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REVIEW ARTICLE

Functional roles of the factor VIII B domain

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Summary. Unravelling the structure, function and molecular interactions of factor VIII (FVIII) throughout its life cycle from biosynthesis to clearance has advanced our understanding of the molecular mechanisms of haemophilia and the development of effective treatment strategies including recombinant replacement therapy. These insights are now influencing bioengineering strategies toward novel therapeutics. Whereas available molecular models and crystal structures have helped elucidate the structure and function of the A and C domains of FVIII, these models have not included detailed structural information of the B domain. Therefore, insights into the role of the FVIII B domain have come primarily from expression studies in heterologous systems, biochemical studies on bioengineered FVIII variants and clinical studies with B domain-deleted FVIII. This manuscript reviews the available data on the potential functional roles of the FVIII B domain. A detailed literature search was performed, and the data extracted were qualitatively summarized. Intriguing emerging evidence suggests that the FVIII B domain is involved in intracellular interactions that regulate quality control and secretion, as well as potential regulatory roles within plasma during activation, platelet binding, inactivation and clearance.

Keywords: B domain, blood coagulation factors, factor VIII, haemophilia A, protein engineering, recombinant proteins

Introduction

Factor VIII (FVIII) is a large, complex, essential glycoprotein coagulation factor with a still incompletely understood life cycle. The primary structure of FVIII was identified only in the early 1980s, when the protein was purified to complete homogeneity, and its cDNA cloned [1,2]. While considerable and steady progress has subsequently been made on unravelling the structure, function and molecular interactions of FVIII throughout its life cycle from biosynthesis to clearance, a complete picture has still not emerged [3–5]. Activated FVIII (FVIIIa) assembles within the 'tenase' complex on a phospholipid surface, such as activated platelets, where it functions as a non-enzymatic cofactor for factor IXa (FIXa) in the activation of factor X (FX). This cofactor function is similar to that of its homologous cofactor, activated factor Va (FVa), which assembles within the 'prothrombinase' complex, exerting cofactor function for FXa for the activation of prothrombin.

The FVIII gene comprises 26 exons, which encode a polypeptide chain for a signal peptide of 19 amino acids and a mature protein of 2332 amino acids [4]. FVIII is synthesized as an inactive single chain with the discrete domain structure of A1-a1-A2-a2-B-a3-A3-C1-C2 (Fig. 1). Domains a1, a2 and a3 are acidic amino acid rich regions between the major structural domains and contain sulfated tyrosine residues. The A domains of FVIII share approx. 40% amino acid identity with each other and to the A domains of FV. The FVIII C domains in turn also exhibit approx. 40% amino acid identity to each other and to the C domains of FV and proteins that bind negatively charged phospholipids, suggesting a role in phospholipid interaction.

Encoded by exon 14, the B domain spans the sequence from amino acids 741–1648. Several characteristics distinguish the B domain. One unusual feature is the encoding of the B domain entirely by a single very large uninterrupted exon. Unlike other

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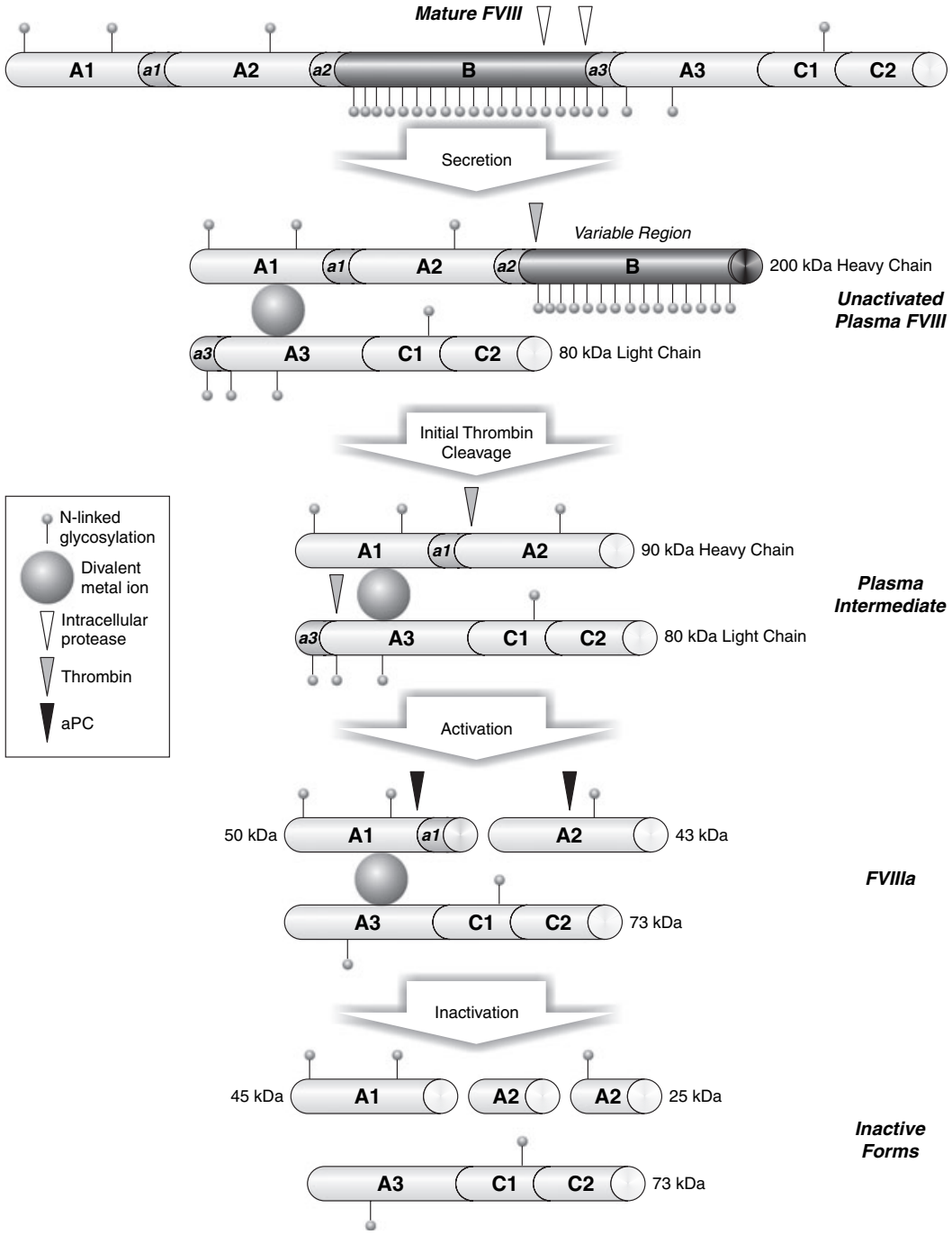

Fig. 1. Domain structure and processing of FVIII. Abbreviations: aPC, activated protein C; FVIII, factor VIII; FVIIIa, activated factor VIII.

portions of the FVIII molecule, the B domain is without amino acid homology to other known proteins, including the B domain of FV. FVIII does share with FV the distinctive property of having extensive glycosylation of B domain asparagine, serine and threonine residues. The B domain contains 19 of the potential 25 asparagine (*N*)-linked glycosylation attachment sites on the entire FVIII molecule [2].

While the B domain is not directly necessary for the central procoagulant activity of FVIII [6,7], more recently it has been shown to play a major role in the intracellular processing and trafficking of FVIII. Moreover, there is emerging evidence that portions of the B domain may have functional influences throughout the life cycle of FVIII (Table 1) [8–20]. This review delineates

Table 1. Functions of the B domain in the life cycle of factor VIII.

Process	Function	References
Intracellular		
Synthesis quality control	Enables interaction of factor VIII with chaperone proteins that distinguish properly folded tertiary structure of proteins; stabilizes folded domains, prevents aggregation	Pipe *et al.* [13]
Secretion	Interacts with cargo-specific sorting receptor complex that enables endoplasmic reticulum to Golgi transport; increases secretion efficiency	Moussalli *et al.* [14]; Cunningham *et al.* [15]; Miao *et al.* [16]; Pipe *et al.* [18]; Zhang *et al.* [19]
Plasma		
Activation	Possibly shields thrombin activation site from premature proteolysis	Eaton *et al.* [8]; Meulien *et al.* [9]; Pittman *et al.* [10]; Pittman *et al.* [11]
Platelet binding	Decreases the affinity of unactivated factor VIII for activated platelets, thus preserving circulating factor VIII	Li and Gabriel [12]
Inactivation	Reduces proteolysis by activated protein C and factor Xa	Khrenov *et al.* [20]
Clearance	May play a further role in factor VIII quality control through interaction with asialoglyprotein receptor	Bovenschen *et al.* [17]

the known intracellular functions of the B domain and its potential roles following secretion into plasma.

Synthesis and intracellular processing of factor VIII

FVIII is believed to be produced primarily in hepatocytes and endothelial cells [4,21]. Thus far, no established or primary cell lines expressing FVIII have been developed, and consequently evidence on the extensive post-translational processing and secretion of FVIII has been generated from expression of the FVIII complementary DNA (cDNA) in transfected mammalian cells, such as Chinese hamster ovary (CHO), African green monkey kidney (COS-1), HeLa, and the human hepatic cell line, SK-HEP1 [13,22]. FVIII is poorly expressed and inefficiently secreted in these heterologous systems. Expression is two to three orders of magnitude lower than that observed with other cDNAs of similar-sized proteins using similar vectors and methods [23]. This has stimulated an extensive effort to delineate the trafficking of FVIII through the cellular secretory machinery and identify the structures and processes necessary for efficient secretion of functional FVIII molecules. The B domain has been found to figure prominently in these processes.

Transcription and translation Reduced FVIII mRNA accumulation has been observed in several cell lines. Deletion analysis within FVIII expression vectors demonstrated that diffuse sequences within the FVIII coding region have a deleterious effect upon RNA accumulation [24]. The FVIII cDNA contains a 305 bp transcriptional silencer that dominantly inhibits its own expression [25]. Shortly after the cloning and subsequent expression of FVIII, it was demonstrated that the B domain was dispensable for its procoagulant activity [6,7]. Removal of the B domain, the equivalent of approx. 38% of the primary cDNA sequence, significantly improved the yield of FVIII [6]. The increased expression resulted from markedly increased levels of mRNA and increased translation [26]. However, detailed studies on the expression of recombinant B domain-deleted forms of FVIII (BDD-rFVIII) indicated that despite an increase in mRNA approaching 20-fold, the yield of secreted BDD-rFVIII was improved by no more than 2-fold [10]. This suggests a role for the B domain during other steps within the FVIII biosynthetic pathway.

Quality control system Secretory proteins follow a path through the cell that leads through the endoplasmic reticulum (ER) to an endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and finally to the Golgi before release into the plasma. After synthesis the FVIII protein, directed by the signal peptide, is translocated into the lumen of the ER, which serves as the major site for the folding and assembly of secretory proteins as well as integral membrane proteins. Facilitated by enzymes and molecular chaperones, newly synthesized proteins fold and assemble into tertiary structures within the ER [13]. The molecular chaperones also serve as an essential part of the ER quality control (QC) system

ensuring export of only properly folded proteins. The QC system recognizes general structural differences between native and non-native proteins and prevents the secretion of non-native proteins that could be non-functional and antigenic.

Cleavage and glycosylation Upon entering the ER, the signal peptide is cleaved off to produce the mature 2332 amino acid FVIII protein. Important structural post-translational modifications occur cotranslationally in which a 14-sugar branched chain is added to the amide nitrogen of consensus asparagine side chains (Figs 1 and 2) [4]. The B domain is the principal region for this modification, accounting for more than three-quarters of the potential 25 *N*-linked glycosylation attachment sites in the FVIII molecule. These carbohydrate moieties not only stabilize folded domains but also provide polar surface groups that prevent aggregation of folding intermediates and allow newly synthesized polypeptide chains to interact with ER chaperones and enzymes. The B domain carbohydrates appear to be important for FVIII secretion, since inhibition of *N*-linked glycosylation by tunicamycin treatment dramatically reduces secretion [26].

Chaperone proteins The high mannose-containing *N*-linked oligosaccharides of the B domain interact with two protein chaperones: calnexin, an integral ER membrane protein, and calreticulin, an ER luminal protein (Fig. 2). The 'calnexin/calreticulin cycle' promotes folding of glycoproteins and retains non-native glycoproteins in the ER until they are correctly folded [27]. Two of three glucose units are rapidly trimmed from the 14-subunit oligosaccharides attached to asparagine residues, allowing specific recognition and binding by calnexin and calreticulin to the cleaved carbohydrate structure.

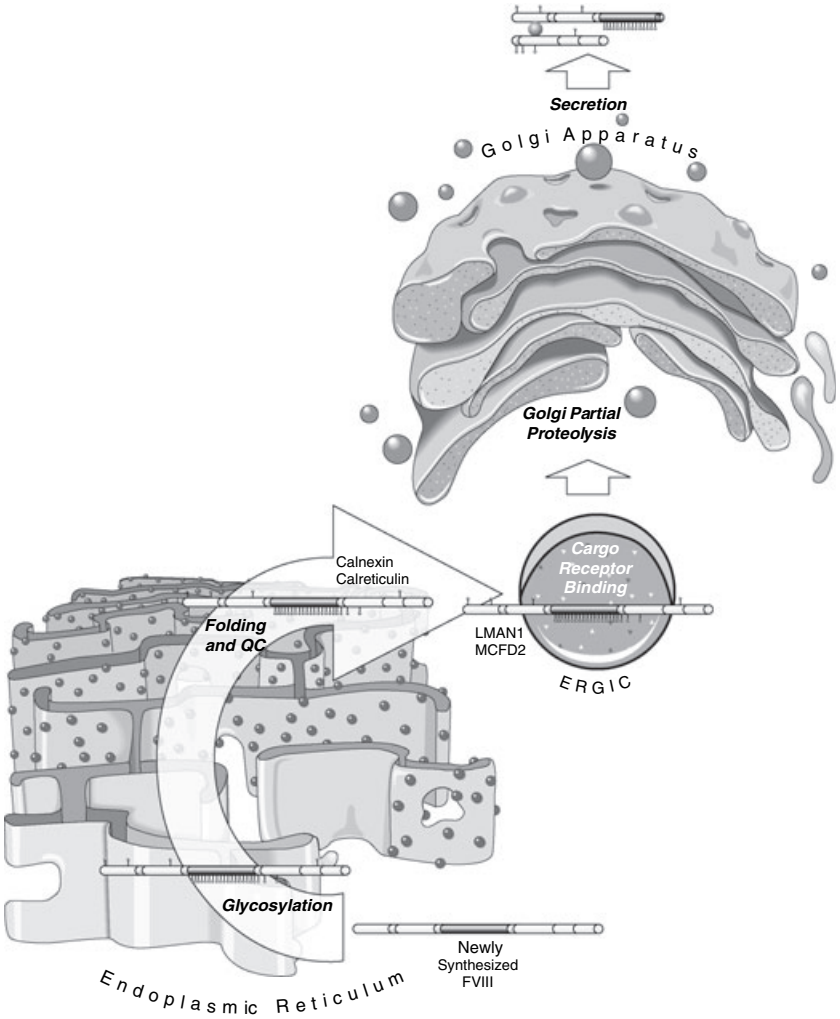

Fig. 2. Intracellular trafficking processes involving the B domain of FVIII. Abbreviations: ERGIC, endoplasmic reticulum Golgi intermediate compartment; FVIII, factor VIII; LMAN1, mannose-binding lectin 1; MCFD2, multiple coagulation factor deficiency protein 2; QC, quality control.

When the third glucose unit is enzymatically removed, FVIII is released from the two chaperone proteins. If the protein is correctly folded, it can leave the cycle and exit the ER. Otherwise, the protein re-enters the cycle or undergoes degradation.

The B domain was shown to be important for this QC function in a study of CHO cells [13]. The interaction with both calnexin and calreticulin of a FVIII fragment lacking most of the B domain (residues 759–1639 deleted) was significantly reduced. Moreover, in the presence of inhibitors of glucose trimming, the interactions of FVIII with calnexin and calreticulin were also attenuated, and the secretion of FVIII inhibited.

Cargo receptor binding After proper folding, FVIII proceeds toward the Golgi apparatus packaged in coated vesicles that uncoat and fuse with each other to form the ERGIC (Fig. 2). A cargo-specific sorting receptor complex consisting of the transmembrane protein mannose-binding lectin 1 (LMAN1, also known as ERGIC-53) and its soluble luminal interaction partner, multiple coagulation factor deficiency protein 2 (MCFD2), are required for this process. Mutations in LMAN1 or MCFD2 cause combined deficiency of FV and FVIII [28]. This disorder is associated with plasma levels of FV and FVIII ranging from 5% to 30% of normal. In an initial study of a tetracycline-inducible HeLa cell line, it was shown that when the cells overexpressed a mutant ERGIC-53 that was unable to exit the ER, the secretion of FVIII, as well as FV, was defective, and that this shuttling of the coagulation factors appeared to be dependent on post-translational modification of *N*-linked oligosaccharides [14]. Subsequent data from the HeLa cell line suggested that FVIII-specific protein sequences also contribute to LMAN1 interaction [15]. In both COS-1 and CHO cells, LMAN1 and MCFD2 interacted specifically with FVIII, and the B domain was the most likely site of the interaction [19]. BDD-rFVIII did not bind to the LMAN1-MCFD2 complex or displayed markedly reduced binding affinity. In contrast to earlier studies, this investigation suggested that the interaction might be independent of the FVIII glycosylation state. Calcium-dependent protein-protein interactions appeared to play a possible role. Further studies are required to pinpoint more precisely elements within the B domain that are important for cargo receptor binding.

Golgi partial proteolysis Once in the Golgi compartment, FVIII is among the many proteins to undergo intracellular proteolysis (Fig. 2). The tertiary structure of FVIII confers on the B domain region a particular susceptibility to proteolysis. The heavy- and light-chain portions of FVIII form a globular domain, while the connecting B domain region forms an exposed extended rod-like structure [29]. Moreover, the middle part and the carboxy-terminal region of the B domain comprise a motif (Arg-X-X-Arg) similar to that recognized by intracellular proteases of the subtilisin-like family [4]. Consequently, the FVIII polypeptide is cleaved in the B domain after residues 1313 and 1648 to generate the heavy chain of 200 kD (A1-a1-A2-a2-B) in a metal ion complex with the light chain of 80 kD (a3-A3-C1-C2). Within the Golgi apparatus, FVIII also undergoes modification of the *N*-linked oligosaccharides to complex-type structures, O-linked glycosylation, and sulfation of specific tyrosine residues.

Secretion efficiency In the absence of the B domain, secretion still occurs but is generally very inefficient. Several BDD-rFVIII species with variable amounts of the B domain deleted have been investigated, such as FVIIIdes-797-1652 [8], FVIIIdes-771–1666 (FVIII-II, 165 kDa) [30] and FVIIIdes-760–1639 (LA-VIII) [10]. The only commercially available BDD-rFVIII protein, moroctocog alfa (ReFacto®; Wyeth Pharmaceuticals, Collegeville, PA, USA), possesses a 14 amino acid B domain sequence linking Ser^{743} to Gln^{1638}, joining the A2 and A3 domains. In each case, the B domain has not been completely deleted, so that small portions are retained. In transfected cells, BDD-rFVIII generally exhibits high levels of mRNA yet the resultant translated proteins are not fully reflected in the amount of secreted protein observed, suggesting intracellular interactions are limiting efficient secretion [10]. The LMAN1/MCFD2 protein complex appears to interact with its cargo primarily through interaction with mannose residues on *N*-linked oligosaccharides. Thus, BDD-rFVIII, missing the majority of the *N*-linked oligosaccharides clustered within the B domain, may not be able to take full advantage of this facilitated transport pathway. The addition of even a small portion of native FVIII B domain (optimally 226 amino acids with six *N*-linked oligosaccharides) to BDD-rFVIII produced a 5- to 10-fold higher secretion efficiency compared to BDD-rFVIII [16]. These studies support a central role of the B domain in the efficient secretion of full-length FVIII.

The B domain is by no means the sole determinant of efficient FVIII secretion process. For example, interaction of elements within the FVIII A1 domain with the protein chaperone BiP in the ER has also been shown to limit FVIII secretion [31]. The relative

contribution of various cellular processes may also depend upon the particular transfected cell line used. Further research is needed to understand more fully the interplay of intracellular chaperones and other intracellular molecules involved in cellular quality control that act in concert to ensure that only functional, properly folded FVIII molecules are secreted.

Fate of factor VIII in circulation

Although the B domain is partially cleaved prior to secretion in the Golgi, a substantial portion persists in the unactivated FVIII molecules secreted into the plasma (Fig. 1). The newly secreted FVIII is a heterodimer linked by a divalent metal ion. Its constituents are an 80 kDa light chain composed of the A3-C1-C2 domains and a heterogeneous 90–210 kDa heavy chain of the A1-A2-B domains [8,30]. Variable lengths of the partially cleaved B domain arising from limited *C*-terminal proteolysis account for the heterogeneity of the heavy chain [32]. An analysis of five currently commercially available FVIII replacement concentrates revealed that B domain terminating at Arg^{1313} was the predominant form of the heavy chain in both plasma-derived FVIII (pdFVIII) and full-length recombinant FVIII (rFVIII) from both CHO or baby hamster kidney cells [33]. Two additional major heavy chain species contained truncations of the B domain that terminated also at Ser^{817} and Lys^{1115}.

Therefore, the predominant form of unactivated FVIII circulating in the plasma after secretion or replacement therapy with full-length FVIII still contains 572 amino acids of the B domain. Moreover, this section of the B domain also retains 15 of the 19 *N*-linked glycosylation sites. Since the B domain contains no site essential for coagulation once FVIII is activated, it has been commonly assumed that the substantial portion of the B domain that remains on the heavy chain of the circulating unactivated FVIII has no function. Nevertheless, some evidence suggests that the retained section of the B domain could modulate the intermolecular interactions of FVIII with various molecules and substrates during the various stages of its life span in the plasma.

Immediately after its release into the circulation, the FVIII heterodimer interacts with its carrier protein, von Willebrand factor (VWF), to form a tight non-covalent complex [21]. Unactivated FVIII is protected from premature clearance or activation by its association with VWF, and this interaction is the most important identified determinant of the survival of FVIII in the circulation. VWF does not bind to the B domain, but rather to two light-chain sites in the A3 and C2 domains [5]. The interaction of FVIII with other molecules such as FIXa and membrane phospholipids in platelets is blocked by VWF [4]. Upon proteolytic activation by thrombin, FVIII achieves a heterotrimeric structure and undergoes a conformational change rendering it capable of binding FIXa and FX on activated platelets.

Activation by thrombin FVIII becomes activated primarily by thrombin (Fig. 1) and participates as a cofactor for FIXa in the formation of the tenase complex on the surface of activated platelets. Additional components of the complex are FX and Ca^{2+}. Thrombin activates FVIII by proteolysis at three residues: Arg^{372}, Arg^{740} and Arg^{1689}. Arg^{372} and Arg^{1689} are known to be essential sites for FVIII activation (Fig. 1) [34]. Cleavage at Arg^{372} bisects the A1 and A2 domains and at Arg^{1689} the acidic a3 region from the light chain to create the FVIIIa heterodimer (A1-a1/ A2-a2/ A3-C1-C2). The ramifications of proteolysis at Arg^{740}, the boundary between the A2 and B domains, have not been as rigorously examined. According to the findings of a recent study, however, thrombin may act in an ordered sequence to accomplish cleavage of FVIII first at Arg^{740}, which then facilitates cleavages at Arg^{372} and Arg^{1689} (Fig. 1) [35]. A number of studies of BDD-rFVIII have indicated altered patterns of thrombin activation, possibly caused by a modified context of the Arg^{740} thrombin cleavage site [8–11]. In contrast, other studies could not detect any differences in thrombin activation between BDD-rFVIII and plasma-derived FVIII (pdFVIII) [36,37]. Moreover, removing approx. 50% of total pdFVIII carbohydrate did not significantly affect thrombin potentiation of clotting activity [38].

Platelet binding The formation of the tenase complex requires the delivery of FVIII to the activated lipid surface of platelets by VWF [12]. The transfer from VWF to platelets and the tenase complex is a complicated, multistep process [39]. Unactivated FVIII does not normally bind to activated platelets in the presence of VWF. However, in the absence of VWF, unactivated FVIII can bind to platelets activated by thrombin, epinephrine or thrombin receptor peptide with a dissociation constant (K_d) of 10.4 nM [12]. The binding affinity of unactivated BDD-rFVIII was found to be increased by two fold, as reflected by a decline in K_d to 5.1 nM. Once full-length FVIII is activated with thrombin, the binding affinity to activated platelets increases even

more (K_d 1.7 nM). Thus, these findings suggest that the B domain may, like VWF, serve as a brake on binding of unactivated FVIII to activated platelets, although the magnitude of this putative modulatory effect is apparently small compared with that of VWF.

Inactivation FVIIIa cofactor activity is rapidly lost either through spontaneous dissociation of the A2 subunit from the rest of the molecule or through proteolytic degradation by a number of enzymes, such as thrombin, FIXa, FXa and activated protein C (aPC). The main physiological inactivator of FVIIIa, aPC (Fig. 1), cleaves FVIIIa in the A1 (Arg^{336}) and A2 domains (Arg^{562}). FXa proteolytically inactivates FVIIIa by cleaving the A1 domain at Lys^{36} and Arg^{336}. In a comparison of full-length FVIII and BDD-rFVIII, proteolysis of A1 and A2 domains of activated BDD-rFVIII was found to proceed 11–13 times faster than that of activated pdFVIII or full-length rFVIII [20]. Inactivation of activated BDD-rFVIII was two to three times faster by aPC and five to six times by FXa. Accelerated inactivation of BDD-rFVIII, as well as accelerated activation by thrombin, would necessitate either higher doses or more frequent dosing. A 2003 meta-analysis did demonstrate increased bleeding incidence associated with the use of BDD-rFVIII compared with full-length FVIII for prophylaxis [40].

Since the last portion of the B domain is cleaved at the time FVIII is activated, it is unclear why the BDD-rFVIII should be more vulnerable to proteolysis than full-length FVIII. Even so, both rFVIII and pdFVIII displayed comparable rates of proteolysis, and the suggestion remains that the initial absence of B domain may render BDD-rFVIII more susceptible to proteolysis. The investigators ruled out the possibility that the free B domain can inhibit the action of aPC and FXa. Thus, addition of purified B domain to the BDD-rFVIII reaction mixture, even at the same molar concentration as FVIII, did not decrease the cleavage rate. Further studies are needed to confirm and clarify this paradoxical observation.

Clearance An aspect of the residence of FVIII in the plasma that is very little understood is the clearance of either the unactivated circulating FVIII molecule or its breakdown subunits resulting from activation [4]. Evidence suggests that the *N*-linked oligosaccharide structures of the B domain may play a role in the catabolism of FVIII [17]. Low-density lipoprotein receptor-related protein (LRP), an endocytic receptor, has been found to contribute to the clearance of FVIII from the circulation [41,42]. Experimental evidence suggests that other pathways play a role as well [43]. A potential candidate is the asialoglycoprotein receptor (ASGPR), a member of the *C*-type family of lectins abundantly expressed in the liver and involved in the binding and endocytic uptake of glycoproteins from the circulation. The B domain has been found to bind with high affinity ($K_d \approx 2$ nM) to ASGPR, and evidence suggests that the *N*-linked oligosaccharides are responsible [17]. Both the intact FVIII heterodimer and the FVIII heavy chain, but not the light chain, exhibited similar high-affinity binding. Treatment with an enzyme that removed the *N*-linked oligosaccharide branches abolished the binding to ASGPR. Finally, BDD-rFVIII was unable to bind ASGPR. The oligosaccharides on the FVIII molecule responsible for the high-affinity interaction with ASGPR remain unknown. Moreover, the physiological significance of the binding is unknown. The investigators proposed that ASGPR may play a physiological role in the QC of FVIII biosynthesis by binding and internalizing incompletely glycosylated FVIII following secretion [17].

Circulating FVIII levels The FVIII levels in normal persons and half-lives of replacement concentrates in patients with haemophilia are both characterized by substantial intraindividual variation. While some explanatory factors, such as ABO blood group, have been identified, the reasons for this variability are still poorly understood. A meta-analysis of 27 pharmacokinetic studies suggested that BDD-rFVIII may have a shorter circulating half-life than full-length FVIII [40]. An acknowledged limitation of the meta-analysis was the scarcity of available data derived from direct comparison between FVIII types in the same study. Two subsequent controlled pharmacokinetic studies have failed to confirm a half-life difference between a reformulated BDD-rFVIII preparation and full-length FVIII [44,45]. Pharmacodynamic differences between the two FVIII types that may affect plasma FVIII levels, as suggested by the recent reports on FVIII activation and clearance, still have not been adequately explored.

Differences in BDD-rFVIII and full-length FVIII have been observed in the results obtained from one-stage clotting assays and chromogenic assays measuring FVIII levels. This assay discrepancy between the two methods occurs *in vitro* as well as *ex vivo* after analysis of plasma from treated patients, and while still unexplained likely reflects biochemical differences, possibly at least in part reflecting conformational changes. BDD-rFVIII activity, as assessed with commercial activated

partial thromboplastin time (aPTT) reagents, is consistently about 50% lower than that measured by the chromogenic assay [46,47]. BDD-rFVIII displays greater sensitivity than full-length FVIII to variations in the composition and concentration of phospholipid [48]. The discrepancy between assays can be abolished by using mixtures of source phospholipid in which the content of phosphatidylserine is maintained below 10% [46]. Interaction of FVIII with phospholipid-containing membranes is critical for the proper function of the tenase complex. The lipid binding region of FVIII resides within the C2 domain (amino acids 2303–2332) of the molecule [49], and perhaps a subtle conformational change resulting from the B domain deletion affects phospholipid binding. The potency estimation of BDD-rFVIII also appears to be sensitive to FX activation time in the chromogenic method, and there have been discrepancies even between different chromogenic assay kits [50]. The authors postulated that differences in the profile of FVIII activation by thrombin may contribute to the discrepancy between BDD-rFVIII and full-length FVIII in results from the two types of assays [50]. In 2003, the assay discrepancies encountered with BDD-rFVIII led to a reformulation of the product with 20% more drug product than the original formulation in order to achieve the stated potency [51,52].

It would also be interesting to determine if and how the B domain affects circulating FVIII levels in the normal population. Individuals with elevated FVIII levels have an increased risk for both venous and arterial thrombosis [53–55]. In a recent study investigating the influence of FVIII polymorphisms on plasma FVIII activity levels, the only single-nucleotide polymorphism (SNP) identified was located in the B domain [56]. The SNP was associated with a substantial increase in FVIII activity. Whether this polymorphism affects the intracellular trafficking of FVIII or its interactions in the plasma remains to be determined. Furthermore, while most evidence suggests that the *N*-linked glycosylation of the B domain accounts for much of its actions and characteristics, the role of its protein component remains unknown. However, a pilot study screening for protein interactions of the FVIII B domain with liver proteins identified several interactions with potential functional implications [57].

Conclusions

Even though it constitutes approx. 40% of the FVIII molecule, the functions of the B domain have not been well characterized. While the strongest evidence of its role is in the intracellular trafficking of FVIII, other intriguing findings and observations also suggest that it may play a modulatory role in the plasma.

Nonetheless, consistent evidence has not emerged to date for a major influence by the B domain on the fate of FVIII in circulation. A possibly subtle modulatory role, on the other hand, is suggested by the above summarized data pertaining to interactions of FVIII with other key elements involved in the tenase complex or modulations of FVIII levels in the circulation. Just before FVIII is secreted, some of the *N*-linked B domain oligosaccharides undergo further modification to bi-, tri-, and tetra-antennary complex-type sugar chains in the Golgi. The purpose served by these modifications is yet to be clarified and deserves investigation. It could be hypothesized that these pre-secretion oligosaccharide modifications might also affect the fate of FVIII in plasma.

Beyond its relevance to replacement therapy for haemophilia A, understanding the range of functions served by the B domain may lead to improved gene therapy constructs. Most gene therapy applications for haemophilia A have relied on BDD-rFVIII due to its advantages of reduced overall cDNA size for packaging into viral vectors and increased mRNA levels. However, *in vivo* results have remained disappointing, with plasma levels typically undetectable (<1%) or in the 1–4% range in a few patients transiently [16]. Incorporating key B domain sequences in the constructs may improve the level of secretion.

Considerable progress continues to be made in unravelling the complex interaction between cells and molecules involved in maintaining the balance needed for effective haemostasis. However, many details of how this fine balance is achieved still remain undiscovered or underappreciated. The full story of the B domain of FVIII is unlikely to have yet been told or its utility fully exploited.

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