDL-receptor–related protein. Pharmacokinetic studies of TFPI<sup>C127</sup> in vivo demonstrate a 10-fold prolongation in the plasma half-life, compared with that of bacterial recombinant TFPI. (Blood. 2000;95:1973-1978)

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Introduction

The extrinsic pathway of coagulation is initiated by the exposure of tissue factor (TF) to the circulating blood. Binding of factor VII to TF promotes the activation of factor VII to factor VIIa. The factor VIIa/TF complex activates its substrates factor IX and factor X, triggering a cascade of events that culminates in the formation of a fibrin clot. Tissue factor pathway inhibitor (TFPI), a 42 kd plasma glycoprotein, plays a key role in the regulation of TF-initiated blood coagulation via its abilities to directly inhibit factor Xa and, in a factor Xa-dependent manner, inhibit the factor VIIa/TF proteolytic activity. Mature TFPI consists of a negatively charged amino terminus, 3 tandem Kunitz-type inhibitory domains, and a positively charged carboxy terminus. The second Kunitz domain binds and inhibits factor Xa, while the first Kunitz domain binds and inhibits the factor VIIa/TF complex. The carboxy terminus of TFPI and a portion of the third Kunitz domain contain heparin-binding sites and are necessary for the anticoagulant function of TFPI in TF-induced coagulation in vitro and for TFPI binding to the cell surface.

TFPI circulates in plasma predominantly bound to lipoproteins. Platelets contain approximately 8% of the total TFPI in blood and release their TFPI after stimulation with thrombin. The major endogenous source of TFPI is thought to be bound to the vascular endothelium and is releasable after heparin infusion, whereupon plasma TFPI levels rise several fold.

Recent animal studies have demonstrated that recombinant TFPI is effective against TF-induced coagulopathy, prevents arterial thrombosis, and reduces mortality from bacterial septic shock. Pharmacokinetic studies after an intravenous bolus injection of recombinant TFPI have shown that TFPI is rapidly cleared from the circulation with a plasma half-life of approximately 2 minutes in rabbits and less than 1 minute in rats. As a result, high doses of recombinant TFPI (approximately 20 mg/kg/d) are required to achieve therapeutic efficacy.

We have recently shown that the rapid clearance of recombinant TFPI from the circulation is in large part dependent on the binding of TFPI to the vascular endothelium and to a lesser degree to its hepatic removal via the endocytic receptor LDL-receptor–related protein (LRP). In this study, we demonstrate that full-length TFPI expressed in mouse C127 cells does not bind to the cell surface nor is it degraded via LRP in vitro. This results in an approximately 10-fold prolongation of the TFPI<sup>C127</sup> plasma half-life in mice. The noncellular binding properties of TFPI<sup>C127</sup> may thus offer additional therapeutic potential.

Materials and methods

Materials

Iodogen was purchased from Pierce (Rockville, IL). [125I]Iodide was from Amersham Corp (Piscataway, NJ). Human factor Xa was from Enzyme Research (South Bend, IN) and Spectrozyme Xa was from Calbiochem-Novabiochem. Affi-gel 10 was from Bio-rad (Hercules, CA). Tissue culture media and plasticware were obtained from Life Technologies, Inc (Rockville, MD). Recombinant TFPI from Escherichia coli was provided by Monsanto Company (St Louis, MO). CHO, SK hepatsoma, and 293 cell-derived TFPI, was purified as described below.

Purification of TFPI<sup>C127</sup>—TFPI<sup>C127</sup> was affinity-purified from the conditioned media of mouse C127 cells that had been transfected with a bovine papilloma virus containing wild-type human TFPI cDNA. In brief, cells producing TFPI<sup>C127</sup> were cultured in serum-free media containing aprotinin (10 µg/mL; Sigma) and the media were replaced every 2 to 3 days. Harvested media were concentrated using an Amicon ultraconcentrator (YM 30; Amicon, Bedford, MA) and the concentrates were absorbed to a 2H8 monoclonal anti-TFPI-affigel 10 column (0.5 × 3 cm) and eluted with 100 mmol/L glycine, pH 2.2. The pH of the eluant was adjusted to pH 7.5 using 1 mol/L Tris-HCl, pH 8.0, and applied to a 1 mL HiTrap
hemin-agarose column (Pharmacia-LKB Biotechnology, Piscatway, NJ), which had been equilibrated in 0.1 mol/L NaCl, 0.05 mol/L Tris-HCl, pH 7.5. The column was developed with a linear NaCl gradient from 0.1 to 1.0 mol, at 0.5 mL/min over 10 column volumes. Fractions were assayed for the presence of TFPIC127 by a modified enzyme linking immunosorbent assay (ELISA) method using 2 independent monoclonal antibodies, 2H8 and 2B12. The desired fractions were pooled, concentrated using Centricon microconcentrators (Amicon), and stored at ~80°C. The protein concentration was determined using the modified ELISA method with TFPI of known concentration as standard.

Amidolytic assays of factor Xa activity

In a 96-well microtiter plate, 1 nmol/L of factor Xa was incubated with various concentrations of TFPI (0.3-3 nmol/L) in 135 µL of 0.1 mol/L NaCl, 0.05 mol/L Tris-HCl, 0.5% bovine serum albumin (BSA), pH 7.5 (TBSA) at room temperature for 30 minutes. Fifteen microliters of 1 nmol/L Spectrozyme Xa chromogenic substrate was then introduced (final concentration 100 µmol/L) and the change in absorbance at 405 nm was determined 10 minutes thereafter.

Tissue factor pathway inhibitor functional assay of Vila/TF inhibition

The end point assay of anti-VIIa/TF activity (American Diagnostica) was used to compare TFPIC127 and bacterially expressed TFPI at equimolar concentrations.

Factor Xa-induced coagulation of plasma

In a fibrometer (BBL, Cockeysville, MD), 50 µL of rabbit brain cephalin prepared as described by the manufacturer (Sigma), 50 µL of CaCl2 (25 mmol/L), 50 µL of various amounts of TFPIC127 (25 ng to 300 ng) in TBSA, and 50 µL of human factor Xa (0.2 nmol/L) were incubated at 37°C. After 30 seconds, 50 µL of normal human plasma (George King Biochemical) were added, and the degree of apparent factor Xa inhibition was determined by comparing the clotting to a factor Xa standard curve.

Cell culture

Human hepatoma HepG2 cells, mouse fibroblast PEA 13 cells, and mouse brain endothelial cells (bend-3) were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal calf serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Cells were incubated at 37°C in humidified air containing 5% CO2.

Protein iodination

Proteins (5-25 µg) were iodinated using the IODOGEN method. Specific radioactivities were typically 1 to 10 x 10^6 cpm/nmol of protein.

Binding and degradation assays

Binding of 125I-TFPI to cells was performed in suspension. Cells were displaced from Petri-dishes by incubation with 3 mmol/L EDTA in phosphate-buffered saline (PBS) at room temperature for 5 minutes. Cells were then pelleted at slow speed (1000 rpm x 5 minutes) on a table top centrifuge (Sorvall RT6000B) to remove EDTA and resuspended in the assay buffer (DMEM/3% BSA). 4 x 10^4 cells in a volume of 300 µL were added with 2 mmol/L of 125I-TFPI to the presence or absence of 100 µL excess of cold ligands. After rocking at 4°C for 2 hours, cell suspensions were gently layered on 900 µL of fetal calf serum in microfuge tubes and spun at 14 000 rpm x 1 minute in a table-top microfuge. The cell pellet was counted in a gamma counter (Cobra II auto-gamma, Packard Instrument, Meriden, CT). Non-specific binding was determined in the presence of excess unlabeled ligand as specified in the figure legends.

Degradation assays were performed by washing cell monolayers (in 12-well dishes) twice with the assay buffer. 0.5 mL of assay buffer containing 2 mmol/L 125I-TFPI was then added to each well. After incubation at 37°C for the indicated periods, the overlying medium was removed and proteins were precipitated by the addition of BSA to 5 mg/mL and trichloroacetic acid to 20%. Degradation of ligand was defined as the appearance of radioative fragments in the overlying medium that were soluble in trichloroacetic acid. Fragmentation of 125I-TFPI at 37°C in cell-free wells was subtracted from the corresponding samples.

For ligand competition binding and degradation assays (Table 2), HepG2 and CHO cells were assayed as monolayers as described previously7,8; 125I-TFPI was at 20 nmol/L for the binding assays and at 0.6 nmol/L for the degradation assays.

Plasma clearance of 125I tissue factor pathway inhibitors in mice

Twelve- to 16-week-old BALB/c mice (weighing 20-25 g) were anesthetized with sodium pentobarbital (1 mg/20 g of body weight) during the course of experiments. Approximately 15 pmol of radiolabeled protein alone or with unlabeled 39 kd protein in 100 µL of sterile saline was injected into the tail vein over 30 seconds. In studies in which heparin was administered, heparin was injected 20 minutes after 125I-TFPI administration. At the indicated times, 40 to 50 µL of blood was collected by periocular bleeding. The blood samples were centrifuged and 15 µL of the plasma fractions was spotted onto 3 MM filter paper (Whatman Inc, Clifton, NJ), precipitated with 10% trichloroacetic acid, rinsed with ethanol, and radioactivity determined. The initial plasma concentration (time = 0) of 125I-TFPI was extrapolated from the measured points. At the end of each experiment, animals were killed and the liver, kidneys, spleen, and lungs were removed, blotted, weighed, and the radioactivity determined.

Metabolic incorporation of 35SO4 and immunoprecipitation of TFPI127

One 75-cm2 flask of C127 cells was labeled for 96 hours with 37 MBq (1 mCi) of Na2[35S]SO4 (Amersham Corp) in 10 mL of sulfate-deficient DMEM (-SO4) supplemented with 2% dialyzed fetal calf serum, in the absence of G418. The culture medium was then concentrated using Centricon microconcentrators (Amicon). Monoclonal antibody 2H8-conjugated affigel-10 beads were added to the concentrates that were then rocked overnight to allow for binding. The beads were subsequently collected by centrifugation, washed 3 times with PBS, boiled in SDS-sample buffer (to release bound TFPI), and the bead-free portion was analyzed by SDS-PAGE.

Electrophoresis and Western blotting

SDS-PAGE was performed using 10% separating and 5% stacking gels and proteins were transferred to nitrocellulose membrane. The membrane was blocked with 5% nonfat milk for 30 minutes, followed by incubation with a 1:100 dilution of rabbit antiserum raised against a synthetic peptide matching the carboxy terminal 12 amino acid residues of the mature TFPI protein. The immunoreactivity was detected using ECL reagents (Amersham Corp).

Results

Purification of recombinant tissue factor pathway inhibitor from mouse C127 cells

Previous studies have shown that TFPI, when expressed in mammalian systems, is susceptible to proteolysis at its carboxy terminus. Thus, mouse C127 cells expressing TFPI were grown in media containing the protease inhibitor, aprotinin. TFPI was purified from the media via TFPI monoclonal antibody-affinity chromatography. This was followed by heparin-agarose affinity which separates the full-length TFPI from the truncated species by charge interactions between the heparin and the positively charged carboxy terminus of TFPI. Figure 1 shows the elution of TFPI127 from the heparin column with a linear NaCl gradient. TFPI127 is a glycosylated protein, which runs at a faster rate than the nonglycosylated form of TFPI. The nonglycosylated TFPI is eluted at approximately 0.5 mol/L NaCl, whereas TFPI127 is eluted at approximately 0.7 mol/L NaCl.
eluted with 2 peaks at NaCl concentrations of 0.4 and 0.6 mol/L, respectively. Because full-length TFPI elutes at 0.6 mol/L salt, only the fractions collected at or greater than 0.6 mol/L NaCl (fractions 12-15) were pooled for subsequent studies. To confirm that the purified TFPI(C127) was full length, TFPI(C127) was subjected to Western blot analysis using antibodies directed against the last 12 amino acid residues of TFPI. As shown in the inset of Figure 1, the TFPI was recognized by these antibodies, confirming that the carboxy terminus was intact.

The activity of TFPI(C127) was assessed in a direct amidolytic assay of factor Xa activity, as well as an end point assay of anti-VIIa/TF activity. As shown in Figure 2, TFPI(C127) was able to inhibit factor Xa activity in a concentration-dependent manner with complete inhibition of factor Xa at approximately 3 nmol/L, which was essentially identical to that of bacterial recombinant TFPI. Using a global anticoagulant assay, TFPI(C127) inhibited factor Xa-induced plasma coagulation with IC50 approximately 10 nmol/L, compared with approximately 4 nmol/L for the bacterial recombinant TFPI. The disparity in IC50 values observed here is consistent with the previous observations that TFPI expressed in prokaryotic systems exhibits higher anticoagulant activities than that expressed in eukaryotic systems. Using the end point assay that measures anti-VIIa/TF activity, on a molar the activity of TFPI(C127) is 108% ± 16% that of bacterially expressed TFPI.

Cellular binding and degradation of tissue factor pathway inhibitor in C127 cells

We have previously shown that bacterial recombinant TFPI binds to human hepatoma HepG2 cells with high affinity and that truncation of sequences distal to the second Kunitz domain abolishes the binding. Surprisingly, the full-length 125I-TFPI(C127) exhibited no binding to hepatoma HepG2 cells, or to mouse vascular endothelial Bend cells, or mouse fibroblast PEA 13 cells (Table 1). Bacterial recombinant 125I-TFPI, however, exhibited abundant binding to these cells (Table 1). Furthermore, 125I-TFPI(C127) was not taken up/degraded by HepG2 cells in contrast to anti-VIIa/TF activity.

<table>
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<th>Cell Line</th>
<th>Specific Binding (fmol/10⁶ cells)</th>
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<tr>
<td>HepG2</td>
<td>Not detectable 590</td>
</tr>
<tr>
<td>Bend</td>
<td>Not detectable 610</td>
</tr>
<tr>
<td>PEA 13</td>
<td>Not detectable 540</td>
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</table>

HepG2, Bend, and PEA 13 cells were incubated in suspension with 2 nmol 125I-TFPI(C127) in the presence or absence of 100 mol excess of cold TFPI(C127) for 2 hours at 4°C. The specific radioactivity associated with cells was determined (open bars). Binding of bacterial recombinant 125I-TFPI to these cells was performed in the identical manner and used as control. Values represent the means of duplicate determinations.
the bacterial recombinant 125I-TFPI, a process mediated via the endocytic receptor LRP (Figure 3). The lack of binding or degradation of 125I-TFPI127 was not the result of iodination of the TFPI127 because noniodinated TFPI127 was unable to compete for bacterial recombinant 125I-TFPI binding or degradation (Table 2). Similar results for TFPI127 were obtained in CHO cells, as well (data not shown).

Clearance of tissue factor pathway inhibitor C127 cells in mice

The rapid clearance of TFPI from the circulation results from both TFPI binding to the vascular endothelium and hepatic removal via LRP.7,18 Because TFPI127 did not bind to the cell surface or was degraded via LRP in vitro, we evaluated its survival in vivo. The plasma 125I-TFPI level from approximately 5% of the initial concentration to less than 2 minutes with approximately 5% remaining at 30 minutes. Bacterial recombinant 125I-TFPI, on the other hand, showed a half-life of less than 2 minutes with approximately 5% remaining at 30 minutes. The tissue distribution (ie, liver, kidneys, lungs, and spleen) of 125I-TFPI127 did not differ significantly from that of bacterial recombinant 125I-TFPI when assessed at 30 minutes after injection (data not shown).

We have previously shown that injection of 50 mg/kg of 39-kd protein, a molecule known to inhibit all ligand interactions with LRP,24 prolongs the beta-phase elimination of bacterial recombinant TFPI. In addition, postinjection of 100 units of heparin raises the plasma 125I-TFPI level from approximately 5% of the initial plasma concentration to approximately 100%.18 When 50 mg/kg of 39-kd protein was coadministered with 125I-TFPI127 to mice, 125I-TFPI127 clearance was unaltered (Figure 4A). Postinjection of 100 units heparin after 125I-TFPI127 administration increased the plasma 125I-TFPI127 concentration only minimally (Figure 4B). These data suggest that neither LRP nor heparin-releasable binding to the vascular endothelium plays a significant role in TFPI127 clearance in vivo.

Tissue factor pathway inhibitor sulfation

Binding of TFPI to the cell surface is primarily due to charge interactions between the positively charged carboxy terminus and negatively charged cell surface heparan sulfate proteoglycans (HSPGs).3,11,20 One possibility to account for the inability of TFPI127 binding to the cell surface is that the carboxy terminus may interact with the negatively charged clusters within TFPI itself such that the carboxy terminus is no longer available for interaction with cell surface HSPGs. It has been reported that TFPI expressed in human kidney 293 cells (TFPI293), but not that expressed in Chinese hamster ovary cells (TFPICHO) or hepatoma cells (TFPIhepatoma), contains the negatively charged sulfate.26 To determine the relationship between TFPI sulfation and its binding property, TFPIs expressed in 293, CHO, and hepatoma cells were examined for cellular binding. As shown in Table 3, sulfated TFPI293 was unable to bind to fibroblast PEA 13 cells, whereas nonsulfated TFPICHO and TFPIhepatoma both exhibited abundant binding.

To examine whether TFPI127 was indeed sulfated, C127 cells were metabolically labeled with 35SO4. TFPI127 was immuno precipitated from the media and analyzed by SDS-PAGE, followed by either autoradiography or Western blot. As shown in Figure 5, a 35SO4-labeled band migrated at the identical position as TFPI127 and was recognized by a TFPI monoclonal antibody, demonstrating sulfate incorporation into TFPI127.

Discussion

Recombinant human TFPI has been studied extensively in terms of its biochemical and biologic properties, as well as its pharmacokinetics. The highly basic carboxyl terminus is necessary for efficient anticoagulant function,4 yet confers on TFPI rapid clearance in vivo.7 Herein we describe a TFPI molecule with an intact carboxy terminus that, nevertheless, exhibits an inability to bind to the cell surface or be internalized and degraded via the endocytic receptor LRP, and displays a markedly prolonged plasma half-life in vivo.

Table 3. Cellular binding of 125I-TFPI expressed in 293, hepatoma, and CHO cells

<table>
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<th>Ligand</th>
<th>Sulfation</th>
<th>Specific Binding (fmol/10⁶ cells)</th>
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<tbody>
<tr>
<td>TFPI293</td>
<td>+</td>
<td>not detectable</td>
</tr>
<tr>
<td>TFPIhepatoma</td>
<td>−</td>
<td>510</td>
</tr>
<tr>
<td>TFPICHO</td>
<td>−</td>
<td>430</td>
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PEA 13 cells were incubated in suspension with 2 nM 125I-TFPI293, or 29I-TFPICHO or 125I-TFPIhepatoma in the presence or absence of 100 mol excess of cold ligands at 4°C for 2 hours and the bound-specific radioactivity determined. Values represent the means of duplicate determinations.

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**Table 2. Lack of effect of TFPI127 on 125I-TFPI binding and degradation in HepG2 cells**

<table>
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<tr>
<th>Competitor (nmol)</th>
<th>125I-TFPI Degraded (fmol/10⁶ c/4 h)*</th>
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<tbody>
<tr>
<td>None</td>
<td>106</td>
</tr>
<tr>
<td>TFPI (600)</td>
<td>33</td>
</tr>
<tr>
<td>TFPI127 (600)</td>
<td>107</td>
</tr>
<tr>
<td>RAP (1000)</td>
<td>22</td>
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*HepG2 cells in monolayer were washed and incubated at 37°C for 4 h with media containing 0.6 nmol 125I-TFPI in the presence or absence of the indicated competitors. Thereafter, the media was removed and assayed for 125I-TFPI degradation products as described in the text. Each value represents the mean of triplicate determinations.

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Binding of full-length TFPI to the cell surface is dependent on its highly positively charged carboxyl terminus that interacts with the negatively charged cell surface HSPGs. We have previously shown that TFPI expressed in bacteria binds with high affinity to hepatoma HepG2 cells, fibroblast PEA13 cells, and microvascular endothelial cells. Similarly, TFPI expressed in hepatoma and CHO cells displays similar binding properties. To our surprise, TFPI expressed in mouse C127 fibroblast cells was devoid of cell surface binding activity but remained biologically active as judged by the inhibition of factor Xa amidolytic activity (Figure 2), of factor Xa-induced plasma coagulation, and of factor VIIa/TF activity. One potential explanation for this loss of binding activity is that secondary to intramolecular interactions, TFPI’s carboxyl terminus is no longer available for interaction with cell surface HSPGs. It is of note that, although TFPI does not bind to cell surface HSPGs, it binds to heparin-agarose (Figure 1) with the same affinity as bacterial recombinant TFPI, i.e., both TFPI species are eluted from heparin-agarose at identical salt concentrations (0.6 mol/L NaCl) (6, data not shown). The fact that the affinity of TFPI is 40-fold greater for heparin than for HSPGs may account for the high affinity binding of both species of TFPI to heparin and their discordant binding to cell surface HSPGs.

Girard et al have shown that serine 2 of TFPI is phosphorylated after its expression in C127 cells. However, TFPI with a serine 2 to alanine mutation, when expressed in these cells, did not regain the binding properties of wild type TFPI (data not shown).

This finding suggests that this negatively charged phosphate group (ie, at serine 2) does not interfere with availability of the carboxyl terminus. TFPI contains 3 potential N-linked and 1 O-linked glycosylation sites. Smith et al have shown that metabolic labeling of 293 cells with [35S]SO4 yielded sulfated TFPI, whereas sulfated TFPI was not produced by CHO cells. They further determined that TFPI carries Asn-linked sulfated oligosaccharides: SO4-GalNAcβ1,2GlcNAcβ1,2Manα. Because TFPI was unable to bind to the cell surface (Table 3), it is possible that sulfated oligosaccharides on TFPI may interact with its own positively charged carboxyl terminus, thus abrogating TFPI’s interaction with the cell surface. Attempts to quantitatively remove linked oligosaccharides or sulfate via incubation of nonadenatured TFPI293 with N-glycanase, O-glycanase or sulfatase and assess biological function have proven unsuccessful. Thus, whether TFPI293 possesses the same sulfation pattern as TFPI293 will require further study.

TFPI293 exhibited a 10-fold increase in plasma half-life compared with bacterial recombinant TFPI (Figure 4A). Because the administration of heparin did not significantly increase the plasma 125I-TFPI293 concentration, secondary to release from the vascular endothelium, or did the administration of the 39-kd protein inhibit its clearance (Figure 4), the prolonged half-life of 125I-TFPI293 is likely due to its inability to bind to either cell surface HSPGs or LRP. These results are consistent with our in vitro findings (Figure 3 and Table 1). However, it is not clear at present whether this observation can be accounted for by alterations in protein sulfation alone, because 125I-TFPI293 and 125I-TFPI293 have similar rates of plasma clearance. Nonetheless, the clearance of 125I-TFPI293, albeit markedly prolonged, did result in elimination of 50% at approximately 20 minutes. Therefore, additional mechanisms (eg, hepatic glycoprotein receptors) may be operative.

Under physiologic conditions, TFPI is primarily synthesized and secreted by vascular endothelial cells. Because the major pool of TFPI in vivo is associated with the endothelial cell surface, it appears that endothelial TFPI maintains the carboxy terminus available for interaction with cell surface HSPGs. Assuming that sulfation does play a role in TFPI binding to cells, TFPI produced by endothelial cells in vivo may well have to be under- or unsulfated to maintain its cellular binding. However, it is not presently known whether endogenous TFPI is sulfated or its degree of sulfation. Of note, human endothelial cells express significantly lower (less than 10%) GalNAc- and sulfotransferase activities than those expressed in 293 cells. These 2 enzymes are the key activities required for the addition of sulfated oligosaccharides to TFPI.

The physiologic significance of the cell-associated TFPI pool may relate to both its rapid mobilization into the plasma as well as its role as a mediator in the cellular uptake and degradation of factor Xa. In addition, it may serve to down-regulate the coagulant activity of factor VIIa/TF complex via transfer to caveolae. Endogenous TFPI within the circulation, however, is likely of less physiological importance, as it is largely truncated at various positions within the carboxy terminal domain and hence exhibits much reduced anticoagulant activity. The nonbinding full-length TFPI we have characterized in this study may therefore offer a therapeutic advantage when high plasma TFPI concentrations are required to effectively modulate TF-induced blood coagulation.
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