University of Louisville
Department of Chemistry

Dino Ablan

Research Seminar

When: February 24, 2022
Time: 12:00 p.m.
Location: CBLL-16

Roles of the Coagulation Factor XIII A Binding Site on the Crosslinking of Fibrinogen αC (233-425)

Abstract:

Fibrinogen (Fbg) is a heterodimeric coagulation protein critical for clot formation. Fibrinogen is converted to fibrin by thrombin, which causes fibrin to aggregate into a fibrous "soft clot" via noncovalent interactions.[1] Coagulation Factor XIII (FXIII) is a calcium-dependent transglutaminase present in plasma and cells such as platelets, macrophages, and osteoblasts. FXIII crosslinks reactive glutamines and lysines between fibrin and other anti-fibrinolytic proteins. The resulting fibrin clots are resistant to fibrinolysis and able to retain red blood cells, which increases clot size.[2] FXIII activity on clots contributes to hemostasis, but unfortunately exacerbates the development of deep vein thrombosis (DVT).[3] In plasma, FXIII is activated proteolytically by thrombin in the presence of Ca^{2+} (FXIII A*), while cellular FXIII is activated non-proteolytically in the presence of Ca^{2+} (FXIII A°).[4-7] Prior work has shown that FXIII A* is more conformationally flexible and catalytically active than FXIII A°.[8] Significant decreases in fibrinolysis and an increase in red blood cell retention is linked to FXIII activity toward the fibrinogen αC region (Fbg Aα 221 – 610).[2, 9] This Fbg Aα region contains several FXIII-reactive glutamines (Q) and lysines (K)[10], as well as a binding site for FXIII A* (Fbg αC 389 – 402) that includes a key binding residue, Fbg αC E396[11-13].

In the current studies, Fbg αC 233 – 425, a "model" αC system that contains three reactive glutamines (Q237, Q328, and Q366) and the FXIII binding site (Fbg αC 389 – 402), was recombinantly expressed and purified. A series of mutations and truncations were subsequently introduced to the αC FXIII binding site region to observe how crosslinking was affected. FXIII A activity was monitored by measuring the crosslinking of lysine mimic glycine ethyl ester (GEE) to αC reactive glutamines over time via MALDI-TOF MS.[14] The effects of Fbg αC 389 – 402 on αC crosslinking catalyzed by FXIII A* versus FXIII A° were examined. FXIII A* activity was significantly more reduced than FXIII A° in mutants where key αC residue E396 was removed (E396A) or the full FXIII binding site was lost altogether (truncated αC 233 – 388). Fbg αC 389 – 402 was thus found to primarily enhance FXIII A* activity toward Fbg. αC, but not FXIII A°.[19] The potential impact of Fbg αC E395 was subsequently explored. αC E395 was proposed to complement αC E396 in binding FXIII A* via a salt bridge with R158 in FXIII A*.[13] However, substituting E395 with residues of various chemical properties (i.e. E395A, E395K, E395S) only had a minimal impact on FXIII A* activity. Further work then explored αC D390 and W391 as residues within Fbg αC 389 – 402 that could enhance αC FXIII A* activity. Prior molecular modeling studies[13] had suggested D390 could form a favorable salt bridge with FXIII A* K156. Additionally, W391 could provide critical hydrophobic contacts with FXIII A* that increase binding affinity. Alanine substitution mutations on these residues significantly reduced αC crosslinking, which identifies D390 and W391 as additional key residues for promoting FXIII A* activity. However, double substitution mutants E396A/D390A and E396A/W391A did not significantly further reduce crosslinking. These results suggest that contributions of D390, W931, and E396 toward promoting FXIII A* activity may not be cumulative, and additional key binding regions have yet to be identified. In summary, Fbg αC 389 – 402 was demonstrated to solely benefit FXIII A* activity. In addition, D390 and W391 were identified alongside E396 as key αC residues that promote FXIII A* activity. The work from this study seeks to aid further drug design towards the treatment of DVT via therapeutic inhibition of FXIII activity on fibrin.

1.Weisel, J. W.; Litvinov, R. I., Mechanisms of fibrin polymerization and clinical implications *Blood* **2013,** *121* (10), 1712-9.
2.Byrnes, J. R.; Duval, C.; Wang, Y.; Hansen, C. E.; Ahn, B.; Mooberry, M. J.; Clark, M. A.; Johnsen, J. M.; Lord, S. T.; Lam, W. A.; Meijers, J. C.; Ni, H.; Ariens, R. A.; Wolberg, A. S., Factor XIIIa-dependent retention of red blood cells in clots is mediated by fibrin alpha-chain crosslinking. *Blood* **2015,** *126* (16), 1940-8.
3.Aleman, M. M.; Walton, B. L.; Byrnes, J. R.; Wolberg, A. S., Fibrinogen and red blood cells in venous thrombosis. *Thromb Res* **2014,** *133 Suppl 1*, S38-40.
4.Polgar, J.; Hidasi, V.; Muszbek, L., Non-proteolytic activation of cellular protransglutaminase (placenta macrophage factor XIII). *Biochem J* **1990,** *267* (2), 557-60.
5.Muszbek, L.; Haramura, G.; Polgar, J., T. *Thromb Haemost* **1995,** *73* (4), 702-5.
6.Walton, B. L.; Byrnes, J. R.; Wolberg, A. S., Fibrinogen, red blood cells, and factor XIII in venous thrombosis. *J Thromb Haemost* **2015,** *13 Suppl 1*, S208-15.
7.Anokhin, B. A.; Stribinskis, V.; Dean, W. L.; Maurer, M. C. *FEBS J* **2017,** *284* (22), 3849-3861.
8.Anokhin, B. A.; Dean, W. L.; Smith, K. A.; Flick, M. J.; Ariens, R. A. S.; Philippou, H.; Maurer, M. C. *FEBS J* **2020,** *287* (3), 452-464.
9.Duval, C.; Allan, P.; Connell, S. D.; Ridger, V. C.; Philippou, H.; Ariens, R. A. *Thromb Haemost* **2014,** *111* (5), 842-50.
10.Schmitt, L. R.; Henderson, R.; Barrett, A.; Darula, Z.; Issaian, A.; D'Alessandro, A.; Clendenen, N.; Hansen, K. C. *J Biol Chem* **2019,** *294* (22), 8773-8778.
11.Procyk, R.; Bishop, P. D.; Kudryk, B., Fibrin--recombinant human factor XIII a-subunit association. *Thromb Res* **1993,** *71* (2), 127-38.
12.Smith, K. A.; Adamson, P. J.; Pease, R. J.; Brown, J. M.; Balmforth, A. J.; Cordell, P. A.; Ariens, R. A.; Philippou, H.; Grant, P. J.*Blood* **2011,** *117* (12), 3460-8.
13.Smith, K. A.; Pease, R. J.; Avery, C. A.; Brown, J. M.; Adamson, P. J.; Cooke, E. J.; Neergaard-Petersen, S.; Cordell, P. A.; Ariens, R. A.; Fishwick, C. W.; Philippou, H.; Grant, P. J. *Blood* **2013,** *121* (11), 2117-26.
14.Mouapi, K. N.; Bell, J. D.; Smith, K. A.; Ariens, R. A.; Philippou, H.; Maurer, M. C. *Blood* **2016,** *127* (18), 2241-8.
15. Syed Mohammed, R.D.; Ablan, F.D.O.; McCann, N.M.; Hindi, M.M.; Maurer, M.C., Transglutaminase Activities of Blood Coagulant Factor XIII are Dependent on the Activation Pathways and on the Substrates, *to be submitted*.