

Blood coagulation

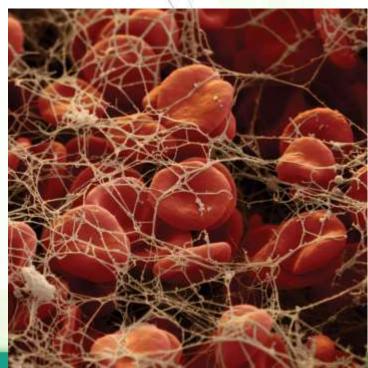
Prof. Mamoun Ahram 2019

What is blood coagulation (clotting)?



It is a coordinated, biochemical process that is initiated as a result of vascular injury where a small area blood of surrounding injury changes from liquid to gel, forming a clot made of fibrin, which results in hemostasis (the cessation of blood loss) followed by

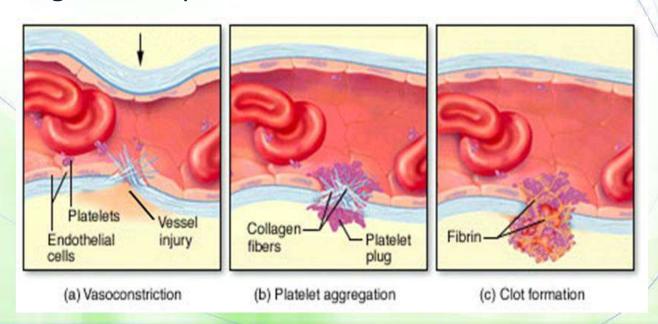
clot dissolution and repair.



Steps of hemostasis



- Vascular constriction limiting blood flow to the area of injury
- Activation then aggregation of platelets at the site of injury, forming a loose platelet plug
- Formation of a fibrin mesh to entrap the plug
- Dissolution of the clot in order for normal blood flow to resume following tissue repair



Players of blood clotting



- The blood clotting process involves a complex array of
 - blood cells,
 - blood proteins (structural and enzymatic),
 - tissue glycoproteins,
 - lipids,
 - ions,
 - vasoactive compounds

Platelets



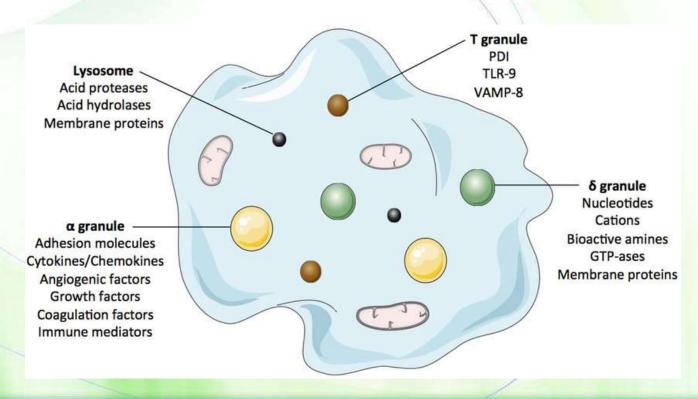
- Small anuclear cell fragments produced from the megakaryocytes.
- Platelets have numerous kinds of surface receptors.
- Platelets also have actin filaments and myosin, which change the shape of the platelet upon activation.
- They also have three types of granules that store substances that are released upon platelet activation.

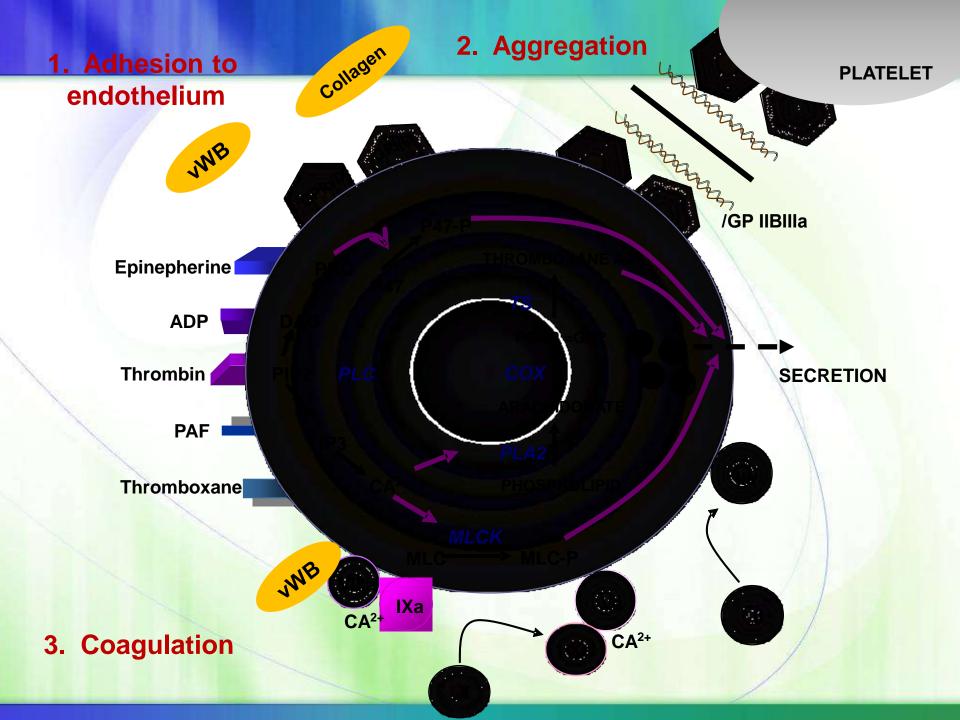


The granules



- Electron-dense granules (calcium ions, ADP, ATP, serotonin)
- α-granule (a heparin antagonist, platelet-derived growth factor, fibrinogen, von Willebrand factor (vWF), clotting factors)
- Lysosomal granule (hydrolytic enzymes)
- During
 activation,
 the contents
 of these
 granules are
 secreted.





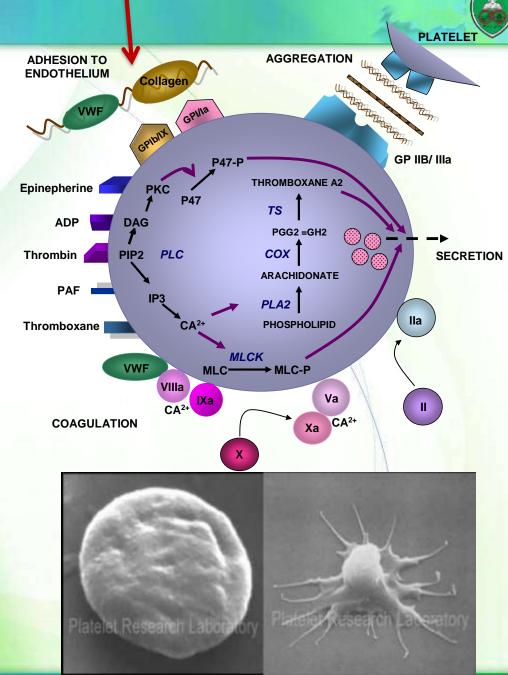
Adhesion

- The endothelial von Willebrand factor (vWF) protein and exposed collagen bind to the platelet glycoproteins (GP).
- Some platelets release substances from the granules:

Bind to

receptors

- ADP
- Serotonin
- Factor V
- ATP
- Calcium
- Fibrinogen
- vWF
- Thrombin
- Thromoxane
- Platelets also change shape allowing for more platelet-platelet interaction and aggregation.



Thrombin receptor



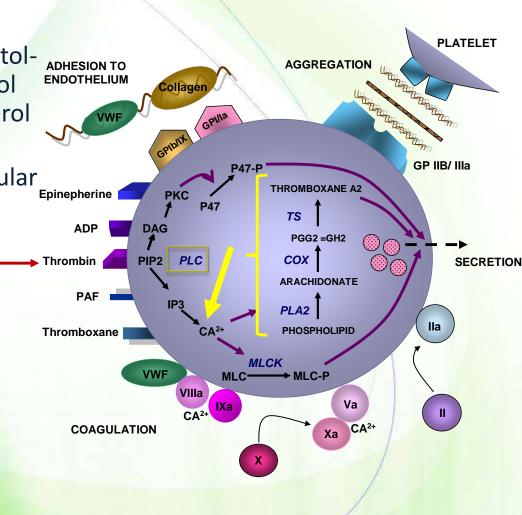
Thrombin receptor activates a Gprotein that activates phospholipase C-γ (PLC-γ).

 PLC-γ hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG).

 IP3 induces the release of intracellular Ca2+ stores, and DAG activates protein kinase C (PKC).

Calcium triggers liberation of arachidonic acid from membrane phospholipids by the enzyme phospholipase A2.

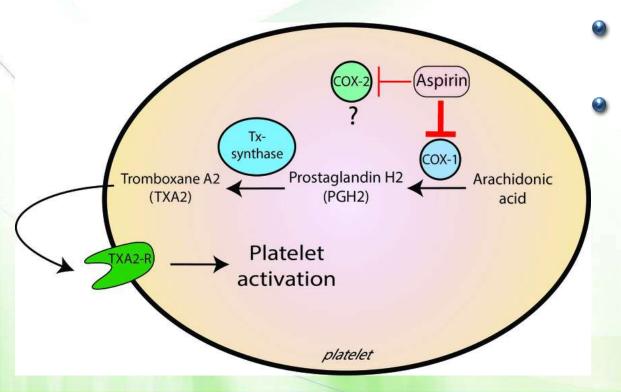
Arachidonate is converted by cyclooxygenase to prostaglandins, which are then converted by thromboxane synthetase to thromboxane A2.



Role of thromboxane (and vasoconstrictors)



- TXA2 is a potent vasoconstrictor and inducer of platelet aggregation.
- Non-steroidal anti-inflammatory drugs inhibit the enzyme cyclooxygenase, accounting for their anticoagulant effects.

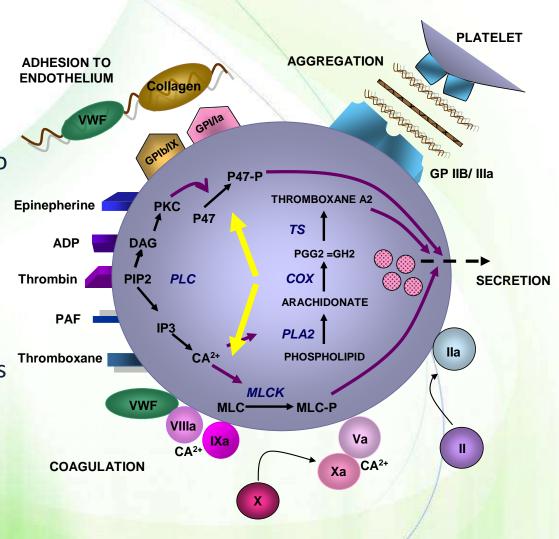


- Serotonin is also a vasoconstrictor.
- PDGF stimulates proliferation of endothelial cells to reduce blood flow.

More release of granular contents



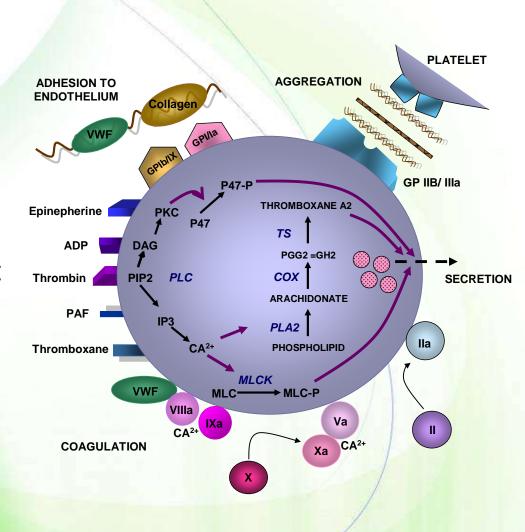
- Intracellular Ca2+ stores is myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin allowing it to interact with actin resulting in altered platelet morphology, induced motility and facilitating release of granules.
- DAG activates PKC, which phosphorylates and activates specific platelet proteins that induce the release of platelet granule contents including ADP.



Role of ADP



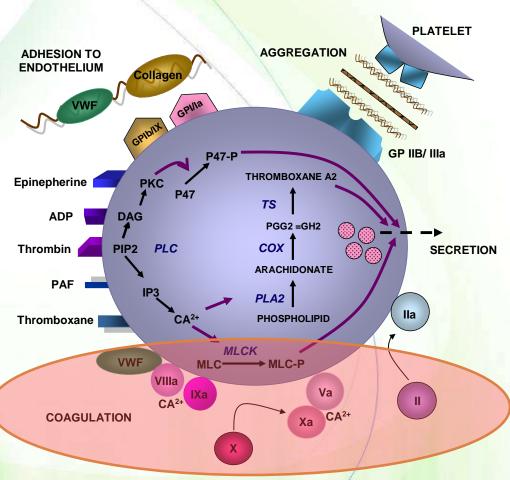
ADP is a platelet activator that binds to its receptor and modifies the platelet membrane allowing fibrinogen to adhere to platelet surface glycoproteins resulting in fibrinogen-induced platelet aggregation.



Role of platelet cell surface



The accumulated platelet plug provides an important surface on which coagulation reactions occur.





Biochemistry of coagulation

Components of coagulation

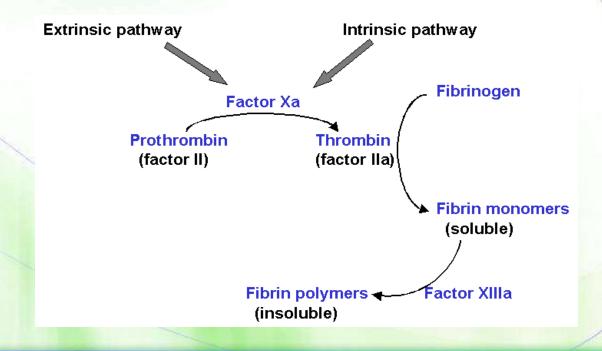


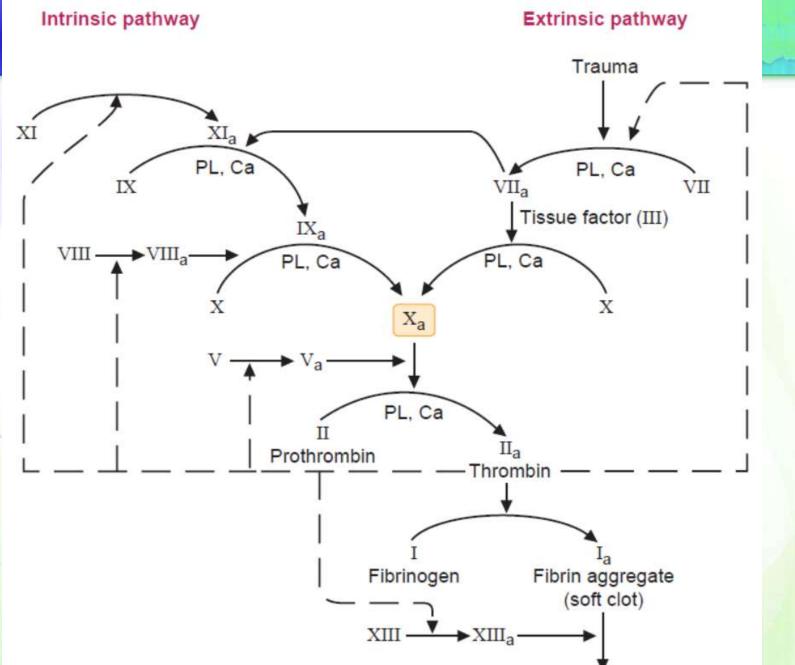
- An organizing surface (platelets)
- Proteolytic zymogens (prekallikrein, prothrombin, and factors XII, XI, IX, VII, and X)
 - These are mainly inactive serine proteases produced and released from hepatocytes.
 - The subscript "a" designates the activated form of a factor
 - e.g., "XIII" is versus "XIIIa"
- Anti-coagulant (protein C, protein S)
- Non-enzymatic protein cofactors (factors VIII, V, and III (tissue factor)
- Calcium ions
- Thrombin
- Fibrinogen

The two imprecise pathways



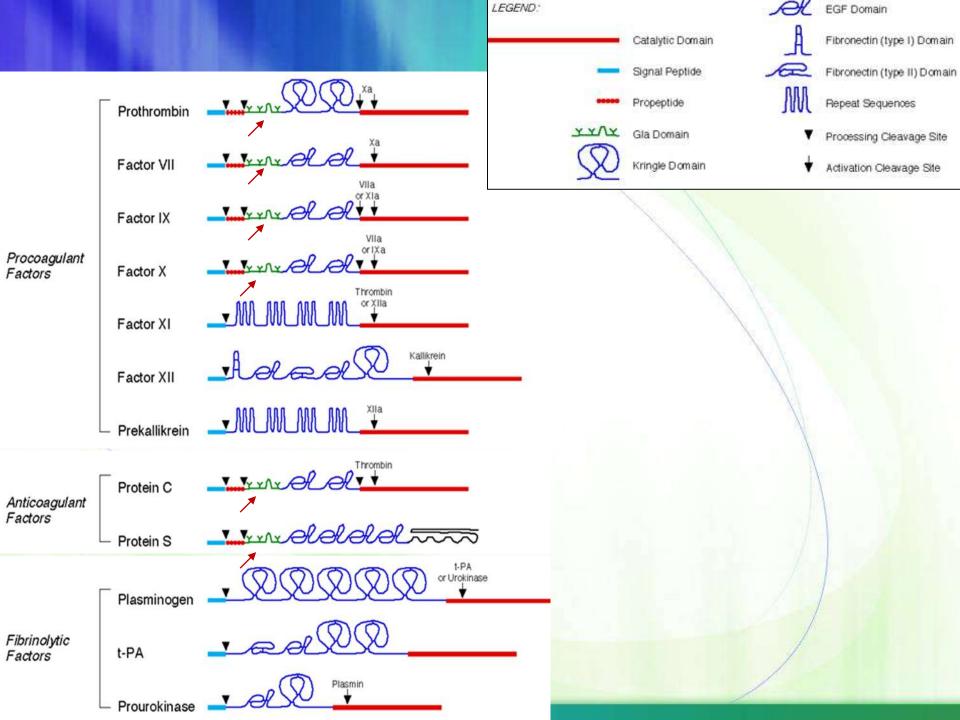
- The intrinsic pathway is initiated when subendothelial surface (i.e., collagen) is exposed.
- The extrinsic pathway is initiated in response to tissue injury.
 - Tissue factor (TF) protein is released.
- However, the two pathways converge on a common pathway.





Cross-linked clot (hard clot)

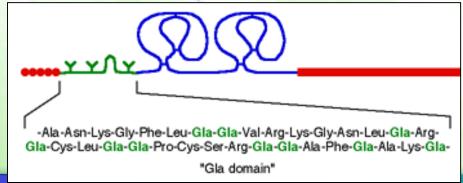


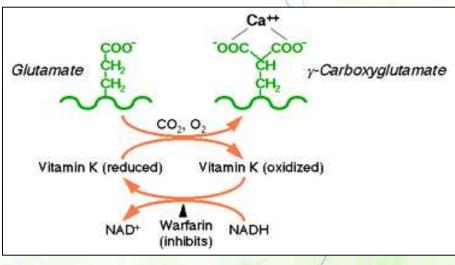


Gla domain



- An ER/Golgi carboxylase binds to prothrombin and factors IX, VII, and X and converts 10≥ glutamate (Glu) residues to γcarboxyglutamate (Gla).
- The Gla residues bind calcium ions and are necessary for the activity of these coagulation factors and formation of a coordinated complex with the charged platelet surface to localize the complex assembly and thrombin formation to the platelet surface.
 - Vitamin K participates in conversion of Glu to γ-Gla
 - Vitamin K becomes oxidized and must be regenerated.

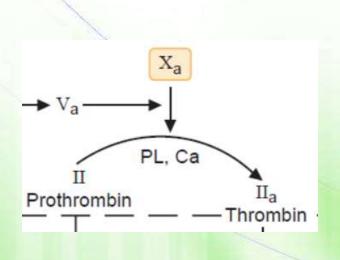


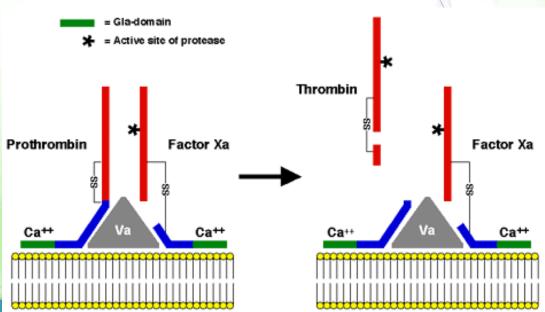


Prothrombin activation

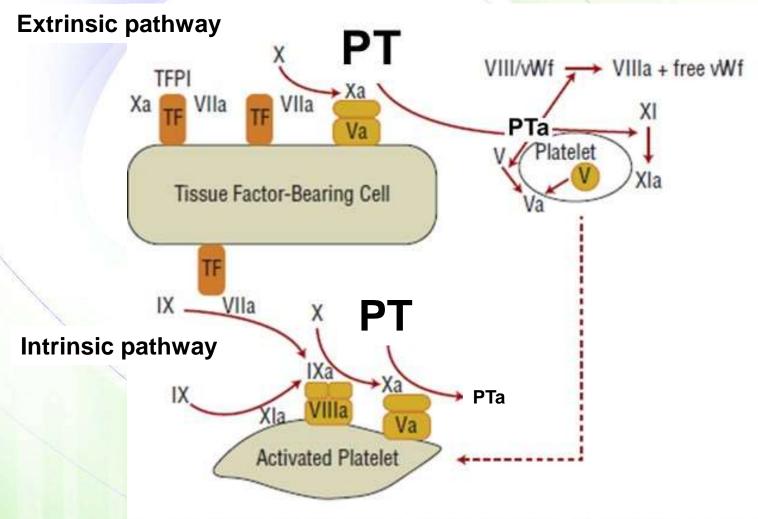


- Factor Xa converts prothrombin to thrombin, which is accelerated by Va, platelets (or phospholipids), and calcium ions.
- Binding of calcium alters the conformation the Gla domains of these factors, enabling them to interact with a membrane surface of platelets.
- Aggregated platelets provide the surface upon which prothrombin activation occurs.









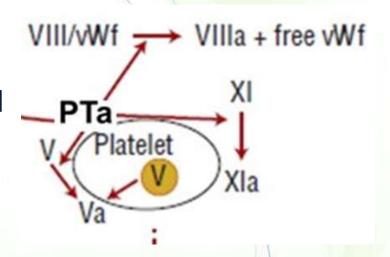
Source: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM: Pharmacotherapy: A Pathophysiologic Approach, 8th Edition: www.accesspharmacy.com

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Factors V and VIII



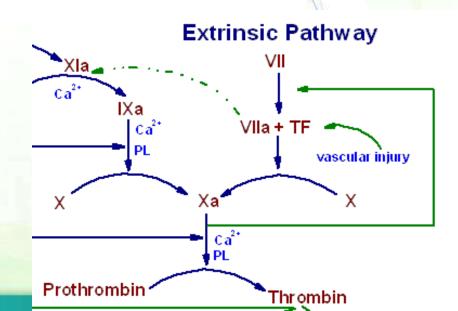
- Va and VIIIa are cofactors that increase the proteolytic efficiency of Xa and IXa, respectively.
- Factors V and VIII are activated by thrombin.
- Factor VIII circulates in plasma bound to von Willebrand factor, which increases VIII half-life, and, when released, it gets activated.
 - von Willebrand factor deficiency is associated decrease in the plasma concentration of factor VII.



Tissue factor



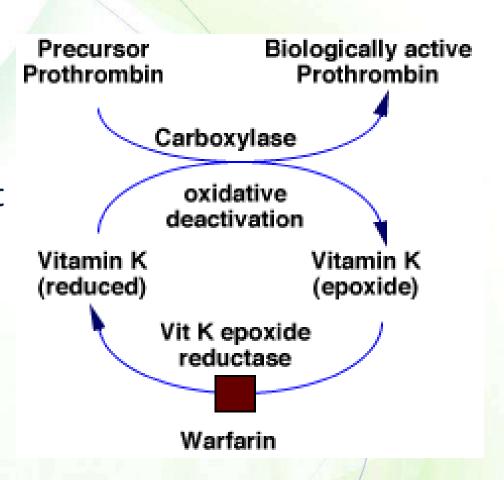
- Tissue factor is an integral membrane protein that is expressed on the surface of "activated" monocytes, endothelial cells exposed to various cytokines, and other cells.
- Tissue factor greatly increases the proteolytic efficiency of VIIa.



Anti-coagulants



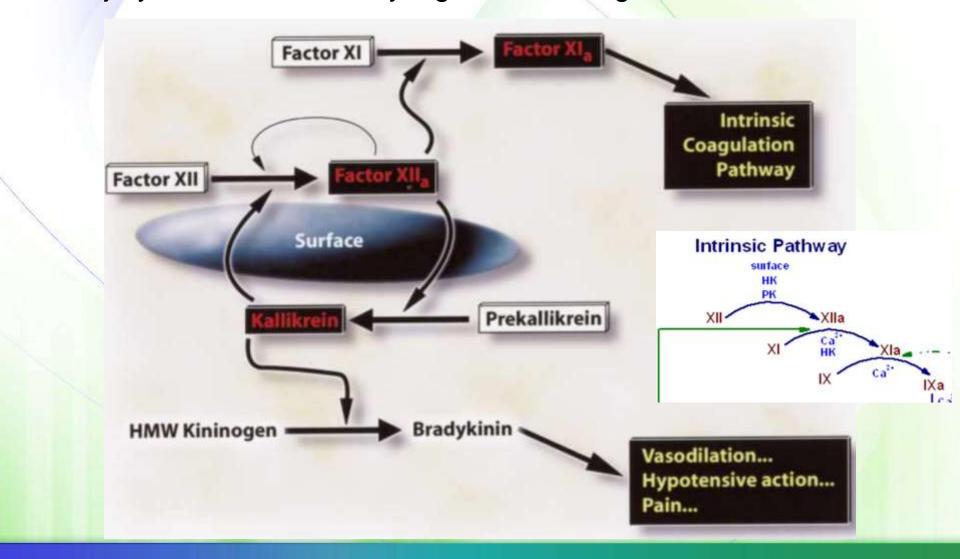
Blood clotting can be prevented by addition of Ca2+ chelators and vitamin K antagonists such as the anticoagulant drug warfarin, which inhibits reduction of vitamin K and thereby prevents synthesis of active prothrombin and factors VII, IX, and X.



The kallikrein-kinin system



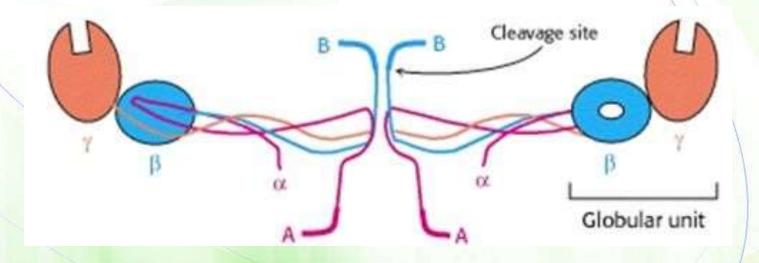
Factor XII binds to exposed collagen at site of vessel wall injury and is activated by high-MW kininogen and kallikrein.



What is fibrinogen?



It is a two triple-stranded helical protein held together by disulfide bonds.

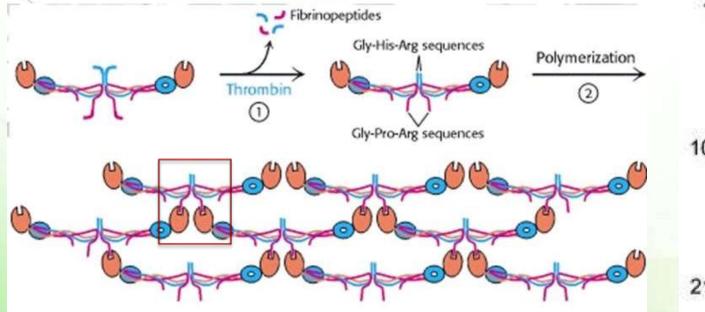


Formation of a fibrin clot



- Thrombin cleaves fibrinogen releasing fibrinopeptides.
- Fibrinopeptides creates electrostatic attractions between the central domain and the end domains facilitating the aggregation of the monomers into a gel consisting of long polymers.

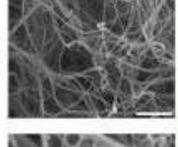
The clot resulting from aggregation of fibrin monomers is referred to as the "soft clot".



1320X

10900X

21600X

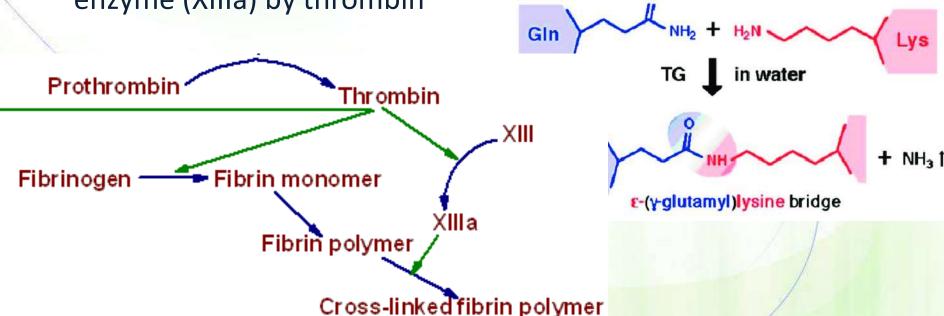


Factor XIII



- Covalent cross-linking of fibrin polymers can be formed by activated factor XIII (XIIIa), a transglutaminase.
- This is required for adequate clot strength and normal wound healing in vivo

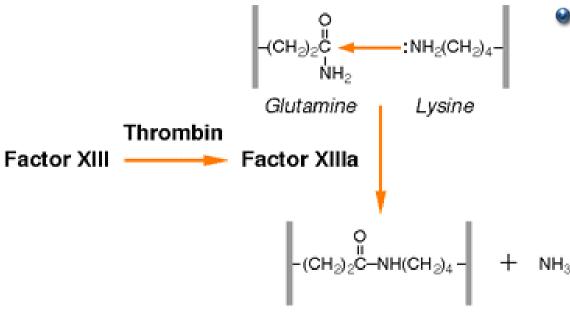
The zymogen form of factor XIII is converted to an active enzyme (XIIIa) by thrombin



Enzymatic mechanism of factor XIII



- Factor XIIIa catalyzes a transglutamination reaction that catalyzes a cross-linking reaction which covalently attaches a glutamine side chain of one fibrin monomer to a lysine side chain of an adjacent fibrin monomer.
- The cross-links strengthen the fibrin mass, forming the "hard clot".

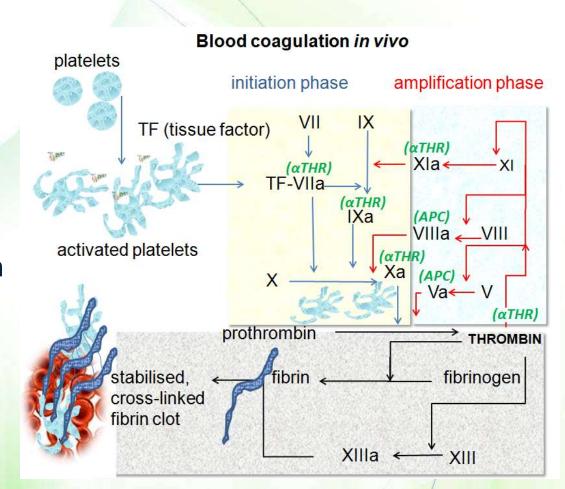


Factor XIIIa also crosslinks the fibrin clot to adhesive proteins on the endothelial tissue and to the platelet surfaces strengthening the platelet plug.

Amplification of coagulation reactions



- The sequential enzymatic activation allows for amplification.
- Amplification also results from positive feedback reactions.
- These include activation of V, VIII, and XI by thrombin.



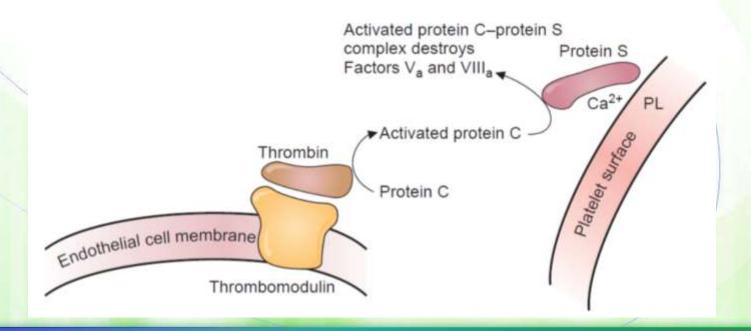


Anti-clotting factors

Antithrombin III



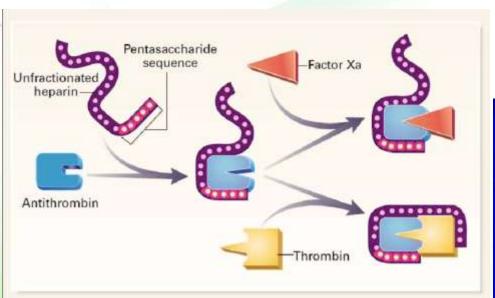
- Thrombin binds to thrombomodulin in the surface of endothelial cells.
- Thrombin can activate protein C, which forms a complex with protein S, both of which are vitamin K-dependent cofactors.
- The complex degrades factors V and VIII.



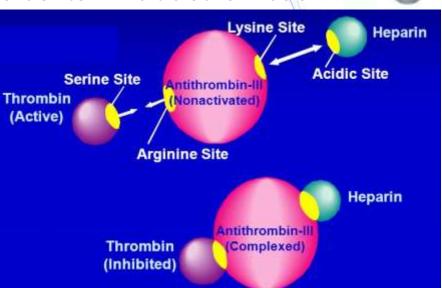
Antithrombin III



- Antithrombin III is a *SerPIn* that binds and inhibits the activity of thrombin as well other clotting factors (IXa, Xa, XIa, and XIIa).
- Heparin, a sulfated polysaccharide synthesized by mast cells, binds to antithrombin III, promoting binding to its substrates.



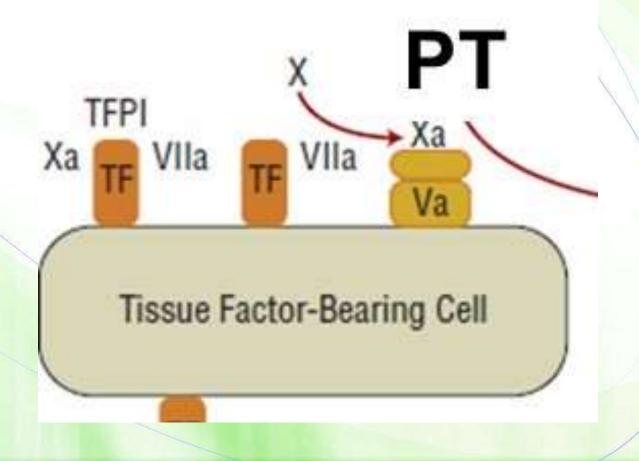
In the clinic, phlebotomy tubes are often treated with heparin in order to inhibit clot formation.



Tissue Factor pathway inhibitor



It inhibits the factor VIIa-Xa complex.





Degradation of the fibrin clot

Clot dissolution

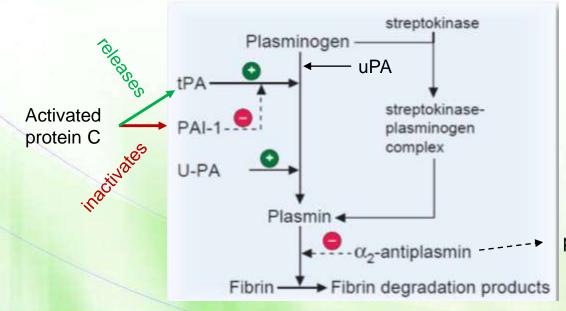


- It is important to prevent clot formation when not needed by anti-clotting factors and to dissolve a clot when formed.
- Clot dissolution starts concomitant with its formation.

The fibrinolytic system



- Plasmin, a serine protease formed from plasminogen, is responsible for fibrinolysis where it catalyzes the hydrolysis of fibrin and fibrinogen to degradation products.
- Plasminogen has a high affinity for fibrin clot.



but not when plasminogen/plasmin are clot-bound

Streptokinase



Streptokinase, a regulatory protein isolated from streptococci, can activate circulating plasminogen to form plasmin in blood, resulting in degradation of fibrinogen as well as fibrin.



Urokinase



- Urokinase, a serine protease is formed from the zymogen prourokinase
- It is synthesized in the kidney and in remodeling tissues
- It is a potent plasminogen activator, and is used clinically