 Novel insights into the regulation of coagulation by factor V isoforms, tissue factor pathway inhibitor, and protein S

B. DAHLBÄCK
Department of Translational Medicine, Lund University, University Hospital SUS, Malmö, Sweden

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Summary. Factor V (FV) is a regulator of both pro- and anticoagulant pathways. It circulates as a single-chain procofactor, which is activated by thrombin or FXa to FVa that serves as cofactor for FXa in prothrombin activation. The cofactor function of FVa is regulated by activated protein C (APC) and protein S. FV can also function as an anticoagulant APC cofactor in the inhibition of FXIIIa in the membrane-bound tenase complex (FIXa/FVIIIa). In recent years, it has become clear that FV also functions in multiple ways in the tissue factor pathway inhibitor (TFPI) anticoagulant pathway. Of particular importance is a FV splice variant (FV-Short) that serves as a carrier and cofactor to TFPIz in the inhibition of FXa. FV-Short is generated through alternative splicing of exon 13 that encodes the large activation B domain. A highly negatively charged binding site for TFPIz is exposed in the C-terminus of the FV-Short B domain, which binds the positively charged C-terminus of TFPIz, thus keeping TFPIz in circulation. The binding of TFPIz to FV-Short is also instrumental in localizing the inhibitor to the surface of negatively charged phospholipids, where TFPIz inhibits FXa in a process that is stimulated by protein S. Plasma FV activation intermediates and partially proteolyzed platelet FV similarly bind TFPIz with high affinity and regulate formation of prothrombinase. The novel insights gained into the interaction between FV isoforms, TFPIz, and protein S have opened a new avenue for research about the mechanisms of coagulation regulation and also for future development of therapeutics aimed at modulating coagulation.

Keywords: blood coagulation; factor V; factor Xa; protein C; protein S; TFPIz.

Coagulation factor V (FV) plays an important role in the regulation of blood coagulation by exhibiting both pro- and anticoagulant functions [1–3]. In normal healthy conditions when coagulation activation is not initiated, FV functions as an anticoagulant cofactor for both protein C and TFPI pathways. In situations when the coagulation system is triggered, FV is converted to a highly effective procoagulant cofactor to activated factor X (FXa), which activates prothrombin to thrombin, which in turn catalyzes fibrin deposition and activates platelets. This review will highlight the novel insights gained into the mechanisms and importance of the reactions involving FV isoforms, TFPIz, and protein S for the regulation of blood coagulation.

Activation of FV

Factor V circulates in blood as a single-chain 330 kDa protein (~20 nm) that exhibits little or no procoagulant function [1,2,4]. It is also present in platelets (~20% of total FV in blood) and is released upon platelet activation and binds to the platelet surface [5]. FV comprises multiple domains (A1-A2-B-A3-C1-C2); the three A domains forming a tight unit with the B domain protruding between A2 and A3 domains (Fig. 1). The B domain is a large carbohydrate-rich activation peptide that keeps FV in a procofactor form [1,4,6,7]. The B domain is flanked by proteolytic cleavage sites, at Arg709 and Arg1545, which are sensitive to cleavage by thrombin. However, these sites can also be cleaved, albeit less efficiently, by FXa in the presence of negatively charged phospholipid surfaces [8]. A further thrombin/FXa cleavage site is located at Arg1018 within the B domain. Recently, a molecular mechanism by which the B domain ensures that FV adopts a procofactor state has been proposed. This involves an interaction between a highly positively charged region (963–1008) located in the beginning of the B domain with a highly negatively charged stretch of amino acid residues at the end of the domain (1493–1537) [4,6,7]. Between these two charged clusters, the B domain contains a bulky, highly carbohydrate-rich region comprising 31 tandemly arranged repeats of nine amino acid residues [9–11].
The activation of FV by thrombin is a carefully regulated process, the three cleavage sites having different sensitivity to the activating enzyme. Arg709 and Arg1018 are highly sensitive to thrombin, whereas the kinetics of proteolysis for the Arg1545 site are less favorable making this the last step in the activation process [12,13]. Cleavage at Arg709 and Arg1018 results in partial FXa cofactor activity, whereas full thrombin-mediated activation requires cleavage at Arg1545 (Fig. 1). Activation of FV by FXa on the surface of negatively charged phospholipids demonstrates similar difference in sensitivity between the three sites, resulting in Arg709 and Arg1018 being cleaved to generate partially active FVa with a retained B domain [8]. In this context, it is interesting to note that an enzyme from the Russell’s Viper venom preferentially cleaves Arg1545 that fully activates FV [14–16]. The venom also contains a FX activator and can thus generate highly efficient prothrombinase (PTase).

Activated FV (FVa) forms a high affinity complex with FXa on the surface of negatively charged phospholipid membranes that are exposed on the surface of activated platelets [17–21]. Both FXa and FVa bind to the membrane as well as each other, the multiple protein–protein and protein–phospholipid interactions contribute to the highly efficient assembly of the complex. The membrane-bound FXa/FVa complex efficiently activates prothrombin to thrombin (Fig. 2).

**Regulation of FVa activity by activated protein C**

The cofactor function of FVa in the PTase complex is regulated by activated protein C (APC), which cleaves three peptide bonds at Arg306, Arg506, and Arg679 (Fig. 2) [2,22–25]. Whereas cleavage after Arg306 and Arg506 is important for the regulation of FVa function, proteolysis after Arg679 site appears to be of less significance. The Arg506 site is more rapidly cleaved than the Arg306 site, but only results in partial loss of FVa activity. Complete loss of FVa activity is achieved after cleavage at both Arg506 and Arg306 sites. By serving as a membrane-bound cofactor to APC, the vitamin K-dependent protein S stimulates the cleavage at Arg306 [26–28]. The cleavage at the Arg506 site is only mildly stimulated by protein S [27,28]. As the Arg506 site appears to form part of the FXa-binding site, this site is protected to a large extent in the assembled PTase complex [17,27]. The Arg306 site can be protected by prothrombin. Therefore, full protection from APC attack of the PTase complex is only lost after consumption of prothrombin [29].

**Point mutations in FV gene causing thrombophilia**

The importance of the Arg506 cleavage site is illustrated by a naturally occurring point mutation in the F5 gene that predicts the replacement of Arg506 with a Gln. The
Cambridge and FV Hong Kong, are described but do not
Two different mutations affecting the Arg306 site, FV
mutations were demonstrated to impart APC resistance. (A512V) was found in six European patients with APC
resistance in a Japanese family [37], whereas FV Bonn
have been described. FV Nara (W1920R) causes APC
Other rare
F5
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commonly found in thrombotic cohorts [2,30,32,34
the mutation is common in the general population, it is
thrombosis risk factor (2- to 3-fold increased risk), but as
ence. The associated APC resistance phenotype is a mild
risk factor as wild-type FV. The defective APC cofactor activity
of FV Leiden contributes to the APC resistance pheno-
type, which is thus due to the combination of impaired inactivation of both FVα and FVIIIα [2,3,23,31,41,43].

**FV and protein S as synergistic APC cofactors in the
regulation of FVIIIa**

The discovery of APC resistance and the characterization
of FV molecular mechanisms lead to the description of
an anticoagulant activity of FV [39–42]. Intact FV has
the capacity to serve as a synergistic APC cofactor
with protein S in the regulation of FVIIIa in the
assembled tenase complex—FIXα/FVIIIa bound to nega-
atively charged phospholipid vesicles (Fig. 2). FVIIIa in
the tenase complex is protected from APC unless both
FV and protein S are present. In model systems using
purified components, the intact FV is cleaved by APC at
Arg506, which stimulates the APC cofactor activity
explaining why FV Leiden is not as efficient APC cofac-
tor as wild-type FV. The defective APC cofactor activity
of FV Leiden contributes to the APC resistance pheno-
type, which is thus due to the combination of impaired inactivation of both FVα and FVIIIα [2,3,23,31,41,43].

**FV as carrier of TFPIβ in circulation**

FV deficiency is a rare autosomal recessive bleeding disor-
der with a variable bleeding phenotype [44–47]. The bleeding problems are milder than expected taking the
very important role of FVα as procoagulant cofactor to
FXα into account. A possible clue to explain the variable
and unexpectedly mild phenotype may be related to the
observation that FV-deficient plasma is also deficient in
TFPIβ, which is an inhibitor of the tissue factor (TF)
pathway [48]. Artificial depletion of FV from plasma
using antibodies against FV also results in the removal of
a large fraction of TFPIβ from plasma, which suggest
that FV and TFPIβ circulate in complex. In binding
experiments using plasma-purified full-length FV and
TFPIβ, a direct interaction with an estimated kDa of
around 13 nm was demonstrated [48]. It was recently
reported that FV stimulates TFPIβ-mediated inhibition of
FXα by 2- to 3-fold, whereas FVα conversely impairs the
reaction [49]. The low levels of TFPIβ in FV deficiency
may contribute, at least in part, to the mild bleeding
phenotype.

**TFPIβ—one of many TFPI isoforms**

Tissue factor pathway inhibitor is a very heterogeneous
protein present on the endothelium and in blood in at
least two splicing isoforms, TFPIβ and TFPIβ [50–53].
TFPIβ is the full-length form of TFPI and is comprised of
def in Kunitz domains and a highly positively charged
C-terminal extension (Fig. 3). TFPIβ is found both in cir-
culation (0.2–0.4 nm) and bound to the endothelium from
where it can be released by heparin [54]. TFPIβ contains

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Fig. 2. Negatively charged phospholipid important in reactions
involving FV/FVa. The C1 and C2 domains of FV/FVa are important
for the localization of the molecule to cell membranes exposing
negatively charged phospholipids. (A) FVa is a highly efficient FXa
cofactor, the two proteins forming a stable complex on the
membrane surface. Two peptide bonds are cleaved in prothrombin resulting
in the generation of thrombin. (B) The heavy chain of FVa contains three peptide bonds that are sensitive to cleavage by APC
in a reaction that is supported by protein S. The three proteins interact
on the surface of negatively charged phospholipid. The Arg306 cleavage is stimulated by the APC cofactor activity of protein S, whereas the Arg506 site is sensitive to APC alone. C, Intact FV has anticoagulant properties serving as a synergistic APC cofactor together with protein S in the cleavage of FVIIIa that is part of the membrane-bound tenase complex (FIXα/FVIIIα).

mutation (FV Leiden), which is the most common genetic
risk factor of venous thrombosis, is the result of a founder
effect, the mutation event being estimated to have occurred around 20–25,000 years ago in a Caucasian
ancestor [23,30–34]. This explains why the mutation is only present in populations with Caucasian gene influence. The associated APC resistance phenotype is a mild
thrombosis risk factor (2- to 3-fold increased risk), but as
the mutation is common in the general population, it is
commonly found in thrombotic cohorts [2,30,32,34–36].
Other rare F5 gene mutations resulting in APC resistance
have been described. FV Nara (W1920R) causes APC
resistance in a Japanese family [37], whereas FV Bonn
(A512V) was found in six European patients with APC
resistance [38]. For both FV Nara and FV Bonn, the F5
mutations were demonstrated to impart APC resistance.
Two different mutations affecting the Arg306 site, FV
Cambridge and FV Hong Kong, are described but do not
result in APC resistance and are not major risk factors for thrombosis [2].

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two Kunitz domains and is linked to the endothelium with a GPI anchor [55]. TFPIα constitutes around 20% of all TFPI in plasma, the remaining major form of TFPI being C-terminally truncated TFPI forms that are bound to the lipoproteins—the molecular mechanisms involved in the truncation and the lipoprotein attachment are incompletely understood [52,53,56]. The anticoagulant actions of TFPI in plasma are due to TFPIα inhibiting FXa in a reaction that is stimulated by protein S [52,53,56–59]. Following inhibition of FXa, the TFPIα/FXa complex very rapidly binds and inhibits FVIIa in the TF/FVIIa complex [60]. FVIIa interacts with the first, FXa with the second and protein S with the third Kunitz domain [52,53,56,61]. The positively charged C-terminal peptide of TFPIα is reported to bind FV/FVa, and it is likely that this region comprises the high affinity-binding region for FV-Short, but more work is needed to fully elucidate the binding site(s) for TFPIα (Fig. 3) [62].

**Rare bleeding disorder reveals novel TFPIα-interacting FV isoform**

A novel FV isoform—FV-Short—was identified when the molecular pathogenesis of a rare bleeding disorder was elucidated [63]. The East Texas bleeding disorder is inherited in an autosomal dominant manner [64]. It was described in a large family, and the affected individuals suffer a moderately severe bleeding phenotype. Evaluation of coagulation parameters revealed prolonged APTT and PT, but all coagulation factor activity assays tested were normal. A linkage analysis using prolonged APTT and/or prolonged PT as the indicator of affected status revealed that the disease was linked to the F5 gene. Sequencing revealed a single nucleotide replacement (A2440G) in the B domain of FV that predicted a serine to glycine shift. In the original report, this mutation was judged unlikely to be the cause of the disease and the molecular mechanism of the disease remained unresolved for several years [64].

In a renewed effort to reveal the pathogenesis of the disease, FV in plasma was re-examined by Western blotting and by partial purification and sequencing. All affected individuals were found to have a shorter variant of FV (FV-Short ~250 kDa) in their plasma, a variant that was not seen on the Western blotting of plasma of unaffected individuals (Fig. 4A) [63]. This FV-Short reacted with monoclonal antibodies against both the heavy and the light chain of FV, but not with an anti-B-domain antibody. Sequencing of the partially purified FV-Short revealed peptides from both A3 and C1 domains. These results suggested that the FV-Short was the result of alternative splicing of the exon encoding the B domain (Fig. 5). This was indeed the case as the A2440G mutation activated a naturally occurring splice donor site that under normal conditions is rarely used. A splice acceptor site is located 2106 base pairs 3′, and the A2440G allele therefore results in a transcript having an in-frame deletion of 2106 base pairs encoding amino acid residues 756–1458 (702 residues deleted), which includes the basic region and the 31 tandemly arranged repeats. This splicing event is infrequent in individuals with the normal allele and qPCR suggested approximately 20-fold up-regulation of the splicing as a result of the mutation [63].

Western blot analysis demonstrated that, in addition to the FV-Short, the affected individuals had similar levels of full-length FV as the unaffected family members [63]. This suggests that the mutant allele is only partially spliced at the activated splice site and that the mutant allele also encodes a full-length transcript. Using ELISAs having different specificities, we could demonstrate that the concentration of full-length FV in affected individuals was essentially normal (~20 nM) and that of FV-Short was around 5 nM. In unaffected individuals, the exact FV-Short concentration is not known, but can be estimated to be 0.2–0.4 nM [63].

**Coagulation inhibitor in plasma of affected individuals proves to be TFPIα**

The prolonged APTT and PT in affected individuals suggested the presence of an inhibitor in their plasma. This concept gained further support following thrombin generation analysis (TGA) of family members [63]. All those that carried the F5 mutation exhibited a prolonged lag phase and decreased generation of thrombin (Fig. 6). Immune depletion of FV from plasma and reconstitution with either recombinant full-length FV or the recombinant FV-Short resulted in normal thrombin generation, which suggested that the inhibitory effect was associated with FV, but that recombinant FV-Short did not confer the inhibitory function. However, specific immune depletion of full-length FV from affected plasma revealed that the FV-Short in their plasma was indeed required for the inhibitory function. This was surprising as the FV-Short
was expected to be intrinsically procoagulant as the basic region (963–1008) as well as the bulky repeat regions were missing [4,6,7]. This raised the possibility that the inhibitory activity was not a property of FV-Short in itself, but that a coagulation inhibitor was associated with the FV-Short. A likely candidate, and the first one we tested, was TFPIα. This was indeed found to be the case as demonstrated by Western blotting of plasma from family members using an antibody against TFPIα (Fig. 4B). Affected individuals demonstrated very strong TFPIα bands, whereas TFPIα in unaffected individuals was barely detected [63]. Quantification with TFPIα-specific ELISA demonstrated an approximate 10-fold increase in TFPIα in affected family members. That TFPIα was indeed the cause of the coagulation inhibition in the affected individuals was demonstrated using thrombin generation assays performed before and after immune depletion of TFPI. Moreover, addition of TFPIα to plasma from unaffected individuals resulted in similar impaired thrombin generation and prolonged lag phases.

**High affinity complexes between FV-Short and TFPIα in plasma**

Immunoprecipitation of TFPI followed by Western blotting for FV demonstrated that FV-Short in both affected and unaffected family members circulated in complex with TFPIα [63]. In the analysis of affected plasma, most of the FV-Short was recovered in the TFPIα immunoprecipitate, whereas there was essentially no full-length FV attached to TFPIα [63]. These results were compatible with the hypothesis that all FV-Short in the plasma of affected individuals is complexed with TFPIα and that the two proteins form a 1 : 1 stoichiometric complex. To demonstrate the presence of FV-Short/TFPIα complexes in normal plasma, larger volumes of plasma were subjected to the immunoprecipitation. The TFPI immunoprecipitate contained both FV-Short and also full-length FV (Fig. 7). As the TFPIα concentration in normal plasma is around 0.2 nM (approximately the same as the FV-Short concentration), whereas full-length FV circulates at around 20 nM, the results suggest that FV-Short and TFPIα interact with much higher affinity than full-length FV and TFPIα—the difference may very well be at least 100-fold. These results are compatible with the reported KD of 13 nM for the interaction between full-length FV and TFPIα and with an estimated KD of
FV Amsterdam, a second case with B-domain deletion and TFPIx complexes

The East Texas family is a large family with 22 identified affected individuals. The A2440G mutation has not been found in other individuals, and the age of the point mutation event is not known. It remains to be elucidated whether other individuals distantly related to the family may carry the mutation. Another family with a splicing event resulting in a short FV-version was recently reported. A small Dutch family with two affected individuals with a similar phenotype to the East Texas family was found to harbor a C2588G mutation in the F5 gene that resulted in the aberrant splicing of 623 amino acids from the B domain [66]. The mutation created a splice donor site that was not normally present, and the splice acceptor site was the same as in the East Texas family. The difference between FV Amsterdam and the FV-Short of the East Texas family is that FV-Short uses a naturally occurring splice donor site, which is activated by the mutation, whereas the FV Amsterdam creates a novel splice donor site. This explains why FV Amsterdam is not normally present, whereas FV-Short is found at low concentrations in normal plasma.

High affinity binding of TFPIx to partially activated FV inhibits formation of PTase

As mentioned above, the three activation cleavage sites in FV have different sensitivities to thrombin and FXa, with the Arg1545 site being the least sensitive. Therefore, partial activation of FV generates intermediates in which the basic cluster (963–1008) is released (after cleavage at Arg709 and Arg1018), but the acidic region (1493–1537) remains attached to the light chain. Such intermediates are generated by FXa-mediated proteolysis of FV in the presence of negatively charged phospholipid membranes [8]. Platelet activation also generates partially proteolyzed FV intermediates that are presented on the platelet surface [67]. The high affinity-binding site for TFPIx is exposed in the plasma- and platelet-derived FV intermediates, and if TFPIx is present, it will bind the FV activation intermediate and inhibit FXa, thus preventing the assembly of the PTase complex [65](Fig. 7). The TFPIx may be derived from the plasma or from platelets, which contain TFPIx that is released upon platelet activation. As the concentration of TFPIx is very low, only small amounts of FXa can be inhibited by this mechanism. However, it may be an important way to inhibit the formation of PTase, possibly primarily on activated platelets as the available TFPIx in plasma is very low [50,53,56,65]. If the stimulus to initiate activation of coagulation is stronger, with generation of FXa exceeding the TFPIx inhibitory capacity, the first thrombin formed will feedback activate FV by cleavage at the Arg1545 site. The high affinity TFPIx binding site in the C-terminus of

around 90 pM for an interaction between TFPIx and a recombinant B-domain truncated FV variant called FV810 [48,65]. The recombinant FV810 variant lacks residues 810–1492 and is thus similar to the FV-Short we identified, and both variants contain the highly acidic region (1493–1537) that constitutes a high affinity-binding site for the C-terminal basic peptide of TFPIx [65]. It is therefore likely that the binding site for TFPIx in the naturally occurring FV-Short is the same acidic region and that results obtained with the FV810 construct is valid also for FV-Short.

Fig. 6. FV A2440G mutation associated with poor thrombin generation and slow FV activation. (A) TGA of plasma from affected individuals demonstrated prolonged lag phase and low thrombin peaks as compared to plasma from unaffected family members. (B) On Western blot analysis of FV on aliquots drawn from the TGA, the unaffected plasma demonstrated rapid FV activation with complete loss of single-chain FV already after 3 minutes, which is just prior to the rapid thrombin generation. (C) Both intact FV and FV-Short are very slowly activated, and full activation is only achieved after around 14 min, which is just prior to the increase in thrombin generation. The intact FV appears to disappear slightly prior to the FV-Short, which may suggest faster cleavage, or be due to inefficient transfer of the intact FV in the Western blotting. The results agree with the concept of an inhibitor of FXa such as TFPI in affected plasma. Modified from Vincent et al. [63].
the B domain is released upon full activation of FV to FVa. This explains why FXa in PTase assembled with fully activated FVa is insensitive to TFPI\(\alpha\) [56,65]. Thus, the cleavage at the Arg1545 site is a crucial regulatory event resulting in the loss of the TFPI\(\alpha\)-cofactor activity of the FVa intermediate. It is interesting to note that this cleavage, as discussed above, also results in the loss of the synergistic APC cofactor activity with protein S in the regulation of the tenase complex [42].

**FV-Short—an important regulator of coagulation functioning in synergy with TFPI\(\alpha\) and protein S**

As a result of the very high affinity-binding site for TFPI\(\alpha\) in the C-terminus of FV-Short (\(K_d = 0.09 \text{ nM}\)), FV-Short will be able to extract TFPI\(\alpha\) from the surface of endothelial cells. Similar negatively charged substances such as heparin, and presumably also polyphosphates, are able to release TFPI\(\alpha\) from the endothelial surface [50,54,56,68,69]. From this, it can be hypothesized that any FV-Short that is released from the liver into the circulation will form a complex with TFPI\(\alpha\). FV-Short will in this manner regulate the level of circulating TFPI\(\alpha\). Full-length FV has lower affinity (\(K_d = 13 \text{ nM}\)), but its higher concentration (\(\sim 20 \text{ nM}\)) makes it competitive as TFPI\(\alpha\) transporter, which explains the observation that immunoprecipitation of TFPI from normal plasma catches mainly FV-Short but also a small fraction of full-length FV (Fig. 7). The lower affinity between TFPI\(\alpha\) and full-length FV suggests an equilibrium between free and bound TFPI\(\alpha\), and that TFPI\(\alpha\) will be released from FV in situations where TFPI\(\alpha\) binders with higher affinity are present, for example, FV-Short or FV activation intermediates. As the affinity of FXa for full-length FV is very low [70], it seems unlikely that the TFPI\(\alpha\)-full-length FV complex is important for inhibition of FXa. The situation for the FV-Short/TFPI\(\alpha\) complex is different as the affinity of FXa for the FV-Short, due to the lack of the basic cluster (963–1008), is very high under the premises that negatively charged phospholipids are present. The plasma concentration of FV-Short/TFPI\(\alpha\) complexes is sub-nM (\(\sim 0.2 \text{ nM}\)), which means that only the initially generated FXa can be inhibited. The FXa-TFPI\(\alpha\) complex inhibits TF-bound FVIIa generating a TF-FVIIa-FXa-TFPI\(\alpha\) complex [1,50,52,53,56,60]. Whether FV-Short is part of such complex remains to be elucidated. Moreover, whether the efficiency of TFPI\(\alpha\) to inhibit TF/FVIIa/FXa is affected by its binding to FV-Short also remains to be elucidated.

Vitamin K-dependent protein S has been demonstrated to function as an important TFPI\(\alpha\) cofactor in the inhibition of FXa on the surface of negatively charged phospholipids/platelets and suggested to be a carrier for TFPI\(\alpha\) in plasma [57,58,71–73]. As most of TFPI\(\alpha\) in plasma is circulating in complex with FV-Short, or with full-length FV, it is an interesting question whether protein S functions as TFPI\(\alpha\) cofactor also when TFPI\(\alpha\) is bound to FV-Short. We have data demonstrating that FV-Short and protein S function as synergistic TFPI\(\alpha\) cofactors in the inhibition of FXa, an activity lost upon

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**Fig. 7.** FV-Short/TFPI\(\alpha\)-complexes present in both affected and unaffected plasma. TFPI\(\alpha\) was subjected to immune precipitation (IP) with anti-TFPI using a magnetic bead approach. The IP and supernatants were analyzed using both anti-FV (top) and anti-TFPI (bottom). Affected plasma contained high concentrations of FV-Short and TFPI\(\alpha\) and a small amount of plasma (0.3 mL) was sufficient for the analysis. In contrast, normal plasma contains low FV-Short and TFPI\(\alpha\), and therefore, a higher starting volume of plasma (5 mL) was used. The IP of affected revealed FV-Short but not intact FV, whereas the IP from normal plasma contained both intact FV and FV-Short, suggesting that in normal circulation, TFPI\(\alpha\) is bound either to FV-Short or to intact FV. Modified from Vincent et al. [63].
Regulation of the FV-Short/TFP1α complex by thrombin

The activity of FV-Short as TFP1α cofactor is dependent on the integrity of the Arg1545 site [74]. Once FV-Short is cleaved at this site, the TFPIα cofactor activity is lost. This raises the question whether the bound TFPIα influences the cleavage at Arg1545 by FXa or by thrombin. The bound TFPIα efficiently inhibits the FXa active site and as long as TFPIα is in molar surplus over FXa the Arg1545 cleavage by FXa is inhibited (B. Dahlbäck, unpublished observation). However, when the FXa concentration exceeds that of TFPIα there is no protection. Thrombin on the other hand is different because TFPIα does not inhibit thrombin. The question then is whether the bound TFPIα blocks the ability of thrombin to cleave the Arg1545 site. It was recently reported that high concentrations of a basic peptide corresponding to the C-terminus of TFPIα inhibit thrombin-mediated cleave at Arg1545 [65,75,76] and that TFPIα bound to FV-Short directly inhibits the Arg1545 cleavage [75]. It will be important to confirm the inhibitory effect of TFPIα on the Arg1545 cleavage, as the thrombin-mediated cleavage of Arg1545 is an efficient way to block the TFPIα cofactor activity of FV-Short and to generate FVa for propagation of coagulation.

Conclusions and future perspectives

Factor V is a fascinating protein that plays multiple roles in the regulation of blood coagulation. Not only is it directly involved in determining the rate of prothrombin activation, but it is also important for regulation of the TFPIα and protein C pathways. Thus, the proteolytic conversion of the procofactor FV to the highly efficient FXa cofactor FVa is a carefully regulated process with different activities of activation intermediates. Proteolysis also regulates the activity of FVa and the anticoagulant APC cofactor activity. The more recent identification of FV-Short splice variants as regulators of TFPIα activity opens a new avenue of research and therapeutic possibilities. Much research is required to fully explore the role of the FV-Short variants for the regulation of blood coagulation. Increased knowledge in this area will facilitate the development of therapeutic agents aiming at either stimulating or inhibiting coagulation. Ongoing efforts to develop TFPIα inhibitors for the treatment of hemophilia will benefit from a detailed understanding of the FV-Short/TFPIα interaction. Another approach to regulate the activity of the FV-Short/TFPIα system is to regulate the alternative splicing event aiming at either up- or down-regulating the plasma levels of FV-Short. Creation of FV-Short variants with modified TFPIα binding and cofactor activities may also be of interest in the effort to develop suitable regulators of blood coagulation.

Disclosure of Conflict of Interests

The author states that he has no conflict of interest.

activation of FV-Short to FVa (to be presented at the 26th ISTH conference in Berlin 2017). However, Wood et al. reported that protein S had no effect on the ability of TFPIα to inhibit PTase containing FXa-activated FV [73]. Future research is required to elucidate the potential role of protein S and FV activation intermediates as synergistic TFPIα-cofactors. Efficient inhibition of FXa by TFPIα requires the assembly of the inhibitory complex on the surface of negatively charged phospholipids. As the concentrations of most of the participating components (FV-Short, TFPIα, and FXa) are very low (sub-nM)—only protein S being present at higher concentration (100 nM free protein S)—a high affinity complex is required. This is ensured by multiple protein–protein and protein–phospholipid interactions. FV-Short, FXa, and protein S all contain binding domains for the phospholipid—FV-Short binds TFPIα and FXa—protein S binds TFPIα and probably FV-Short and FXa. The combination of all these interactions ensures high affinity assembly of the inhibitory complex and rapid inhibition of FXa (Fig. 8).

Fig. 8. Proposed reaction scheme for inhibition of FXa by TFPIα in the presence of FV-Short and protein S. TFPIα in circulation interacts with FV-Short, intact FV, and protein S. In situation with low-grade FX, a generation and the presence of negatively charged phospholipids a FXa inhibitory complex comprising FV-Short, protein S, and TFPIα is assembled on the phospholipid surface, FV-Short binding the C-terminus of TFPIα, protein S interacting with Kunitz 3, and FXa being inhibited by Kunitz 2.

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