

# Feedback activation of FVIII in plasma

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## Feedback activation of FVIII in plasma

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The proteolytic backbone of the coagulation mechanism is well known, but it is important to remember that it contains a 'sidestep', FVII/thromboplastin activating FIX (Fig. 1).

There is a pulse of FVII/thromboplastin activity that is shut down by the EPI mechanism. After this shut-down, the only way to activate FX is via FIX, which does not decay in plasma when there is no heparin present.

The proteolytic backbone is not the whole story, because the coagulation factors need to be adsorbed on phospholipids, next to their helper proteins. For example, FXa activates prothrombin with the help of FVa, and FVIIIa is the helper enzyme for FIX.

Helper enzymes are not active during circulation in the plasma, but must be activated from the plasma precursors, usually by thrombin. FXa can also activate FVIII, but this does not occur in plasma. The feedback reaction from FV, which might be thought to be as important as that from FVIII, has little influence in the usual clotting tests. This may be because FVIII must be freed from von Willebrand Factor. Thrombin activates FVIII, which loses its affinity to vWF, then can attach to the phospholipid, where FIX is waiting for it.

FVIIIa has a double function in this complex. The first is kinetic. Comparison of the apparent  $K_m$  and  $V_{max}$  of factor X activation by FIXa; FIXa plus calcium; FIXa, calcium and phospholipid (PL); and FIXa, calcium, PL and FVIII, shows that the apparent  $K_m$  drops as soon as PL is present, and  $V_{max}$  rises as soon as FVIII is present (Table).

By adding FIXa to a fixed amount of PL with or without FVIIIa, the  $K_d$  of FIX for PL with and without FVIIIa can be calculated when the kinetic constants are known.  $K_d$  in the presence of FVIIIa is much smaller than in its absence.

The major functions of activated FVIIIc, therefore, are to increase  $V_{max}$  of

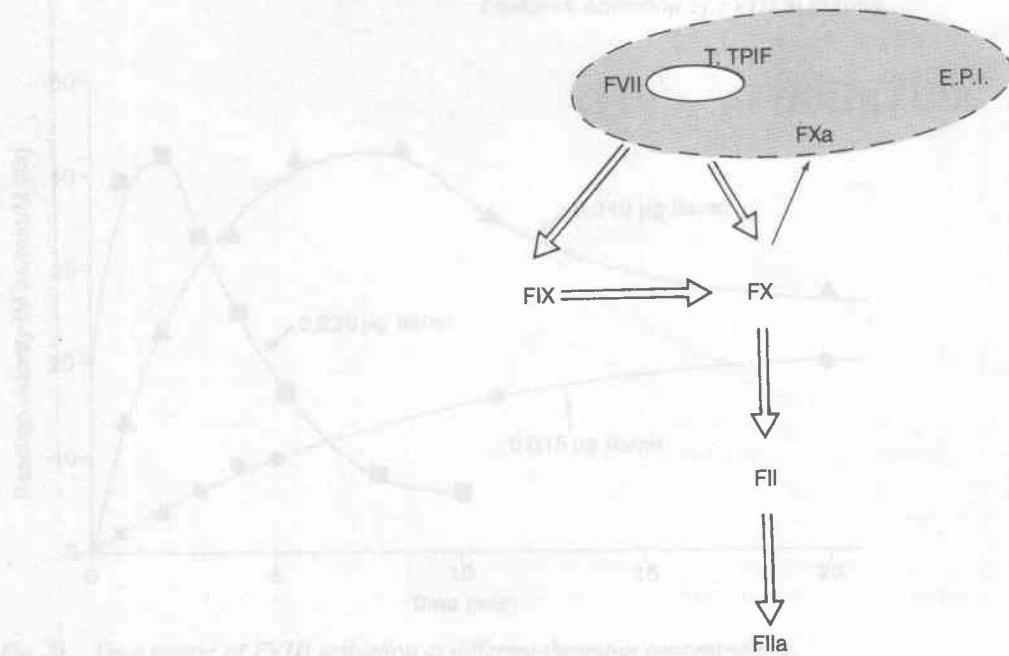


Fig. 1: The proteolytic 'backbone' of coagulation.

FX formation by about 10,000-fold, and to promote the assembly of FIXa and FVIIIa at the PL.

Adding thrombin to FVIII increases its activity and decreases its stability. The decrease is interesting, and in a way depends on the amount of thrombin available (Fig. 2). This is important in plasma, as the amount of FXa formed after activation by FIXa is subject to a lag phase which is abolished by adding



Fig. 2: Thrombin generated by FIXa activated plasma.  $\square$ - $\square$  = Control;  $\circ$ - $\circ$  = 10  $\mu$ M Aprotinin;  $\Delta$ - $\Delta$  = 10  $\mu$ M Heparin

Table: Effects of the accessory components on kinetic parameters of FX activation.

Factor X activating mixture	$K_m^{app}$ ( $\mu$ M)	$V_{max}$ (mole Xa $\cdot$ min $^{-1}$ $\cdot$ mole IXa $^{-1}$ )
IXa	299	0.0022
IXa, CaCl <sub>2</sub>	181	0.0105
IXa, CaCl <sub>2</sub> , PL (10 $\mu$ M)	0.058	0.00247
IXa, CaCl <sub>2</sub> , PL (10 $\mu$ M), VIIIa (11 U/ml)	0.063	500

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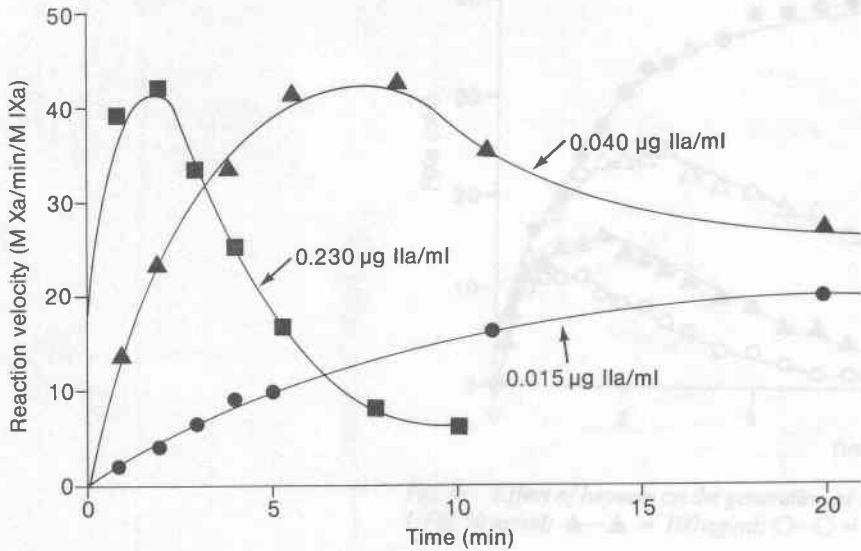


Fig. 2: Time course of FVIII activation at different thrombin concentrations.

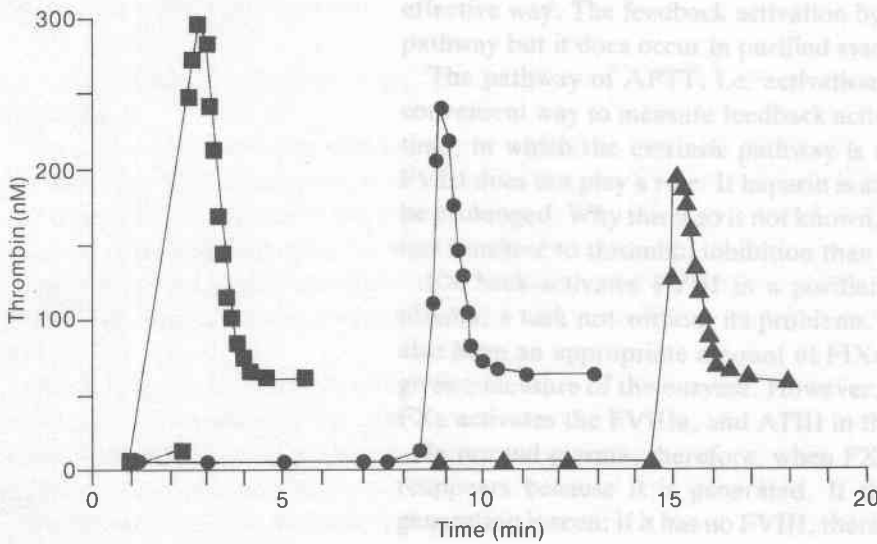


Fig. 3: Thrombin generation by FIXa activated plasma. ■—■ = Control; ●—● = 150 µM Hirudin; ▲—▲ = 300 nM Hirudin.

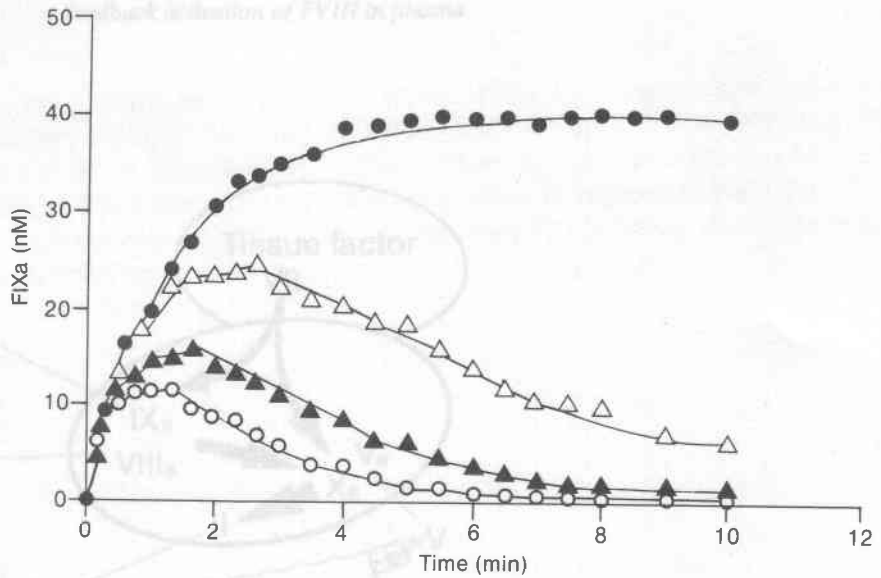


Fig. 4: Effect of heparin on the generation of FIXa in plasma. ●—● = Control; △—△ = UFH 50 ng/ml; ▲—▲ = 100 ng/ml; ○—○ = 175 ng/ml.

thrombin. The rise in activity is itself abolished by inhibiting thrombin by hirudin (Fig. 3).

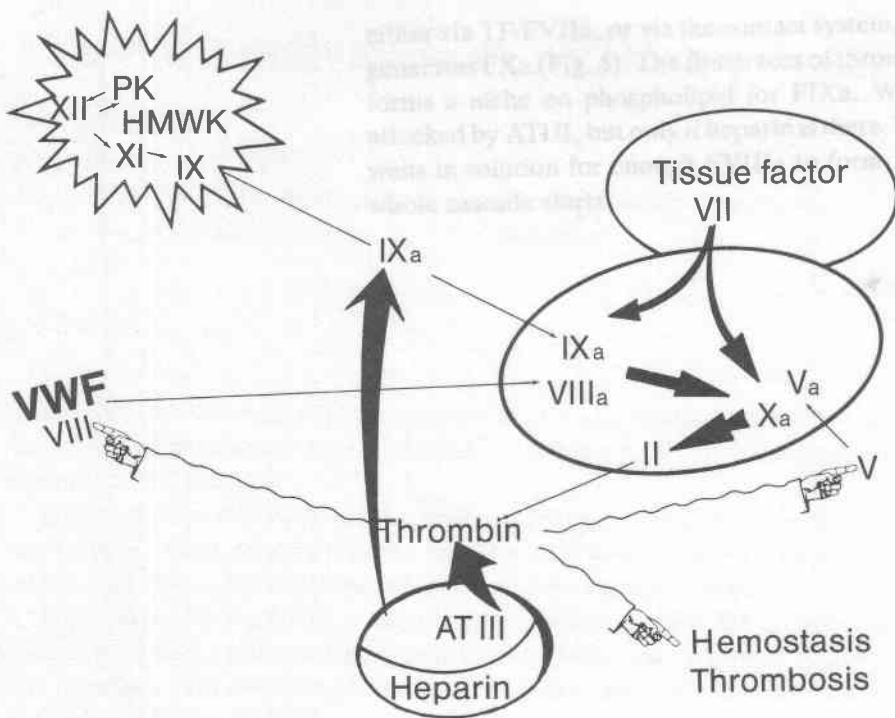
In plasma, hirudin shuts off the feedback activation of FVIII in a very effective way. The feedback activation by FX in plasma is not a physiological pathway but it does occur in purified systems.

The pathway of APTT, i.e. activation of FIX by the contact factors, is a convenient way to measure feedback activation of FVIII. In the prothrombin time, in which the extrinsic pathway is triggered by tissue thromboplastin, FVIII does not play a role. If heparin is added there, the lag phase will hardly be prolonged. Why this is so is not known, but FV feedback activation is much less sensitive to thrombin inhibition than activation of FVIII.

FX back-activates FVIII in a purified system. We measured FVIIIa in plasma, a task not without its problems. Plasma samples containing FVIIIa also have an appropriate amount of FIXa and PL. Subsampling on FX then gives a measure of the enzyme. However, there are 2 complicating reactions: FXa activates the FVIIIa, and ATIII in the sample inactivates the FXa.

In normal plasma, therefore, when FXa is added, it first disappears, then reappears because it is generated. If the system has no FVIII, there is only the decay. These complications

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*Fig. 5: The initiation of coagulation.*

ing reactions must be get rid of in any system in which FVIIIa is to be measured in plasma.

We achieved this in a one-step system, by adding an excess of chromogenic substrate for FXa which avoids the inactivation of FXa by ATIII or the activation of FVIII. There is a quadratic rise in colour, which can be analyzed to give a good standard curve for FVIII estimation in plasma.

Some drugs act directly on activation of FVIII, one being pentosan polysulphate. This drug also inactivates FVa, and boosts heparin co-factor II and ATIII.

One way to show the importance of FVIII feedback is the mode of action of heparin in the intrinsic system. The appearance of FIXa with time, with and without heparin, is crucial (Fig. 4). FIXa will not decay in the presence of ATIII or other plasma inhibitors. When the pulse of thromboplastin FVIIa activation is over, FIX is the only way to activate FX: it is not broken down unless heparin is present.

The general picture, therefore, of the importance of FVIII feedback, is that

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either via TF/FVIIa, or via the contact system, FIXa is generated, which then generates FXa (Fig. 5). The first traces of thrombin activate FVIII, and FVIIIa forms a niche on phospholipid for FIXa. When it does not, FIXa can be attacked by ATIII, but only if heparin is there. When there is no heparin, FIXa waits in solution for enough FVIIIa to form, then FXa is activated, and the whole cascade starts.

The proteolytic backbone of the coagulation mechanism is well known, but it is important to remember that it contains a 'bottleneck'. FVIII/thromboplastin activates FIX (Fig. 1).

There is a pulse of FVIII/thromboplastin activity that is shut down by the EPI mechanism. After this shut-down, the only way to activate FX is via FIX, which does not decay in plasma when there is no heparin present.

The proteolytic backbone is not the whole story, because the coagulation factors need to be adsorbed on phospholipids, next to their helper proteins. For example, FXa activates prothrombin with the help of FVa, and FVIIa is the helper enzyme for FIX.

Most enzymes are not active during circulation in the plasma, but must be activated from their plasma precursors, usually by thrombin. FXa can also activate FVIII, but this does not occur in plasma. The feedback reaction from FV, which might be thought to be as important as that from FVIII, has little influence in the usual clotting tests. This may be because FVIII must be freed from von Willebrand Factor. Thrombin activates FVIII, which forms hexamers, then can attach to the phospholipid, where FIX is waiting for it.

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