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Blood coagulation factors V and VIII: Molecular Mechanisms of Procofactor Activation

Mettine H.A. Bos[1] and **Rodney M. Camire**[1,2]

[1]Department of Pediatrics, Division of Hematology, The Children's Hospital of Philadelphia

[2]The University of Pennsylvania, School of Medicine, Philadelphia, PA, USA

Abstract

A hallmark of hemostasis is that cells and proteins involved in the formation of a blood clot remain in a quiescent state and are only activated following an appropriate stimulus. The homologous proteins factors V and VIII cannot participate to any significant degree in their macromolecular enzyme complexes and are thus considered procofactors. Activity is generated following limited proteolysis, indicating that the conversion of the procofactors to factor Va and factor VIIIa must result in structural changes that impart cofactor function. The proteolytic events that lead to the activation of these proteins have been extensively characterized over the past three decades. However, a fundamental understanding of the mechanism(s) by which these proteins are kept as inactive procofactors and how specific bond cleavage facilitates the conversion to the active cofactor state is only starting to become known. These molecular processes undoubtedly play critical regulatory roles, evolved to maintain normal hemostasis since factor Va and factor VIIIa have a tremendous influence on thrombin generation. This review will detail our current understanding of the molecular process of procofactor activation and highlight structural features that play a major role in factor V and factor VIII activation.

Keywords

Factor V; Factor VIII; prothrombin activation; FX activation; procofactor; proteolytic activation; hemostasis

Introduction

The majority of blood coagulation factors are synthesized as inactive precursors that only express activity following discrete and limited proteolysis. A defined conformational change ensues which allows these proteins to assemble on cellular surfaces localized at the site of injury where they optimally function. This molecular strategy allows for a high level of temporal and spatial regulation as well as protection against naturally circulating inhibitors which typically target the active protein conformation. It is well established that the serine protease zymogens of coagulation (e.g. FVII, FIX, FX, prothrombin, etc.) follow a general activation strategy which is shared by all serine proteases and is typified by trypsinogen and chymotrypsinogen [1]. The process requires cleavage following Arg^{15} (the bond between

Correspondence: Rodney M. Camire, Ph.D., The Children's Hospital of Philadelphia, Division of Hematology, 5018 Colket Translational Research Building, 3501 Civic Center Boulevard, Philadelphia, PA 19104, Tel: 215-590-9968, Fax: 215-590-3660, rcamire@mail.med.upenn.edu.

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Arg^{15} and Ile^{16}) which generally removes an activation peptide and exposes a new N-terminus in the catalytic domain beginning with Ile^{16}. The new N-terminal sequence then folds back into the catalytic domain and inserts into the N-terminal binding cleft in a sequence-specific manner forming a salt bridge between the α-NH_2 group of Ile^{16} and Asp^{194} in the interior of the catalytic domain. This transition is associated with numerous changes in the structure and ultimately leads to the maturation of the active serine protease. In contrast to this molecular strategy, the mechanism underlying the activation of the two major procofactors in blood coagulation, factor V (FV) and factor VIII (FVIII) are substantially different.

Factor V and FVIII are structurally and functionally homologous proteins and play a central role in the hemostatic process. Once activated these proteins serve analogous functions as cofactors in the blood coagulation system [2]. Activated FV (FVa) assembles with the serine protease factor Xa (FXa) while activated FVIII (FVIIIa) is a cofactor for factor IXa (FIXa); these protein complexes assemble in the presence of Ca^{2+} ions on a negatively charged membrane surface to form the prothrombinase and intrinsic tenase complexes, respectively [3,4]. The prothrombinase complex catalyzes the conversion of prothrombin to thrombin, while intrinsic Xase catalyzes the proteolytic conversion of FX to FXa; both pivotal steps in the coagulation cascade [5]. The individual contribution of the serine proteases FIXa and FXa to overall thrombin generation is relatively minor, as incorporation of FVa and FVIIIa into the macromolecular enzyme complexes enhances the reaction rate by several orders of magnitude [5]. The importance of these cofactors is further underscored by clinical findings, which indicate that FV and FVIII deficiency states lead to parahemophilia and hemophilia A, respectively [6,7].

Following the discovery of FV and FVIII in the 1930-40s, it was quickly recognized that they require proteolytic activation to fully participate in coagulation [8-11]. Over the past three decades considerable effort and progress has been made defining their mode of activation [12-14]; however, key mechanistic details remain to be uncovered. This review focuses on our current understanding of FV and FVIII activation and discusses the various structural elements that assist in keeping these proteins in an inactive procofactor state.

The Factor V procofactor to cofactor transition

Factor V is a large (M_r = 330,000), multidomain (A1-A2-B-A3-C1-C2), single chain glycoprotein that circulates in blood at a concentration of ~20 nM (~10 μg/mL) [12,14]. Of the total FV pool in whole blood, ~20% is stored in the α-granules of platelets and secreted upon platelet activation [15]. At physiological plasma concentrations, the procofactor FV cannot assemble or function in the prothrombinase complex; proteolytic processing within the B domain is an absolute requirement for the expression of cofactor function [12,16-19]. Thrombin is considered the key physiological activator of FV and cleaves three peptide bonds (Arg^{709}, Arg^{1018}, and Arg^{1545}) within the B domain, thereby facilitating B domain removal (Figure 1) [16,20-22]. While not completely defined, thrombin appears to interact with FV through the heavy and light chains [23,24]. The binding site within the light chain is thought to reside within the C2 domain and appears important for proteolysis at all three thrombin cleavage sites [24]. The resulting active cofactor species, FVa, is a heterodimer composed of a heavy chain (A1-A2; M_r = 105,000) and a light chain (A3-C1-C2; M_r = 71/74,000), which are associated through Ca^{2+} ions (Figure 1) [16,20-22,25]. The heavily glycosylated B domain, spanning amino acids 710-1545, is released as two large fragments (M_r = 71,000 and M_r = 150,000) [16,21,22,26].

In addition to thrombin, various proteases have been identified that cleave FV to generate a cofactor species with variable amounts of activity. For example, several groups have

established the FXa activates FV in a membrane- and Ca^{2+}-dependent fashion following cleavage at or near Arg^{709} and Arg^{1018}, and possibly at other sites in the light chain depending on reaction conditions [19,27,28]. Other proteases include, to name a few, activators from *Daboia russelli* (Russell's viper), *Daboia lebetina, and Naja naja oxiana* venoms [16,29-32], calpain [33]}, plasmin [34], platelet proteases [35,36], meizothrombin [37], as well as elastase and cathepsin G [38-41]. These later two proteases are of potential significance as they are released from polymorphonuclear leukocytes at extravascular tissue sites and could amplify thrombin generation through FV activation. This in combination with FV released from activated platelets may play a major role in the initiation phase of cell-based coagulation events.

While previously appreciated, the laboratories of Mann and Esmon firmly established that FV requires proteolytic processing at multiple sites to effect activation [22,42]. In the following decades, numerous studies have attempted to define the contribution of the individual cleavage sites to the development of FV cofactor activity. Although somewhat conflicting results have been obtained, it is generally acknowledged that proteolysis by thrombin follows a kinetically preferred order of bond cleavage: Arg^{709} is cleaved first, followed by cleavage at Arg^{1018}, and Arg^{1545}. Furthermore, most data support the finding that maximal activation of FV requires proteolysis at Arg^{1545}. Cleavage at Arg^{709} and Arg^{1018} yields a FV derivative with significant, but partial cofactor activity [21,26,43,44], whereas individual cleavage at these sites does not lead to any substantial increase in cofactor activity [28,43,44]. Support for the contribution of cleavage at Arg^{1545} to maximal FV cofactor activity came from mutagenesis studies, which demonstrated that isolated cleavage at this site is sufficient for complete activation [28,43,44]. This is also consistent with the observation that proteolysis by Russell's viper venom (RVV-V) and *Daboia lebetina* venom (LVV-V), which cleave FV at Arg^{1545}, results in full activation [16,32,45-47]. Thus release of the B domain from the FV light chain appears to be a necessary requirement for the expression of cofactor activity, which is facilitated by proteolysis of the two preceding activation sites in the B domain. Collectively, the results suggest that the FV B domain somehow keeps FV in an inactive state and its removal by proteolysis contributes to the activation mechanism.

The B domain preserves the procofactor state of factor V

The human FV B domain is 836 amino acids long, comprises ~50% of the mass of the protein, and has no homology to any other known protein, including the FVIII B domain (Figure 1) [20,48]. The B domain is heavily glycosylated and has unusual regions of tandem repeats, of which the function remains to be elucidated. Electron microscopy and physical studies have suggested that the B domain appears as a bulky extension to a globular core, assumed to be the heavy/light chains [49,50]. Because of these unusual properties and lack of importance to FVa procoagulant activity, less attention has been paid to investigating its functional significance. Some studies have suggested that the B domain may play a role in the anticoagulant function of FV by stimulating the APC-mediated inactivation of FVIIIa (reviewed here [51]). As suggested above, one role must be to maintain FV as an inactive procofactor. Recent work from our laboratory as well as others has shed some light on how the FV B domain regulates the function of FV. An important first observation came from the Kane laboratory who showed that a B-domainless derivative of FV (FVdes811-1491 or FV-810) has constitutive, but partial activity compared to FVa [43,52]. These studies suggested that the B domain somehow prevents expression of procoagulant activity prior to proteolytic processing [43]. More recently, our laboratory has further investigated the molecular properties of this B domain-deleted FV variant. We found that purified FV-810 as well as a thrombin-resistant derivative interact with membrane-bound FXa with high affinity and are functionally equivalent to FVa in the absence of intentional proteolysis [53]. These

findings indicate that proteolysis within the B domain, while necessary, is incidental to the mechanism by which cofactor function is actually realized. Instead, proteolytic activation of FV simply eliminates steric and/or conformational constraints imposed by the B domain that interfere with discrete binding interactions essential to the FVa cofactor activity, for example the FXa binding site. Removal of these inhibitory constraints through recombinant truncation bypasses the requirement for proteolysis to activate the molecule. Using a panel of progressively finer B domain-truncated variants, we were able to identify a discrete region of the B domain that appears to play a critical role in stabilizing the procofactor state [54]. Part of this B domain region (residues 963-1008) is unusually basic with 18 out of 46 residues being Arg or Lys and is well conserved across the vertebrate lineage [55]. As expected, disruption of this B domain region by mutagenesis or through deletion yielded derivatives with cofactor-like properties in the absence of intentional proteolysis, indicating that the length of the B domain *per se* is not a primary factor in preserving the procofactor state. Thrombin-mediated proteolysis of FV facilitates removal of these inhibitory B domain sequences; however, it is likely that other, as yet to be identified components of the B domain also play a role in preserving the procofactor state.

Insight into B domain function from non-mammalian forms of FV

Whereas most of the B domain sequence is highly variable throughout vertebrate evolution, several short motifs are strongly conserved, including the basic region 963-1008 detailed above [55-57]. An exception to these findings has been found in an unusual form of FV derived from the venom of some Australian Elapidae family members (*O. microlepidotus, P. textilis*, and *O. scutellatus*), which are among the most venomous snakes in the world [58]. A unique feature of these snakes is that approximately 5-40% of their venom consists of a large prothrombin activating complex comprising a cofactor FVa-like subunit and a serine protease FXa-like subunit, which share high sequence homology with mammalian FVa and FXa (55-60%) [59-63]. Remarkably, the FV homologues expressed in the elapid venom have extraordinarily short B domains: 46 versus ~600-800 residues in mammals, and they lack the basic region. This intriguing observation prompted us to assess the functional properties of purified recombinant venom-derived *P. textilis* FV (pt-FV) [59]. Consistent with our previous observations, we were able to show that the absence of the basic region correlates with the expression of procoagulant FV activity, indicating that venom FV is expressed as a constitutionally active FV variant [59]. As such, this is the first FV species observed thus far that exists as an active cofactor. Notably, this protein can also function in the absence of anionic membranes and is completely resistant to inactivation by APC, despite APC-mediated proteolysis within the heavy chain at the equivalent Arg^{506} and Arg^{709} sites [59]. We speculate that a unique disulfide bond between the A2 and A3 domains of pt-FV enhances its structural stability and prevents dissociation of the A2 domain upon APC cleavage [59]. Thus, pt-FV represents an exceptional example of a protein that has adapted into a potent biological weapon for host defense and envenomation of prey.

Mutations in Factor V associated with disease states

Some mutations in FV have been reported to increase the risk of developing thrombosis (for a review see [64]), whereas others can lead to a bleeding disorder (reviewed in [65]). No mutations or polymorphisms associated with disease states have been observed at the FV thrombin cleavage sites [66].

APC-resistant factor V

One of the most commonly observed genetic risk factors for thrombosis is FV^{Leiden}, which is partially resistant to inactivation by APC due to a Gln substitution at the APC cleavage

site Arg^{506} [67-71]. Individuals who are heterozygous for this mutation have a 3-8-fold increased risk of developing thrombosis, whereas the risk for homozygous individuals is 50-80-fold higher as compared to individuals with normal FV [72,73]. Several other mutations and polymorphisms that have been shown to also result in some degree of APC resistance are reviewed elsewhere [74].

Parahemophilia

Factor V deficiency (parahemophilia) is an autosomal recessive bleeding disorder which was first described in the 1940s by Paul Owren in Norway [10]. It is a rare bleeding disorder that affects one in a million individuals and is characterized by low or undetectable FV activity and/or antigen levels (reviewed in [75]). Patients with undetectable levels of FV (<1%) due to homozygous nonsense, frameshift, or missense mutations exhibit the gamut of phenotypes from asymptomatic to severe bleeding. Two thirds of all mutations causing FV deficiency are nonsense mutations in the FV gene [65]. Even though mRNA containing a premature stop codon is normally degraded by nonsense mediated decay, it has been suggested that trace amounts of FV may be expressed following ribosomal slippage or somatic inversion events [76]. Of the reported missense mutations, most are clustered in the A and C domains, while none are found in the B domain [66]. Deficiency in FV presents a conundrum because the phenotype is variable and correlates unexpectedly poorly with FV levels in plasma [65,77-81]. These observations are generally not consistent with the fundamental role played by FV in coagulation and with findings in the FV knockout mice which have a lethal phenotype [82]. However, observations made in the past two years point to an important role for platelet FV that may help explain these observations.

Approximately one fifth of the total FV pool is stored in the α-granules of platelets from which it is secreted upon platelet activation [15]. While megakaryocytes can synthesize FV [83-85], the vast majority of platelet FV is endocytosed from the plasma pool by megakaryocytes [86-88]. Following endocytosis via a specific receptor-mediated process [89,90], FV is modified intracellularly such that it is functionally unique compared to its plasma-derived counterpart [88,91]. Recently, Duckers et al. were able to correlate the levels of platelet FV with thrombin generation in FV-deficient patients. They showed that patients deficient in FV resulting from missense mutations have sufficient functional FV in their platelets to guarantee thrombin generation and protect them against major bleeding [92]. Furthermore, the same group demonstrated that the FV requirement for thrombin generation is considerably lower in these patients due to markedly reduced levels of the anticoagulant protein tissue factor pathway inhibitor (TFPI) in FV-deficient plasma [93]. Whether the disease phenotype correlates with the platelet FV levels and whether a similar mechanism may explain the relatively mild phenotype in patients that have an introduced stop codon in the FV gene remains to be determined.

The procofactor to cofactor transition of factor VIII

Factor VIII circulates as a large (M_r ≈ 330,000), multidomain (A1-A2-B-A3-C1-C2) heterodimer resulting from limited proteolysis at the B-A3 junction and at additional sites in the B domain [13]. This heterodimer consists of a variably sized heavy chain (A1-A2-B; 200-90 kDa) and a light chain (A3-C1-C2; 80 kDa) that are noncovalently associated (Figure 2). The A domains are bordered by short segments (~30-40 amino acids) of negatively charged residues, known as the acidic regions a1 (337-372), a2 (711-740), and a3 (1649-1689) (Figure 2). Whereas regions a2 and a3 are more or less well conserved in FV (Figure 3), the a1 region of FVIII is absent from both FV and ceruloplasmin [94-96], the latter being a ferroxidase with an A1-A2-A3 domain structure that originates from the same ancestral protein as FV and FVIII [97,98]. The FVIII acidic regions are thought to function,

in part, as binding sites for thrombin [13,99,100]. In addition, the a3 region and regions within both C domains have been suggested to mediate the tight interaction of the FVIII heterodimer with its carrier protein von Willebrand factor (VWF) [101-104]. This interaction serves various important roles in FVIII physiology, as it has been reported to stabilize the heterodimeric structure of FVIII as well as to prevent proteolysis by FXa and APC [105].

The FVIII heterodimer is an inactive procofactor and must be subjected to limited proteolysis to effect full cofactor activity [11,106-112]. Factor VIII can be proteolytically activated by both thrombin and FXa [13]. Thrombin cleaves three peptide bonds at Arg^{372}, Arg^{740}, and Arg^{1689}, thereby generating FVIIIa, which is a heterotrimer composed of the A1 (50 kDa; 1-372), A2 (43 kDa; 373-740), and the light chain (A3-C1-C2; 73 kDa; 1689-2332) (Figure 2) [112,113]. Activation of FVIII results in a transient ~20-50-fold increase in biological activity which decays over a short period of time due to A2 domain dissociation from A1/A3-C1-C2, a mechanism which contributes to the regulation of FVIIIa cofactor activity [114-116].

Numerous studies have examined the role of the individual thrombin cleavage sites in the expression of FVIIIa cofactor activity. Similar to thrombin-mediated activation of FV, the evidence obtained supports an ordered cleavage pathway, with cleavage at Arg^{740} occurring first, followed by cleavage at Arg^{1689}, and subsequently at Arg^{372}. The specific role of the individual cleavage sites in the expression of FVIIIa cofactor activity will be discussed in the following sections.

Cleavage at Arg^{740} and Arg^{1689}

Although cleavage at Arg^{740} appears to be of little consequence to the development of cofactor function [117], it is thought to facilitate subsequent proteolysis at Arg^{372} and Arg^{1689} [118]. Surprisingly however, its significance in FVIII activation is not reflected in the hemophilia A patient population, as there have been no reports of missense mutations at position 740 (http://hadb.org.uk). Cleavage of the light chain at Arg^{1689} results in dissociation of FVIII from VWF which allows for association of the cofactor with anionic phospholipids and FIXa [119,120]. Whether proteolysis at this site directly contributes to the potentiation of FVIIIa cofactor activity remains controversial. There is some evidence that this cleavage partially increases cofactor activity [121,122]; however, Pipe and Kaufman have shown using a single chain FVIII derivative (IR8) that cofactor activity can be obtained even in the absence of the Arg^{1689} cleavage site [123].

Cleavage at Arg^{372}

The results obtained with various FVIII derivatives as well as other biochemical studies and naturally occurring mutations indicate that cleavage at Arg^{372} is essential to procofactor activation [117,124-127]. Biochemical data suggest that cleavage at this site exposes a functional FIXa binding site which promotes rapid FX activation by cofactor-bound FIXa [128]. Based on these and other observations, it was suggested that acidic region a1 and possibly a portion of the a3 region (1649-1689) could obscure functionally important surface areas such as a FIXa binding site; results that are in line with functional studies [128]. Interestingly, acidic region a1 of FVIII is noticeably absent from FV (missing from exon 7) possibly pointing to a unique function in FVIII (Figure 3) [94,95]. Furthermore, recent structural data on B domain-deleted FVIII indicate that this part of FVIII is highly flexible as no electron density was observed in this region [129,130]. Alternatively, cleavage at Arg^{372} could induce a change in conformation that is critical for the expression of FVIIIa cofactor function. Evidence for this comes from studies employing cross linking agents and apolar probes as well as circular dichroism experiments. These studies support the idea that

there are subtle, yet measureable changes in conformation in the vicinity of the A2 domain when FVIII is activated to FVIIIa [131-133]. Future biochemical and structural studies are needed to resolve the precise mechanism by which cleavage at Arg^{372} facilitates the FVIII procofactor to cofactor transition.

The Factor VIII B domain

The FVIII B domain, like that of FV, is very large (908 residues), encoded by a single exon, heavily glycosylated, and is also removed following thrombin-mediated proteolysis; however it does not share sequence homology with the FV B domain. Yet, unlike FV, several groups have established that removal of most of the B domain yields a derivative that remains as an inactive procofactor [123,134-137]. Thus, the FVIII B domain does not appear to play a role in preserving FVIII as a procofactor. As such, the molecular mechanisms that regulate or prevent the potential cofactor activities of FV and FVIII are surprisingly different.

Mutations in Factor VIII associated with disease states

In contrast to the dual effects that mutations in FV can have on the hemostatic balance (e.g. procoagulant vs. anticoagulant), no FVIII mutations have been described thus far that are linked to a prothrombotic state. Even though APC resistance is strongly correlated with an enhanced risk of developing thrombosis, no mutations at the equivalent APC inactivation sites in FVIII have been observed in a cohort of patients with venous thrombosis [138]. In addition, recombinant FVIII variants carrying substitutions at one of the two APC cleavage sites did not show an APC resistant phenotype [139]. It was found, though, that high levels of FVIII are associated with an increased risk of venous thrombosis (reviewed here [140]). To date, no genetic variation in the FVIII gene has been identified that might account for this phenotype.

Hemophilia A

A deficiency or functional defect in FVIII is at the basis of the X-linked congenital bleeding disorder known as hemophilia A (for a review see [141]), which has an incidence of one in 5,000 in the general population. Hemophilia A is categorized as severe (<1%), moderate (1-5%), or mild (5-20%), according to the relative amount of FVIII activity in the patient's plasma. The classic symptoms of hemophilia include bleeding episodes that affect joints, muscles, internal organs, and the brain. Whereas approximately one third of all hemophilia A cases are due to intron 22 inversions [142,143], missense mutations have been observed to be the most frequent mutation type and are almost exclusively responsible for the mild/moderate hemophilia A phenotypes [144]. In addition, stop codons account for ~10% of all hemophilia A mutations, and one third of the identified FVIII gene defects other than intron inversions represent new mutations [144]. Most reported mutations have been registered in the hemophilia A mutation database (http://hadb.org.uk), and a graphic representation of the number of missense mutations per FVIII region is given in Figure 4.

As expected, missense mutations at or near some of the thrombin activation sites have been reported to result in hemophilia A (Figure 4). Two different substitutions have been observed at position 1689 in a total of 35 mild to severe hemophilia A patients. Furthermore, there has been one report of a substitution at the P1′ position Ser^{1690} resulting in mild hemophilia. At position 372, four different missense mutations have been observed in a total of 22 reported cases of mild to severe hemophilia A, and missense mutations at P1′ Ser^{373} have been found in two mild hemophilia A patients. Remarkably, there have been no reports of missense mutations at or near position 740, which may suggest that mutations at this site do not result in hemophilia A.

Concluding remarks

The molecular process of maintaining FV and FVIII as inactive procofactors plays a critical regulatory role which has evolved to limit the expression of cofactor activity. Despite its significance, clear mechanistic insight by which the various proteolytic events lead to expression of FVa and FVIIIa procoagulant activity has proven difficult to pinpoint. Despite the similarities in structure and function of factors V and VIII, their molecular mechanisms of activation have been shown to differ substantially. For FV, the B domain plays a fundamentally important role as discrete conserved B domain sequences are involved in the mechanism by which FV persists as an inactive procofactor. The data suggest that the FV B domain serves an inhibitory function which, under normal physiological conditions, is efficiently removed upon proteolytic processing. In contrast, the FVIII B domain does not appear to be involved in regulating cofactor activity. Rather, cleavage between the A1 and A2 domains at position Arg^{372} is critical for the procoagulant activity of FVIII. The precise mechanism by which cleavage at Arg^{372} facilitates the transition to the active cofactor state remains to be determined. Collectively these studies lay the groundwork for further uncovering the precise molecular mechanism by which FV and FVIII transition from the procofactor to cofactor state.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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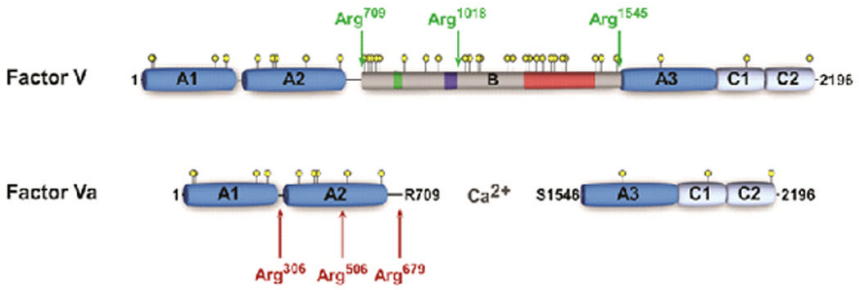

Figure 1. Schematic representation of FV and FVa

Schematic A1-A2-B-A3-C1-C2 domain representation of human FV and FVa. Thrombin cleavage sites are indicated by green arrows and APC cleavage sites by red arrows. Yellow circles represent potential N-linked glycosylation sites, the green box indicates a 2× 17 amino acid repeat region, the dark blue box corresponds to the basic sequence 963-1008 implicated in preserving the FV procofactor state, and the red box represents a 31× 9 amino acid tandem repeat region.

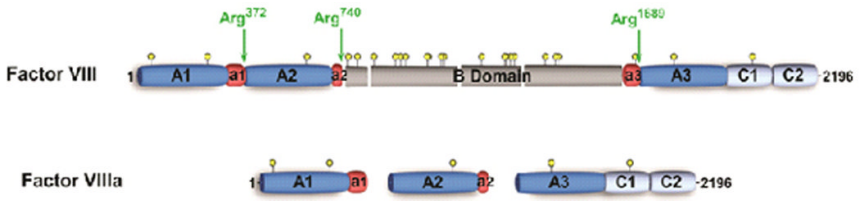

Figure 2. Schematic representation of FVIII, FVIIIa, and FVIII-SQ

Schematic A1-A2-B-A3-C1-C2 domain representation of human FVIII and FVIIIa. The acidic regions denoted by a1, a2, and a3 are indicated in red, thrombin cleavage sites are indicated by green arrows, and yellow circles represent potential N-linked glycosylation sites.

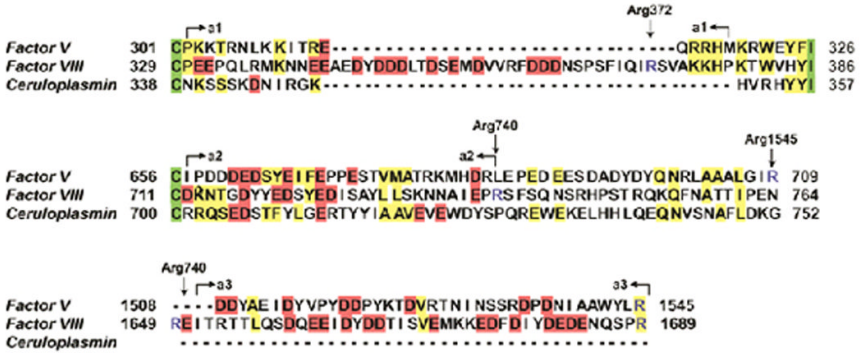

Figure 3. Alignment of the acidic regions a1, a2, and a3 of FVIII

The acidic regions a1, a2, and a3 from human FVIII were aligned with human FV and human ceruloplasmin (AlignX Module; Invitrogen Carlsbad, CA, USA). Residues fully conserved between all three molecules are shown in green, partially conserved amino acids are indicated in yellow, and the negatively charged residues characterizing the acidic regions are shown in red. The boundaries of the acidic regions are indicated by arrows and thrombin cleavage sites are indicated by arrows and depicted in blue.

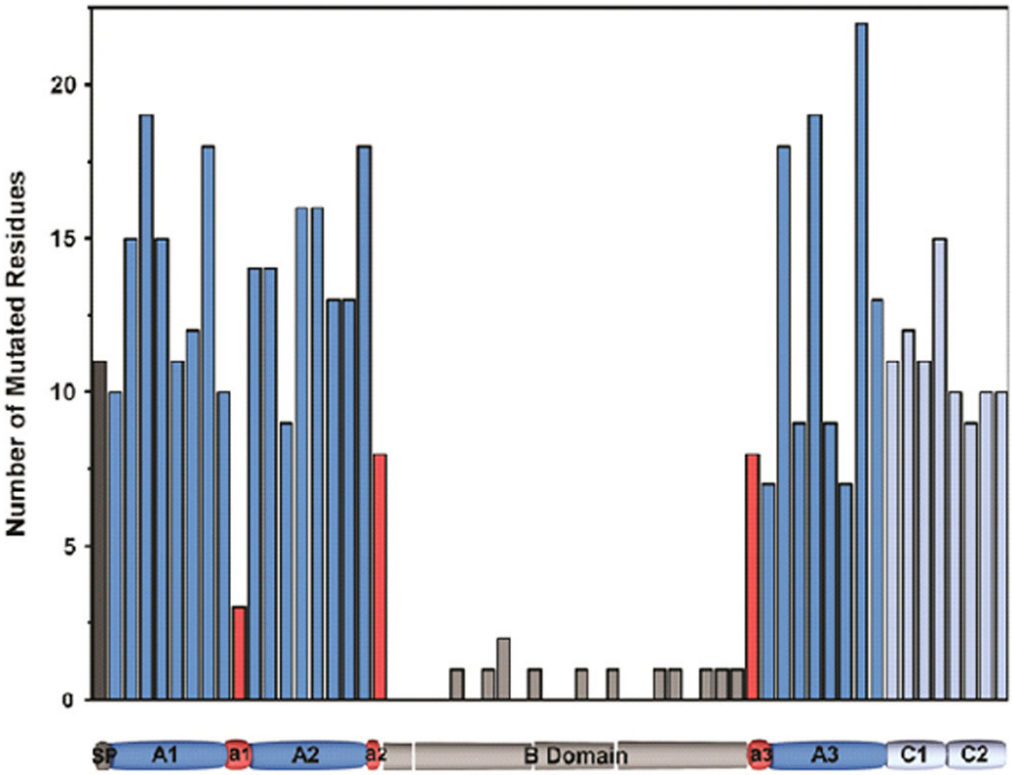

Figure 4. Graphic representation of missense mutations per FVIII

The number of hemophilia A causing mutations in FVIII was determined in 40 residue blocks, including the signal peptide. Mutations were derived from the Haemophilia A Mutation Database (http://hadb.org.uk), and mutations resulting in introduction of a stop codon were excluded. A schematic domain representation of FVIII is shown, indicating the location of the 40 residue stretches.