Factor VIII Structure and Function

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Abstract

Factor VIII, a non-covalent heterodimer comprising of a heavy chain (A1-A2-B domains) and light chain (A3-C1-C2 domains), circulates as an inactive procofactor in complex with von Willebrand factor. Metal ions are critical to the integrity of factor VIII, with Cu and Ca ions stabilizing the heterodimer and generating the active conformation, respectively. Activation of factor VIII catalyzed by thrombin appears dependent upon interactions with both anion-binding exosites I and II, and converts the heterodimer to the active cofactor, factor VIIIa. This protein, comprised of A1, A2, and A3-C1-C2 subunits, is labile due to weak affinity of the A2 subunit. Association of factor VIIIa with factor IXa to form the intrinsic factor Xase complex is membrane-dependent and involves multiple inter-protein contacts that remain poorly characterized. This complex catalyzes the conversion of factor X to factor Xa, a reaction that is essential for the propagation phase of coagulation. The role of factor VIIIa in this complex is to increase the catalytic efficiency for factor Xa generation by several orders of magnitude. Mechanisms for the down-regulation of factor Xase focus upon inactivation of the cofactor and include dissociation of the A2 subunit as well as activated protein C-catalyzed proteolysis.

Key words: Factor VIII; Factor VIIIa; Factor IXa; Intrinsic factor Xase; Factor X; Catalytic rate constant; Thrombin

1. Introduction

Factor VIII serves as a cofactor for the serine proteinase factor IXa in the conversion factor X to factor Xa during the propagation phase of blood coagulation. The essential role of factor VIII in coagulation is indicated by hemophilia A, the most common of the severe bleeding disorders, which results from a defect or a deficiency in this protein. Although the primary structure of factor VIII is known, and homologies with similar proteins have produced model structures, little information from high-resolution structures of factor VIII is available. Similarly, regions of factor VIII critical for cofactor function and specific mechanisms for catalytic rate enhancement remain poorly understood. This review focuses on recent information related to understanding the structure and function of this essential blood coagulation factor.

2. Factor VIII Structure

Factor VIII is synthesized primarily by liver sinusoidal endothelial cells [1] as a large (~300 kDa; 2332 residues) single chain protein [2,3]. Internal sequence homologies indicate a triplicated “A” domain homologous to the A domains in factor V and ceruloplasmin, a duplicated “C” domain, and a large “B” domain that is not homologous to other known structures [2,4]. Segments (~30-40 amino acids; designated by a lowercase “a”) possessing a high concentration of acidic residues directly follow the A1 (residues 337-372) and A2 domains (residues 711-740) and precede the A3 domain (residues 1649-1689). The domain structure of factor VIII is ordered as: (NH$_2$) A1-a1-A2-a2-B-a3-A3-C1-C2 (COOH) (Figure 1). N-linked glycosylation, which heavily modifies the B domain as well as several sites in the A1 domain, appears dispensable for factor VIII activity. Post-translational modification includes sulfation of specific tyrosine residues in the acidic (“a”) regions. Sulfated tyrosine residues in the a2 and a3 acidic segments contribute to cofactor function and von Willebrand factor (VWF) binding, respectively [5].

 Structural information on factor VIII is limited. Homology modeling of the A domains based upon the crystal structure of ceruloplasmin [6], which shows ~35% identity with
factor VIII A domains, has provided useful information for predicting interactive sites for ligands and macromolecules, as well as mapping mutations. The x-ray structure of the C2 domain [7] represents the only current high-resolution structure for a factor VIII domain and provides a mechanism for cofactor binding to membranes that involves both hydrophobic and electrostatic interactions. Studies on 2-D crystals of factor VIII bound to phospholipids have yielded gross information (at 15 Å resolution) on inter-domain orientation and have been employed to construct a 5-domain model (A plus C domains) of factor VIII [8]. The recent x-ray structure of the bovine factor Va A1/A3-C1-C2 dimer [9], obtained following cleavage of factor Va by activated protein C, may provide added insights into the interactions of these domains in factor VIIIa, because models derived from this structure would be superior to the current models derived from homology to ceruloplasmin A domains.

Factor VIII circulates as a series of heterodimers formed as a result of proteolysis at the B-A3 junction plus additional cleavages at sites within the B domain (see Ref. [10] for review). Thus factor VIII contains a constant sized light chain, composed of the A3-C1-C2 domains, and a heavy chain, minimally represented by the A1-A2 domains but variable in size due to the presence of some or all of the contiguous B domain (Figure 1). Binding of the 2 chains is noncovalent and requires a metal ion-dependent linkage, with the residues responsible contained within the A1 and A3 domains. This metal ion is likely copper, and 1 mol of copper ion (Cu+, [11]) has been identified in factor VIII [12]. Reconstitution of isolated factor VIII chains showed that Cu ions significantly increase the inter-chain affinity [13]. Three putative Cu-coordination sites exist in factor VIII [6], a type 1 site in the A1 (His257/Cys310/His315/Met320) and A3 (His1954/Cys2000/His205/Met2010) domains and a type 2 site that spans residues in the A1 (His99) and A3 (His1957) domains. Sites for Cu ion binding in factor VIII have not been rigorously determined and the location of the metal ion(s) remains controversial. One study examining the activity of transiently expressed factor VIII molecules possessing point mutations in the putative sites suggested a role for the type 1 site in the A1 domain in coordinating the Cu ion [11]. However, other studies assessing the reconstitution of factor VIII activity employing fluorophore-modified factor VIII at Cys residues predicted to participate in Cu binding (Cys310 and Cys2000) supports a role for the type 2 site in promoting chain association [13].

Although Cu ions facilitate inter-chain affinity, Ca2+ [13,14] or Mn2+ [15] is essential to yield active factor VIII. Ca2+ coordination within both the factor VIII heavy and light chains is necessary for generation of maximal specific activity [14]. Ca2+ (or Mn2+) appears to modulate activity by altering protein conformation [13,14]. Site-directed mutagenesis of acidic residues in factor VIII sequence 110-126, a region homologous to a putative Ca2+ binding site in factor V [16], suggested that Glu110, Asp116, Glu122, Asp125, and Asp126 likely coordinate Ca2+ whereas Asp116 and Asp125 may also contribute to Mn2+ coordination [17]. Recently, a novel point mutation within this A1 domain Ca2+ binding site was described that possessed ~2-fold greater specific activity than wild type factor VIII [18]. The basis for this gain-of-function is not clear but may relate to the ~4-fold enhanced affinity for factor IXa observed on physiological membrane surfaces. This observation reinforces the role for Ca2+ sites in modulating cofactor function.

Factor VIII circulates in noncovalent complex with VWF, a large, multimeric protein comprised of identical ~220 kDa subunits. Factor VIII binds VWF in a high affinity interaction (Kd ~0.3 nM) mediated by residues in the factor VIII light chain. Critical sites for this interaction have been localized to the N-terminal acidic region preceding the A3 domain (a3, residues 1649-1689) and the C2 domain (see Ref. [19] for review). Recent insights provided by the factor VIII model suggest that the a3 segment and the C1 and C2 domains form an extended surface for interaction with VWF [20]. Interactive residues for binding VWF also appear to overlap with those required for the association of factor VIII with anionic phospholipids, thus blocking premature association with thrombogenic surfaces. The association of factor VIII in this complex results in several positive attributes that include increased circulatory half-life and protection from proteolysis. This latter attribute, largely a result of inhibition of the interaction of factor VIII with phospholipid surfaces as well as direct competition with protease binding sites [21], is particularly relevant in that it limits catalysis by surface-dependent

![Figure 1](image-url). Factor VIII and factor VIIIa. The A and C domains are in pink and gold, respectively. Acidic rich segments a1, a2, and a3 are in green. The B domain in silver is not to scale. Heavy and light chains of the factor VIII heterodimer are linked by a metal ion (Cu)-dependent bridge depicted by light blue circles. Activating cleavage sites are shown in green arrows and inactivating cleavages are shown in red. Approximate locations for macro-molecular and metal ion interactive sites are denoted by the blue lollipops. Electrostatic association of A1 and A2 is denoted by the plum dashed line. Modified from Ref. [59], reprinted with permission.
activators and/or inactivators such as factor Xa and activated protein C.

3. Activation of the Procofactor

Conversion of procofactor factor VIII to active factor VIIIa by thrombin (or factor Xa) is associated with cleavages (Figure 1, arrows) in the heavy chain at Arg372 (A1-A2 junction) and at Arg740 (A2-B junction), and the light chain is cleaved near its NH\textsubscript{2} terminus at Arg1689 [22]. Molecular mechanisms by which thrombin, considered the physiologically active factor VIII, catalyzes this conversion are not fully understood. Significant evidence indicates that multiple structures in thrombin, including anion binding exosites I and II as well as the Na\textsuperscript{+} site, contribute to the interactions of enzyme with the factor VIII substrate, based upon inhibition studies using ligands for the exosites (see Ref. [10] for review). Ala-scanning mutagenesis of residues contained in these structures [23], and the Na\textsuperscript{+}-dependence of selected binding interactions [24]. Complementary binding sites in factor VIII are not well defined. Cleavage of the factor VIII light chain appears to require thrombin binding to the C2 domain based upon inhibition of this reaction by a recombinant C2 domain [25]. Recent evidence based upon binding studies employing isolated A1 and A2 subunits suggests that thrombin binds with high affinity (K\textsubscript{d} ~5 nM) to the A1 domain of heavy chain, and that this binding is modulated by the exosite I and requires occupancy of the Na\textsuperscript{+} site, whereas a lower affinity interaction (K\textsubscript{d} ~100 nM) is observed for A2 domain that is exosite II-dependent, but independent of the Na\textsuperscript{+} site [24]. That study also suggested that an acidic residue-rich region (residues 389-394) in factor VIII A2 domain interacts with thrombin, facilitating cleavage at Arg740 during procofactor activation. Similar sites in factor VIII heavy chain that facilitate cleavage at Arg372 have not been identified.

Factor Xa has been shown to attack factor VIII at identical sites as those cleaved by thrombin. Although both proteases bind the C2 domain, the binding sites are not identical [26]. Functional differences for these interactions are also observed. Interaction of the C2 domain with factor Xa regulates in part cleavage of heavy chain, whereas C2 interaction with thrombin shows no influence on this cleavage [25]. Furthermore, Tyr346Phe mutants are efficiently cleaved by factor Xa, indicating that tyrosine sulfation at this site does not influence the binding of factor Xa [27]. On the other hand, factor Xa, but not thrombin, was affected by the cluster mutation converting the Asp residues at positions 361-363 to Ala. These observations suggest significant mechanistic differences in the interactions of the two proteases during cofactor activation [28].

4. Factor Xase and Factor VIIIa Function

Assembly of the factor Xase complex is mediated by protein-lipid and protein-protein interactions (see Ref. [10,29] for review). Factor VIII binds anionic phospholipid membranes via hydrophobic and electrostatic [7] interactions that are mediated by the C2 domain. The former interaction occurs via 2 hydrophobic spikes comprised of Met2199/Phe2200 and Leu2151/Leu2152 that penetrate the lipid bilayer and associate with the fatty acyl hydrocarbon chains. Electrostatic interactions are mediated by a ring of basic residues that include Arg2215, Arg2220, Lys2227, and Lys2249, localized just above the hydrophobic residues, which form salt linkages with the anionic polar head groups of phosphatidyl serine.

Inter-protein interactions between factor IXa and VIIIa likely occur over an extended interface. Two domains of factor VIII (VIIIa) appear to be critical for this interaction. The high affinity (K\textsubscript{d} ~15-50 nM) of the isolated factor VIII light chain for factor IXa [30,31] is consistent with the A3-C1-C2 subunit of factor VIIIa providing the majority of the binding energy for this interaction. Peptide inhibition studies mapped a minimum sequence required for this interaction to Glu1811-Lys1818, although more recent mutagenesis studies employing a chimera where sequences of factor VIII were substituted for the homologous sequences in factor V have proposed residues 1803-1810 as factor IXa-interactive [32]. Studies examining the stimulation of factor IXa activity by the isolated A2 subunit indicate that the interaction of this subunit with the protease is of relatively weak affinity (K\textsubscript{d} ~300 nM [33]).

Similar approaches to those employed above have been used to identify interactive regions in the A2 subunit. One region is represented by a loop comprising residues 558-565 [34]. Mutagenesis of residues within the 558-loop and subsequent characterization of the recombinant proteins have shown a specific role for this region in contributing to the kcat effect, whereas these mutations showed little if any reduction in the inter-protein affinity [35]. Two other sites in the A2 subunit bear mentioning. Residues 484-509 represent a primary epitope for inhibitor antibodies [36] and appear to participate in factor VIIIa-dependent stimulation of factor IXa activity [37,38]. A recent study evaluating the effects of Ala-scanning of charged residues in this sequence showed that 1 cluster mutant where residues Arg489, Arg490, and Lys493 were replaced with Ala showed significantly reduced rates of catalysis that were not observed when these residues were individually changed to Ala [39]. These observations suggested a role for the basic electrostatic potential of this region in facilitating catalysis. Recent results have also suggested that the C-terminal region of A2 contributes to the factor IXa interface. Asp712 likely makes direct contact with factor IXa based upon a ~7-fold decrease in the inter-protein affinity when this residue is replaced by Ala [31]. These regions of inter-protein interaction are illustrated in Figure 2.

The mechanisms by which factor VIIIa enhances catalytic efficiency (kcat/Km) for factor Xa generation remain poorly understood. The primary kinetic component affected by factor VIIIa is kcat, which is increased by several orders of magnitude (see Ref. [10] for review). Studies using isolated subunits of factor VIIIa have shown that although neither A1 nor the A3-C1-C2 subunit affect the activity of factor IXa in catalyzing the conversion of factor X to factor Xa, the isolated A2 subunit enhanced the kcat for this reaction by as much as 100-fold [33]. A subsequent study examining interactions of isolated A2 subunit with mutant factor IXa forms was used to identify and model a binding surface involving the 330-helix of the protease and the 558-loop of the cofactor [40] (see Figure 2). This region of factor IXa, which links
Figure 2. Factor Xase. Factor VIIIα (left) and factor IXα (right) are drawn in ribbon format based upon the 5-domainal model of factor VIII [20] and the crystal structure of factor IXα [41], respectively. Factor IXα is shown in green with the 330-helix in red. Spheres indicate the alpha carbon positions of the active site residues. Factor VIII domains are coded as A1 (blue), A2 (cyan), and A3 (red) and C domains (copper). Spheres indicate alpha carbon positions for the indicated factor IXα-interactive sites. Reprinted from Ref. [59] with permission.

directly to residues in the extended substrate binding site [41], has been shown by kinetic and mutational studies to contribute to the modulation of the factor IXα structure yielding altered enzymic activity [42]. That study also demonstrated that mutations in this region, which yield hemophilia B phenotypes, perturb the interactions of the proteinase with factor VIIIα. It has been suggested that factor VIIIα binding close to factor IXα residue Tyr345, which stabilizes the 99-loop and promotes the “zymogen-like” conformation of factor IXα [43], releases the locked 99-loop conformation and that subsequent binding of substrate factor X rearranges this structure, yielding access of the active site region [44].

Although the phospholipid membrane surface makes a primary contribution to Km by restricting the reactants to a 2-dimensional surface, factor VIIIα also reduces the Km for substrate factor X about 5-fold and this effect appears significant at plasma concentrations of factor X. Information on the interaction of factor X with factor VIIIα is limited. Solid phase assays revealed that the A1 subunit C-terminal region (residues 336-372) contains a binding site for factor X [45]. Affinity determinations estimated a moderate affinity (Kd ~1-3 μM), however, non-equilibrium methods were used in this approach. Studies using a zero length crosslinking reagent showed that the association between factors VIII and X was primarily electrostatic and mediated by formation of a salt bridge(s) [46]. Recent functional studies have confirmed the importance of this C-terminal region of A1 in factor Xase catalysis. Cleavage at Arg336 by activated protein C (see below) to liberate this fragment yielded a truncated factor VIIIα that, when recombinated with factor IXα and phospholipid, yielded a ~5-fold increased Km for factor X [47]. Furthermore, mutation of a cluster acidic region in this segment of A1 (residues 361-363) to alanine resulted in an increased Km for factor X [48]. Taken together, these results suggest that the C-terminal region of the A1 subunit contributes to substrate binding in factor Xase.

5. Factor VIIIα Inactivation and Xase Dampering

The activity of factor VIIIα is unstable and decays spontaneously, reflecting the dissociation of the A2 subunit (see Ref. [10] for review). The observed “self-damping” of factor Xase is primarily attributed to this process, based upon kinetic analysis of reconstituted factor Xase using a cleavage resistant factor VIII molecule [49]. This property likely contributes a regulatory role in coagulation. The physiological importance of this weak inter-subunit affinity is reflected by a class of hemophilia A mutations referred to as possessing 1-stage assay (measure of factor VIII procofactor activity) versus 2-stage assay (measure of factor VIIIα cofactor activity) discrepancy [50,51]. Several point mutations exhibit less relative activity in the latter assay compared with the former, as noted in the hemophilia A database [52], reflecting a greater rate of A2 dissociation than observed in native factor VIIIα. Furthermore, novel, recombinant factor VIII reagents have been developed that possess enhanced cofactor stability by preventing dissociation of A2 subunit in the factor VIIIα heterotrimer. In one construct, a largely B-domainless factor VIII molecule retains a short length of B domain contiguous with the A3 domain but with the thrombin cleavage site at 1689 eliminated. Thus cleavage at Arg372 results in separation of the A1 and A2 domains, but the latter remains covalent with the (B segment)-A3-C1-C2 subunit [53]. A second recombinant factor VIII makes use of a novel disulfide bridge to covalently bond A2 subunit within the factor VIIIα molecule [54]. In this factor VIII mutant, residues Tyr664 in A2 and Thr1826 in A3, postulated to be in close proximity according to the A domain homology model [6], were both changed to Cys to allow for disulfide bonding. Assays revealed little or no loss in activity for either factor VIIIα during extended reaction time courses.

A second pathway for down-regulation relies on proteolytic inactivation of factor VIIIα catalyzed by activated protein C. Cleavage of factor VIIIα occurs at Arg562, bisecting the A2 subunit, and Arg336, preceding the C-terminal acidic region of the A1 subunit, and both cleavages affect the factor VIIIα-dependent modulation of the factor IXα active site. Cleavage of Arg562 occurs within a critical factor IXα inter-active site (the 558-loop) and thus would directly alter the interaction of cofactor with factor IXα. Recent studies have provided additional details to inhibitory mechanisms attributed to proteolysis at the A1 site. Cleavage at Arg336 alters the interaction between the A1 and A2 subunits reflecting reduced kcat values [55], as well as resulting in a several-fold increase in Km of factor Xase for substrate factor X [47]. Although activated protein C action is largely responsible for down-regulation of prothrombinase by attack of factor Va (reviewed in Ref. [56]), kinetic analysis in purified systems using reconstituted factor Xase components [57,58] indicates that this is not the primary pathway for down-regulation of factor Xase.
6. Conclusions

Investigation of structure and function of factor VIII/ VIIIa regarding mechanisms for molecular recognition, association and modulation of subunits within the cofactor, and catalytic rate enhancement is critical to our understanding of this essential coagulation protein. Definition of these issues will yield valuable insights into the biochemistry of factor VIII and have implications for understanding defects in hemophilia that may potentially assist in the design and development of improved therapeutics for the treatment of this disorder.

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References


