



COMMENTARY

Factor XIII: What does it look like?

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Coagulation factor XIII (FXIII) is a stepchild among clotting factors. As opposed to all other zymogenic clotting factors, it is not the precursor of a proteolytic enzyme but of a transglutaminase. It is of tetrameric structure consisting of two types of subunits (FXIII-A₂B₂). In the final step of the coagulation cascade the A subunit (FXIII-A) is transformed into an active enzyme by the concerted action of thrombin and Ca²⁺. Thrombin cleaves off an activation peptide (AP-FXIII) of 37 amino acids from the N-terminal end of FXIII-A. Subsequently, in the presence of Ca²⁺, the protective/inhibitory B subunits (FXIII-B) dissociate from the cleaved A' subunits, which then assume an active configuration (FXIII-A*). Activated FXIII is a transglutaminase, the main function of which is the cross-linking of fibrin α and fibrin γ chains and the attachment of α_2 -plasmin inhibitor to fibrin through $\epsilon(\gamma$ -glutamyl) lysyl isopeptide bonds. This way FXIII is involved in mechanically strengthening the fibrin clot and protecting it from fibrinolytic degradation. A dimer of FXIII-A lacking the B subunits is also expressed in several cell types, including platelets, monocytes, macrophages, osteoblasts, chondrocytes, and preadipocytes in which it exerts diverse functions. The intracellular activation of FXIII-A₂ does not need limited proteolytic involvement. The elevation of the Ca²⁺ level in the cytoplasm is sufficient to convert FXIII-A₂ to an active configuration (FXIII-A°). (For comprehensive reviews see references [1–5].)

X-ray crystallography revealed the atomic resolution structure of FXIII-A₂ and demonstrated that the FXIII-A monomer contains four domains (β -sandwich, catalytic core, β -barrel 1, and β -barrel 2) and AP-FXIII

[6,7]. The crystallographic structure of FXIII-B is not available. This protein is of mosaic structure, it consists of 10 short tandem repeats (sushi domains) each containing about 60 amino acids and held together by a pair of disulfide bonds [8]. Our knowledge on the structure of FXIII and on structural changes during FXIII activation was enriched by important studies using electron microscopy, size exclusion chromatography, analytical ultracentrifugation, H-D exchange, mass spectrometry, and molecular modeling [2,9–13]. The use of high-resolution single-molecule atomic force microscopy (AFM) in the study of Protopopova *et al.*, published in this issue of *JTH*, provides a new dimension to this extensive line of research [14]. This technique visualizes the surface topology/contour of the molecule rather than the protein backbone, and this way an additional aspect of structural information is revealed. The authors investigated the morphology of the tetrameric FXIII-A₂B₂ complex and the individual subunits. The structural changes induced by thrombin and Ca²⁺ were also demonstrated.

The structure of FXIII and its subunits

Based on early electronmicroscopic studies it was suggested that two FXIII-B subunits are wrapped around the FXIII-A dimers [9]. This hypothesis was supported by the finding that the presence of FXIII-B protects the A subunits from proteolytic inactivation [10,14]. The AFM pictures produced by Protopopova *et al.* provide a remarkably novel insight into the structure of FXIII-A₂B₂. In the published study it is clearly demonstrated that within the tetrameric structure, FXIII-A₂ is represented by a globular part of moderate ovality. In the majority of cases two strands corresponding to monomeric FXIII-B extend from the globular part; however, there were also minor structural variants with only one or no thin flexible extensions. This structural diversity very likely reflects the equilibrium between association and dissociation of FXIII-B subunits from the A₂ dimer. According to the AFM data, 26% of the B subunits dissociate from the globular core, while 74% are connected to the globules, which is in excellent agreement with the values calculated from the actual FXIII concentration

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(~6.3 nm) and the K_d (4.17×10^{-10} M) [15]. Earlier reports suggested that the first two N-terminal sushi domains of FXIII-B are involved in the association to FXIII-A₂ [10,15], which was confirmed by the AFM pictures. As expected, the individual FXIII-A monomers in the complex are not resolved by AFM. The location of FXIII-B in the complex is somewhat surprising. As discussed later, the individual free FXIII-B molecules form dimers, and it is not clear how the FXIII-B dimer falls apart into monomers to be able to combine with an individual FXIII-A molecule. The binding site for FXIII-B on FXIII-A also needs to be specified to explain how the two FXIII-B monomers enter the compact/closed structure of FXIII-A₂. Such investigations might be relevant for FXIII-A-deficient patients receiving recombinant FXIII-A₂ for prophylaxis: in these patients the binding of recombinant FXIII-A₂ to the patient's FXIII-B is essential for the formation of heterotetramers, which in contrast to uncomplexed FXIII-A₂ have a long half-life in the plasma.

Earlier reports concerning the dimeric or monomeric state of free FXIII-B were controversial. Sedimentation analysis and low-resolution electronmicroscopic images suggested free FXIII-B to be a flexible and kinked monomeric structure [9], while gel filtration experiments suggested the protein to be a dimeric structure [10,16]. In the present study the dimeric nature of free FXIII-B was clearly confirmed. It has been shown that the measured length of FXIII-B dimer corresponded to a previously suggested model, in which the two FXIII-B subunits have an antiparallel orientation, which is ensured by the interaction of sushi domains 4 and 9 [10]. Accepting this model, further experiments are needed to explain the mechanism of complex formation considering that the two N-terminal sushi domains are positioned at the opposite ends of the dimeric strand. One possibility is that the two opposite ends of FXIII-B₂ bind to FXIII-A molecules present in distinct FXIII-A dimers. The other possibility is that the binding of

a FXIII-B subunit to FXIII-A₂ induces the dissociation of the FXIII-B₂ and the released FXIII-B monomer becomes available for combining with FXIII-A₂. Obviously such speculations and others call for experimental support.

Structural changes upon FXIII activation

Earlier reports suggested that after activation of FXIII-A₂B₂ and FXIII-A₂ by thrombin and Ca^{2+} the two active subunits stick together (FXIII-A₂*) and express transglutaminase activity. Most recently this paradigm has been challenged. The dissociation of FXIII-A₂ into FXIII-A* monomers upon both proteolytic and non-proteolytic activation was demonstrated by analytical ultracentrifugation, and FXIII-A* also remained monomeric upon binding of substrate analogs [11]. Protopopova *et al.* provided direct evidence on the monomeric form of FXIII-A* by AFM [14]. Using volume calculations the FXIII-A₂ dimer was found to be 2.3-fold larger than FXIII-A*. Molecular modeling also supported a monomeric form for FXIII-A* [13,17]. The monomeric structure of all other active transglutaminases is in line with these findings [2,18]. Based on the results of Protopopova *et al.* and on earlier findings a schematic representation of FXIII activation is proposed in Fig. 1. The transformation of FXIII-A does not induce drastic conformational changes within a particular domain. However, the changes in the position of individual domains indicate considerable structural transitions, usually termed as transition from closed/compact to open/extended structure (reviewed in references [2,19]). The resolution of single-molecule AFM does not seem sufficient to capture such a difference.

The introduction of high-resolution single-molecule AFM opens new perspectives in FXIII structural research. It helps in evaluating controversial issues and might confirm or contradict existing paradigms. New findings by this technique contribute to our better understanding of the structural-functional relationship of this

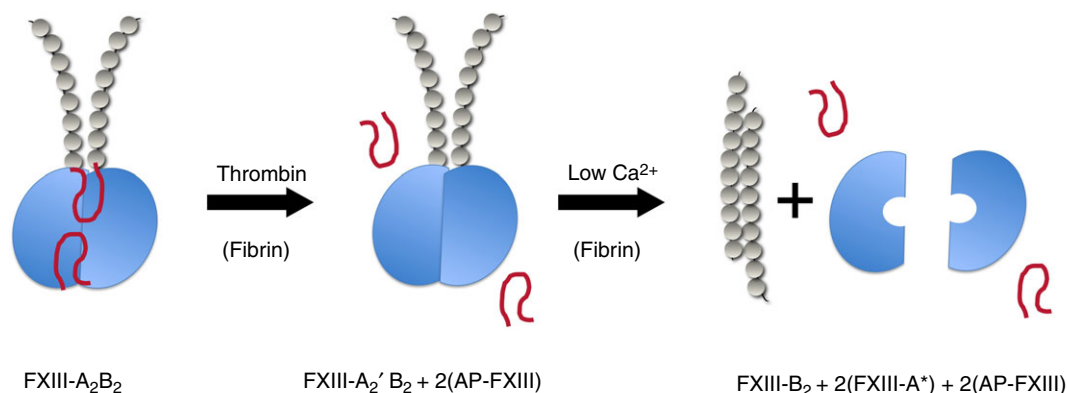


FIGURE 1. Schematic representation of proteolytic FXIII activation. The A subunit of (FXIII-A) is depicted in blue. Elongated strands consisting of gray pearls represent the FXIII B subunit (FXIII-B) consisting of sushi domains. Activation peptides (AP-FXIII) are shown as red loops. FXIII-A', FXIII-A cleaved by thrombin; FXIII-A*, FXIII activated by thrombin; Ca^{2+} (active transglutaminase).

unique coagulation factor and its role in the hemostatic mechanism.

Addendum

The authors were equally involved in writing and constructing the commentary.

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