RESEARCH COMMUNICATION

Structural requirements for the interaction between Tissue Factor and Factor VII: characterization of chymotrypsin-derived Tissue Factor polypeptides

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Tissue Factor (TF) is the cellular receptor for coagulation Factor VII/VIIa (FVII/VIIa). TF binds to FVIIa and promotes the rapid activation of the zymogen substrates Factors IX and X (FIX and FX) to the respective serine proteinases. In order to probe structure–function relationships in TF, we have subjected the truncated membrane-bound variant, TF 1–243, to proteolytic digestion in SDS-containing gels. Three major polypeptide fragments were generated by proteolysis of TF 1–243 with chymotrypsin, producing cleavages C-terminal to residues 34, 76 and 103. All three polypeptides, TF 35–243, 77–243 and 104–243, bound biotinylated human FVII in a highly specific ligand blot assay. High-performance electrophoretic chromatography was used to isolate chymotrypsin-derived fragments of TF. These

purified fragments bound FVII in ligand blots, and two of the three polypeptides exhibited much reduced, but significant, procoagulant activity in a chromogenic assay for the generation of Factor Xa in the presence of FVIIa and Ca^{2+} . The smallest chymotrypsin-derived TF polypeptide, TF 104–243, showed reduced binding of FVII in ligand blot analyses, inhibited the activity of the full-length molecule, but had no procoagulant activity. These data suggest that a part of the binding site for FVII is contained within the TF sequence 104–243. The sequence TF 1–34 either contains a part of the FVII-binding domain or its removal leads to dysfunctional folding, disrupting binding sites elsewhere in the molecule.

INTRODUCTION

Tissue Factor (TF) is the cellular receptor for blood coagulation Factor VII and Factor VIIa (FVII/VIIa). The complex of TF and FVIIa catalyses the activation of the zymogen substrates FIX and FX, leading to prothrombin activation [1]. Exposure of TF to plasma initiates the coagulation cascade and may be responsible for the fibrin deposition associated with various pathological states, including Gram-negative sepsis and atherosclerosis [2,3]. Analysis of TF cDNAs indicates that TF comprises three domains: a hydrophilic extracellular domain, a hydrophobic transmembrane-spanning segment and a cytoplasmic domain [4–7]. It has been postulated that the binding of FVIIa to TF induces allosteric changes in the enzyme, enhancing the catalytic function of the complex [8]. The precise structural requirements for the TF–FVIIa interaction are not known, but the isolated extracellular domain of the receptor binds to FVIIa and promotes hydrolysis of peptidyl substrates by the proteinase [9]. There are two disulphide bonds in this part of the molecule [10], with the integrity of the cystine at positions 186–209 being essential for the binding of FVII to the cofactor [11]. Both N-terminal and C-terminal TF extracellular domain sequences have been implicated in receptor function by monoclonal-antibody epitope mapping and by cross-linking studies [12,13].

We have subjected a truncated recombinant TF variant to chymotryptic digestion in SDS/PAGE gels and have isolated polypeptides which show reduced binding to FVII in ligand blots and are minimally functional. These studies suggest that the N-terminal part of the TF extracellular domain is essential for the expression of activity of the TF–FVIIa complex, and demonstrate

that the C-terminal part of the TF extracellular domain plays a role in FVII binding.

MATERIALS AND METHODS

Recombinant human TF 1–243 was generously provided by Dr. D. Higgins, Genentech Inc., South San Francisco, CA, U.S.A. TF 1–243 lacks the cytoplasmic domain of the full-length molecule, but is wild-type-identical in *in vitro* assay systems. The protein was expressed in *Escherichia coli* and purified as described elsewhere [14]. FVII concentrate was the gift of Dr. J. Smith, Plasma Fractionation Laboratory, Oxford, U.K. Purified FX was from Enzyme Research Laboratories, Swansea, Wales, U.K. Sulpho-*N*-hydroxysuccinimide ester (NHS)–biotin and streptavidin–horseradish peroxidase (sHRP) were from Pierce Europe, Luton, U.K. Enhanced chemiluminescence (e.c.l.) detection reagent was from Amersham International, Amersham, Bucks., U.K. Materials for SDS/PAGE were from Bio-Rad Laboratories, Richmond, CA, U.S.A. All other reagents were from Sigma, Poole, Dorset, U.K. and were reagent-grade or better. Protein estimations were performed by Micro BCA assay (Pierce Europe) according to the manufacturer's instructions. BSA was used as standard. The normal plasma used in functional assays was collected from ten normal donors, pooled, snap-frozen and stored at -70°C .

Purification of FVII

FVII was purified as previously described from freeze-dried FVII concentrate [15]. The purified protein had a specific activity of 2500 units of FVII clotting activity/mg by one-stage FVII

Abbreviations used: FVII etc., Factor VII etc.; TF, Tissue Factor; sHRP, streptavidin–horseradish peroxidase; e.c.l., enhanced chemiluminescence; PVDF, poly(vinylidene difluoride); hGHbp, human-growth-hormone receptor; h.p.e.c., high performance electrophoretic chromatography; NHS, *N*-hydroxysuccinimide ester.

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coagulation assay and 2000 units of FVII antigen/mg by FVII e.l.i.s.a. (Asserachrom FVII; Diagnostica Stago, Asnières, France). The protein was resolved as a single band on silver-stained SDS/PAGE gels. Labelling of FVII with biotin was carried out as follows: 7 µg of FVII was incubated with 22 µg of sulphy-NHS-biotin in 50 µl of 0.2 M imidazole/HCl/0.15 M NaCl/50 % glycerol, pH 8.0, for 30 min on ice. The solution was then dialysed against 0.05 M Tris/HCl/0.15 M NaCl, pH 7.4 (TBS), containing 10 % glycerol, for 2 h and the resulting biotin-labelled FVII made 50 % (v/v) with respect to glycerol and stored at -20 °C.

Ligand blot analysis of TF digests

Recombinant TF was subjected to proteolytic digestion using the method of Cleveland [16]. TF was diluted into 0.0625 M Tris/HCl (pH 6.8)/10 % glycerol/0.1 % SDS and various concentrations of chymotrypsin or control proteinases. Samples were applied to SDS/15 %-polyacrylamide gels. Current was applied until the proteins had entered the stacking gel. Electrophoresis was then halted for the 30 min to allow digestion of the TF. Samples were re-electrophoresed and gels were stained with Coomassie Blue or electroblotted for further analysis. Ligand blot analysis was performed by a modification of the method described previously [15]. Membranes were blocked for 2 h in NET buffer [0.05 M Tris (pH 7.4)/0.15 M NaCl/0.05 % Triton X-100/0.25 % gelatin] and incubated for 2 h with FVII-biotin in NET buffer containing 10 mM CaCl₂. After three washes in the same buffer, membranes were probed with sHRP conjugate and a signal generated using e.c.l. reagent. Densitometric scanning of polyacrylamide gels and nitrocellulose membranes stained with Coomassie Blue revealed that each of the polypeptides generated by digestion of TF electroblotted with equal efficiency in a quantitative manner.

N-terminal amino acid sequencing of TF digests

For N-terminal amino acid sequence analysis, TF digest products were resolved on SDS/15 %-polyacrylamide gels and then electroblotted on to poly(vinylidene difluoride) (PVDF) membranes (Applied Biosystems, Warrington, Cheshire, U.K.) using a TransBlot apparatus (Bio-Rad Laboratories). The membranes were stained with Coomassie Blue for 1 min, destained for 30 min and soaked in distilled water before drying. Individual bands were excised and placed in a tangential-flow cartridge for sequencing in an ABI 473A protein sequencer. The pulsed liquid delivery of reagents was optimized for the altered geometry of the cartridge. Data from the on-line liquid chromatograph were analysed with ABI 610A software to identify and quantify amino acid phenylthiohydantoin derivatives in each cycle of Edman degradation. Vapour-phase cleavage of Coomassie Blue-stained protein on PVDF membranes was carried out as follows: 50 mg of CNBr was added to 1.4 ml of formic acid and 0.6 ml of water in a test tube attached to a vacuum pump and a nitrogen line. The membrane was applied to a sintered-glass filter in the tube, which was then sealed, evacuated, flushed with nitrogen and left overnight at room temperature. The filter was then washed with ethyl acetate/hexane and dried by suction. The cleaved protein was then sequenced as described above.

High-performance electrophoretic chromatography (h.p.e.c.)

H.p.e.c. was carried out using an ABI 230A instrument (ABI, Warrington, Cheshire, U.K.). The Tris/glycine/SDS buffer sys-

tem of Laemmli was used throughout [17]. Gel dimensions were 3.5 mm × 5 cm. Polyacrylamide and cross-linker (PDA; Bio-Rad) concentrations were 12.5 % and 1 % respectively. Gels were pre-run for 230 min at 1.2 mA constant current with voltage limits set at 350 V. A portion (100–250 µg) of TF was applied to the gel in a maximum volume of 75 µl. The sample buffer was 0.0625 M Tris/HCl, pH 6.8, containing 10 % glycerol. Samples of TF contained bovine pancreatic chymotrypsin (Calbiochem) at 1 % (w/w). Gels were electrophoresed for 230 min at 1.2 mA constant current. The elution buffer flow rate was 25 µl/min; 5 min fractions were collected. On-line monitoring of the eluted material was performed at 280 nm. Peaks were routinely analysed by slab-gel electrophoresis.

Assay for TF activity

Procoagulant activity of TF and TF polypeptides was assayed following re-lipidation in mixtures containing 5 µl of TF or TF polypeptide, 30 µl of 0.05 M Tris/0.15 M NaCl/0.1 % BSA, pH 7.4 (TBSA), 15 µl of mixed brain phospholipids (rabbit brain cephalin; Sigma) in 0.25 % sodium deoxycholate and 10 mM CdCl₂. Samples were incubated for 10 min at room temperature prior to assay in microtitre plate wells as follows: 5 µl of re-lipidated TF was added to 25 µl of TBSA, 25 µl of purified FVII (2.5 µg/ml), 50 µl of purified FX (10 µg/ml) and 25 µl 25 mM CaCl₂. Samples were incubated for 20 min at 37 °C; 25 µl of the chromogenic substrate S2222 (*N*-benzoyl-Ile-Glu-Gly-Arg *p*-nitroaniline hydrochloride methyl ester) was then added and the change in absorbance monitored continuously at 450 nm. Since treatment of TF with SDS reduced the procoagulant activity of the cofactor, relative activities of the fragments were measured with reference to undigested h.p.e.c.-purified TF. For inhibitor studies the same assay was used, but the FVII concentration was reduced to 0.25 µg/ml and 1 µl was added per assay to obtain a rate-limiting concentration of the enzyme; 5 µl of TF 1–243 was added to each well, along with various concentrations of TF 104–243. Final volumes were adjusted to parity with TBS, pH 7.4. Following the addition of FX, 25 µl CaCl₂ was added and the samples were assayed as described above.

RESULTS

Proteolytic digestion of TF in Cleveland gels

Chymotryptic digestion of TF in Cleveland gels generated three major polypeptides of relative molecular masses 29, 24 and 18 kDa (Figure 1). N-terminal-amino-acid-sequence analyses indicated that these polypeptides resulted from N-terminal proteolysis, generating fragments starting at residues Thr-35, Ser-77 and Leu-104 respectively. The relative mobilities of these polypeptides were consistent with N-terminal cleavage alone. In order to obtain the C-terminal amino acid sequence, PVDF-immobilized polypeptides were cleaved with CNBr and sequenced. TF contains a single methionine residue at position 210, and thus sequence analysis of the CNBr-cleaved 18 kDa fragment yielded two N-termini and two residues at each cycle. The most C-terminal residue identified unequivocally was Phe-221, but tentative assignments were possible to Val-228.

Since the TF 35–243 and the TF 77–243 polypeptides required re-lipidation before procoagulant activity could be detected, it is most likely that these polypeptides include the transmembrane-spanning segment and thus comprise TF 35–243 and 77–243. The smallest of the polypeptides extended from position 104 to at least position 228. It is likely that this polypeptide was also

Figure 1 N-terminal-amino-acid-sequence analysis of chymotrypsin-derived TF polypeptides

TF was subjected to digestion with chymotrypsin as described in the Materials and methods section. The gel on the left-hand side of the Figure shows the polypeptides which were blotted, excised and sequenced. Molecular-mass (*M*, kDa) markers are shown in the left-hand side of the gel. The 18 kDa polypeptide (4) was cleaved with CNBr and the resulting two sequences are shown. Assignments for residues 222–228 were tentative, owing to low repetitive yield. In. Y refers to the initial yield of amino acid phenylthiohydantoin derivatives identified following Edman degradation. R. Y refers to the repetitive yield obtained. X denotes a position where no assignment could be made.

generated following N-terminal cleavage alone and will be referred to as '104–243'.

Ligand blot analyses

Previous studies revealed that ligand blots using biotinylated TF or FVII to probe the receptor or ligand are highly specific for the FVII–TF interaction [18]. Densitometric scanning of lanes stained with Coomassie Blue and luminography revealed a linear relationship between FVII binding and TF protein dye binding (results not shown). FVII–ligand blot analyses were performed on chymotryptic digests of TF where TF had been simultaneously electrophoresed with increasing concentrations of the enzyme (Figure 2). Biotinylated FVII bound to residual uncleaved TF and to the three major polypeptides at 29, 24 and 18 kDa. None of the lower-molecular-mass species reacted with biotin-FVII in the ligand blot. In control digests trypsin was used to effect the digestion of TF in Cleveland gels, and none of the polypeptides generated bound to FVII (Figure 3, lanes C and D). Cleveland-gel electrophoresis performed using TF and plasmin or thrombin also resulted in the generation of TF polypeptides which did not bind to FVII (results not shown). Thus FVII binds specifically to chymotrypsin-derived TF polypeptides. Quantification by densitometric scanning of Coomassie Blue-stained gels and ligand blots revealed the sequential N-terminal processing of TF was associated with progressive loss of FVII binding (Figure 4). A direct comparison of identical digests on Coomassie Blue-stained gels (Figure 4a) and ligand blots (Figure 4b) revealed that, on a molar basis, TF 35–243 showed binding of 33.4 % relative to the full-length molecule; for TF 77–243 and TF 104–243 the values were 29.9 and 16.1 % respectively. Thus while the minimal sequence 104–243 was capable of binding to FVII, progressive loss of binding was associated with sequential deletion of the N-terminal part of the molecule. The greatest absolute loss of FVII binding was associated with the removal of the first 34 amino acids.

Procoagulant activity of TF polypeptides

TF was also subjected to chymotryptic digestion in preparative-electrophoresis gels and the fragments purified by h.p.e.c. (Figure 5). Identical cleavages were detected C-terminal to residues 34, 76 and 103. These fragments bound biotinylated FVII in non-reducing, but not reducing, ligand blot analyses. This is consistent with the observation that an intact intramolecular disulphide bond is a prerequisite for the FVII–TF interaction [11]. In

Figure 2 Ligand blot analysis of TF in Cleveland gels

(a) 15% Polyacrylamide Cleveland gel stained with Coomassie Blue. Molecular-mass (*M*, kDa) markers are shown at the left of the gel. Lane 1, 3.5 μ g of TF + 0.01 μ g of chymotrypsin; lane 2, 3.5 μ g of TF + 0.02 μ g of chymotrypsin; lane 3, TF + 0.05 μ g of chymotrypsin. (b) The same samples subjected to ligand blotting with biotinylated FVII (see the Materials and methods section).

Figure 3 Cleveland gels of TF, using chymotrypsin and trypsin

A 3.5 μ g portion of TF was incubated with 0.05 μ g of trypsin and subjected to Cleveland gel digestion as described in the Materials and methods section. After electrophoresis, lanes were either stained with Coomassie Blue (lanes A and C) or subjected to electrotransfer and ligand blotting with biotin-FVII (lanes B and D). Lanes A and B, TF + chymotrypsin; lanes C and D, TF + trypsin. Note: enzymes themselves were added at concentrations which were too low for detection by Coomassie Blue staining.

addition, h.p.e.c.-purified polypeptides resolved as single bands on reduced SDS/PAGE, indicating that chymotrypsin produced no intra-disulphide cleavages in the molecule. Individual

TF polypeptide (sequence)	Concentration (scan area)	FVII binding (scan area)	Relative binding (%/mol)
1-243	198777.9	42375.6	100.0
35-243	10680.4	8852.8	33.4
77-243	4709.6	4468.2	29.9
104-243	6453.1	3956.7	16.1

Figure 4 Densitometric scans of chymotryptic digests of TF

A 3.5 μg portion of TF 1-243 was electrophoresed with 0.01 μg of chymotrypsin in two separate lanes. After electrophoresis one lane was stained with Coomassie Blue and the other electroblotted and subjected to ligand blotting as described in the Materials and methods section. Gels and films were then densitometrically scanned using a Shimadzu flying-spot densitometer. (a) Coomassie Blue-stained digest; (b) FVII ligand blot of digest. Binding to the undigested TF 1-243 was taken as 100%. Relative binding of FVII to the three polypeptides was corrected for molecular mass.

TF polypeptides were assayed in a chromogenic assay for TF procoagulant activity. Residual undigested TF and the three purified chymotrypsin-derived fragments were re-lipidated at equimolar concentrations prior to assay in a chromogenic assay for TF in the presence of FVII, FX and the chromogenic substrate S2222. Progress curves for each fragment and the undigested TF are shown in Figure 6. TF 35-243 and TF 77-243 both enhanced the generation of FXa by FVIIa, whereas TF 104-243 had virtually no cofactor activity. Activity was measured as the initial rate of S2222 hydrolysis in the FX activation assay and corrected for FVIIa activity in the absence of TF (Table 1). Undigested h.p.e.c.-purified TF was arbitrarily ascribed a specific activity of 1000 TF procoagulant units/nmol. The relative specific activities of the chymotrypsin-derived polypeptides ['Rel. Act.

Figure 5 SDS/PAGE analysis of h.p.e.c.-purified TF polypeptides

The Figure shows 15%-polyacrylamide-slab-gel electrophoresis of fractions eluted from h.p.e.c. of a TF/chymotrypsin digest. M_r markers (M , kDa) are shown on the left of the gel, which was stained with Coomassie Blue.

Figure 6 Procoagulant activity of h.p.e.c.-purified TF polypeptides

Equimolar concentrations (20 nmol) of full-length h.p.e.c.-purified TF and three chymotrypsin-derived fragments were re-lipidated and assayed in a chromogenic assay for FXa in the presence of FVII and Ca^{2+} . Key to symbols: ●, 1:10 dilution of 20 nmol of re-lipidated full-length TF (TF 1-243); ▼, 1:20 dilution of TF 1-243; □, 20 nmol of re-lipidated TF 35-243; △, 20 nmol of re-lipidated TF 77-243; ◇, 20 nmol of re-lipidated TF 104-243; ○, re-lipidation buffer.

(%)'] are shown in Table 1. TF 35-243 had a specific activity of 77.4 ± 1.00 units/nmol and TF 77-243 had a specific activity of 33.94 ± 0.87 unit/nmol. TF 104-243 was non-functional in this assay (0.77 ± 0.88 units/nmol), suggesting that, while it retained the ability to bind to TF in ligand blots, it was unable to act as a cofactor for FVIIa-mediated FX hydrolysis. Binding of FVII to TF 104-243 was independently confirmed using the chromogenic assay to evaluate the inhibitory effect of TF 104-243 on the undigested TF 1-243. A fixed concentration of FVII, FX and three concentrations of TF 1-243 was incubated

Table 1 Procoagulant activity of chymotrypsin-derived TF polypeptides

Abbreviations: mAU, milli-absorbance units; 'Rel. Act. (%)' is defined in the text.

Polypeptide	Activity		Polypeptide (nmol)	Specific activity (units/nmol $\pm$ S.E.)	Rel. Act. (%)
	(mAU/min $\pm$ S.E.)	(TF units)			
TF 1-243	26.64 ± 0.35	1000.00	1	1000.00 ± 13.10	100
TF 35-243	41.24 ± 0.53	1548.00	20	77.40 ± 1.00	7.74
TF 77-243	18.08 ± 0.46	678.70	20	33.94 ± 0.87	3.39
TF 104-243	0.41 ± 0.47	15.50	20	0.77 ± 0.88	0.08

Figure 7 Dixon plot of the inhibition of TF 1-243 by TF 104-243

A TF activity assay was performed on various concentrations of re-lipidated TF 1-243 in the presence of increasing concentrations of re-lipidated TF 104-243 (see the Materials and methods section). Initial rates of S2222 hydrolysis were measured and their reciprocals plotted against TF 104-243 concentration. Lines were fitted by a linear least-squares method.

with various concentrations of TF 104-243, and initial rates were determined by continuously monitoring the change in absorbance at 405 nm (Figure 7). Dixon plots demonstrated that the TF 104-243 polypeptide inhibited the procoagulant activity of the full-length TF 1-243 (K_i 36 nM).

DISCUSSION

Two indirect methods have been employed in previous studies to investigate structure-function relationships in TF. Ruf and co-workers [12], using antibody mapping of the TF extracellular domain, implicated two regions of the molecule in cofactor function and high-affinity binding of FVII. Non-overlapping epitopes of two inhibitory monoclonal anti-TF antibodies were mapped within the TF sequences Thr-106-Lys-165 and Thr-40-Val-83. These authors speculated that the ligand-binding domain on TF comprises either two remote sequences forming a complex contiguous region of the folded protein or two spatially separate areas which interact with different sites on the FVII molecule. In another approach FVII coupled to a photo-activatable cross-linking reagent was allowed to interact with and label TF [13]. Two regions of the TF extracellular domain were identified as cross-linking sites; an N-proximal site between residues 44 and 84 and a C-proximal site between residues 129 and 169. These studies support the hypothesis that at least two parts of the TF molecule are required for binding to FVII/FVIIa and expression of procoagulant activity.

Since steric effects could account for the inhibition seen with monoclonal antibodies, and long cross-linker-spacer-arm lengths could allow transfer of label to non-interactive parts of the receptor, we chose an independent direct method to probe structure-function relationships in TF and have identified three chymotrypsin-derived polypeptides which bind to FVII. Two of these exhibit weak TF procoagulant activity. The largest extends from position 35 to 243, binds to FVII in ligand blots and, on a molar basis, has approx. 7-8% of the procoagulant activity of the wild-type TF molecule. TF 77-243 also bound to biotinylated FVII and was minimally functional (3.39% of the activity of the wild-type). A third polypeptide, TF 104-243, bound to FVII, but had no cofactor activity. This smaller polypeptide inhibited the activity of the TF 1-243 molecule (K_i 36 nM), indicating that, while it bound to FVII, it was unable to promote FVII procoagulant activity. From this study it may be inferred that three regions of amino acid sequence, namely 1-34, 76-103 and

104-243, are required for TF activity. The sequence 104-243 is the minimum sequence that can support FVII binding, but this appears by semi-quantitative analysis to be of lower affinity than the full-length molecule. It is evident that the removal of the first 34 N-terminal residues is associated with the greatest absolute loss of procoagulant activity and FVII binding. This sequence has not been implicated in receptor-ligand binding by cross-linking or epitope-mapping studies, which identified an N-proximal (44-84) and a C-proximal site (129-169) in the hydrophilic extracellular domain [12,13]. The former sequence (44-48) is contained within the largest chymotryptic fragment that binds FVII in Cleveland gels, but is only marginally represented in the second fragment (77-103) and is not part of the smallest binding polypeptide (104-243).

It has been proposed that the TF extracellular domain comprises a pair of immunoglobulin-like β -strand-rich modules [18], based on sequence similarity to the cytokine receptor family [19], and that these may represent two separate interactive sites on the receptor. The structure of a member of this family of proteins, the human-growth-hormone receptor (hGHbp) has recently been solved in complex with the ligand, hGH [20]. The C-terminal β -strand module in hGHbp is involved in receptor dimerization, whereas the N-terminal domain is responsible for ligand binding. TF does not require dimerization for function, although it may form dimers through transmembrane domain interactions [21]. The results presented here suggest that, in contrast with hGHbp, TF binds FVII, at least in part, through the TF C-terminal domain.

Following functional analysis of the binding of des-(1-38)-FVII to TF it has been speculated that the FVII Gla domain interacts with an N-proximal TF site, whereas other FVII domains effect binding to the C-terminal module [13]. Data presented here show that the binding of FVII to TF and the expression of procoagulant activity is highly dependent on the presence of the first 34 amino acids of the mature TF molecule. It remains to be established whether this sequence is essential for enhanced binding of FVII to the C-terminal domain, maintenance of the folding of an N-proximal ligand-binding domain, or whether it represents a ligand recognition site *per se*. Both the C-terminal FVII binding site and the N-terminal part of the receptor are required for functional expression of the enzyme-receptor complex, since the binding of FVIIa to the isolated C-proximal domain is not, itself, sufficient to promote FX hydrolysis. Since TF expression occurs in inflammation and may be responsible for the fibrin deposition associated with atherosclerosis, the development of specific inhibitors of the TF-FVII interaction has important therapeutic potential. The data described here identify two distinct regions of the molecule that could be targets for therapeutic inhibition of TF procoagulant activity.

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