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Structure-function relationship of the interaction between tissue factor and factor VIIa

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Abstract

Interactions between tissue factor and factor VIIa are the primary initiators of coagulation in hemostasis and certain thrombotic diseases. Tissue factor, an integral membrane protein expressed extensively outside of the vasculature, is the regulatory protein cofactor for coagulation factor VIIa. Factor VIIa, a trypsin-like serine protease homologous with other blood coagulation proteases, is weakly active when free in solution and must bind its membrane-bound cofactor for physiologically-relevant activity. Tissue factor allosterically activates factor VIIa by several mechanisms such as active site positioning, spatial stabilization, and direct interactions with the substrate. Protein-membrane interactions between tissue factor, factor VIIa, and substrates all play critical roles in modulating the activity of this enzyme complex. Additionally, divalent cations such as Ca^{2+} and Mg^{2+} are critical for correct protein folding, as well as protein-membrane and protein-protein interactions. The contributions of these factors towards tissue factor-factor VIIa activity are discussed in this review.

Keywords

Tissue factor; factor VIIa; protein-membrane interactions; extrinsic tenase; metal ions

INTRODUCTION

The “extrinsic tenase” complex, comprised of tissue factor (TF) and factor VIIa (FVIIa), is a two-subunit enzyme that initiates the coagulation cascade under most *in vivo* conditions (depicted in Figure 1).[1,2] The regulatory subunit of this complex, TF (also known as thromboplastin, CD142, or coagulation factor III), is a cell-surface, transmembrane protein of the class II cytokine receptor family that is extensively expressed amongst adventitial and epithelial tissues; however, tissues exposed to the vessel lumen such as endothelial cells, platelets, and leukocytes constitutively express little or no TF.[3–5] The enzymatic subunit is a plasma protein, FVIIa, that is a trypsin-like serine protease demonstrating homology with several other coagulation proteins, including its cognate substrates factors IX (FIX) and X (FX). Total FVII (active enzyme and zymogen) circulates in plasma at a concentration of approximately 10 nM; however, only about 1% is in the active form.[6] Due to its poor

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enzymatic activity, free FVIIa in plasma largely escapes recognition by protease inhibitors and therefore circulates with a half-life of ~90 minutes.[7,8] The extended half-life afforded to FVIIa may serve an important function, as low basal concentrations of pre-formed FVIIa may serve to "prime" the coagulation cascade for a rapid response to injury.[6]

Under homeostatic conditions, TF and blood are physically separated, but vascular injury exposes plasma (which contains FVII and FVIIa) to a variety of TF-expressing cells. Once zymogen FVII binds to TF, it is rapidly converted to FVIIa by limited proteolysis, thereby generating the active TF-FVIIa complex. Formation of the TF-FVIIa complex greatly increases the enzymatic activity of FVIIa via allosteric interactions between TF and FVIIa, as revealed by about a 20- to 100-fold increase in the rate of hydrolysis of small, chromogenic peptidyl substrates (termed its amidolytic activity),[9,10] and nearly a million-fold increase in the rate of activation of the macromolecular substrates, FIX and FX.[11] Subsequently FIXa, in association with its regulatory subunit FVIIIa, further activates FX; FXa in turn complexes with its cofactor FVa, to proteolytically cleave prothrombin to thrombin, leading to fibrin clot formation. Widespread, constitutive expression of TF on cells surrounding organs, blood vessels, and skin serves to create a "hemostatic envelope" that initiates clotting upon vascular injury.[3]

An additional point of modulation of TF-FVIIa is via the phospholipid bilayer. In concert with allosteric activation of the active site of FVIIa upon binding to TF, formation of the extrinsic tenase on a suitably procoagulant phospholipid bilayer increases the rate of FIX or FX activation, in a Ca^{2+}-dependent manner, an additional 1,000-fold.[11] In fact, nearly all reactions of the coagulation cascade are reliant upon exposure of phosphatidyl-L-serine (PS) on membrane surfaces.[12] The roughly million-fold overall increase in FX activation by the TF-FVIIa-phospholipid complex relative to free FVIIa is critical regulatory point for the coagulation cascade.[13] The roles of TF-FVIIa in hemostasis, thrombosis and other biological processes are numerous, and the structural underpinnings comprising the foundation of their activities continue to be a point of focus and investigation.

STRUCTURE

Tissue Factor

TF, a 263 amino acid glycoprotein with a molecular weight of ~46kDa and member of the cytokine class II receptor family, is composed of three domains: a 219 amino acid N-terminal extracellular domain (residues 1–219, whose crystal structure is shown in Figure 1); a 22 amino acid transmembrane domain (residues 220–242); and a 21 amino acid cytoplasmic C-terminal tail (residues 242–263). The cytoplasmic tail contains two phosphorylation sites at Ser^{253} and Ser^{258}, and one S-palmitoylation site at Cys^{245}. Removal of the cytoplasmic domain has no deleterious effects on TF coagulant activity. The TF transmembrane domain is composed of a single-spanning alpha-helix, the precise identity of which has been shown unimportant for TF procoagulant function;[14] anchoring of a histidine-tagged extracellular domain of TF to the membrane using nickel-chelating lipids resulted in full restoration of procoagulant activity of TF.[15] The extracellular domain of TF (sTF) is composed of two fibronectin type III domains, and is connected to the transmembrane domain through a six-amino acid linker. This linker likely exhibits sufficient flexibility to

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conformationally decouple the extracellular domain of TF from the transmembrane and cytoplasmic domains.[14,16] The fibronectin type III domain structure, composed mainly of beta-strands connected by β-loops, is a member of the immunoglobulin-like family of protein folds and is conserved amongst a wide variety of extracellular proteins.[17]

The procoagulant activity of TF does not necessarily correlate with its levels of cell-surface expression. Much of the TF expressed on a cell surface is 'encrypted', and must first be 'decrypted' to participate fully in coagulation reactions. The process by which this occurs has yet to be fully explained, and is likely a combination of several mechanisms. One clear contributor is exposure of anionic phospholipids. Healthy cells actively sequester anionic phospholipids such as PS to the inner leaflet of the plasma membrane,[18,19] but following cellular damage, activation, or increased levels of cytosolic Ca^{2+} this bilayer asymmetry is lost, resulting in increased PS exposure on the outer leaflet which increases the specific activity of cell-surface TF-FVIIa complexes. PS exposure is well known to decrease the apparent K_m for activation of FIX and FX, but additional mechanisms could include conformational rearrangement of TF or the TF-FVIIa complex and subsequent exposure of substrate binding sites.[16,20] Expression levels of GRP78, a molecular chaperone protein, have also been shown to mediate TF procoagulant activity in a Ca^{2+}-dependent manner and thus may also play a role in its decryption.[21,22]

A fascinating suggestion is that disulfide linkages play a role in TF encryption/decryption, and in particular, that the membrane-proximal cysteine pair in TF (Cys^{186}-Cys^{209}) is an 'allosteric' disulfide that is subject to redox control, leading to TF encryption/decryption.[23–25] Others have disputed this conclusion, however, and have proposed alternative explanations for the observed effects (reviewed in [26,27]). Though the importance of TF disulfide bond formation towards its cofactor activity has yet to be resolved, FVIIa binding to TF is not dependent on the oxidation state of Cys^{186} and Cys^{209}.[23] A number of insightful and detailed reviews focus on controversies surrounding TF decryption are available.[28–32]

Factor VII/VIIa

The trypsin-like serine protease FVII (in the inactive precursor, or zymogen form) is a ~50KDa, single-chain polypeptide consisting of 406 residues, with an N-terminal γ-carboxyglutamate-rich (GLA) domain, two epidermal growth factor-like domains (EGF1 and EGF2), and a C-terminal serine protease domain.[33–36] Activation of FVII to FVIIa is accomplished via specific proteolytic cleavage of the Ile^{153}-Arg^{152} bond in the short linker region between the EGF2 and protease domain, with the resultant light and heavy chains held together by a single disulfide bond (Cys^{135}-Cys^{262}). The crystal structure of FVIIa is shown in Figure 1. FVII has significant structural and sequence homology to coagulation factors IX, X, and protein C.[37,38]

FVIIa binds the phospholipid membrane in a Ca^{2+}-dependent manner through its N-terminal GLA-domain. Containing 10 vitamin K-dependent, posttranslationally modified γ-carboxyglutamate (Gla) residues, GLA-domains coordinate 7–9 divalent metal ions such as Ca^{2+} and Mg^{2+}, inducing conformational rearrangements that are requisite for interaction with membrane surfaces.[39,40]

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Immediately C-terminal to the GLA domain is an aromatic stack and two epidermal growth factor (EGF) domains (EGF1 and EGF2). The aromatic stack connects the GLA to EGF1 domain, which binds a single Ca^{2+} ion with moderately high affinity.[41,42] Occupancy of this Ca^{2+}-binding site increases FVIIa amidolytic activity and TF association.[43] The FVIIa heavy chain comprises the trypsin-like protease domain, which is also homologous to other coagulation serine proteases such as FX, FIX, protein C, and prothrombin. The catalytic triad consists of His^{193}, Asp^{242} and Ser^{344}, and binding of a single Ca^{2+} ion within the FVIIa protease domain is critical for catalytic activity.[41,44] Additionally, proteolytic activation of FVII to FVIIa frees the newly formed amino terminus at Ile^{153} to fold back and insert into the activation pocket, forming a salt bridge with the carboxylate of Asp^{343} to generate the oxyanion hole.[45] Formation of this salt bridge is critical for FVIIa activity; indeed, FVIIa with the mutation V154G is cleaved to the two-chain form normally and with wild-type macromolecular substrate affinity,[46] but with significantly reduced ability of the resultant FVIIa to activate FX.[46,47] Reduced N-terminal hydrogen-deuterium exchange upon TF binding to FVIIa supports the hypothesis that the N-terminal Ile^{153} is not fully inserted into the activation pocket when free in solution.[48] Additionally, unlike most other serine proteases, oxyanion hole formation in free FVIIa does not occur upon proteolytic activation, but instead upon substrate interaction.[49] As a result, FVIIa circulates in a zymogen-like state that is poorly recognized by plasma protease inhibitors,[49] allowing it to circulate with a half-life of approximately 90 minutes.[7,8] This is far longer than other coagulation enzymes such as FIXa, FXa and thrombin, whose plasma half-lives are on the order of seconds to minutes.[50,51]

STRUCTURE-FUNCTION RELATIONSHIP

Association of TF with FVIIa allosterically activates the protease, creating what is essentially a dimeric enzyme in which TF is the regulatory subunit and FVIIa the catalytic subunit. The ability to cleave very small, tripeptidyl-amide substrates (amidolytic activity) of FVIIa is increased approximately 50-fold upon binding of TF, with the largest change being increased k_{cat},[9,10] indicating that TF association induces conformational changes within the active site of the FVIIa protease domain.[45,52–54] Additionally, pKa values of the catalytic triad are altered upon TF binding.[55] It has been hypothesized that FVIIa in solution exists in an equilibrium between two states; one in which the heavy-chain N-terminus has inserted into the active site pocket, and one that is more zymogen-like with the N-terminus incorrectly or incompletely inserted. TF may preferentially bind FVIIa when it is in the catalytically active form,[45] shifting the equilibrium towards the active (N-terminal buried) conformation. Hydrogen-deuterium exchange experiments coupled with mass spectrometry have demonstrated that several loop regions within the protease domain of FVIIa are stabilized upon binding to TF, through rearrangement and strengthening of an extensive hydrogen bonding network.[48,52] These structural changes are not limited to the protease domain but extend throughout most of FVIIa,[48] indicating widespread allosteric modulation of FVIIa by TF.

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Active Site Positioning

In vivo enzymatic activity of the TF-FVIIa complex occurs exclusively on the phospholipid membrane surface, and is dependent upon the interaction of FVIIa, FIX and FX with the membrane through their membrane-binding GLA-domains. Fluorescence resonance energy transfer (FRET) experiments indicate that free FVIIa adopts a stable, extended structure when bound to the membrane, with its active site positioned ~80 Å above the membrane surface.[56] This distance is in good agreement with those seen for the homologous proteins FIXa[57] and FXa[58]. Upon FVIIa binding to TF, the FVIIa active site is repositioned ~6 Å closer to the membrane, a modulation that may aid in proper alignment of the FVIIa catalytic triad with the target substrate cleavage site.[56] In comparable FRET experiments using GLA-domainless FVIIa, the active site was still positioned a similar distance above the membrane, demonstrating that TF is able to fully support FVIIa active site positioning even in the absence of FVIIa-membrane interactions.[59] Further, experiments using multiple approaches have shown that TF supports full FVIIa proteolytic activity as long as the TF extracellular domain is tethered in some way to the membrane surface, while the exact nature of this membrane tether is essentially irrelevant.[14,15,60] In contrast, raising the active site of FVIIa greater than 80 **Å** above the membrane surface using TF/P-selectin chimeras greatly reduced the ability of the TF-FVIIa complex to activate FX, but did not diminish TF-FVIIa amidolytic activity. This indicates that TF-mediated positioning of the FVIIa active site above the membrane surface is important for its activity towards cognate substrates.[61]

Molecular dynamics (MD) simulations of TF-FVIIa in the presence of membrane surfaces indicate TF reduces FVIIa inter-domain flexibility. Both in solution and on the membrane surface, the hinge-like motion of FVIIa and its Cα RMSD values are significantly reduced in the presence of TF.[16]

Spatial Stabilization

Free FVIIa is an inherently dynamic molecule, with MD simulations, fluorescence anisotropy, and hydrogen-deuterium exchange data indicating intra- and inter- domain flexibility.[16,52,62–64] A major component of TF allosteric modulation of FVIIa activity is the stabilization and reduced flexibility of FVIIa upon TF binding. In the protease domain, stabilization of the 170-loop located near the TF interaction site appears to be important for FVIIa amidolytic activity.[63,64] Replacement of the loop with that of a similar but truncated loop from trypsin results in an increase in FVIIa amidolytic activity even in the absence of TF, suggesting that stabilization of the 170-loop plays a significant role in TF-mediated allosteric activation of FVIIa.[65] Additionally, hydrogen exchange experiments and MD simulations show that TF binding aids the insertion of Ile^{153} into the activation pocket of FVIIa and stabilizes its structure, even after removal of the N-terminal insertion.[52,63] Specific TF and FVIIa residues have been identified that contribute to stabilization of FVIIa within the TF-FVIIa complex. Alanine scanning mutagenesis studies[66] and crystallography data[41] demonstrated that Met^{306} in FVIIa plays a pivotal role in TF interactions, restricting the flexibility of FVIIa's 170-loop upon FVIIa-TF complex formation. The crystal structure of free FVIIa,[67] in which the 170-loop and precluding α-helix (containing Met^{306}) are more disordered, also supports this idea.

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TF-Substrate Interactions

In addition to the role of TF in allosterically activating FVIIa, binding interactions between the 'exosite' region of TF and macromolecular substrates are also implicated in TF-FVIIa catalytic activity (shown in Figure 1C). The known physiologic substrates of TF-FVIIa are FVII, FIX, FX and certain protease activated receptors (PARs). TF mutational analysis has identified a number of residues that, when mutated, support full FVIIa amidolytic activity towards small peptidyl substrates but are deficient in their ability to support macromolecular substrate (FVII, FIX, FX) activation.[68–70] Several crystal structures have shown disorder in the TF loop region at residues 159–165,[41,49] and residues in or adjacent to this flexible loop have been shown to be especially critical for proteolytic activity of the TF-FVIIa complex, thereby defining the proposed substrate-binding exosite region of TF that is quite distant from the FVIIa active site.[68,69] Interestingly, mutation of Gly^{164} of TF to a marginally more bulky alanine significantly impairs TF-FVIIa proteolytic activity, suggesting the flexibility afforded by glycine is critical for macromolecular substrate recognition.[68,70]

TF residues Lys^{165} and Lys^{166} have also been demonstrated to be important for substrate recognition and binding; mutation of either of these residues to alanine results in a significant decrease in the cofactor function of TF.[68,69,71,72] However, TF with mutations at K165A and K166A activated GLA-domainless FX at rates comparable to that of the wild-type TF, and utilization of GLA-domainless FVIIa greatly muted the effects of these TF mutations on FX activation.[69] Crystal structures have indicated that Lys^{165} and Lys^{166} face away from each other, with Lys^{165} pointing towards FVIIa in most TF-FVIIa structures, and Lys^{166} pointing into the substrate binding exosite region.[41,73] Putative salt bridge formation between Lys^{165} of TF and Gla^{35} of FVIIa would support the notion that TF interaction with the GLA-domain of FVIIa modulate substrate recognition.[73] Taken together, these results suggest that the C-terminal portion of the TF ectodomain directly interacts with the GLA-domains (and possibly the adjacent EGF1 domains) of FIX and FX, and that the presence of the FVIIa GLA-domain may modulate these interactions, either directly or indirectly. Furthermore, the TF residues involved in substrate interactions with FIX and FX are similar or identical to those that interact with FVII during TF-mediated FVII autoactivation,[53] indicating a similar mechanism of substrate binding.

A number of monoclonal anti-TF antibodies have been raised, with the vast majority blocking association between TF and FVII.[74] However, two monoclonal antibodies, TF8-5G9 and TF8-11D12 (which came from the same fusion and which are probably identical) were shown not to inhibit TF-FVIIa binding, but to strongly inhibit activation of FIX and FX by TF-FVIIa.[75,76] Crystal structures of TF in complex with the antibody TF-5G9[77] indicates that the epitope on TF for this antibody overlaps significantly with the substrate interaction (exosites) region identified by Kirchhofer et al.[70] More recently, two additional monoclonal anti-TF antibodies (D3 and 5G6) with similar properties have also been reported.[78]

Monoclonal antibody TF9-10H10 binds to TF but does not inhibit its procoagulant activity, which is unusual as almost all anti-TF antibodies are inhibitory.[74] More recent studies using this antibody have shown that it does inhibit the ability of the TF-FVIIa complex to participate in signaling.[79] These results suggest that TF-FVIIa signaling via integrins and

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PAR-2 is mediated by exosite-like interactions on TF distinct from those involved in TF-FVIIa procoagulant functions.

Protein-Membrane Interactions

Lipid bilayer composition plays an important role in activity of the TF-FVIIa complex. GLA-domain containing coagulation proteins are well-known to preferentially bind anionic phospholipids in general, and PS in particular.[12] PS is, however, actively sequestered to the inner leaflet of the plasma membrane, serving as an important point of regulation of blood clotting.[12,18,19,80] Exposure of PS on the outer leaflet occurs either via physical disruption of the cell membrane as a consequence trauma, or via regulated cellular processes such as those that occur upon platelet activation. Interestingly, despite the high degree of sequence homology between GLA-domains of different clotting proteins, their membrane binding affinities vary by three orders of magnitude,[81,82] with FVIIa and activated protein C (APC) displaying the weakest affinities for PS-containing bilayers. We recently reported that FVIIa and APC preferentially bind to bilayers containing phosphatidic acid (PA), a minor anionic lipid in cell membranes.[83] PA has minimal effect on TF-FVIIa activity *in vitro*, however, likely due to the fact that protein-protein interactions between TF-FVIIa dominate the recruitment of FVIIa to the membrane.[84] Further, when used pharmacologically to treat bleeding disorders, the mechanism of action of recombinant FVIIa has been shown to be independent of TF.[85,86] Thus, the membrane binding characteristics of “free” (non-TF bound) FVIIa may be an important component of its *in vivo* efficacy, especially when high concentrations of recombinant FVIIa are employed to treat bleeding.

Direct interactions between the TF ectodomain and the membrane surface may also contribute to the activity of the TF-FVIIa complex. It is thought that there is considerable freedom of motion and autonomy of the TF ectodomain relative to the membrane due to the structural flexibility of the short peptide linker between the ectodomain and the transmembrane domain.[14] Furthermore, MD simulations have identified a number of TF residues in the C-terminal portion of the ectodomain that directly contact the phospholipid membrane surface. These residues maintained association of sTF (i.e., the isolated ectodoman) with the membrane surface, suggesting that TF residues may associate directly with PS headgroups.[16] Additionally, simulations suggest the orientation of TF with respect to the membrane is altered upon FVIIa binding, with TF leaning toward FVIIa. As a result, the TF residues in contact with the phospholipid membrane are proposed to change.[16] This region of putative membrane-interacting TF residues is immediately adjacent to the proposed substrate-binding exosite, and alanine scanning mutagenesis studies have identified mutations in this region that alter the ability of membrane-anchored TF-FVIIa to activate FX.[20] Interestingly, increasing the PS content of TF-liposomes partially overcomes these deficiencies, suggesting that direct PS-TF interactions may either stabilize the complex or induce ideal conformational arrangements, promoting interaction of FIX and FX with the TF exosite.[16,20]

Biochemical studies of protein-membrane interactions in blood clotting often utilize liposomes with non-physiological membrane compositions. Thus, it typically requires 30% or more PS to achieve maximal TF-FVIIa enzymatic activity *in vitro*,[87] while only ~10% of

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the total plasma membrane bilayer is composed PS in eukaryotic cells.[88] Incorporation of phosphatidylethanolamine (PE, a plasma membrane phospholipid that is much more abundant than PS) into TF-liposomes markedly decreases the required PS content, although PE by itself does little to promote TF activity.[87] Other lipids such as phosphatidic acid, phosphatidylglycerol, and phosphatidylinositol also reduce the PS requirement, indicating 'synergy' between PS and PE is not a unique property of PE but a more broadly encompassing mechanism. This 'ABC hypothesis' (Anything But Choline) postulates that any lipid not containing the bulky choline headgroup of PC or sphingomyelin can 'synergize' with PS and decrease the required PS content for maximal enzymatic activity.

Divalent Metal Ions

Calcium ions are required for assembly and function of TF-FVIIa. Although TF lacks any known divalent metal ion binding-sites, Ca^{2+} can occupy up to nine metal binding sites within FVIIa (Figure 1). Of these, seven reside in the GLA-domain of FVIIa and are critical for both structure and function of this domain. In particular, Gla^{7} and Gla^{9} coordination of Ca^{2+} induces formation of the ω-loop, which exposes hydrophobic residues that are believed to insert into the bilayer.[87,89–91] Recent crystallographic, enzymatic, and equilibrium dialysis studies have indicated that the FVIIa GLA-domain actually binds a combination of 4–5 Ca^{2+} and 2–3 Mg^{2+} under physiologic divalent metal ion conditions.[49,73] Ca^{2+} is absolutely required for GLA-domain structure and function, and can occupy all GLA-domain metal binding sites when it is the only divalent metal ion present (especially at supraphysiologic concentrations of Ca^{2+}), but Mg^{2+} alone is unable to induce the correct GLA-domain structure. This suggests that a subset of metal binding sites in GLA domains are occupied with Mg^{2+} *in vivo*,[40,73] and occupancy of these sites by Mg^{2+} even in the presence of vast excesses of Ca^{2+} suggest these sites preferentially bind Mg^{2+} in plasma.[49,73] The reasons for differential metal ion specificity of GLA domains remain unclear, but are likely due to differences in coordination geometries of each binding site along with the 'hardness' properties of Ca^{2+} *versus* Mg^{2+}.[73,92] Mg^{2+} has been demonstrated to modulate both the membrane binding[73] and enzymatic properties[93] of FVIIa and FIX[94].

One Ca^{2+} binds to the EGF1 domain of FVIIa, for which Mg^{2+} cannot substitute. [49] This Ca^{2+} is implicated in optimizing TF-FVIIa binding interactions, likely through modulating the orientation of the FVIIa GLA-domain relative to the EGF1 domain.[95] The protease domain of FVIIa also contains one Ca^{2+} binding site; its occupancy results in allosteric activation through reregistration of the Ca^{2+} binding loop.[52] Additionally, two Zn^{2+} binding sites have been identified in the protease domain, although Zn^{2+} occupancy of these sites inhibits both FVIIa enzymatic activity and TF binding.[49,96]

The Ca^{2+} binding sites in FVIIa are incompletely saturated at plasma concentrations of free Ca^{2+} (~1.25 mM).[97,98] However, using the plasma concentrations of free Ca^{2+} and Mg^{2+} (~1.25 mM and ~0.6 mM, respectively) restores activity to maximal levels, indicating that Mg^{2+} likely plays a role *in vivo*.[73,93,94,99] The GLA-domains of both FVIIa and FX mediate enzymatic rate enhancements due to Mg^{2+}, suggesting that conformational changes of the FX GLA-domain upon Mg^{2+} occupancy of metal binding sites modifies its interactions with TF.[99]

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Signaling

The role of TF in cellular signaling is incompletely understood, though it is known to play roles in tumor growth,[79] metastasis,[100,101] angiogenesis, and anti-apoptotic signaling;[102] additionally, TF is known to be upregulated in many malignant tumor cell types.[103–105] FVIIa binding to TF has been implicated in directly mediating PAR-2 cleavage via mechanisms both dependent[106–109] and independent[110,111] of the cytoplasmic domain of TF.[112] Phosphorylation of the TF cytoplasmic domain subsequent to TF-FVIIa cleavage of PAR-2 is thought to release TF-dependent negative regulation of PAR-2 mediated signaling.[79,103,108] This results in mitogen-activated protein kinase (MAPK) pathway activation and downstream effects, including the upregulation of cytokines and proangiogenic factors.[103,113] Activation of the p44/42 MAPK pathway, as well as JAK2, p70/p85^{S6K}, and p90^{RSK}, can occur independent of the TF cytoplasmic domain.[110,114,115] Though the cytoplasmic domain is not necessary for activation of these proteins, proteolytically active FVIIa is required. In addition, TF can also mediate signaling through the enzymatic activity of FXa in complex with TF-FVIIa, which plays an important role in the regulation of several pathways. TF-FVIIa-FXa-mediated cleavage of PARs, particularly PAR-2, has been shown to up-regulate IL-8 expression, resulting in increased cell migration.[101] A number of detailed reviews can be found regarding TF-FVIIa-mediated signaling.[101,103,116,117]

CONCLUSION

The interaction between TF and FVIIa is a critical component of hemostasis. TF is expressed extensively on cells outside the vasculature, creating a ‘hemostatic envelope.’ Disruption of the blood vessel endothelium results in exposure of blood coagulation proteins, including FVIIa, to TF-bearing cells. Subsequent to TF-FVIIa complex formation, two blood clotting zymogens, FIX and FX, are proteolytically activated, propagating the coagulation cascade and resulting in fibrin deposition and clot formation. The physical separation of TF from the plasma clotting enzymes is undoubtedly a regulatory mechanism; without its protein cofactor, FVIIa is a very weakly enzyme and will not initiate coagulation at physiologic concentrations. Expression of TF inside the vasculature results in aberrant coagulation cascade activity and is believed to play an important role in many thrombotic disorders.

TF modulates FVIIa activity through a number of mechanisms. FVIIa interaction with TF has been demonstrated to position its active site ~75 Å above the membrane surface, ~6 Å closer than FVIIa in the absence of TF. Additionally, MD simulations have suggested that TF restricts both FVIIa inter- and intra- domain flexibility, particularly within the protease domain. TF has been shown to stabilize a number of loop regions in the protease domain of FVIIa as well as facilitate the insertion of the N-terminus of the FVIIa heavy chain into the activation pocket, which is critical for its enzymatic activity. In addition, an extended substrate binding exosite has been identified on TF, which has been shown to interact directly with both extrinsic tenase complex substrates, FIX and FX. The importance of the phospholipid membrane in mediating the activity of TF-FVIIa cannot be understated; recruitment of FX to the membrane surface is dependent upon the exposure of anionic

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phospholipids, particularly PS, which may interact with substrates (FIX and FX), enzyme (FVIIa) and protein cofactor (TF). All the interactions between TF, FVIIa, protein substrates and membrane surfaces are dependent upon divalent cations. Although several structures of TF-FVIIa have been solved, as of yet no tertiary TF-FVIIa-FX or TF-FVIIa-FIX structures have been determined, nor has the role of the membrane surface in the formation of these complexes been determined structurally. Such results would provide significant information regarding the structure-function relationship of the extrinsic tenase in blood clotting.

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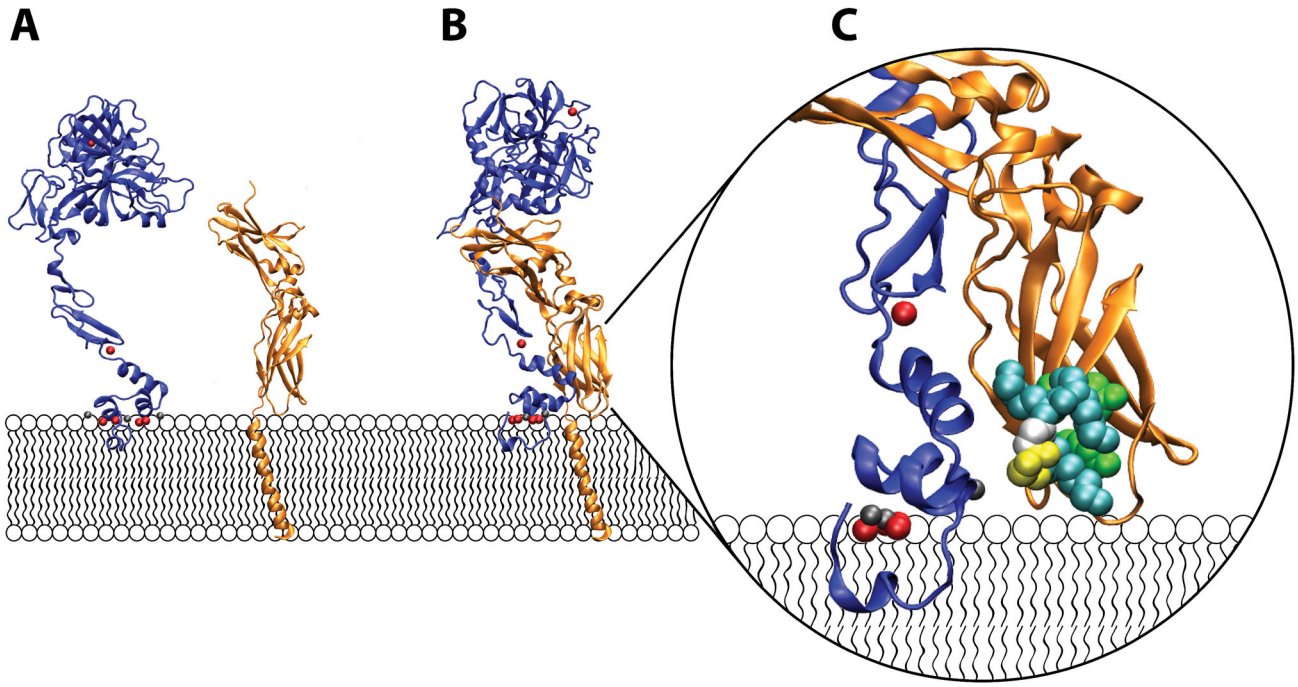

Figure 1.

Crystal structures of FVIIa and sTF arranged on a membrane surface. A) FVIIa (blue) has an extended conformation and binds to anionic phospholipids in membrane bilayers through its N-terminal GLA-domain (depicted here in contact with the membrane). Coordination of divalent cations such as Ca^{2+} (red spheres) and Mg^{2+} (gray spheres) by the GLA domain is critical for proper domain folding and function. In addition, a Ca^{2+} ion is bound to the first EGF-like domain of FVIIa and also to the protease domain of this protein (the domain farthest from the membrane). The isolated ectodomain of TF (sTF, orange) is depicted here as anchored to the membrane surface via a single transmembrane helix, which has been modeled in. Full-length TF also contains a 21 amino acid-long cytoplasmic tail (not shown) which is implicated in interactions with the cytoskeleton. B) Crystal structure of the sTF-FVIIa complex, with the transmembrane helix of TF modeled in. FVIIa interacts extensively with sTF, with a binding interface that spans all domains of FVIIa and sTF. C) Close-up of TF residues putatively involved in substrate recognition (i.e., the substrate-binding exosite region of TF). In addition to allosterically activating FVIIa, TF is thought to directly interact with the protein substrates, FIX and FX, through membrane-proximal residues. Thus, TF residues Tyr157, Lys159, Ser163, Gly164, Lys165, Lys166, and Tyr185 (shown as van Der Waals radii and colored according to identity as follows; Teal: Lysine, White: Glycine, Yellow: Serine, Green: Tyrosine) contribute significantly to interactions with substrate as demonstrated by mutagenesis studies. (Panel C is rotated ~45° from panel B.) The structure of the sTF-FVIIa complex in panels B and C is rendered from pdb file 3TH2[73] using VMD Molecular Graphics Viewer.[118] The isolated structures of FVIIa and sTF shown in panel A are a separation of the two from the TF-FVIIa complex. The transmembrane helix attached to sTF is adopted from pdb file 1A11.[119]