Receptor-mediated Endocytosis of Coagulation Factor Xa Requires Cell Surface-bound Tissue Factor Pathway Inhibitor*

(Received for publication, December 26, 1995, and in revised form, February 6, 1996)

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Coagulation factor Xa is a plasma serine protease that catalyzes prothrombin to thrombin conversion, which, in turn, leads to the generation of the fibrin clot. Of the several parameters that govern the plasma level of factor Xa, control of its catabolism is of crucial importance. However, little is known regarding the mechanisms by which factor Xa is catabolized. In the present study we examine the cellular basis for the uptake and degradation of factor Xa. 125I-Factor Xa was degraded by hepatic cell surface and embryonic fibroblasts via a process which required cell surface-bound tissue factor pathway inhibitor (TFPI), a potent inhibitor of factor Xa. Uptake and degradation of cell-surface-bound 125I-TFPI was also markedly stimulated in response to factor Xa binding. The intracellular kinetics of 125I-factor Xa and cell-surface-bound 125I-TFPI display a strikingly similar pattern, suggesting that factor Xa and cell-surface-bound TFPI are taken up as a bimolecular complex. Using cell lines either deficient in low density lipoprotein receptor-related protein, an endocytic receptor that mediates the degradation of uncomplexed TFPI (Warshawsky, I., Broze, G. J., Jr., and Schwartz, A. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6664–6668), or deficient in tissue factor (TF), an integral membrane protein capable of forming quaternary complexes with factor Xa, TFPI, and factor VIIa, we demonstrated that the receptor that mediates the uptake and degradation of factor Xa-TFPI complex was neither low density lipoprotein receptor-related protein nor TF. As the vascular endothelial cell surface retains a substantial pool of TFPI (Sandset, P. M., Allidgaard, U., and Larsen, M. L. (1988) Thromb. Res. 50, 803–813; Novotny, W. F., Brown, S. G., Mileitch, J. P., Rader, D. J., and Broze, G. J., Jr. (1991) Blood 78, 387–393), our data suggest that endothelial cell surface TFPI may be actively involved in the clearance of factor Xa from the circulation via mediated uptake and degradation.

Factor X, a plasma glycoprotein involved in the blood coagulation cascade, can be converted to its active serine protease form factor Xa by both the intrinsic (factor XIa) and extrinsic (factor VII and tissue factor) pathways (Jackson and Nemer-son, 1980). Factor Xa as the activator of prothrombin occupies a central position linking the two blood coagulation pathways. Control of factor Xa levels by its catabolism and by plasma protease inhibitors may therefore be pivotal in the regulation of the coagulation process.

While little is known about factor Xa catabolism, a number of plasma serine protease inhibitors are thought to inhibit factor Xa activity. These include α1-antitrypsin (Colman et al., 1982), α2-macroglobulin (Jackson and Nemer-son, 1980), antithrombin III (Kurachi et al., 1976), and the recently characterized TFPI (reviewed in Broze et al. (1990)). TFPI is unique among coagulation inhibitors in having multiple protease inhibitory domains. It contains an acidic amino terminus followed by three tandem Kunitz-type protease inhibitory domains and a basic carboxyl terminus (Wun et al., 1988). The second Kunitz domain of TFPI mediates its binding to and inhibition of factor Xa, whereas the first Kunitz domain of TFPI is required for its inhibition of the factor VIIa/tissue factor catalytic complex (Girard et al., 1989). The carboxyl-terminal region of TFPI, including at least a portion of the third Kunitz domain, is required for its binding to heparin and for optimal inhibition of factor Xa (Wesselschmidt et al., 1992, 1993). The carboxyl terminus of TFPI is also necessary for its binding to the cell surface (Warshawsky et al., 1995).

There are three sources of TFPI in vivo: plasma TFPI, which predominantly associates with lipoproteins and has a mean plasma concentration of 2.5 nM (Novotny et al., 1991); platelet TFPI, which is sequestered from the circulation and represents 10% of the plasma TFPI level (Novotny et al., 1988); and endothelial TFPI, which is proposed to associate with endothelial glycosaminoglycans. Plasma levels of TFPI rise severalfold following heparin infusion (Sandset et al., 1988; Novotny et al., 1991). The physiological roles of these three pools are not well defined. Platelet TFPI is released following thrombin stimulation and may exert an antithrombotic effect at local sites (Novotny et al., 1988). However, to date it has not been possible to demonstrate a direct correlation between plasma levels of TFPI and thromboses. The fact that patients with homozygous abetalipoproteinemia and hypobetalipoproteinemia have very low levels of plasma TFPI and do not suffer from thrombosis (Novotny et al., 1989) suggests that the TFPI associated with lipoproteins may not be essential. Perhaps the large endothelial cell surface pool of TFPI is of greater physiological importance.

In order to begin to elucidate the function of cell surface-associated TFPI, we have investigated its role in factor Xa catabolism. Our data show that the uptake and degradation of 125I-factor Xa by human hepatoma cells and mouse embryonic fibroblasts require cell surface TFPI and reciprocally, the uptake and degradation of cell-surface-bound 125I-TFPI is enhanced in response to factor Xa binding. Because of the simi-
larity of the intracellular kinetics of factor Xa and TFPI, it is likely that they are taken up by cells as a complex. Our data further indicate that there is a specific receptor responsible for TFPI-factor Xa degradation and that this receptor is not the low density lipoprotein receptor-related protein (LRP), an endocytic receptor that mediates the uptake and degradation of uncomplexed TFPI (Warshawsky et al., 1994), nor tissue factor, an integral membrane glycoprotein capable of forming a quaternary complex with TFPI, factor Xa, and factor VIIa (Girard et al., 1989; Gemmell et al., 1990). Thus, our data suggest that in vivo endothelial cell surface TFPI may clear activated factor Xa from the circulation by mediating its cellular uptake and degradation.

EXPERIMENTAL PROCEDURES

Materials—Iodogen was purchased from Pierce. ([125I])Iodide was from Amersham Corp. Prones, bovine serum albumin, and dansyl-L-glutamyl-glycyl-arginine chloromethyl ketone (DNS-GGACK) were obtained from Calbiochem-Novabiochem (La Jolla, CA). Factors Xa and X were from American Diagnostica (Greenwich, CT). Tissue culture media and plasticware were obtained from Life Technologies, Inc. An inhibitory rabbit anti-mouse soluble TF polyclonal antibody will be described separately.2

Proteins—Recombinant human full-length TFPI and the 39 kDa protein were expressed in and purified from Escherichia coli (Warshawsky et al., 1993, 1994). DNS-GGACK inactivated factor Xa was achieved by incubating factor Xa at room temperature for 1 h in a buffer containing 20 mM Tris-HCl, pH 7.3, 100 mM NaCl, and a 100-fold molar excess of DNS-GGACK.

Protein Iodinations—Proteins (20–50 μg) were iodinated using the iodogen method (Bu et al., 1992). Specific radioactivities were typically 0.5–3 × 106 cpm/μg of protein.

Cell Culture—HepG2 cells were as described (Owensby et al., 1988). PEA 10 and PEA 13 cells were obtained from J. Herz (Willnow and Herz, 1994). TF (−/−) mouse embryonic fibroblasts were derived from TF (−/−) mouse embryonic fibroblasts and NIH3T3 cells. Detailed methods and characterization will be described separately.2

All cell lines were cultured in Dulbecco's modified Eagle's medium (with glutamine) supplemented with 10% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml. Cells were incubated at 37°C in humidified air containing 5% CO2.

Binding and Degradation Assays—Cells were seeded in 12-well dishes 1 day prior to assay. Cell monolayers were generally used at 70–90% confluence. Assay buffers were Dulbecco's modified Eagle's medium containing 3% bovine serum albumin. Binding assays were carried out by washing cell monolayers with prechilled assay buffer twice and binding was initiated by adding 0.5 ml of 4°C assay buffer containing the indicated concentrations of [125I]-labeled proteins. After incubation at 4°C for 2 h, assay buffer containing unbound ligand was removed, and the cells were washed three times with 4°C assay buffer. The cells were then lysed in 62.5 mM Tris-HCl, pH 6.8, 0.2% SDS, and 10% glycerol. The radioactivity of cell lysates was determined in a γ counter (model C5304; Packard Instruments, Meriden, CT). Non-specific binding was determined in the presence of excess unlabelled ligand as specified in the figure legends (Owensby et al., 1989).

Degradation assays were generally carried out at 37°C for 3 h in 0.5 ml of assay buffer containing the indicated concentrations of [125I]-labeled proteins. Thereafter, the medium overlaying the cell monolayers was removed and proteins were precipitated by addition of bovine serum albumin to 5 mg/ml and trichloroacetic acid to 10%. Degradation of ligand was defined as the appearance of radioactive ligand fragments in the overlying medium that were soluble in trichloroacetic acid. Degradation of [125I]-ligand in parallel dishes that did not contain cells was subtracted from the total degradation (Owensby et al., 1989).

Single-cycle Endocytosis Assays—Cells were seeded into 12-well dishes. After the cells had been washed twice with 4°C assay buffer, 0.5 ml of assay buffer containing the indicated concentrations of [125I]-labeled proteins were added. After binding for 30 min at 4°C, cells were washed three times with 4°C assay buffer to remove unbound ligand. Cells were then warmed rapidly to 37°C by adding prewarmed assay buffer and incubated at 37°C for selected time intervals. The overlying medium was removed at each time point, and proteins were precipitated with trichloroacetic acid to assess the degradation. Cell monolayers were then washed three times with 4°C assay buffer and incubated at 4°C for 30 min in assay buffer containing 0.25% Pronase to remove cell surface radioligand and also detach cells from the culture wells. The detached cells were separated from the buffer by centrifugation and radioactivity associated with the cell pellet (i.e., internalized radioligand) was determined.

RESULTS

Uptake and Degradation of Coagulation Factor Xa Are Mediated by Cell Surface-bound TFPI—Human factor Xa has been reported to bind to human hepatoma HepG2 cells in a specific and saturable manner, while the zymogen factor X failed to bind to these cells (Sakai and Kisiel, 1990). Using chemical cross-linking and immunoprecipitation techniques, this group subsequently showed that TFPI was the major factor Xa-binding protein on the cell surface (Kazama et al., 1993).

To investigate the role of cell surface-bound TFPI in factor Xa degradation, HepG2 cells were preincubated at 4°C in the presence or absence of TFPI. Following warming to remove the unbound ligand, the cell monolayers were incubated with various concentrations of [125I]-factor Xa. Degradation of [125I]-factor Xa was carried out at 37°C for 3 h, and the overlying medium containing the resultant degradation products was assayed following trichloroacetic acid precipitation. As shown in Fig. 1, at each concentration of [125I]-factor Xa added, its degradation was enhanced about 10-fold in cells which had been preincubated with TFPI compared with those without prior TFPI exposure. This result suggests that TFPI mediates the uptake and degradation of factor Xa by HepG2 cells. The small amount of [125I]-factor Xa degradation seen in cells without exogenous TFPI addition may have resulted from either a small amount of endogenous TFPI or more likely from fluid-phase endocytosis (Fig. 1).

Degradation of Factor Xa Is Not Mediated through the LRP Endocytic Pathway—LRP is a member of the low density lipoprotein receptor family involved in the endocytosis of several circulating plasma proteins and heteromeric protein complexes

2 J. R. Tooney and G. J. Braze, J. r., unpublished data.
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To confirm this observation, 125I-factor Xa required cell surface-bound TFPI, we examined whether LRP served as an endocytic receptor for factor Xa. To this question directly, LRP-negative embryonic fibroblasts (PEA 10), isolated from LRP-heterozygous knockout (Willnow and Herz, 1994), were utilized. Mouse embryonic fibroblasts (PEA 12), isolated from mice homozygous for LRP gene

We first evaluated the binding of 125I-factor Xa to PEA 10 and PEA 13 cells as a function of cellsurface-bound TFPI. Cells were preincubated at 4°C with increasing concentrations (0–16 nM) of TFPI. After washing to remove the unbound ligand, the cell monolayers were incubated with 2 nM 125I-factor Xa at 4°C for 2 h for binding assays. Fig. 2A shows that at zero concentration of TFPI, the specific binding of 125I-factor Xa to both cell lines was minimal. As the concentration of TFPI in preincubation buffer increased, binding of 125I-factor Xa to the cells increased accordingly (Fig. 2A). This result indicates that binding of factor Xa to these cells is TFPI-dependent, supporting the observation that TFPI is the major binding protein for factor Xa on cells (Kazama et al., 1993). The observation that binding of 125I-factor Xa to both PEA 10 and PEA 13 cells was indistinguishable (Fig. 2A) indicates that LRP does not play a role in factor Xa binding. Fig. 2B shows that binding of 125I-factor Xa to PEA 10 cells was saturable at fixed levels of prebound TFPI (similar data were obtained using PEA 13 cells, data not shown), confirming the specific nature of the TFPI-mediated factor Xa binding to cells.

Previously we showed that the uptake and degradation of TFPI by hepatoma cells is blocked by anti-LRP antibody as well as by the 39 kDa protein, an LRP-binding protein that inhibits all known ligand interactions with LRP (Warshawsky et al., 1994). These observations led to the conclusion that LRP is the endocytic receptor for TFPI. To confirm this observation, 125I-TFPI degradation was evaluated in LRP-positive PEA 10 and LRP-negative PEA 13 cells. As shown in Fig. 3, 125I-TFPI degradation occurred in PEA10 cells, but not PEA 13 cells. There was an approximately 7-fold difference in 125I-TFPI degradation between these two cell lines (Fig. 3).

PEA 10 and PEA 13 cells are phenotypically different only in their expression of LRP. Thus using these two cell lines, we next examined to what extent LRP contributed to TFPI-dependent 125I-factor Xa degradation. Cells were processed in an identical manner as that described in Fig. 1. As shown in Fig.
To determine at which step(s) factor Xa observed effect in association with cell surface-bound TFPI is required for the cells. These results clearly demonstrate that active factor Xa in association with cell surface-bound TFPI is required for the observed effect.

Factor Xa-stimulated TFPI Degradation Results from Cellular Ligand Uptake—To determine at which step(s) factor Xa affect the cellular fate of cell surface-bound 125I-TFPI, a single-cycle endocytosis analysis (Owensby et al., 1989) was performed. This analysis was carried out with PEA 13 cells, such that LRP does not contribute to the observed results. Fig. 7A shows that in the absence of factor Xa, there was essentially no internalization of cell surface-bound 125I-TFPI, even after 60 min of incubation at 37 °C. However rapid internalization of 125I-TFPI was observed upon binding to factor Xa. This indicates that factor Xa induces the uptake of cell surface-bound 125I-TFPI. Fig. 7B shows that the degradation of cell surface-bound 125I-TFPI was also enhanced in the presence of factor Xa, apparently as a consequence of the increased internalization.

It is of note that the internalized pool of 125I-TFPI was sustained intracellularly over a period of at least 60 min (Fig. 7A). This feature is distinct from that observed with ligands internalized by the constitutive endocytic receptor LRP (Owensby et al., 1988; Bu et al., 1992; Underhill et al., 1992; Warshawsky et al., 1994), in which ligand internalization peaks at 10–15 min and declines precipitously thereafter. To determine whether factor Xa followed similar intracellular kinetics to that of TFPI, the fate of a cohort of 125I-factor Xa was examined during single cycle endocytosis in TFPI-prebound PEA 13 cells. As shown in Fig. 7C, similar internalization and degradation kinetics were observed for 125I-factor Xa. These results, taken together with previous observations that factor Xa-stimulated TFPI degradation required association with factor Xa, strongly suggest that factor Xa and TFPI are internalized as a complex.

Tissue Factor Is Not the Receptor for the Factor Xa-TFPI Complex Degradation—Data seen in Fig. 7 imply that there is a specific receptor which mediates the internalization and degradation of factor Xa-TFPI complex. One obvious potential candidate is tissue factor (TF). TF is an integral membrane protein capable of forming quaternary complexes with TFPI, factor Xa, and factor VIIa (Girard et al., 1989; Gemmell et al., 1991). To examine this possibility we evaluated the effect of preincubation of cells with anti-TF polyclonal antibodies. When analyzed in LRP-negative PEA 13 cells, no significant inhibition of factor Xa-stimulated 125I-TFPI degradation was observed in the presence of anti-TF antibody (data not shown). This observation suggested that TF did not play a significant role in factor Xa-stimulated TFPI degradation; however, alternative explanations were possible. Therefore, in order to definitively eliminate a role for TF in this process, we took advantage of TF-negative fibroblasts that were generated by gene

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Fig. 4. 125I-factor Xa degradation by PEA 10 and PEA 13 cells is comparable. Treatment of cells in the presence or absence of unlabeled TFPI and the subsequent degradation of 125I-factor Xa by the cells were performed as described in the legend to Fig. 1. Symbols are the means of duplicate determinations.

Fig. 5. Factor Xa stimulates the degradation of 125I-TFPI bound to both PEA 10 and PEA 13 cells. Cells were incubated with 16 nM 125I-TFPI at 4 °C for 30 min and unbound radioligand removed. Cells were then incubated with increasing concentrations of unlabeled factor Xa (0–16 nM) at 4 °C for 30 min to allow binding followed by incubation at 37 °C for 3 h to initiate 125I-TFPI degradation. Degradation of 125I-TFPI via LRP was derived from the difference between the amounts degraded by PEA 10 and PEA 13 cells. Symbols represent the means of five independent experiments.

FIG. 5. Factor Xa stimulates the degradation of 125I-TFPI bound to both PEA 10 and PEA 13 cells. Cells were incubated with 16 nM 125I-TFPI at 4 °C for 30 min and unbound radioligand removed. Cells were then incubated with increasing concentrations of unlabeled factor Xa (0–16 nM) at 4 °C for 30 min to allow binding followed by incubation at 37 °C for 3 h to initiate 125I-TFPI degradation. Degradation of 125I-TFPI via LRP was derived from the difference between the amounts degraded by PEA 10 and PEA 13 cells. Symbols represent the means of five independent experiments.
TFPI, is crucial in factor Xa catabolism that the vascular endothelium, through its surface-bound presence of factor Xa, degradation of cell surface-bound $^{125}$I-

binding, is also accelerated. Since the intracellular kinetics of $^{125}$I-factor Xa and $^{125}$I-TFPI are quite similar, it is likely that targeting and demonstrate a complete absence of TF expression.$^2$ As seen in Table I, TF-negative cells, in the absence of factor Xa, degraded $^{125}$I-TFPI at 122 fmol/10$^6$ cells. Degradation was completely abrogated by incubation with the 39-kDa protein, indicating that uncomplexed TFPI was degraded via LRP as has been shown (Warshawsky et al., 1994). In the presence of factor Xa, degradation of cell surface-bound $^{125}$I-TFPI was further enhanced to 215 fmol/10$^6$ cells. This indicates that TF-negative cells were capable of markedly increasing degradation of $^{125}$I-TFPI in response to factor Xa addition. Furthermore, this increased degradation was not blocked by the 39-kDa protein. Thus LRP did not contribute to the enhancement, as observed in other cells (e.g. see above). Taken together, these data demonstrate that TF is not the endocytic receptor for the factor Xa-TFPI complex.

**DISCUSSION**

In the current study we show that $^{125}$I-factor Xa is actively endocytosed and degraded by various cell lines in a manner that requires cell surface-bound TFPI. The uptake and degradation of cell surface-bound $^{125}$I-TFPI, in response to factor Xa binding, is also accelerated. Since the intracellular kinetics of $^{125}$I-factor Xa and $^{125}$I-TFPI are quite similar, it is likely that they are taken up by cells as a complex. Furthermore, our data indicate that there is a specific endocytic receptor responsible for the TFPI-factor Xa degradation and that this receptor is neither LRP nor TF.

TFPI interacts with factor Xa in 1:1 stoichiometry; however, the interaction is reversible (Broze et al., 1987, 1988). Thus one would expect an eventual transfer of factor Xa from this complex to its major plasma inhibitors, antithrombin III, $\alpha_2$-proteinase inhibitor, and $\alpha_2$-macroglobulin, which form irreversible bonds with factor Xa (Pratt and Pizzo, 1986; Colman et al., 1987). Our data shown here indicate that rather than targeting factor Xa to other plasma protease inhibitors, cell surface-bound TFPI directly inactivates factor Xa by mediating its cellular degradation. Considering the fact that the endothelial cell surface provides a substantial reservoir of TFPI (Sandez et al., 1988; Novotny et al., 1991), it is reasonable to speculate that the vascular endothelium, through its surface-bound TFPI, is crucial in factor Xa catabolism in vivo.

We showed previously that cellular degradation of $^{125}$I-TFPI is inhibited by antibodies directed against LRP and by the 39-kDa protein (Warshawsky et al., 1994). These observations led to the conclusion that LRP is the endocytic receptor for TFPI. In addition, recent studies with adenoviral mediated delivery of the 39-kDa protein to mice in vivo further confirm the role of LRP as the predominant receptor governing TFPI uptake and degradation (Narita et al., 1995). In the present study we demonstrate that $^{125}$I-TFPI degradation occurs only in LRP-positive PEA 10 cells, but not in LRP-negative PEA 13 cells (Fig. 3). This result thus confirms and extends our previous findings. Using these two cell lines in degradation assays, we have observed that TFPI-mediated $^{125}$I-factor Xa degradation, however, is independent of the LRP endocytic pathway (Fig. 4).

Several lines of evidence suggest that factor Xa and cell surface-bound TFPI are internalized as a bimolecular complex. First, binding and degradation of $^{125}$I-factor Xa are mediated by cell surface-bound TFPI (Figs. 1, 2, and 4). Second, the cell surface-bound $^{125}$I-TFPI, upon association with factor Xa, is also internalized (Fig. 7). Last, the intracellular kinetics of $^{125}$I-TFPI and $^{125}$I-factor Xa are similar if not identical (Fig. 7). The above observations also indicate that a specific endocytic receptor is responsible for the uptake of the complex. It is interesting to note that $^{125}$I-factor Xa or $^{125}$I-TFPI internalized by the putative receptor does not share the internalization kinetics typical of that observed for ligands internalized by the endocytic receptor LRP (Owensby et al., 1988; Bu et al., 1992; Underhill et al., 1992; Warshawsky et al., 1994). Rather than rapidly declining after 10–15 min at 37 °C, $^{125}$I-factor Xa or $^{125}$I-TFPI is sustained intracellularly at plateau levels for a significantly prolonged time. The underlying mechanism is not clear at present. Whether this is due to an unusual intracellular pathway traversed by the TFPI-factor Xa complex or to resistance of the complex or its individual component proteins to proteolysis within the vacuolar system, as is the case for PAI-1 of the t-PA-PAI-1 complex (Underhill et al., 1992), remains to be discerned.

**FIG. 7. Uptake and degradation of $^{125}$I-TFPI or $^{125}$I-factor Xa by PEA 13 cells during a single cycle of endocytosis.** A and B, cells were incubated with 2 nM $^{125}$I-TFPI at 4 °C for 30 min and unbound radioligand removed. Cells were then incubated at 4 °C for 30 min in the presence (●) or absence (○) of 2 nM unlabeled factor Xa followed by incubation at 37 °C for selected intervals. At the indicated times, the overlying medium was removed for analysis of $^{125}$I-TFPI degradation (B), while the cell monolayers were incubated with Pronase at 4 °C for 30 min to remove cell surface $^{125}$I-TFPI. The Pronase-resistant radioligand associated with cells, defined as the internalized fraction, was then determined (A). C, cells were incubated with 2 nM TFPI at 4 °C for 30 min and unbound ligand removed. Cells were then incubated with 2 nM $^{125}$I-factor Xa at 4 °C for 30 min. After washing to remove unbound radioligand, cells were placed at 37 °C for selected intervals for the uptake (●) and degradation (○) of $^{125}$I-factor Xa, determined as described above. Symbols represent means of two independent experiments.

**TABLE I**

| Factor Xa (fmol/10$^6$ cells) | 39-kDa protein | $^{125}$I-TFPI-degraded |
|------------------------------|----------------|-------------------------|
| 0                            | 0              | 122                     |
| 0                            | 1000           | 7                       |
| 8                            | 0              | 215                     |
| 8                            | 1000           | 112                     |
In an effort to identify the receptor for the TFPI-factor Xa complex, we explored the possibility of TF as a potential candidate. TF is an integral membrane protein capable of forming quaternary complexes with TFPI, factor Xa, and factor VIIa (Girard et al., 1989; Gemmell et al., 1990). Adventitial fibroblasts beneath the vascularendothelium constitutively express TF (Wilcox et al., 1989; Drake et al., 1989; Fleck et al., 1990). On the contrary, endothelial cells that are in intimate contact with blood, under physiological conditions, either do or do not express very little TF (Wilcox et al., 1989; Drake et al., 1989; Fleck et al., 1990). Assuming that the endothelium serves as a potential site for degradation of the TFPI-factor Xa complex, one may not predict that TF serves as this receptor due to its potential site for degradation of the TFPI-factor Xa complex, we explored the possibility of TF as a potential candidate. TF is an integral membrane protein capable of forming quaternary complexes with TFPI, factor Xa, and factor VIIa (Girard et al., 1989; Gemmell et al., 1990).

Kazama et al. (1993) reported that in addition to binding to cell TFPI, 30% of 125I-factor Xa could be cross-linked to protease nexin-1 on HepG2 cells. Hence they proposed that nexin-1 is a receptor for factor Xa as well. Protease nexin-1 is secreted by many anchorage-dependent cells, including fibroblasts, cardiac muscle cells, and kidney epithelial cells (Eaton and Baker, 1983). However secretion by endothelial cells has not been reported. Whether nexin-1 plays a role in factor Xa catabolism is not presently known, nor is its physiological significance in this matter. These issues will certainly deserve further study. As will the molecular identity of the TFPI-factor Xa clearance receptor.

Acknowledgment—We thank Dr. Guojun Bu for providing the recombinant 39-kDa protein and for critical reading of this manuscript.

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J. Biol. Chem. 1996, 271:9497-9502.
doi: 10.1074/jbc.271.16.9497

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