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ORAL ABSTRACTS

321.COAGULATION AND FIBRINOLYSIS: BASIC AND TRANSLATIONAL

First Validated Model of Zymogen Factor XII Provides Insights into Protein Assembly and Activation

Aleksandr Shamanaev, PhD[1], Mao-Fu Sun, BSc[1], Michal Ponczek, PhD[2], Jonas Emsley, PhD[3], David Gailani, MD[4]

[1] Vanderbilt University Medical Center, Nashville, TN
[2] University of Lodz, Lodz, Poland
[3] University of Nottingham, Nottingham, United Kingdom
[4] Department of Pathology, Immunology and Microbiology, Vanderbilt University Medical Center, Nashville, TN

Factor XII (FXII), the zymogen of the plasma protease FXIIa, contributes to thrombo-inflammatory processes. As part of the kallikrein-kinin system, FXIIa converts prekallikrein to the protease kallikrein (PKa), which then cleaves high-molecular-weight kininogen (HK) to release the vasoactive peptide bradykinin. In the disorder hereditary angioedema, dysregulation of FXII activation leads to excessive bradykinin production. When blood is exposed to artificial surfaces, such as during cardiopulmonary bypass or ECMO, FXII is rapidly converted to FXIIa by contact activation, and contributes to thrombus formation by activating factor XI. FXII circulates in plasma in a "closed" form that is resistant to activation by PKa and other proteases. Surface-binding "opens" the FXII structure, facilitating activation. Relatively little is known about the structural features that maintain the closed FXII conformation as the full-length protein has been refractory to crystallization. Recently, a crystal structure was reported for part of the non-catalytic portion of FXII. An intriguing feature of the structure is an interaction between the fibronectin type 2 (FN2) and kringle (KNG) domains at opposite ends of the polypeptide that forms a ring-like structure. Functional studies by our group and others indicate these domains are key to maintaining the closed conformation. Here we describe a model for FXII prepared with the program AlphaFold (AF), and its validation using site-directed mutagenesis to disrupt interactions predicted to maintain the closed conformation.

The human FXII model in the AF database was modified by removing residues 300-316 from the proline-rich region and by reference to crystal structures of the FXIIa catalytic domain (CD) bound to inhibitors. Molecular graphics and interdomain interaction analyses were performed in ChimeraX. The refined model predicts 15 interdomain bonds involving residues in FN2, KNG, and the CD. Of the 22 amino acids involved in these bonds, 15 are highly conserved in FXII across 25 vertebrate species. We expressed human FXII in which these residues were individually changed to alanine (Table). Alanine replacements for amino acids not predicted to be involved in interdomain interactions were prepared as controls. Four variants (FXII-D253A, FXII-W257A, FXII-W268A and FXII-E502A) were activated in cell culture and one (FXII-R61A) did not express. FXII-R61A, FXII-W257A, and FXII-W268A were not studied further. FXII-D253A was replaced with FXII-D253K. The active site serine of FXII-E502A was replaced with alanine to prevent autocatalysis (FXII-E502A,S544A).

In the absence of a surface, FXII-R36A, FXII-E225A, FXII-D253K, and FXII-K346A were activated by PKa (~15 fold) faster than wild type FXII (FXII-WT) in a chromogenic substrate assay, consistent with an open conformation. Using densitometric analyses of stained SDS- polyacrylamide gels, we noted that these four FXII variants and FXII-E502A,S544A were converted to FXIIa by PKa more rapidly than FXII-WT and other variants. FXII-R36A, FXII-E225A, FXII-D253K, and FXII-K346A bound to prekallikrein with higher affinity than FXII-WT in a plate-binding assay, consistent with a change in conformation. When the four variants were added to human plasma in the absence of a surface, they caused a rapid cleavage of HK by accelerating reciprocal activation with prekallikrein.

Our functional analyses indicate that non-covalent bonds between R36 on FN2 and E502 on the CD; R36 and E225 on KNG; and D253 on KNG and K346 adjacent to the activation cleavage site are critical for maintaining FXII in a closed conformation. The instability of FXII variants with substitutions for tryptophans at positions 257 and 268 also implicate interactions between these residues and K346. In the AF model these bonds appear to maintain FXII in a conformation in which access to the activation cleavage site is limited. Our binding studies also suggest the closed conformation mask a binding site for prekallikrein and PKa. These bonds are likely disrupted when FXII binds to a surface, as part of the contact activation mechanism that enhances FXII activation.

Disclosures Gailani: *Anthos Therapetuics:* Consultancy, Honoraria; *Aronora:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: Factor XI and factor XII inhibitors; *Bristol-Myers Squibb:* Consultancy, Honoraria; *Ionis:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria.

FXII Domain Interactions	Validated bonds	Screened FXII Mutants
FN2-CD	R36 ↔ E502	FN2: R36A CD: E502A*, V359A
		FN2 Controls: K41A, K45A, R47A CD Controls: Q501A
FN2-KNG	R36 ↔ E225	FN2: R36A, D61A* KNG: L266A, E225A, D264A
		FN2 Controls: K41A, K45A, R47A KNG Controls: N230A
KNG-CD	D253 ↔ K346	KNG: D253A*, D253K, W257A*, R265A, W268A* CD: K346A, R362A, E411A, E551A
		KNG Controls:N230A CD Controls: R343A, R345A

* - alanine replacement led to FXII activation in cell culture or no expression.

Figure 1

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