Influence of Complement factor H on Factor XIIIA activation: A preliminary study

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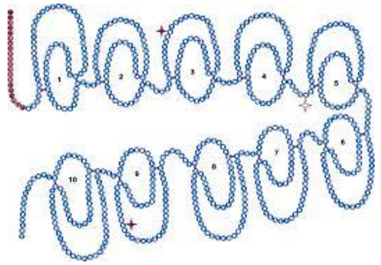
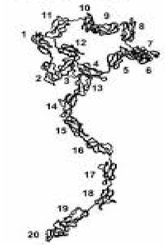
Introduction and Objective

Fibrogammin P, the plasma concentrate of Coagulation Factor XIII (FXIII), is used for treating FXIII deficient patients. Biochemical content characterization of Fibrogammin P has shown Complement factor H (CFH) to be one of its major constituents. Since CFH is a structurally homologous molecule to FXIIIB, the natural partner of FXIIIA, we contemplate that CFH might influence the functional profile of FXIII. We performed mixing studies, in order to test this hypothesis.



FXIIIB (10 CCP modules)



Complement Factor H (20 CCP modules)

FIBROGAMMIN P: Commercially available source of Human FXIII (Plasma concentrate of Healthy donors)

References

1.. Dodt J, Volkers P, Seitz R. Factor XIIIa generation assay: a tool for studying factor XIII function in plasma. Anal Biochem. 2013 Aug 15;439(2):145-51.

2. Souri M, Osaki T, Ichinose A. The Non-catalytic B Subunit of Coagulation Factor XIII Accelerates Fibrin Cross-linking. J Biol Chem. 2015 May 8;290(19)

Materials and Methods

In order to purify $FXIIIA_2B_2$, series of **Size exclusion chromatography** was performed till single, monodispersed homogenous peak of the $FXIIIA_2B_2$ heterotetramer was obtained. The peak fractions were ran onto SDS PAGE, and bands were confirmed by gel tryptic digestion followed by mass spectrometry (**Figure 1**).

FXIIIAa generation[1] **monitored for different plasmatic combinations (mixing studies).** To analyse the effect of presence/absence of CFH on FXIII generation in Standard vs. FXIII deficient plasma

rFXIIIA: Purified from *Pichia Pink*. **rFXIIIB**: Commercially available (Zedira)

Complement Factor H: Isolated from Fibrogammin P (**Figure 1**)

The resulting activation curves (**Figure 2**) were evaluated based on a bi-exponential, mathematical model with first order absorption and elimination. Furthermore, the data was fitted to the equation: $\mathbf{C(t) = c*k_a/ (K_a\text{-}k_b)*\{[exp(-k_b*(t\text{-}t_{lag})]\text{- }[exp(-k_a*(t\text{-}t_{lag})]\}}$, where, k_a is constant of absorption (activation) which describes the development of active FXIIIAa species, and k_b is the elimination (deactivation) constant (**Figure 3**).

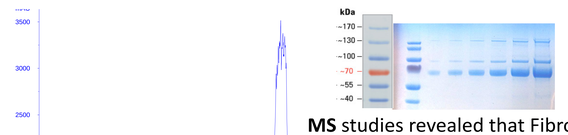
Results

1. In agreement with an earlier report[2] the generation assay results reveal that, rate of activation of FXIIIA is accelerated in the presence of FXIII B ($Ka_{(FXIIIDP+\ FXIIIA)}$ is 0.12, $Ka_{(FXIIIDP+\ FXIIIA+FXIIIB)}$ is 0.54)**.** (**Figure 3c**)
2. However, the rate of depleteion of activated species is reduced in the presence of FXIIIB subunit and a similar effect is observed with CFH(**Figure 3f**).
3. The lag time (**Figure 3d**), which represents thrombin accessibility to FXIIIA molecule in the assay is also mildly influenced by both CFH and FXIIIB.

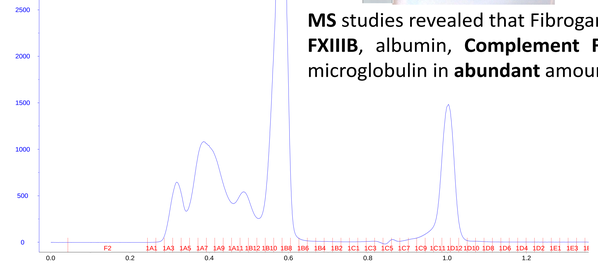
Conclusion

FXIIIB accelerates the rate of FXIIIA activation in plasma. Both CFH and FXIIIB appears to influence the rate of FXIIIAa depletion. Both CFH and FXIIIB additively control access to thrombin and therefore of thrombin mediated cleavage of FXIIIA.

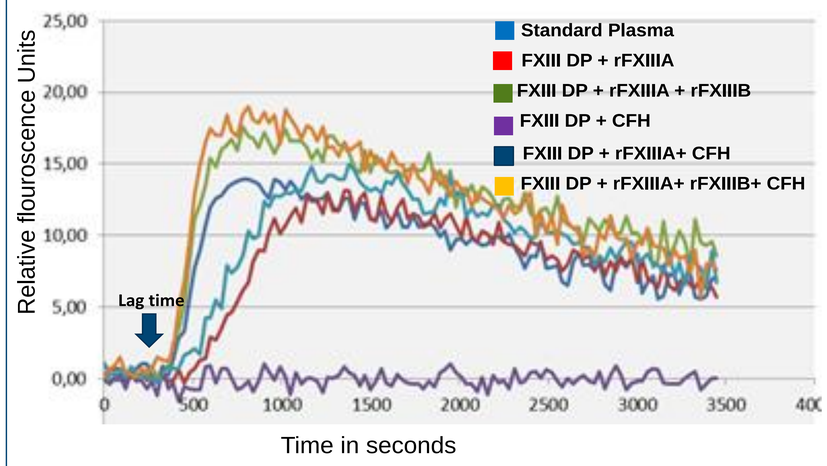
1. Size Exclusion chromatography for separation and purification of biochemical constituents of Fibrogammin P



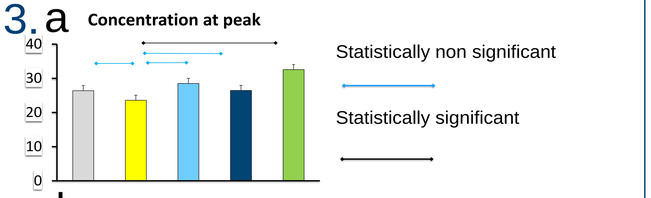
MS studies revealed that Fibrogamin P contains **FXIIIa, FXIIIB**, albumin, **Complement Factor H**, and alpha microglobulin in **abundant** amounts



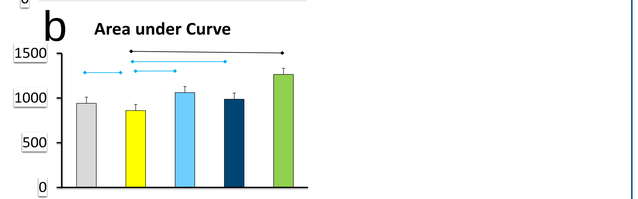
2. FXIIIAa (activated FXIIIA) Generation assay [1]



3. a



b



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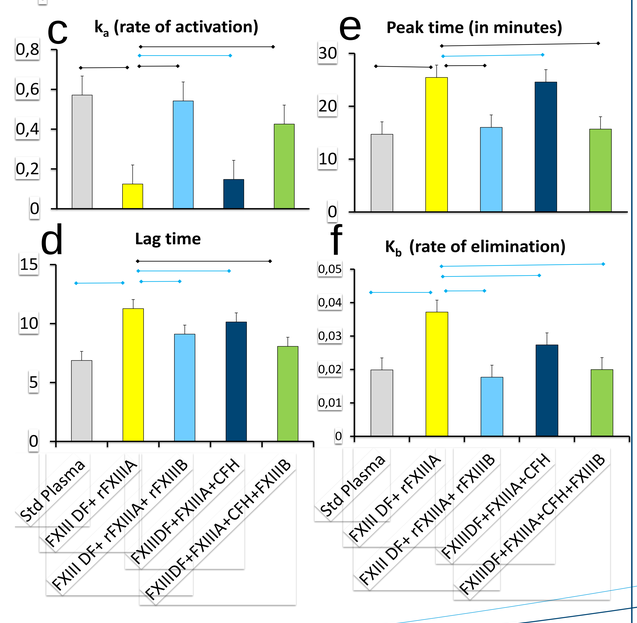

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