Role of the Gla-Domain in Phospholipid-Independent Activation of Human Factor X

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Introduction

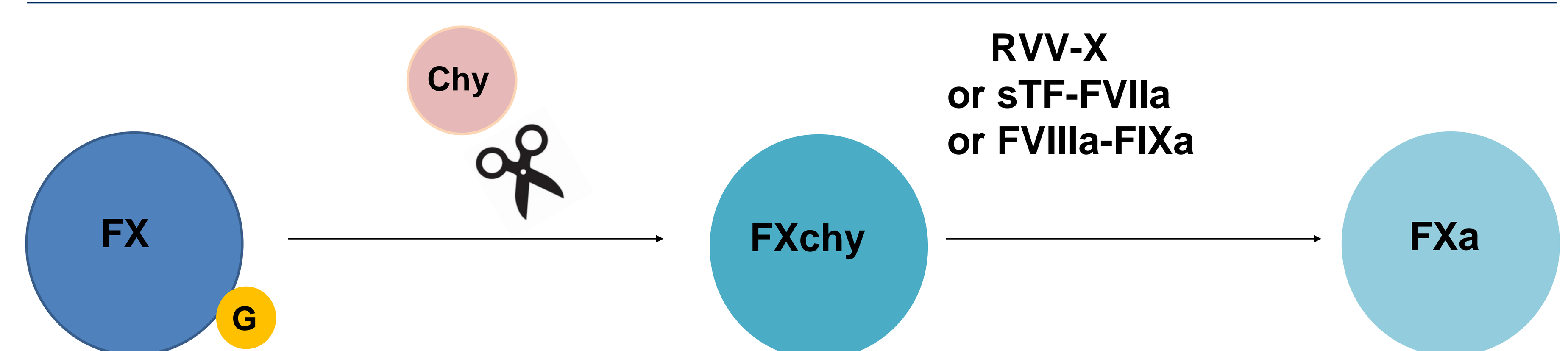
- **Factor (F) X is a critical blood coagulation protein. Once activated to FXa, it ultimately leads to the formation of clot.**
- **The γ-carboxyglutamic acid (Gla)-rich domain of FX is essential for Ca^{2+} dependent binding to negatively charged phospholipid, optimizing its orientation within tenase complexes for activation.**
- **Published data indicates that chymotrypsin can cleave the Gla-domain of bovine Factor X without generating additional cleavage[1]. Bovine FX lacking the Gla domain exhibits a reduced activation rate by RVV in the presence of Ca^{2+} and phospholipids[1]. However, studies on Gla-domain-less human FX remain unexplored.**
- **In this preliminary study, we investigated the role of the Gla-domain in human FX activation under phospholipid-free conditions to ascertain its role in protein-protein interactions.**

Hypothesis

- **The Gla-domain differentially participates in protein-protein interactions within the various tenases and is required for optimal activation of human FX in phospholipid–free condition.**

Experimental Design and Methods

- ***Chymotrypsin treatment:*** **Purified human FX (28uM) was treated with chymotrypsin (136nM) at room temperature, Coomassie blue staining was used to identify FX cleavage pattern.**
- ***N-terminal sequencing:*** **Chymotrypsin treated human FX (FXchy) was transferred to PVDF membrane, Coomassie blue stained bands were excised for Edman N-terminal sequencing to detect cleavage site.**
- ***Human FX activation:*** **Three different purified tenases were used for human FX activation in the presence of Ca^{2+}: 1) Russell's Viper Venom FX Activator (RVV-X, a direct activator of FX). 2) soluble recombinant tissue factor-FVIIa, (sTF-FVIIa, extrinsic pathway of coagulation). 3) FVIIIa-FIXa, (intrinsic pathway of coagulation). 2-step chromogenic assay was used to measure FXa generation.**



Results

Figure 1

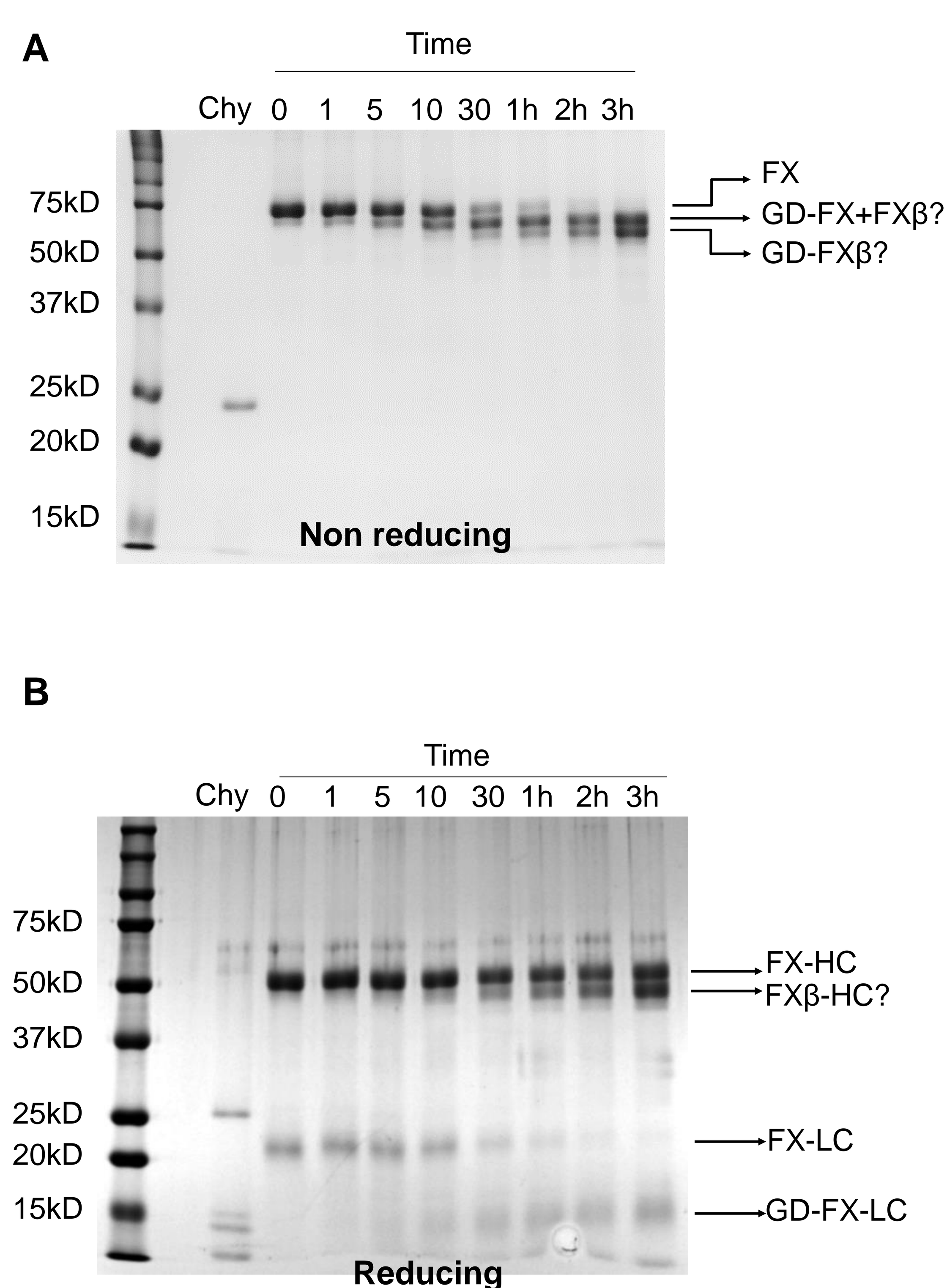


Figure 1. Time-dependent of human FX cleavage by chymotrypsin. (A) Non-reduced SDS-PAGE analysis of the human FX after chymotrypsin treatment. (B) Reduced SDS-PAGE analysis of the human FX after chymotrypsin treatment.

Figure 2

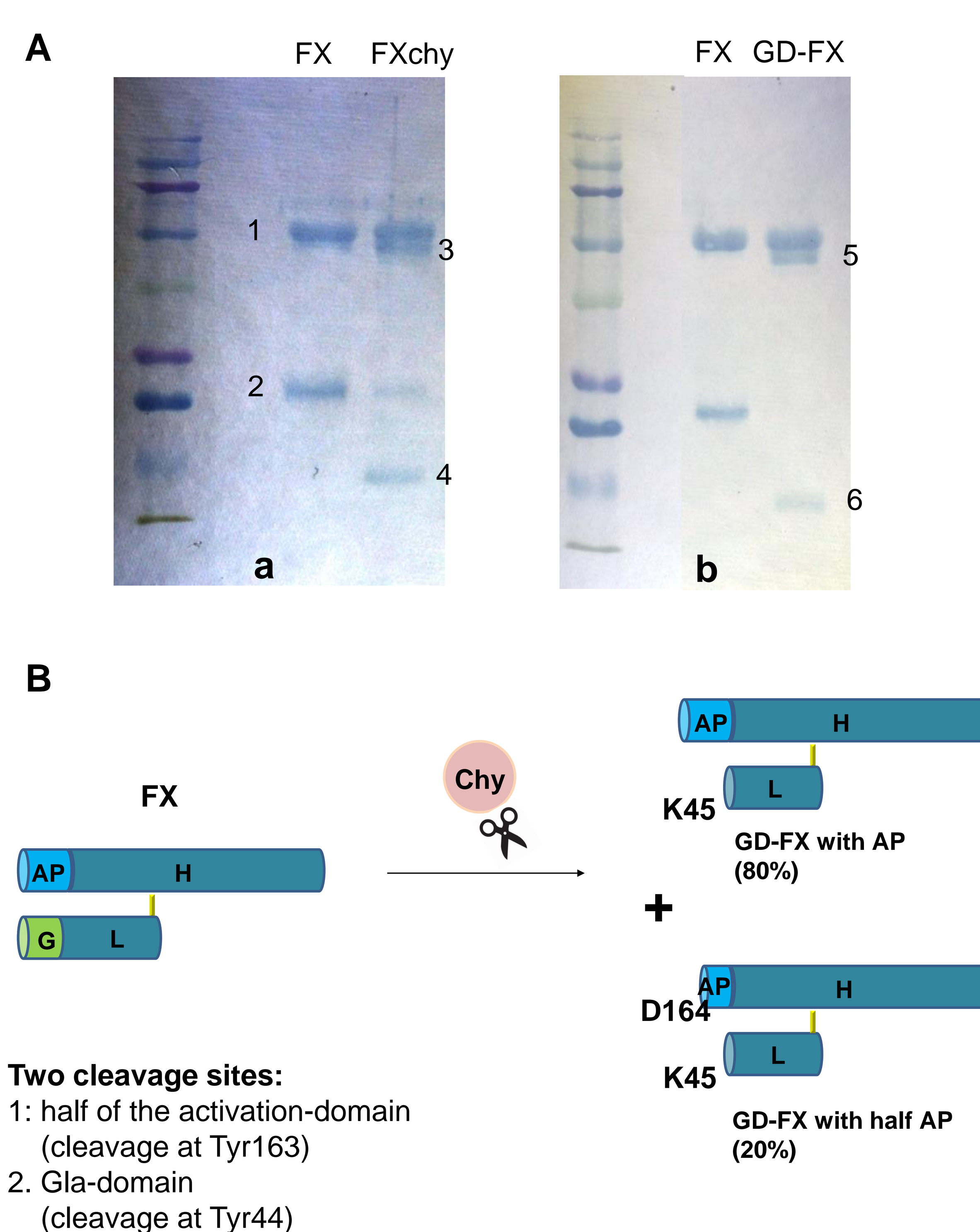


Figure 2. N-terminal sequencing for Fxchy and commercial Gla-domain less FX (GD-FX). (A) Representative images of bands for N-terminal sequencing. a: Fxchy in our lab; b: commercial GD-FX. (B) Schematic diagram illustrating the cleavage sites of chymotrypsin in human FX.

Figure 3

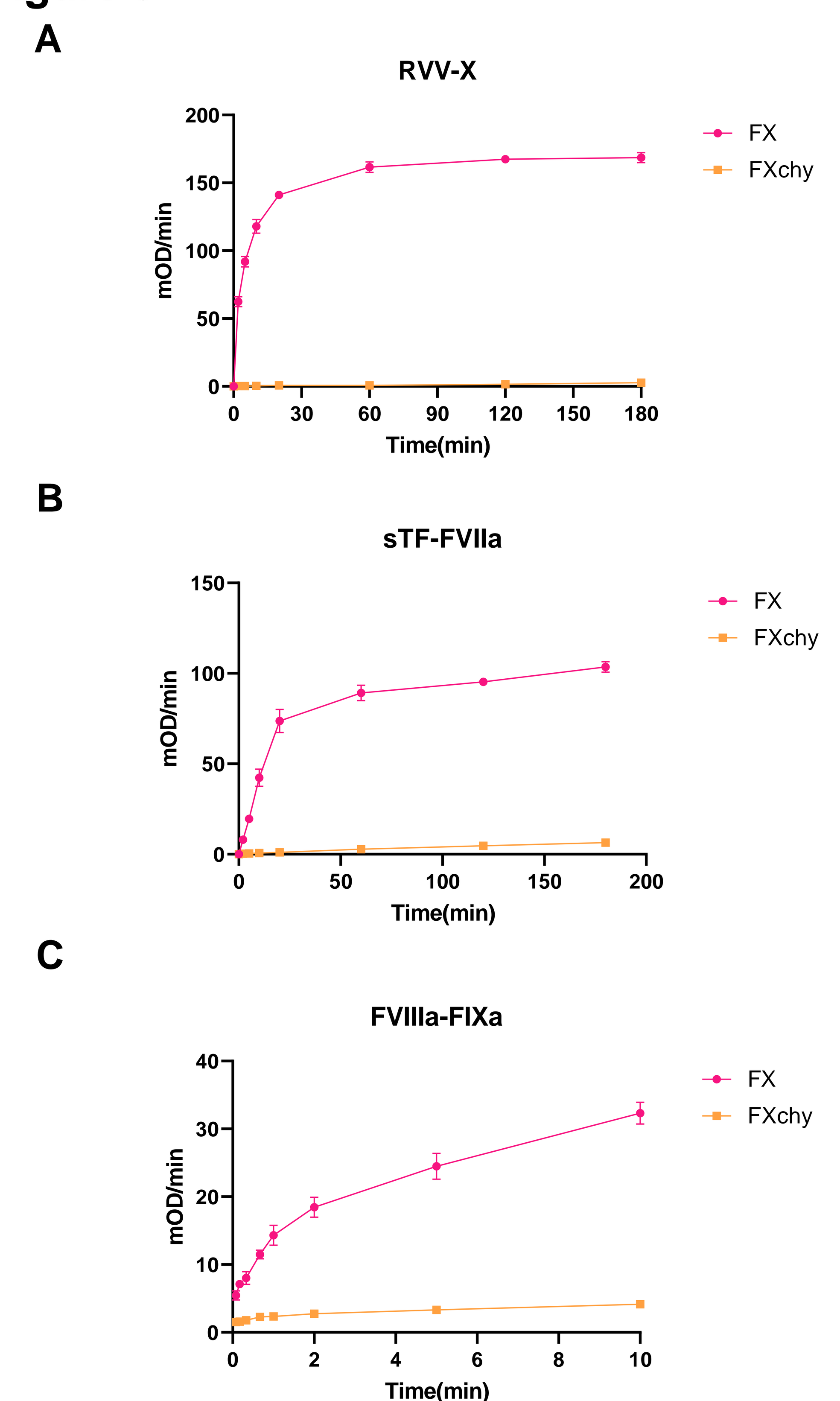


Figure 3. Comparation of human FX activation by two-step chromogenic assay. (A) Activation of FX by RVV-X. (B) Activation of FX by sTF-FVIIa. (C) Activation of FX by FVIIIa-FIXa.

Conclusions

- **A novel cleavage site was identified in human FX treated with chymotrypsin, excluding the Gla domain.**
- **The FX Gla-domain participates in the protein-protein interactions within tenases under phospholipid free condition.**
- **The efficiency of FXa generation was differentially affected, with greatest effect in the order, RVV>sTF/VIIa>VIIIa/Ixa, suggests that the substrate FX presents itself uniquely to each tenase, which may have implications for tailoring anticoagulant design for specific branches of coagulation and understanding coagulopathy.**

Acknowledgments and funding

References

1. Morita T, Jackson CM. Preparation and properties of derivatives of bovine factor X and factor Xa from which the gamma-carboxyglutamic acid containing domain has been removed. J Biol Chem. 1986 Mar 25;261(9):4015-23.