[Frontiers in Bioscience 16, 3156-3163, June 1, 2011]

Allosteric activation of coagulation factor VIIa

Egon Persson, Ole Hvilsted Olsen

Haemostasis Biochemistry, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Maaloev, Denmark

TABLE OF CONTENTS

1. ABSTRACT

Coagulation factor VIIa (FVIIa) is present at subnanomolar concentration and represents a small percentage of the total amount of FVII in the circulation. FVIIa is poised to initiate blood clotting when it encounters its pivotal cofactor tissue factor (TF) which becomes exposed to blood upon vascular rupture. The requirement for complex formation with TF in order for FVIIa to express procoagulant activity ensures thrombin and fibrin generation at the right time and place. Thus TF acts as a guardian of safety of paramount importance to blood coagulation by providing localization to the site of injury and at the same time inducing maturation of zymogen-like free FVIIa to the active cofactor-bound enzyme. This review gives an account of the accumulated knowledge about the structure, function and TF dependence of FVIIa to arrive at a plausible allosteric mechanism by which TF induces maturation of the active conformation of FVIIa.

2. INTRODUCTION

The complex between FVIIa and TF initiates blood coagulation upon perforating injury to a blood vessel by activating the zymogens factor IX (FIX) and X (FX). The activated factors are key catalysts in the ensuing amplification and propagation phases of the blood coagulation cascade, which results in the formation of a fibrin mesh that stabilizes the haemostatic plug (1-5). In some pathological conditions, blood cells are also stimulated to express TF. In addition, FVIIa-TF complex formation results in intracellular signaling which leads to cell migration and angiogenesis through cleavage of protease-activated receptors, events that also require the proteolytic activity of FVIIa (5-8).

FVIIa consists of an N-terminal, membrane-interactive domain rich in gamma-carboxyglutamic acid (Gla) residues, two domains homologous to the epidermal

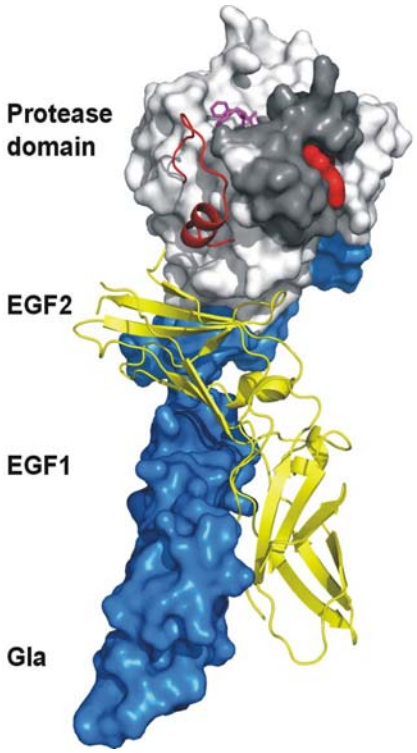

Figure 1. Overview of the structure of the FVIIa·TF complex (Protein Database entry code: 1dan). The structure of FVIIa is shown in a surface-rendered representation (blue: light chain, grey: protease domain (heavy chain)), except for the TF-binding helix and 170 loop which are shown as a ribbon model in red. The darker grey area shows the activation domain and the red surface represents the inserted N-terminal tail. A covalent inhibitor (fFR-cmk, magenta stick model) occupies the active site. The domain names are given to the left. The structure of TF is shown as a yellow ribbon model.

growth factor (EGF) and a disulfide-linked C-terminal serine protease domain constituting a separate polypeptide chain (9). The separation into two chains, a light and a heavy (the protease domain), and the concomitant generation of a second, new N-terminus occur upon activation of FVII to FVIIa. The light chain anchors FVIIa to cell surface TF (Figure 1), whereas the protease domain provides exosites that make essential contributions to define substrate specificity and contains the active site machinery (10,11). It has been hypothesized that TF recognition by the protease domain is the first step in the formation of an active complex (12,13) followed by light chain-mediated consolidation of the FVIIa-TF complex. Under all circumstances, tethering to TF positions the protease domain at the appropriate distance above the membrane surface (14,15), which is important to allow the interactions with TF that induce enzyme maturation (Figure 2) and to facilitate subsequent macromolecular substrate cleavage, including autoactivation (16). In addition, as a consequence of the extensive interactions between FVIIa and TF, the global structural fluctuations in the FVIIa molecule are reduced upon complex formation with the much more rigid cofactor, something that probably aids substrate recognition and processing (17-19). The protease domain of FVIIa is unique compared with that of homologous proteases, such as the archaetype enzyme trypsin (20), by its striking inability to spontaneously insert its N-terminus into what is termed the activation pocket and consequent failure to gain intrinsic activity. The biological activities of FVIIa, i.e. the FVIIa-catalyzed proteolytic cleavages, totally depend on the ability of TF to eventually facilitate the insertion of the N-terminus of the protease domain (21). Here, we present our current understanding of the underlying allosteric mechanism by which TF exerts its stimulatory effect in the light of extensive structural and functional characterization of FVIIa alone and bound to TF. Other important cofactors for FVIIa, such as metal ions (primarily Ca^{2+}), are not explicitly mentioned but assumed to be present at optimal concentrations (22). Ca^{2+} binding to FVIIa is crucial for the physiological interaction with cell surface TF (both the phospholipid surface and the TF protein itself). Amino acid residues are identified by both FVIIa and chymotrypsinogen numbering throughout the article (the latter in brackets with a prefix c).

3. ALLOSTERIC ACTIVATION OF FVIIa BY TF

3.1. Structural contributions to our understanding of the allostery

Free FVIIa circulates in a zymogen-like state with very low intrinsic enzymatic activity. Moreover, FVIIa has a relatively low affinity for procoagulant membrane surfaces. Therefore, FVIIa needs TF to provide both stimulation and localization in order to express biological activity. It is obvious that the integral membrane receptor TF, with its high affinity for FVIIa, acts as a magnet and mediates localization of FVIIa to anionic, procoagulant membranes. It is also well established that TF stabilizes the active form of FVIIa and thereby shifts the conformational equilibrium of FVIIa away from the zymogen-like state. The latter is lucidly illustrated by measuring the activity of FVIIa using a low-molecular-weight, chromogenic substrate in the absence and presence of the extracellular domains of TF (23). However, the means by which TF accomplishes this is not known in detail.

The X-ray crystallographic structure of the complex between FVIIa and TF shows the active FVIIa conformation and pinpoints the contact points between the two proteins (Figure 1), including the interactions potentially responsible for the allosteric effect of TF on the protease domain of FVIIa (24-26). The involved FVIIa residues (together with others) are also picked up in a comprehensive alanine scanning mutagenesis study (27). However, in order to identify the TF-induced changes in the FVIIa conformation one needs a snapshot of free FVIIa in the zymogen-like state for comparison. The available crystal structures of FVIIa alone are all obtained in the presence of an active site inhibitor, covalently attached (28,29) or reversible (30), which is unfortunate because many inhibitors, just like TF, stabilize the active conformation of the protease domain of FVIIa. This includes facilitation of the insertion of the N-terminus and

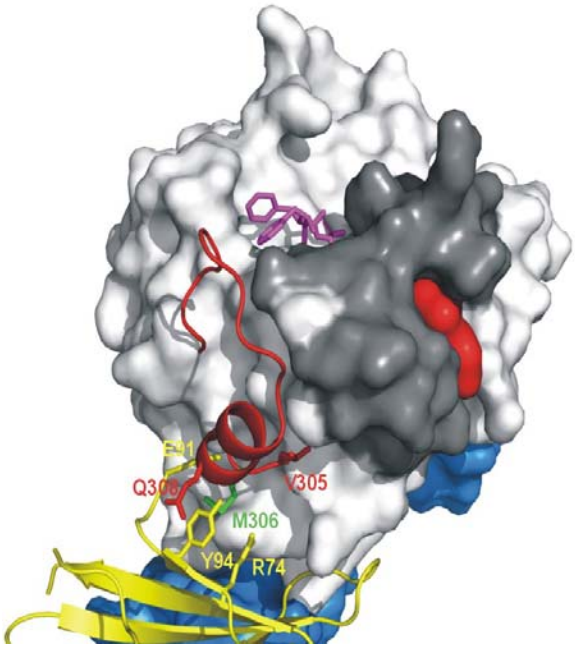

Figure 2. Close-up of the protease domain of FVIIa and part of its interface with TF. The protease domain sidechains Val-305 {c163}, Met-306 {c164} and Gln-308 {c166}, and TF sidechains Arg-74, Glu-91 and Tyr-94 are shown as stick models. The models were drawn using PyMol v0.99.

formation of a stable salt bridge between the terminal amino group and Asp-343 (31), as well as stabilization of the 170 and 99 loops (32,33) and the preceding TF-interactive region which results in an increased TF affinity of inhibited FVIIa (34,35). Indeed, labeling of TF with spectroscopic probes at positions known to interact with FVIIa has shown that active site inhibitors affect the gross conformation of the protease domain of FVIIa in a way which is sensed by the probes as an environmental change at the interface (36,37). Furthermore, when looking more broadly at the conformation of FVIIa by measuring hydrogen exchange kinetics, it appears that TF and an active site inhibitor have very similar global effects on the protease domain (33). Even in the case of benzamidine, which is not one of the powerful stabilizers of the active conformation, the crystal structure of free FVIIa is very similar to that of TF-bound FVIIa. Moreover, primarily local changes are observed when benzamidine is soaked out of the FVIIa crystal, whereas the salt bridge involving the inserted N-terminus, a hallmark of the catalytically competent conformation, persists (30). The explanation for this is presumably found in the crystal packing. In conclusion, a large amount of data corroborates an inhibitor-induced active conformation and explains why structures of free (inhibited) and TF-bound FVIIa are barely distinguishable. Available structures do not reveal the conformation of zymogen-like FVIIa, nor do any structural comparisons expose the changes in the protease domain induced by TF association.

A FVIIa activation mechanism based on the structure of zymogen FVII, involving the reregistration of beta strands, has been proposed (38). This mechanism builds on an observed 3-residue shift toward the C-terminus of beta strand B2 in FVII, a process facilitated by a duplicated Leu-X-Val motif at positions 297-302 {c155-c160}. This movement would seriously affect the interaction with TF by altering the TF-interactive region of the FVIIa protease domain and introducing steric clashes. Moreover, the N-terminal tail is kept out of the activation pocket by hydrogen bonds with Glu-296 {c154}. However, details concerning the crystal packing make the structural interpretation ambiguous (39). Moreover, data from mutagenesis and hydrogen exchange studies of FVIIa have failed to support the reregistration hypothesis (33,40) and it is not discussed further.

3.2. Functional contributions to our understanding of the allostery

Until the zymogen-like FVIIa structure becomes available, our knowledge of the allosteric activation mechanism employed by TF is based on functional and indirect structural information. This eagerly longed for structure might turn out to be evasive due to great inter- and intradomain flexibility, including disorder within the protease domain, and possible degradation of FVIIa in the absence of an inhibitor. One should bear in mind that the successful crystallizations of FVIIa, with and without TF, have all been done in the presence of an inhibitor. The crystal structures of the FVIIa-TF complex show interactions between the cofactor and areas centered at Arg-277 {c134} and Met-306 {c164}/Asp-309 {c167} in the protease domain. There is little doubt that Met-306 {c164} is the entry port for the influence of TF on the conformation and activity of FVIIa, even though both contact areas are needed for optimal stimulation of FVIIa (27). Of the three residues in the protease domain of energetic importance for TF binding, only Met-306 {c164} mediates an interaction required for the appropriate communication between TF and the active site of FVIIa or *vice versa* (34,41). When Met-306 {c164} in FVIIa is replaced by an aspartic acid residue, the effects of TF on the active site, the 170 loop and the N-terminus of the protease domain as well as the effect of an active site inhibitor on FVIIa's affinity for TF are virtually abolished (41,42).

The allosteric FVIIa activation journey starts at Met-306 {c164}, which fits into a small surface cavity on TF (Figure 2), and ends with maturation of the substrate-binding cleft and active site (Figure 3, path I and II, respectively).

3.2.1. Path I

The adjacent TF-binding helix (residues 307-312 {c165-c170}) with its own interactions with TF is stabilized upon complex formation (step I). This has been inferred from a structural comparison of free and complexed FVIIa (28). Several lines of evidence suggest that the stabilizing effect is transmitted to the neighboring 170 loop (residues 313-321 {c170A-c170I}, step II). This loop is shorter in trypsin, thrombin, FXa and FIXa than in FVIIa, and it constitutes an extra button that needs to be pushed in order to activate this particular enzyme. A FVIIa

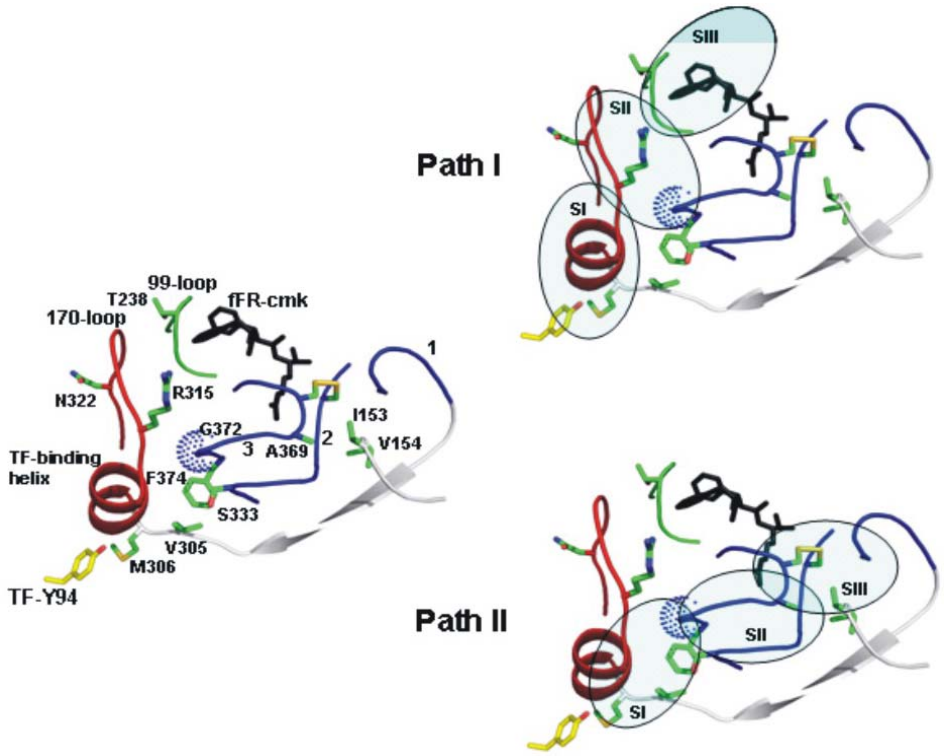

Figure 3. Graphical illustration of the structural elements involved in the proposed allosteric pathways. Left: Tyr-94 of TF (TF-Y94, yellow) depicts the eliciting interaction with TF. The TF-binding helix and the 170 loop are shown in red, the 99 loop in green and the three activation loops, constituting the activation domain, in blue. The β strand connecting activation loop 1 to the TF-binding helix is in grey. The active-site inhibitor fFR-cmk (in black) interacts with the 99-loop and defines the location of the S3 to S1 substrate binding sites. Sidechains referred to in the text are shown as stick models (green carbon atoms). Right, top: Path I, the allosteric maturation of the substrate binding cleft divided into three steps (SI-SIII, see text). Right, bottom: Path II, the allosteric maturation of the catalytic apparatus divided into three steps (SI-SIII, see text). FVIIa residues: 153 {c16}, 154 {c17}, 238 {c98}, 305 {c163}, 306 {c164}, 315 {c170C}, 322 {c175}, 333 {c185}, 369 {c221A}, 372 {c223}, 374 {c225}. The models were drawn using PyMol v0.99.

variant in which the shorter loop from trypsin was substituted for the native loop indeed possessed enhanced activity (43). A hydrogen bond between the 170 loop (Arg-315 {c170C}) and activation loop 3 (Gly-372 {c223}) of the activation domain is stabilized by TF (32). This aligns and tethers the TF-binding helix and 170 loop to the body of the protease domain and leads to maturation of substrate subsites S1-S3 (step III) as evident from a stabilization of the 99 loop (33) and an increased affinity for low-molecular weight substrates and the S1 probe inhibitor *p*-aminobenzamidine (44,45).

3.2.2. Path II

The adjacent Leu-305 {c163} and its interaction partner Phe-374 {c225} are stabilized in favorable orientations (step I). The positioning of Phe-374 {c225} induces a conformational change in activation loop 3 (residues 365-372 {c215-c223}) located in its vicinity, which in turn influences activation loop 2 (residues 331-342 {c188A-c193} containing the oxyanion hole), the activation pocket and the S1 subsite (step II). Gly-372 {223} in activation loop 3 appears to play a pivotal part as an interaction hub because TF binding stabilizes main-chain hydrogen bonds to Ser-333 ({c185}. activation loop 2) and Arg-315 ({c170C}, 170 loop) (46). The N-terminus (Ile-153 {c16}) can now insert into the activation pocket and establish an electrostatic interaction with Asp-343 {c194}, which further stabilizes the activation domain (step III). A link is formed between Val-154 {c17} and Ala-369 {c221A}, connecting the N-terminus to the activation pocket, and it continues to the S1 pocket through the Cys-368-Cys-340 {c220-c191} disulfide bond (32). This eventually results in well-defined substrate recognition subsites and oxyanion hole in a catalytically competent FVIIa conformation.

The two routes appear to spread in slightly different directions from Met-306 {c164}, their common point of origin. However, they are interdependent and overlap in space. For instance, the 170 loop is locked in the 'active' conformation by a combined effort from both pathways and the 340-368 {c191-c220} disulfide bond mediates a certain degree of communication.

TF binding presumably not only influences FVIIa's activation domain, as evident from the insertion of N-terminus, and the recognition of substrate residues adjacent to the scissile peptide bond. There are indications

that TF binding also is linked to the specificity-determining macromolecular substrate exosites on FVIIa (10,11), defined as areas critical for protein substrate processing but without a direct involvement in or effect on TF binding as judged by alanine substitutions (27). More precisely, the binding of a monoclonal antibody with an epitope on FVIIa that contains Glu-296 {c154} and overlaps with an exosite was affected by TF binding (as well as by active site occupancy and zymogen activation) (47,48). An alternative explanation is that antibody binding is indirectly influenced when the salt bridge between the N-terminal Ile-153 {c16} and Asp-343 {c194} is formed, i.e. by N-terminal insertion, whose stability in turn would be influenced by antibody binding.

4. PERSPECTIVE

The structure of free and uninhibited FVIIa (or FVII) has not been solved and the conformation of zymogen-like FVIIa (and zymogen FVII) remains unknown. There is little doubt that free FVIIa is in equilibrium between a zymogen-like and an active conformation, perhaps with participation of relatively stable intermediate conformations, but with a great majority of FVIIa molecules in the zymogen-like state. Perhaps a corresponding equilibrium exists for zymogen FVII. These assumptions are compatible with the great similarity between the solution conformations of FVII and FVIIa as judged from hydrogen exchange kinetics (33), the very similar interaction patterns of FVII and FVIIa with TF (49), and the high degree of stimulation of the amidolytic activity of FVIIa by soluble TF (23). Free (zymogen-like) FVIIa probably closely resembles trypsinogen considering nature's tendency to exploit successful designs. However, in sharp contrast to the FVII-to-active FVIIa transition, the trypsinogen-to-trypsin transition is assumed to be virtually complete and to occur spontaneously upon removal of the activation peptide. Interestingly, another homologous enzyme believed to be (much) shifted towards the active conformation, thrombin, appears to rely heavily on cofactors and/or active site occupancy to fully attain the active state (50). Hence, once generated in the blood from its inactive precursor, prothrombin, thrombin plays two roles (51). Firstly, it acts as a procoagulant factor when it orchestrates the amplification phase of blood coagulation preceding the thrombin burst by proteolytic activation of factors V, VIII, XI and protease-activated receptors, and eventually ends up converting fibrinogen into an insoluble fibrin clot. Secondly, thrombin, in combination with thrombomodulin, acts as an anticoagulant agent by activation of protein C which in turn inactivates factors Va and VIIIa. These two apparently opposite functions are governed by the allosteric action of Na^+. In the absence of cofactors, thrombin, like FVIIa, can be considered zymogen-like as judged from NMR experiments (50). Binding of Na^+ to thrombin generates the so-called fast form (constituting 60% *in vivo*) which is responsible for its procoagulant function, while the Na^+-free slow form (40%) bound to thrombomodulin efficiently activates protein C. A combination of site-directed mutagenesis and fluorescence spectroscopy measurements was used to identify the tryptophan residues whose contribution to the intrinsic fluorescence emission changed as a consequence of the allosteric Na^+ activation of thrombin (52). The environments of Trp-141 and Trp-215 (which are conserved in FVII and correspond to residues 284 {c141} and 364 {c215}) appeared to be drastically altered upon Na^+ binding as compared to those of the remaining seven tryptophan residues in thrombin. Interestingly, Trp-364 {c215} in FVIIa defines one rim of the S3-S2 substrate binding site which is the endpoint of the suggested allosteric path I, while the mainchain atoms of Trp-284 {c141} constitute part of the activation pocket which is the endpoint of the suggested allosteric path II. Because the starting point for the TF-induced allosteric process in FVIIa, Met-306 {c164}, is very close to its putative Na^+ site (26) which is structurally similar to that of thrombin, it is tempting to speculate that the allosteric mechanisms in the two enzymes are (very) similar.

In the activation of FVIIa, there is a biological need to introduce an extra control mechanism in the form of a cofactor (TF) to ensure safety. In order to maintain the zymogen-like state in the absence of TF, the protease domain of free FVIIa is plausibly more disordered and plastic than its relatives, including a longer 170 loop. Unfortunately, this might in turn preclude crystallization and make FVIIa more susceptible to proteolytic autodegradation during crystallization trials. In the mean time, a recent area of research which might be able to teach us something about FVIIa allostery concerns FVIIa variants with enhanced enzymatic activity. Building blocks or features assumed to be involved in the TF-induced allosteric activation mechanism have been introduced into FVIIa by mutagenesis and indeed resulted in higher intrinsic (TF-independent) activity. This includes the stabilization of the 170 loop, the repositioning of Phe-374 {c225}, and facilitation of N-terminal insertion (43,45,53-55).

5. REFERENCES

1. Davie EW, K Fujikawa and W Kisiel: The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry* 30, 10363-10370 (1991)

2. Kalafatis M, N A Swords, M D Rand and K G Mann: Membrane-dependent reactions in blood coagulation: role of the vitamin K-dependent enzyme complexes. *Biochim Biophys Acta* 1227, 113-129 (1994)

3. Monroe D M, M Hoffman and H R Roberts: Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol* 22, 1381-1389 (2002)

4. Butenas S, T Orfeo and K G Mann: Tissue factor in coagulation. Which? Where? When? *Arterioscler Thromb Vasc Biol* 29, 1989-1996 (2009)

5. Konigsberg W, D Kirchhofer, M A Riederer and Y Nemerson: The TF:VIIa complex: clinical significance, structure-function relationships and its role in signaling and metastasis. *Thromb Haemost* 86, 757-771 (2001)

6. Monroe D M and N S Key: The tissue factor-factor VIIa complex: procoagulant activity, regulation, and multitasking. *J Thromb Haemost* 5, 1097-1105 (2007)

7. Rao L V M and U R Pendurthi: Tissue factor-factor VIIa signaling. *Arterioscler Thromb Vasc Biol* 25, 47-56 (2005)

8. Albrektsen T, B B Sørensen, G M Hjortø, J Fleckner, L V M Rao and L C Petersen: Transcriptional program induced by factor VIIa-tissue factor, PAR1 and PAR2 in MDA-MB-231 cells. *J Thromb Haemost* 5, 1588-1597 (2007)

9. Hagen F S, C L Gray, P O'Hara, F J Grant, G C Saari, R G Woodbury, C E Hart, M Insley, W Kisiel, K Kurachi and E W Davie: Characterization of a cDNA coding for human factor VII. *Proc Natl Acad Sci U S A* 83, 2412-2416 (1986)

10. Shobe J, C D Dickinson, T E Edgington and W Ruf: Macromolecular substrate affinity for the tissue factor-factor VIIa complex is independent of scissile bond docking. *J Biol Chem* 274, 24171-24175 (1999)

11. Baugh R J, C D Dickinson, W Ruf and S Krishnaswamy: Exosite interactions determine the affinity of factor X for the extrinsic Xase complex. *J Biol Chem* 275, 28826-28833 (2000)

12. Österlund M, E Persson, M Svensson, U Carlsson and P-O Freskgård: Transition state analysis of the complex between coagulation factor VIIa and tissue factor: suggesting a sequential domain-binding pathway. *Biochem Biophys Res Commun* 327, 789-793 (2005)

13. Österlund M, E Persson, U Carlsson, P-O Freskgård and M Svensson: Sequential coagulation factor VIIa domain binding to tissue factor. *Biochem Biophys Res Commun* 337, 1276-1282 (2005)

14. McCallum C D, R C Hapak, P F Neuenschwander, J H Morrissey and A E Johnson: The location of the active site of blood coagulation factor VIIa above the membrane surface and its reorientation upon association with tissue factor. *J Biol Chem* 271, 28168-28175 (1996)

15. McCallum C D, B Su, P F Neuenschwander, J H Morrissey and A E Johnson: Tissue factor positions and maintains the factor VIIa active site far above the membrane surface even in the absence of the factor VIIa Gla domain. *J Biol Chem* 272, 30160-30166 (1997)

16. Waters E K, S Yegneswaran and J H Morrissey: Raising the active site of factor VIIa above the membrane surface reduces its procoagulant activity but not factor VII autoactivation. *J Biol Chem* 281, 26062-26068 (2006)

17. Soejima K, Y Kurihara, K Kamiya and H Umeyama: Dynamic character of the complex of human blood coagulation factor VIIa with the extracellular domain of human tissue factor: a normal mode analysis. *FEBS Lett* 463, 19-23 (1999)

18. Mosbæk C R, D Nolan, E Persson, D I Svergun, J T Bukrinski and B Vestergaard: Extensive small-angle X-ray scattering studies of blood coagulation factor VIIa reveal interdomain flexibility. *Biochemistry* 49, 9739-9745 (2010)

19. Ohkubo Y Z, J H Morrissey and E Tajkhorshid: Dynamical view of membrane binding and complex formation of human factor VIIa and tissue factor. *J Thromb Haemost* 8, 1044-1053 (2010)

20. Fehlhammer H, W Bode and R Huber: Crystal structure of bovine trypsinogen at 1.8 Å resolution. II. Crystallographic refinement, refined crystal structure and comparison with bovine trypsin. *J Mol Biol* 111, 415-438 (1977)

21. Higashi S, H Nishimura, K Aita and S Iwanaga: Identification of regions of bovine factor VII essential for binding to tissue factor. *J Biol Chem* 269, 18891-18898 (1994)

22. Persson E: Ca^{2+} binding to proteins containing gamma-carboxyglutamic acid residues. *Methods Mol Biol* 172, 81-95 (2002)

23. Pedersen A H, O Nordfang, F Norris, F C Wiberg, P M Christensen, K B Møller, J Meidahl-Pedersen, T C Beck, K Norris, U Hedner and W Kisiel: Recombinant human extrinsic pathway inhibitor. *J Biol Chem* 265, 16786-16793 (1990)

24. Banner D W, A D'Arcy, C Chène, F K Winkler, A Guha, W H Konigsberg, Y Nemerson and D Kirchhofer: The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. *Nature* 380, 41-46 (1996)

25. Zhang E, R St Charles and A Tulinsky: Structure of extracellular tissue factor complexed with factor VIIa inhibited with a BPTI mutant. *J Mol Biol* 285, 2089-2104 (1999)

26. Bajaj S P, A E Schmidt, S Agah, M S Bajaj and A Padmanabhan: High resolution structures of *p*-aminobenzamidine- and benzamidine-VIIa/soluble tissue factor: unpredicted conformation of the 192-193 peptide bond and mapping of Ca^{2+}, Mg^{2+}, Na^{+}, and Zn^{2+} sites in factor VIIa. *J Biol Chem* 281, 24873-24888 (2006)

27. Dickinson C D, C R Kelly and W Ruf: Identification of surface residues mediating tissue factor binding and catalytic function of the serine protease factor VIIa. *Proc Natl Acad Sci U S A* 93, 14379-14384 (1996)

28. Pike A C W, A M Brzozowski, S M Roberts, O H Olsen and E Persson: Structure of human factor VIIa and its implications for the triggering of blood coagulation. *Proc Natl Acad Sci U S A* 96, 8925-8930 (1999)

29. Kemball-Cook G, D J D Johnson, E G D Tuddenham and K Harlos: Crystal structure of active site-inhibited human coagulation factor VIIa (des-Gla). *J Struct Biol* 127, 213-223 (1999)

30. Sichler K, D W Banner, A D'Arcy, K-P Hopfner, R Huber, W Bode, G-B Kresse, E Kopetzki and H Brandstetter: Crystal structures of uninhibited factor VIIa

link its cofactor and substrate-assisted activation to specific interactions. *J Mol Biol* 322, 591-603 (2002)

31. Higashi S, N Matsumoto and S Iwanaga: Molecular mechanism of tissue factor-mediated acceleration of factor VIIa activity. *J Biol Chem* 271, 26569-26574 (1996)

32. Olsen O H, K D Rand, H Østergaard and E Persson: A combined structural dynamics approach identifies a putative switch in factor VIIa employed by tissue factor to initiate blood coagulation. *Protein Sci* 16, 671-682 (2007)

33. Rand K D, T J D Jørgensen, O H Olsen, E Persson, O N Jensen, H R Stennicke and M D Andersen: Allosteric activation of coagulation factor VIIa visualized by hydrogen exchange. *J Biol Chem* 281, 23018-23024 (2006)

34. Sørensen B B, E Persson, P-O Freskgård, M Kjalke, M Ezban, T Williams and L V M Rao: Incorporation of an active site inhibitor in factor VIIa alters the affinity for tissue factor. *J Biol Chem* 272, 11863-11868 (1997)

35. Dickinson C D and W Ruf: Active site modification of factor VIIa affects interactions of the protease domain with tissue factor. *J Biol Chem* 272, 19875-19879 (1997)

36. Österlund M, R Owenius, K Carlsson, U Carlsson, E Persson, M Lindgren, P-O Freskgård and M Svensson: Probing inhibitor-induced conformational changes along the interface between tissue factor and factor VIIa. *Biochemistry* 40, 9324-9328 (2001)

37. Carlsson K, E Persson, U Carlsson and M Svensson: Inhibitors of factor VIIa affect the interface between the protease domain and tissue factor. *Biochem Biophys Res Commun* 349, 1111-1116 (2006)

38. Eigenbrot C, D Kirchhofer, M S Dennis, L Santell, R A Lazarus, J Stamos and M H Ultsch: The factor VII zymogen structure reveals reregistration of beta strands during activation. *Structure* 9, 627-636 (2001)

39. Perera L and L G Pedersen: A reconsideration of the evidence for structural reorganization in FVII zymogen. *J Thromb Haemost* 3, 1543-1545 (2005)

40. Olsen O H, P F Nielsen and E Persson: Prevention of beta strand movement into a zymogen-like position does not confer higher activity to coagulation factor VIIa. *Biochemistry* 43, 14096-14103 (2004)

41. Persson E, L S Nielsen and O H Olsen: Substitution of aspartic acid for methionine-306 in factor VIIa abolishes the allosteric linkage between the active site and the binding interface with tissue factor. *Biochemistry* 40, 3251-3256 (2001)

42. Rand K D, M D Andersen, O H Olsen, T J D Jørgensen, H Østergaard, O N Jensen, H R Stennicke and E Persson: The origins of enhanced activity in factor VIIa analogs and the interplay between key allosteric sites revealed by hydrogen exchange mass spectrometry. *J Biol Chem* 283, 13378-13387 (2008)

43. Soejima K, J Mizuguchi, M Yuguchi, T Nakagaki, S Higashi and S Iwanaga: Factor VIIa modified in the 170 loop shows enhanced catalytic activity but does not change the zymogen-like property. *J Biol Chem* 276, 17229-17236 (2001)

44. Higashi S, H Nishimura, S Fujii, K Takada and S Iwanaga: Tissue factor potentiates the factor VIIa-catalyzed hydrolysis of an ester substrate. *J Biol Chem* 267, 17990-17996 (1992)

45. Persson E, H Bak, A Østergaard and O H Olsen: Augmented intrinsic activity of factor VIIa by replacement of residues 305, 314, 337 and 374; evidence of two unique mutational mechanisms of activity enhancement. *Biochem J* 379, 497-503 (2004)

46. Persson E and O H Olsen: Activation loop 3 and the 170 loop interact in the active conformation of coagulation factor VIIa. *FEBS J* 276, 3099-3109 (2009)

47. Dickinson C D, J Shobe and W Ruf: Influence of cofactor binding and active site occupancy on the conformation of the macromolecular substrate exosite of factor VIIa. *J Mol Biol* 277, 959-971 (1998)

48. Shobe J, C D Dickinson and W Ruf: Regulation of the catalytic function of coagulation factor VIIa by a conformational linkage of surface residue Glu 154 to the active site. *Biochemistry* 38, 2745-2751 (1999)

49. Kelley R F, J Yang, C Eigenbrot, P Moran, M Peek, M T Lipari and D Kirchhofer: Similar molecular interactions of factor VII and factor VIIa with the tissue factor region that allosterically regulates enzyme activity. *Biochemistry* 43, 1223-1229 (2004)

50. Lechtenberg B C, D J D Johnson, S M V Freund and J A Huntington: NMR resonance assignments of thrombin reveal the conformational and dynamic effects of ligation. *Proc Natl Acad Sci U S A* 107, 14087-14092 (2010)

51. Di Cera E: Thrombin. *Mol Aspects Med* 29, 203-254 (2008)

52. Bah A, L C Garvey, J Ge and E Di Cera: Rapid kinetics of Na^+ binding to thrombin. *J Biol Chem* 281, 40049-40056 (2006)

53. Persson E, H Bak and O H Olsen: Substitution of valine for leucine 305 in factor VIIa increases the intrinsic enzymatic activity. *J Biol Chem* 276, 29195-29199 (2001)

54. Persson E, M Kjalke and O H Olsen: Rational design of coagulation factor VIIa variants with substantially increased intrinsic activity. *Proc Natl Acad Sci U S A* 98, 13583-13588 (2001)

55. Olsen O H and E Persson; Cofactor-induced and mutational activity enhancement of coagulation factor VIIa. *Cell Mol Life Sci* 65, 953-963 (2008)

Abbreviations: FVII(a), FIX(a), FX(a): (activated) factor VII, IX and X, respectively; Gla: gamma-carboxyglutamic acid; EGF: epidermal growth factor

Key Words: factor VIIa, Tissue Factor, Allostery, Allosteric Activation, Procoagulant Activity, Extrinsic Xase complex, Review

Send correspondence to: Egon Persson, Haemostasis Biochemistry, Novo Nordisk A/S, Novo Nordisk Park, G8.1.61, DK-2760 Maløv, Denmark, Tel: 45-44434351, Fax: 45-44663450, E-mail: egpe@novonordisk.com

http://www.bioscience.org/current/vol16.htm