Tissue factor pathway inhibitor (TFPI) is a Kunitz-type serine proteinase inhibitor that down-regulates tissue factor-initiated blood coagulation. The most biologically active pool of TFPI is associated with the vascular endothelium, however, the biochemical mechanisms responsible for its cellular binding are not entirely defined. Proposed cellular binding sites for TFPI include nonspecific association with cell surface glycosaminoglycans and binding to glycosyl phosphatidylinositol-anchored proteins. Here, we report that TFPI binds specifically and saturably to thrombospondin-1 (TSP-1) purified from platelet α-granules with an apparent \( K_d \) of \( \sim 7.5 \) nM. Binding is inhibited by polyclonal antibodies against TFPI and partially inhibited by the B-7 monoclonal anti-TSP-1 antibody. TFPI bound to immobilized TSP-1 remains an active proteinase inhibitor. Additionally, in solution phase assays measuring TFPI inhibition of factor VIIa/tissue factor catalytic activity, the rate of factor Xa generation was decreased 55% in the presence of TSP-1 compared with TFPI alone. Binding experiments done in the presence of heparin and with altered forms of TFPI suggest that the basic C-terminal region of TFPI is required for TSP-1 binding. The data provide a mechanism for the recruitment and localization of TFPI to extravascular surfaces within a bleeding wound, where it can efficiently down-regulate the procoagulant activity of tissue factor and allow subsequent aspects of platelet-mediated healing to proceed.

Blood clotting is initiated following a vascular injury when blood is exposed to tissue factor (TF)\(^*\) present on the surface of perivascular smooth muscle cells and fibroblasts. TF is a 50 kDa membrane-associated protein that binds to plasma factor VII-VIIa forming a catalytic complex that initiates the blood coagulation cascade through activation of factors IX and X, which lead to thrombin generation and fibrin formation. TF procoagulant activity is regulated, in part, by tissue factor pathway inhibitor (TFPI). TFPI is a trivalent, Kunitz-type serine proteinase inhibitor that inhibits the active site of factor Xa with the second Kunitz domain and the active site of the factor VIIa-TF catalytic complex with the first Kunitz domain. The third Kunitz domain does not have an identified function (1). Following the third Kunitz domain, TFPI has a highly basic C-terminal region that is required for rapid inhibition of factor Xa by the second Kunitz domain (2–4) and for its association with cell surfaces (5). Although antithrombin has also been shown to inhibit factor VIIa \textit{in vitro} (6), TFPI is the only proteinase inhibitor that down-regulates TF procoagulant activity at physiologically relevant rates (7–9). When used as a therapeutic agent, TFPI has been shown to prevent disseminated intravascular coagulation and death from \textit{Escherichia coli} sepsis in baboons (10) and to attenuate endotoxin-induced coagulation in humans (11).

It appears that the majority of TFPI is produced by endothelial cells and remains associated with the endothelial surface (12, 13). This pool of TFPI contains an intact basic C-terminal region and is thought to be localized and oriented on the cell surface in a manner that allows it to simultaneously inhibit factor VIIa and factor Xa prior to dissociation of the newly activated factor X from the factor VIIa-TF catalytic complex (14). Thus, it is likely that TFPI is most effective as a surface-bound inhibitor of blood coagulation. However, the mechanisms responsible for TFPI binding to the endothelium are not entirely defined. Because heparin infusion results in a 2- to 10-fold increase in the circulating TFPI concentration (15–17), nonspecific interactions with glycosaminoglycans are often cited as a primary mode of cell surface association. However, there is a growing body of evidence indicating that a portion of endothelial-associated TFPI is bound to glycosyl phosphatidylinositol (GPI)-anchored proteins in a manner that is not dependent on glycosaminoglycans or altered by heparin (13, 18, 19). We have previously shown that glypiccan-3, a GPI-anchored proteoglycan, binds specifically to TFPI and that the binding is likely mediated by its protein core (20).

In addition to being associated with the endothelium, TFPI is also present in circulating plasma and within platelets. The plasma form of TFPI is largely associated with lipoproteins and is variably C-terminally truncated (21). Because of the reduced anticoagulant activity of circulating TFPI, it is not thought to be an important \textit{in vivo} inhibitor of TF initiated coagulation (21, 22). The TFPI in platelets represents about 8% (8 ng/ml) of the total TFPI in whole blood and is released after platelet activation. Platelet TFPI has an intact basic C-terminal region and may account for the increasing TFPI concentrations found in blood samples obtained from the site of a template bleeding time wound (23).

Because TFPI is thought to be a surface-associated inhibitor...
of coagulation, we investigated the mechanisms through which TFPI may down-regulate factor VIIa-TF catalytic activity in the extravascular space after vascular injury. We examined the interaction of TFPI with platelet α-granule proteins and found that TFPI binds specifically and saturably to thrombospondin-1 (TSP-1). TSP-1 accounts for about 25% of the protein within platelet α-granules and is secreted when platelets are activated at sites of vascular injury (24). After secretion, TSP-1 is a transient component of the inflammatory extravascular matrix of healing wounds (25, 26) and also binds to several cell surface integrins (27–30), thereby acting as a “molecular bridge” between activated platelets and other cells within the wound (31). The binding interaction between TFPI and TSP-1 described here suggests that TSP-1 released from platelet α-granules also acts to localize TFPI to surfaces within the extravascular space, where it can efficiently down-regulate TF-initiated coagulation after vascular injury.

**EXPERIMENTAL PROCEDURES**

**Proteins—**Recombinant full-length human TFPI produced in *Escherichia coli* was a gift of the Chiron Corp. (Emeryville, CA) and the Sunshine Pharmaceutical Corporation (Rockville, MD). The non-radioactive TFPI-160 molecule, after Gly-160, was produced in *E. coli* and purified as described previously (5). Rabbit polyclonal and 2H8 monoclonal anti-TFPI antibodies and K1K2C, an altered form of TFPI containing the first two Kunitz domains and the basic C-terminal region, were gifts of Dr. George Broze, Jr. (Washington University, St. Louis, MO). Specifically, the K1K2C form of TFPI contains a Met-Ala-Asp-Ser sequence connected to Glu-15 at the N terminus. The protein is then truncated at Gly-150 following the second Kunitz domain, and the basic C-terminal region (amino acids from Phe-243 to Met-276) is attached. Rabbit polyclonal anti-TSP-1 antibodies were from NeoMarkers (Freemont, CA). The anti-TSP-1 B-7 monoclonal antibody and rabbit polyclonal anti-fibronecrtin antibodies were from Sigma Chemical Co. (St. Louis, MO). Human factors VIIa and X were from Enzyme Research (South Bend, IN). Recombinant TF was from Ortho Diagnostic Systems Inc. (Raritan, NJ). Bovine serum albumin (A3912) was from Sigma. Thrombin was from Hematologic Technologies (Essex Junction, VT). Human Type I collagen was a gift of Dr. David Brand, Veterans Affairs Medical Center (Memphis, TN). Fresh frozen plasma was obtained from the Memphis Veterans Affairs Hospital Blood Bank.

Platelet α-granule proteins were obtained by thrombin stimulation of 6-day-old (freshly outdated) apheresis platelets (LifeBlood, Memphis, TN) as described by Frazier and Santoro (32). In brief, the platelets were washed three times in phosphate-buffered saline containing 5.5 mM glucose at 23 °C and resuspended in the same buffer at a concentration of 1 × 10^8 platelets/ml. Thrombin was added to 0.5 unit/ml, and the platelets were rocked gently. Phenylmethylsulfonyl fluoride was added to 1 mM final concentration immediately after visual observation of platelet aggregation, which typically occurred 1–2 min after the addition of thrombin. Calcium chloride was added to 1 mM final concentration after Gly-160, was produced in *E. coli* and purified as described previously (5).

**RESULTS**

**Demonstration of TFPI Binding to TSP-1 in Ligand Blots after SDS-PAGE of α-Granule Proteins—**Platelet activation and the secretion of α-granule proteins is a key component of hemostasis and vascular wound healing. We hypothesized that one or more platelet α-granule proteins may function to localize TFPI to extravascular surfaces after vascular injury. TFPI ligand blots of platelet α-granule proteins separated by non-
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TSP-1 is the primary TFPI binding protein in the platelet heparin binding domain. Because there are multiple platelet TSP-1 as well as fragments of TSP-1 that lack the N-terminal, identical to that seen in the TFPI ligand blot (compare This revealed a pattern of high molecular mass bands nearly identical to that seen in the TFPI ligand blot (compare lanes 2 and 3 in Fig. 1) and indicated that TFPI bound to full-length TSP-1 as well as fragments of TSP-1 that lack the N-terminal, heparin binding domain. Because there are multiple platelet α-granule proteins that did not bind to TFPI in the ligand blot assay (compare lanes 1 and 2 in Fig. 1), these data suggest that TSP-1 is the primary TFPI binding protein in the platelet α-granule preparations. It is possible that a second high molecular weight protein with a migration pattern similar to TSP-1 also bound to TFPI, however, no proteins other than TSP-1 were detected in TFPI ligand blot analysis of platelet α-granule proteins fractionated by either heparin agarose or ion exchange (MonoQ) chromatography (data not shown).

Demonstration of TFPI Binding to Purified TSP-1 in Ligand Blots after SDS-PAGE and in Slot Blots—When the TFPI ligand blot was repeated using purified TSP-1, binding of TFPI was again observed (Fig. 2A, lane 4). Purified TSP-1, under reducing and non-reducing conditions, is shown in lanes 1 and 2 of Fig. 2A to demonstrate that there are no detectable contaminating proteins that migrate similar to non-reduced TSP-1 present in the purified material. Thus, the binding of TFPI to non-reduced TSP-1 observed in lane 4 is not due to a contaminating protein. When the ligand blot was performed under reducing conditions, TFPI binding was not reliably detected. To determine the effect of reduction and SDS treatment, TFPI binding to purified TSP-1 after various treatments was measured in a slot blot assay (Fig. 2B). In this assay, binding to reduced and boiled TSP-1 was consistently observed, whereas binding to reduced TSP-1 in 1% SDS was greatly reduced. Thus, it appeared that TFPI bound to the reduced subunits of TSP-1 but not in the presence of SDS.

Demonstration of 125I-TFPI Binding to Immobilized TSP-1 in a Microtiter Plate Assay—When increasing amounts of 125I-TFPI are added to wells containing immobilized TSP-1, binding appeared to saturate at ~40 nM (Fig. 3A). Analysis of these data with a double reciprocal plot yielded an estimated appar-

![Fig. 1. Binding of TFPI to platelet α-granule proteins separated by SDS-PAGE. α-granule proteins were obtained by thrombin stimulation of washed platelets and separated by non-reducing, non-boiled SDS-PAGE. Lane 1, α-granule proteins stained with Coomassie Blue; lane 2, TFPI ligand blot of α-granule proteins; lane 3, Western blot of α-granule proteins using a polyclonal antibody against TSP-1. Molecular weight standards are as indicated.](image1)

![Fig. 2. Binding of TFPI to purified TSP-1. A, SDS-PAGE. lane 1, reduced, boiled TSP-1 stained with Coomassie Blue; lane 2, non-reduced, non-boiled TSP-1 stained with Coomassie Blue; lane 3, Western blot of non-reduced, non-boiled TSP-1 using a polyclonal antibody against TSP-1; lane 4, TFPI ligand blot of non-reduced, non-boiled TSP-1. B, slot blot demonstrating TFPI binding to: 1, 5% BSA; 2, non-boiled, non-reduced TSP-1; 3, non-boiled, reduced TSP-1; 4, boiled, non-reduced TSP-1; 5, boiled, reduced TSP-1; 6, non-boiled, reduced TSP-1 in 1% SDS; 7, boiled, reduced TSP-1 in 1% SDS. The increased binding observed to boiled, reduced TSP-1 (5) present in this slot blot was not a consistent finding.](image2)
The Binding of 125I-TFPI to Immobilized TSP-1—Commercially prepared material (data not shown). Observed between TSP-1 purified in our laboratory and the from Hematologic Technologies. No differences in binding were observed between TSP-1 and the very low concentration of TSP-1 present in circulating blood. When purified TSP-1 was added to plasma and proteins, the binding of 125I-TFPI to TSP-1 in the presence of increasing amounts of unlabeled TFPI. The apparent $K_d$ estimated from the point where binding of 125I-TFPI was decreased by 50%, is $\sim 7.5$ nM (Fig. 3B).

Binding of 2.5 nM 125I-TFPI to immobilized TSP-1 was compared with that of immobilized plasma and immobilized human Type I collagen. As shown in Fig. 4A, significantly more (5- to 10-fold) 125I-TFPI binding occurs in the TSP-1-coated wells than to either collagen- or plasma-coated wells. Binding of TFPI to plasma proteins was further investigated by performing slot blot analysis of plasma fractionated by Superose 6 gel filtration chromatography and TFPI ligand blot analysis of plasma proteins separated by non-reducing SDS-PAGE. These assays did not demonstrate any proteins present in plasma that bound exogenously added TFPI. Endogenous plasma TFPI bound to plasma lipoproteins was detected in these assays (data not shown). These findings are consistent with the low binding of 125I-TFPI to plasma observed in the microtiter plate assay and the low concentration of TSP-1 present in circulating blood. When purified TSP-1 was added to plasma and the mixture separated by non-reducing SDS-PAGE, TFPI bound to TSP-1 in the ligand blot assay (data not shown).

Binding of 125I-TFPI was confirmed using TSP-1 purchased from Hematologic Technologies. No differences in binding were observed between TSP-1 purified in our laboratory and the commercially prepared material (data not shown).

The Effects of Antibodies, Soluble TSP-1 and Calcium on the Binding of 125I-TFPI to Immobilized TSP-1—Polyclonal anti-TFPI antibodies and the monoclonal 2H8 anti-TFPI antibody blocked the binding of 5 nM 125I-TFPI to TSP-1 in the microtiter plate assay by 71% and 57%, respectively. Polyclonal anti-TSP-1 antibodies did not block binding, however, the monoclonal B7 anti-TSP-1 antibody reduced 125I-TFPI binding by 33% (Fig. 4B). A 100-fold molar excess of soluble TSP-1 reduced the binding of 125I-TFPI to immobilized TSP-1 by 51% (Fig. 4B). Because TSP-1 undergoes a conformational change upon binding to calcium, 1 mM calcium chloride was included in all buffers used in the microtiter plate assay. When the binding interaction was examined in the presence of 5 mM EDTA, 125I-TFPI binding was not affected (data not shown).

Effect of Heparin on the Binding of 125I-TFPI to Immobilized TSP-1—Because both TFPI and TSP-1 are heparin binding proteins, the binding of 125I-TFPI to TSP-1 in the presence of varying heparin concentrations was measured. As demonstrated in Fig. 5, 125I-TFPI binding in the presence of heparin concentrations ranging from 0.0001 to 0.1 unit/ml was not significantly different from that observed in the absence of heparin. However, in the presence of 1 unit/ml heparin, binding was reduced to 20%, and 10 units/ml heparin reduced binding to slightly below the background binding observed in the presence of 5% BSA with no TSP-1 adsorbed to the plate.

Effect of Altered Forms of TFPI on the Binding of 125I-TFPI to Immobilized TSP-1—The ability of heparin to block binding suggests that the basic C-terminal region of TFPI is required for binding to TSP-1. Two altered forms of TFPI were used to further investigate the role of the C-terminal domain. TFPI-160 is truncated after Gly-160 and contains the first two Kunitz domains and the C-terminal region but lacks the third Kunitz domain. SDS-PAGE of full-length TFPI, TFPI-160, and K1K2C is shown in Fig. 6A. Western analysis, using an antibody that recognizes only the C-terminal region of TFPI, confirmed that the full-length TFPI and K1K2C contain the C-terminal region, whereas the TFPI-160 does not (Fig. 6A). The K1K2C has a larger predicted molecular weight than TFPI-160, but it migrates more rapidly in SDS-PAGE. The reason for this behavior...
is not known. A 100-fold molar excess of TFPI-160 had no effect on the binding of $^{125}$I-TFPI to TSP-1, whereas a 100-fold molar excess of K1K2C decreased binding to 15% (Fig. 6B), demonstrating that the C-terminal region of TFPI has a critical role in the binding of TFPI to TSP-1.

**TFPI Bound to Immobilized TSP-1 Remains an Active Inhibitor of Factor VIIa-TF Catalytic Activity**—The microtiter plate assay was performed using unlabeled TFPI. In these experiments the relative amount of TFPI bound to either immobilized TSP-1 or BSA was measured using the TF inhibition assay. In wells coated with TSP-1, there was a 60% reduction in the amount of factor Xa generated compared with wells coated with 5% BSA (data not shown). These data demonstrate that the TFPI binding to TSP-1 observed in the microtiter plate assay is not an artifact induced by the radiolabeling of TFPI and, importantly, they indicate that TFPI bound to immobilized TSP-1 remains an active proteinase inhibitor.

**Soluble TSP-1 Enhances the Inhibition of Factor VIIa-TF Catalytic Activity by TFPI**—To determine the effect of soluble TSP-1 on TFPI inhibitory activity, rates of factor Xa generation by factor VIIa-TF were measured in the presence of 5 nM TFPI and a range of TSP-1 concentrations from 0 to 100 nM (Table I). When TSP-1 and TFPI were at equimolar (5 nM) concentration, there was no effect on the rate of factor Xa generation. However, when TSP-1 was present at 50 nM, the rate of factor Xa generation decreased by over 50%. This appeared to be a saturating amount of TSP-1, because the rate of factor Xa generation did not slow further in the presence of 100 nM TSP-1.

**DISCUSSION**

TSP-1 is a 450-kDa protein with affinity for cell surfaces and extracellular matrix proteins. It consists of three identical 150-kDa subunits linked by disulfide bonds. Each subunit is made up of a linear series of functional domains, including an ~30-kDa N-terminal heparin binding domain, regions homologous to procollagen, properdin, and epidermal growth factor, a calcium binding domain, and a C-terminal domain (36). Cultured endothelial cells (37), fibroblasts (38), and monocytes (39) synthesize and secrete TSP-1. In vivo, it is transiently expressed in skin wounds and is incorporated into the extracellular matrix of healing tissues (25, 26). As a result of its complex structure and properties, multiple potential functions have been proposed for TSP-1.

We have demonstrated that TFPI binds to TSP-1 purified from platelet α-granules in three separate assays, a ligand blot after SDS-PAGE, a slot blot assay, and a microtiter plate assay. Additionally, TFPI bound to immobilized TSP-1 remains functionally active and soluble TSP-1 enhances the ability of TFPI to inhibit factor Xa generation by the factor VIIa-TF catalytic complex. Although we cannot absolutely rule out binding to another high molecular weight α-granule protein, the data suggest that TSP-1 is the only TFPI binding protein present in either platelet α-granules or plasma. In plasma, a C-terminally truncated form of TFPI circulates associated with lipoproteins. However, exogenously added, full-length TFPI does not bind to plasma lipoproteins with high affinity (40), and we could not identify any plasma protein to which TFPI binds in the ligand blot after SDS-PAGE, slot blot assay, or microtiter plate assays. Additionally, TFPI does not bind to human Type I collagen, a prominent protein in the extracellular matrix that could potentially compete with TSP-1 for binding TFPI. The affinity of TFPI for numerous other extracellular matrix proteins remains to be determined. The binding of TFPI to immobilized TSP-1 is saturable with an estimated apparent $K_D$ of ~7.5 nM. This $K_D$ indicates that TFPI binds to TSP-1 more avidly than it does to its cellular degradation receptor on hepatocytes, the low density lipoprotein receptor-related protein, (apparent $K_D$ ~30 nM) (41) and is within the physiological range of TFPI concentration that would be present at the site of a vascular wound.

**TABLE I**

| TFPI | TSP-1 | Factor Xa generated/min |
|------|------|-------------------------|
| nM   | pmol |                         |
| 0    | 0    | 565                     |
| 0    | 100  | 405                     |
| 5    | 5    | 24.3                    |
| 5    | 50   | 26.6                    |
| 5    | 100  | 10.7                    |

**FIG. 5.** Effect of heparin on the binding of $^{125}$I-TFPI to TSP-1 in the microtiter plate assay. Comparison of the binding of 10 nM $^{125}$I-TFPI to TSP-1 in the presence of the indicated concentrations of heparin. When heparin was present at 10 units/ml, the specific binding to immobilized TSP-1 was lower than to wells coated with 5% BSA. The data points represent the average of experiments performed at least in triplicate and standard deviation.

**FIG. 6.** Effect of TFPI-160 and K1K2C on the binding of $^{125}$I-TFPI to TSP-1. A, reducing SDS-PAGE of the purified forms of TFPI stained with Coomassie Blue. Lane 1, full-length TFPI; lane 2, TFPI-160; lane 3, K1K2C. Western blot with a polyclonal antibody specific for the C-terminal region of TFPI following reducing SDS-PAGE, lane 4, full-length TFPI; lane 5, TFPI-160; lane 6, K1K2C. B, the binding of 5 nM $^{125}$I-TFPI to TSP-1 in the microtiter plate assay in the presence of either 500 nM TFPI-160 or 500 nM K1K2C. Data were normalized with binding to TSP-1 in the absence of binding inhibitors representing 100%. The data points represent the average of experiments performed at least in triplicate and standard deviation.
The binding of 125I-TFPI to immobilized TSP-1 is readily blocked by both polyclonal anti-TFPI antibodies and the monoclonal 2H8 anti-TFPI antibody. However, anti-TSP antibodies are much less effective at blocking binding. This suggests that TFPI may preferentially bind to surface-associated TSP-1, perhaps via a cryptic epitope of TSP-1 that is fully exposed after surface binding. This hypothesis is supported by the high concentration of soluble TSP-1 (500 nM) required to block 50% of the binding of 5 nM TFPI to immobilized TSP-1 (Fig. 4B) and the 50-fold excess of TSP-1 necessary for accelerated inhibition of factor Xa generation by TFPI in the solution phase TF inhibition assay (Table I).

Experiments were performed to define structural characteristics of TSP-1 and TFPI that are important for the binding interaction. Although TSP-1 undergoes a distinct conformational change upon binding calcium (42, 43), 125I-TFPI binding was not affected when the microtiter plate assay was performed in 5 mM EDTA. This is similar to the binding of plasminogen, fibrinogen, and fibronectin to TSP-1 which also are not dependent on calcium (44, 45) but different from TSP-1 binding to cellular binding sites, which tend to be calcium-dependent (28). It appears that TFPI binds to the individual 150-kDa subunits of TSP-1, because TFPI bound to reduced TSP-1 in the slot blot assay, however, the binding domain on TSP-1 remains to be localized. Heparin, at concentrations above 0.1 unit/ml, greatly reduced the binding of 125I-TFPI to TSP-1 in the microtiter plate assay. This is likely due to heparin blocking an interaction between the basic C-terminal region of TFPI and TSP-1 based on the following interpretation of the data. First, the experiments with the altered forms of TFPI strongly indicated that the C-terminal region of TFPI was required for binding to TSP-1. K1K2C, a form of TFPI that is missing the third Kunitz domain but has the C-terminal region, competed with 125I-TFPI for binding to TSP-1 in the microtiter plate assay, whereas TFPI-160, a form of TFPI that is missing both the third Kunitz domain and the C-terminal region, did not (Fig. 6B). Second, it appeared that the heparin binding domain of TSP-1 was not required for binding to TFPI. In the TFPI ligand blot of platelet α-granule proteins, TFPI bound to partially degraded forms of TSP-1 (Fig. 1). However, none of the degraded forms of TSP-1 bound to heparin agarose, suggesting that the thrombin used to activate the platelets cleaved the N-terminal, heparin binding domain of these fragments (46, 47). Therefore, a portion of TSP-1, other than the heparin binding domain, is likely involved in binding TFPI. Although it appears that the majority of endogenously bound TFPI on cultured endothelial cells is associated with a GPI-anchored protein and is not released from the cell surface with heparin (13), TSP-1 is made by endothelial cells in culture and TFPI bound to endothelial TSP-1 may account for a portion of the TFPI released into the circulation after heparin infusion.

It is well established that TFPI is a key regulator of TF-induced coagulation in vivo. The in utero death of mice lacking the first Kunitz domain of TFPI due to disseminated intravascular coagulation demonstrates that TFPI has a critical role in maintaining the anticoagulant properties of the endothelium (48). The intravascular function of TFPI is likely down-regulation of factor VIIa-TF activity transiently present on endothelial cells or monocytes that have been stimulated by inflammatory cytokines. However, under normal conditions, TF is predominantly expressed in extravascular locations surrounding the blood vessels where TFPI is not typically located. Because plasma TFPI is largely truncated at the C terminus and a poor inhibitor of blood clotting (21, 22), down-regulation of extravascular TF initiated coagulation by TFPI most likely requires the release of TFPI from activated platelets or the transfer of endothelial associated TFPI into the extravascular space.

Because TFPI accounts for approximately 25% of the platelet α-granule protein secreted at sites of vascular injury (24), it is likely that TFPI contributes to hemostasis within the wound site, but its exact role is not clear. TFPI-deficient mice do not have bleeding diatheses, and their platelets aggregate normally in response to thrombin (49). However, TFPI is involved in the early organization of the extracellular matrix of healing wounds (25, 26, 50). We propose that TFPI-secreted by platelets plays an important role in recruiting and localizing TFPI to surfaces within the extravascular matrix. Once localized, it can efficiently down-regulate the procoagulant activity of TF, which initiates blood clotting within the wound, and allow subsequent aspects of platelet-mediated healing to proceed. As mentioned above, TFPI is an adhesive, multifunctional protein with many proposed functional roles. The in vitro data presented here indicate that a binding interaction between TFPI and TFPI-1 likely occurs at the site of a bleeding wound and that binding to TSP-1 enhances TFPI inhibitory activity. Further characterization of its in vivo importance is warranted.

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