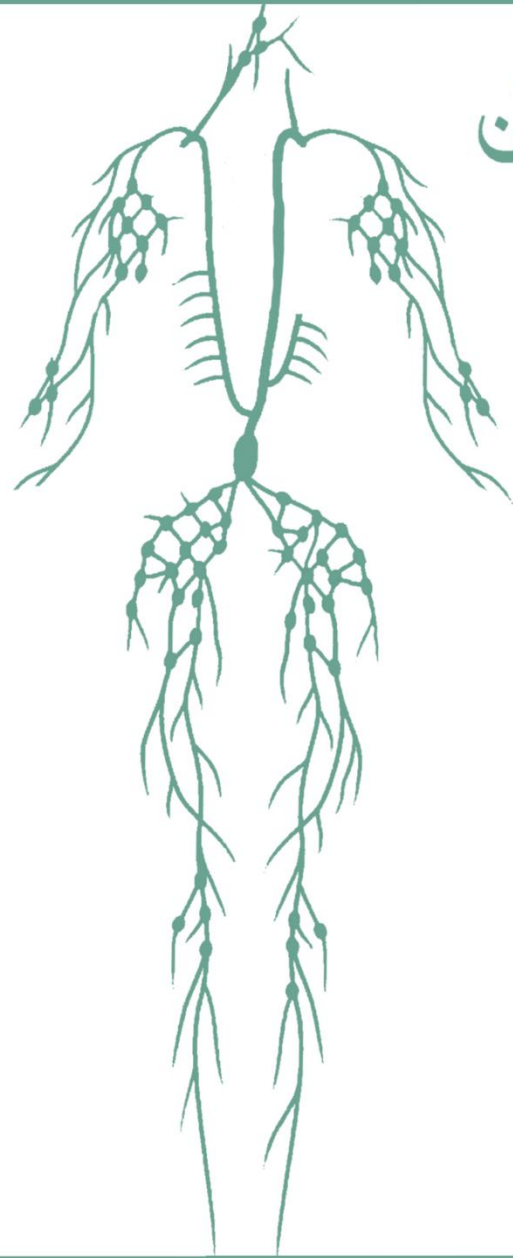
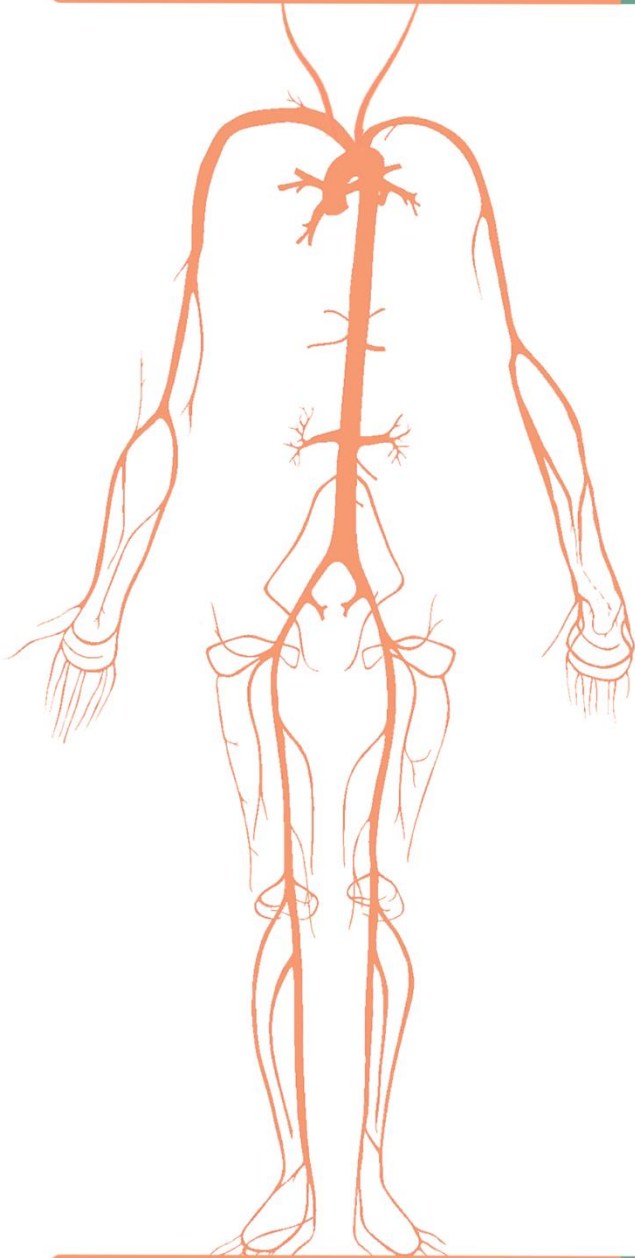


# Biochemistry HematoLymphatic



الجهاز الليمفاوي

**Title:** Sheet 7

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## Blood coagulation

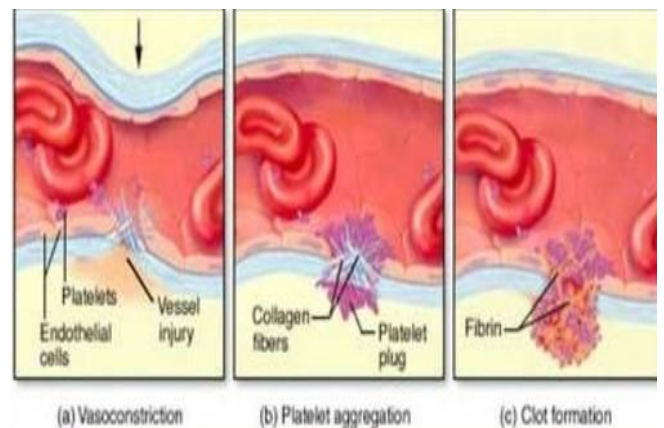
### -> What is blood coagulation (clotting)?

it describes an **orchestrated**, 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 (**hemo: blood, stasis: stoppage; the cessation of blood loss**) followed by clot dissolution and repair.

the word **orchestrated** means that it's almost like a classical music, so it starts **slow** and then it goes **up and up and up** until it reaches a climax then you see the music is going **down** reaching the end. it's basically a symphony.

### -> Steps of hemostasis

- 1- Vascular constriction limiting blood flow to the area of injury. (**physiological effect**)
- 2- Activation then aggregation of platelets at the site of injury, forming a **loose platelet plug**.
- 3- Formation of a fibrin mesh to entrap and solidify the plug, so it is called a **hard clot**. (**cellular & biochemical process**)
- 4- Dissolution of the clot for normal blood flow to resume following tissue repair.



### -> platelets are a major player

platelets are small anuclear cell fragments produced from the megakaryocytes. they have:

- ✓ plasma membrane.
- ✓ numerous kinds of surface receptors.
- ✓ actin filaments and myosin which change the shape of the platelet upon activation.
- ✓ three types of granules that store substances which are released upon platelet activation.

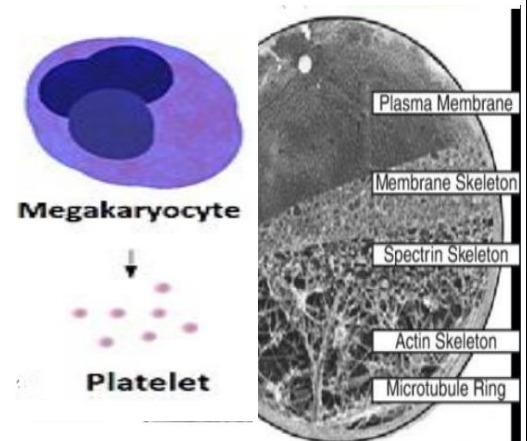
### -> granules:

- ❖ **Electron-dense granules** (calcium ions, ADP, ATP, serotonin)

why do we have ATP and ADP inside these vesicles?

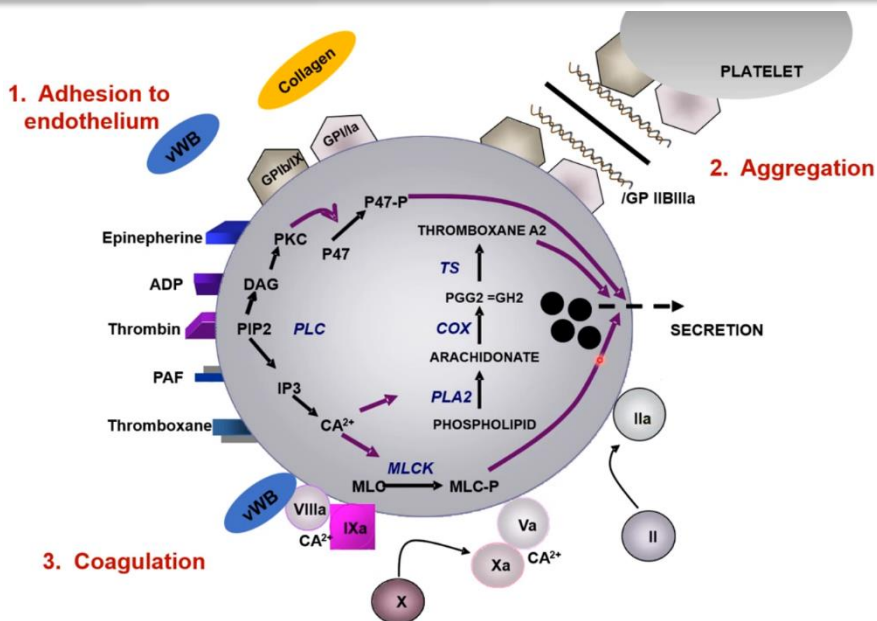
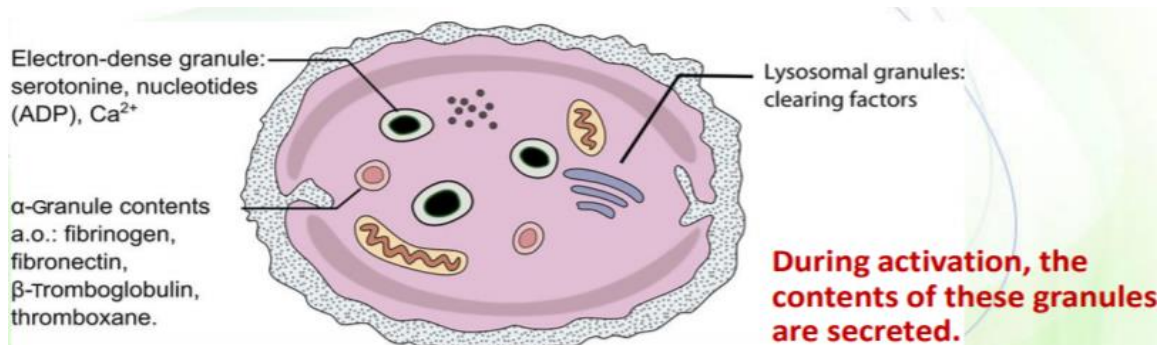
They are not used as source of energy rather they are used as signaling molecules.

- ❖  **$\alpha$ -granule** {a heparin antagonist, platelet-derived growth factor (**signaling molecule**), fibrinogen (**structural protein**), von Willebrand factor -vWF - (**regulatory protein**), clotting factors}
- "Heparin is an anticoagulant."



- ❖ **Lysosomal granules** (hydrolytic enzymes) → necessary for removal of the clot and activation of different proteins.

Again, once these platelets are activated upon vascular injury → these granules fuse with the plasma membrane releasing their contents.



Notice the numerous receptors on the platelet surface [for example, there are receptors for epinephrine, ADP and thrombin]. All of these receptors activate G-protein.

There are a lot of glycoproteins which are important for interacting with vWF, collagen and fibrinogen and for forming aggregation of platelets at the site of injury.

Also, on the platelet surface, the process of blood coagulation takes place.

Now let's go deeply to what exactly happens at the time of vascular injury:

### 1- Adhesion to endothelium:

Whenever there is a vascular injury, a protein known as **Von Willebrand factor (from endothelial cells)** and the collagen (subendothelial collagen) are exposed. This leads to the binding of the platelet glycoproteins with this factor activating the platelets [= generating a series of signaling reactions inside the platelets resulting in the secretion of several factors from the granules of the platelets].

\*\* These factors are ADP, Serotonin, Factor V, ATP, Calcium, Fibrinogen, **vWF**, Thrombin, Thromboxane. → these factors bind to specific receptors.

=> **Note: once the platelets are activated, their shape changes allowing for more platelet-platelet interaction and aggregation.**





## 2- Aggregation:

As we mentioned earlier the **glycoproteins** on the platelet surface are important for forming aggregation of platelets at the site of injury.

## 3- Coagulation

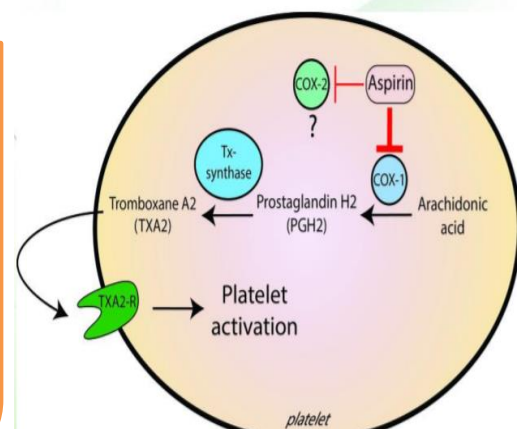
Some important molecules are involved in the process of coagulation:

### 1- Thrombin receptor:

- ❖ Thrombin receptor activates a **G-protein** that activates phospholipase C- $\gamma$  (**PLC- $\gamma$** ).
  - ❖ **PLC- $\gamma$**  hydrolyzes phosphatidylinositol-4,5- biphosphate (**PIP2**) into inositol trisphosphate (**IP3**) and diacylglycerol (**DAG**).
  - ❖ **IP3** induces the release of intracellular **Ca<sup>2+</sup>** stores, and **DAG** activates protein kinase C (**PKC**).
  - ❖ **Calcium** activates the enzyme **phospholipase A2** that triggers liberation of **arachidonic acid** from membrane phospholipids.
  - ❖ **Arachidonate** is converted by **cyclooxygenase 1** to **prostaglandins**, which are then converted by **thromboxane synthetase** to **thromboxane A2**.
- ⇒ Once **thromboxane A2** is released, it has vasoconstrictor activities (limiting blood flow) and it is a platelet activator (helps in the aggregation of the platelet.)
- ⇒ **It acts in autocrine and paracrine manners:** it can bind to its receptor on the cell surface of the same cell that secreted it , thus activating signal transduction pathway inside, it can also act on neighboring places as well.

As we said, **cyclooxygenase1** converts **Arachidonate** into **prostaglandins** leading to the release of **thromboxane A2** . (all of these are 20-carbon fatty acids)

-> **Non-steroidal anti-inflammatory drugs (like Aspirin)** inhibit the enzyme **cyclooxygenase1&2**, accounting for their anticoagulant effects. → preventing the formation of **atherosclerosis**.



in addition to activation of phospholipase A2 and release of arachidonate from phospholipids, **Ca<sup>2+</sup> ions** activate myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin allowing it to interact with actin resulting in a number of effects: **altering platelet morphology** (more aggregation), **inducing motility** (because they modify the actin cytoskeleton) , and increasing the **release of granules**.

**2- Serotonin** (vasoconstrictor)

**3- PDGF (Platelet-derived growth factor)** which stimulates proliferation of endothelial cells to reduce blood flow.

## 4-ADP

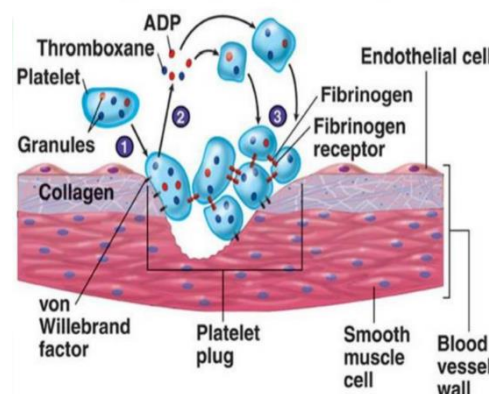
Remember that, **DAG** activates **PKC**, which phosphorylates and activates specific platelet proteins (including P47) that induce the release of **platelet granule contents** including **ADP**.

### the 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, called **platelet plug**.

### Role of platelet surface

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



## Biochemistry of coagulation

### Components of coagulation they vary in size

- 1- An organizing surface (platelets) we've already talked about
- 2- Proteolytic zymogens (prekallikrein, prothrombin, and factors VII, IX, X, XI, XII, and XIII)

\*remember that zymogens are enzymes that require proteolytic cleavage in order to be active, example: trypsinogen which is converted to trypsin when it is cleaved

\*factors are mostly proteins designated with roman numbers

These are mainly serine propeptases released from hepatocytes.

The subscript "a" designates the activated form of a factor. e.g., once factor "XIII" is activated it's called "XIIIa"

- 3- Anti-coagulants (protein C, protein S)
- 4- Non-enzymatic protein cofactors (factors VIII, V, and tissue factor)
- 5- Calcium ions
- 6- Vitamin K
- 7- Fibrinogen

Clotting factor number	Clotting factor name	Function	Plasma half-life (h)
I	Fibrinogen	Clot formation	90
II	Prothrombin	Activation of I, V, VII, VIII, XI, XIII, protein C, platelets	65
III	TF	Co factor of VIIa	-
IV	Calcium	Facilitates coagulation factor binding to phospholipids	-
V	Proaccelerin, labile factor	Co-factor of X-prothrombinase complex	15
VI	Unassigned		
VII	Stable factor, proconvertin	Activates factors IX, X	5
VIII	Antihemophilic factor A	Co-factor of IX-tenase complex	10
IX	Antihemophilic factor B or Christmas factor	Activates X: Forms tenase complex with factor VIII	25
X	Stuart-Prower factor	Prothrombinase complex with factor V: Activates factor II	40
XI	Plasma thromboplastin antecedent	Activates factor IX	45
XII	Hageman factor	Activates factor XI, VII and prekallikrein	
XIII	Fibrin-stabilizing factor	Crosslinks fibrin	200
XIV	Prekallikrein (F Fletcher)	Serine protease zymogen	35
XV	HMWK- (F Fitzgerald)	Co factor	150
XVI	vWf	Binds to VIII, mediates platelet adhesion	12
XVII	Antithrombin III	Inhibits IIa, Xa, and other proteases	72
XVIII	Heparin cofactor II	Inhibits IIa	60
XIX	Protein C	Inactivates Va and VIIIa	0.4
XX	Protein S	Cofactor for activated protein C	

HMWK – High molecular weight kininogen; vWf – Von Willebrand factor; TF – Tissue factor

This is a list of the different clotting factors, their names, and what their functions are. Please refer to it after finishing the sheet to summarize the whole process.

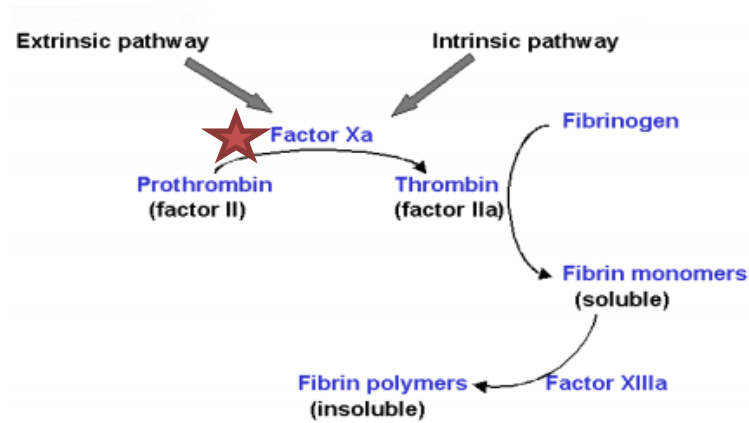
**These factors are not all proteins. Notice that factor IV is Ca.**

There are two pathways included in coagulation:

- 1- **Intrinsic pathway:** is initiated when subendothelial surface (i.e., collagen) is exposed. **That is as a result of internal effect like an inflammation.**
- 2- **Extrinsic pathway:** is initiated in response to tissue injury.  
So, here tissue factor (TF) protein is released.

These two pathways were thought to be independent, but later it was discovered that they are not totally independent, there is a bridge that connects them together, so whenever the extrinsic pathway is activated, the intrinsic is activated too.

- 3- However, the two pathways converge on a **common pathway**.★



#### Extrinsic pathway:

Vascular injury (trauma) → exposure of collagen and vWF → activation of factor VII **(with the help of tissue factor)** → activation of factor X

#### Intrinsic pathway:

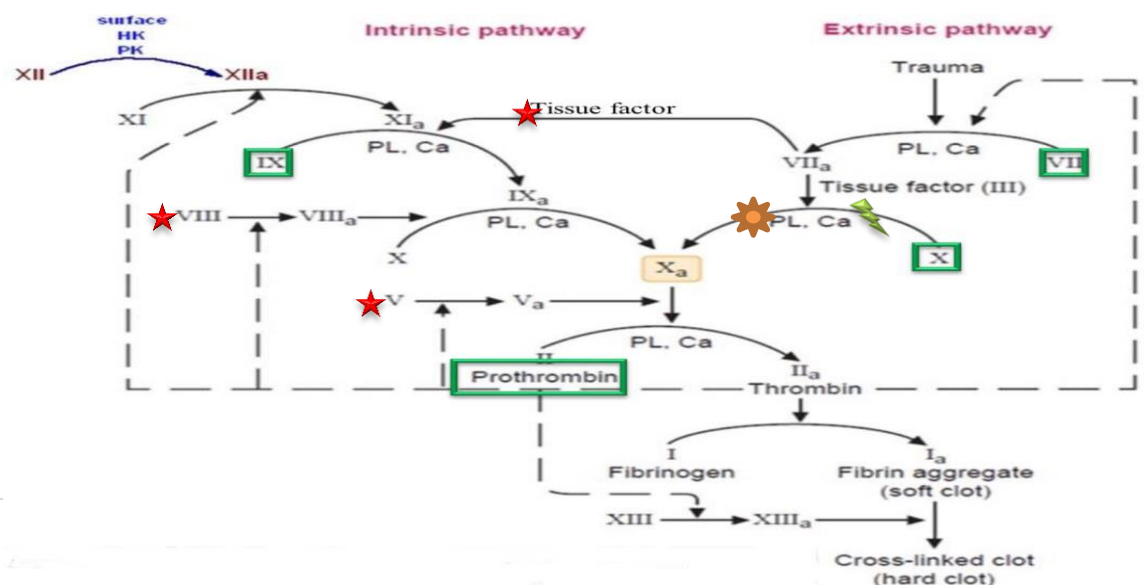
In case of inflammation (there is also activation of the [kallikreins-kinins pathway](#)), there is activation of factor XII → activation of factor XI → activation of factor IX

\* tissue factor also plays an important role in the intrinsic pathway; it forms a bridge whereby factor VII can activate factor IX → activate factor X

#### common pathway:

activation of factor X → activation of prothrombin → formation of the fibrin network

notice the involvement of the **non-enzymatic protein cofactors**, **platelet surface** and **Ca**.



## Gla domain:

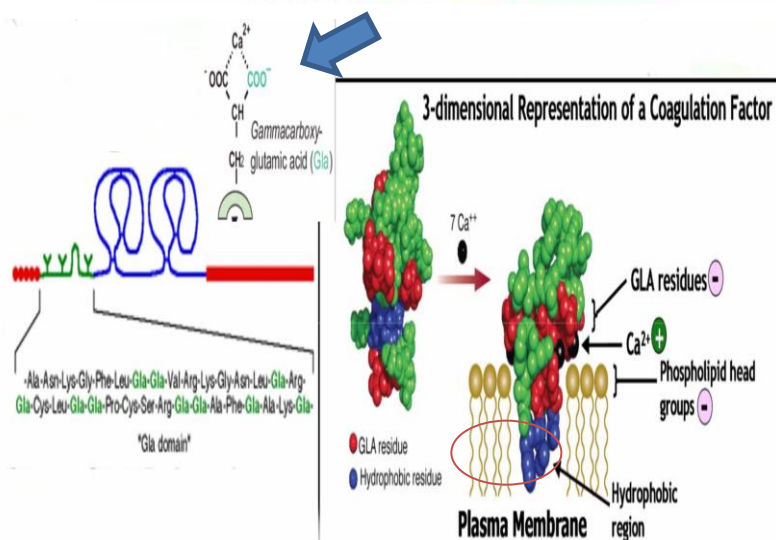
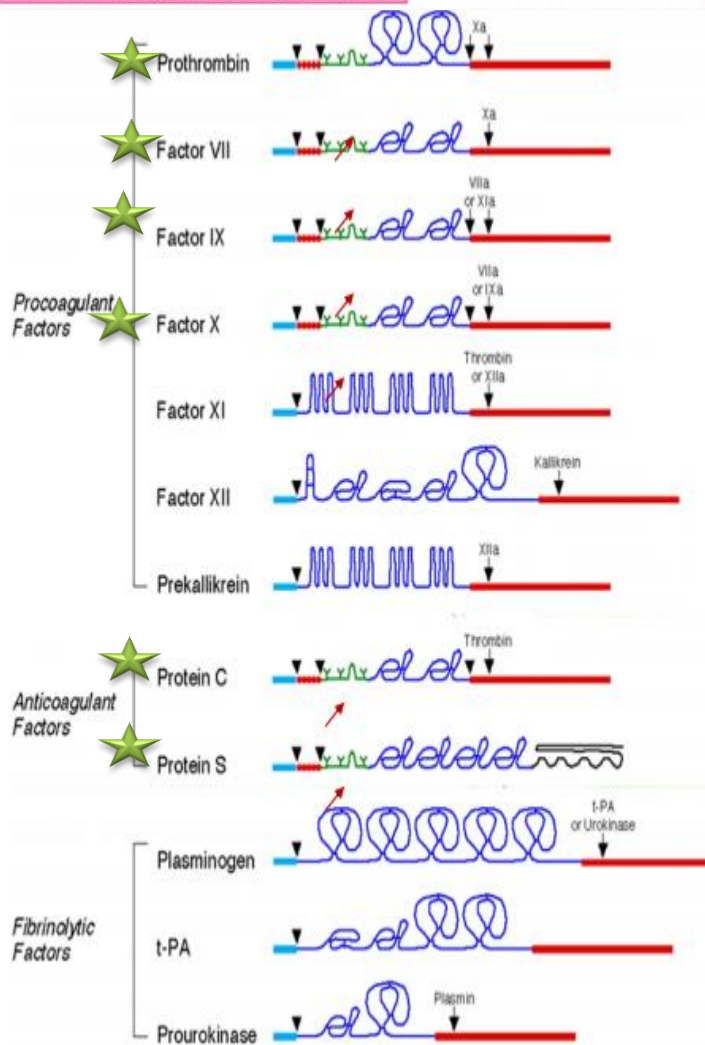
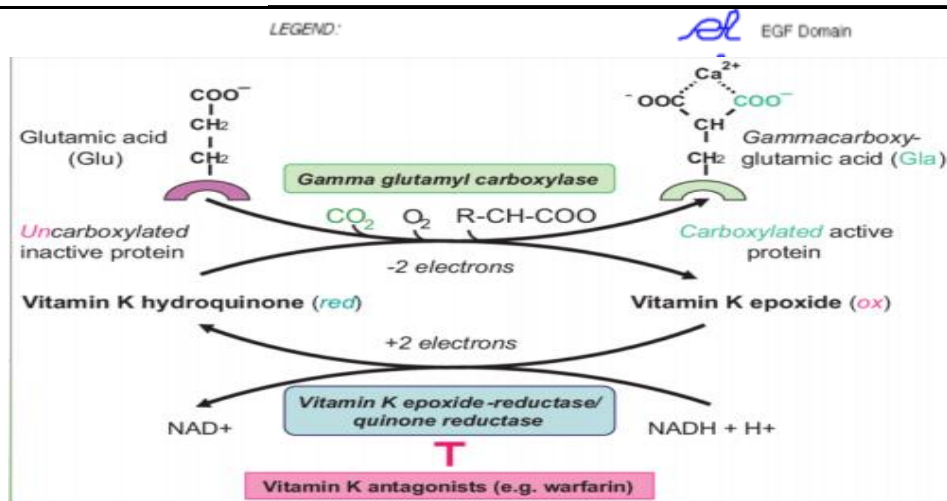
-> a domain that contains 9-12 glutamate residues and it is part of the primary structure of prothrombin, factors (VII, IX, X), protein C and protein S. It is a substrate for an enzyme known as **carboxylase**.

-> An ER/Golgi **carboxylase** binds to prothrombin and factors IX, VII, and X and **converts 10≥ glutamate (Glu) residues to γ-carboxyglutamate (Gla)**, by adding another carboxyl group to each Glu.

-> The Gla residues **bind Ca ions** and are necessary for **the activity of these coagulation factors** and formation of a coordinated **complex with the charged platelet surface** to localize complex assembly and thrombin formation to the platelet surface.

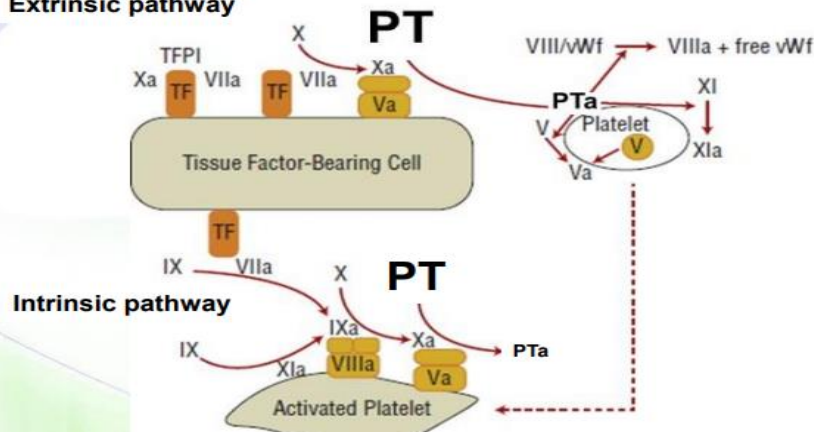
-> notice that this domain is followed by a small (10 amino acid) hydrophobic region which strengthens the interactions between these factors and the plasma membrane of platelets where biochemical processes of coagulation take place.

**Vitamin K** participates in conversion of Glu to γ-Gla. (It is needed for carboxylation as a source of electrons).





### Extrinsic pathway



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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**Vitamin K** becomes oxidized to vitamin K epoxide and must be regenerated by an enzyme known as **vitamin K epoxide reductase** and this requires NADH as a source of electrons. This enzyme is the target for vitamin K antagonist (Warfarin).

### Newborns are at risk for early

### vitamin K deficiency bleeding. Why? (signs and symptoms: gum bleeding, formation of bruises)

- 1- The placenta is a poor passage channel for fat-soluble compounds, including vitamin K.
- 2- Neonates are born with an immature liver that impairs coagulation factor synthesis and GLA modifications.
- 3- Breast milk is a poor source of vitamin K.
- 4- Intestinal flora, the main source of vitamin K, is not established yet.

### Prothrombin activation

- The complex of factor Xa/Va is the “**prothrombinase complex**”. factor V also interacts with prothrombin. (due to interaction with Ca ions) → factor V brings factor X and prothrombin close together.

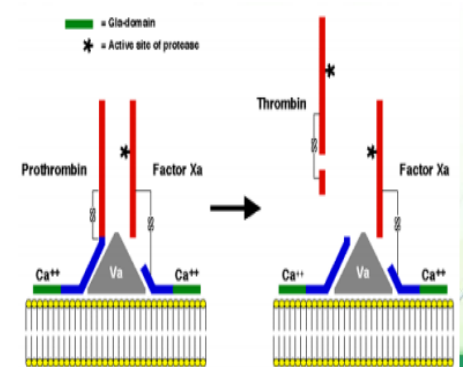
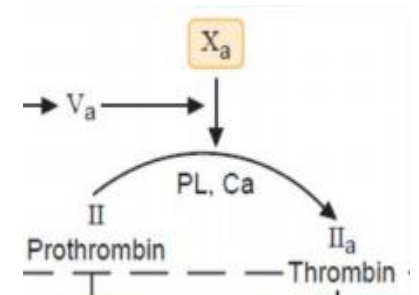
- **Factor Xa converts prothrombin to thrombin**, which is accelerated by Va, platelets (or phospholipids), and calcium ions.

- 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.

in the extrinsic pathway prior to activation of factor X, you have the activation of factor VII and with the help of TF.

In the intrinsic pathway, you have the involvement of TF which activates factor IX and helps in activation of factor X by activation of factor VIII. (factor VIIIa and factor IXa form complex that activates factor X), the complex called “tenase





complex”.

### **Factors V and VIII**

→ Va and VIIIa are cofactors that increase the proteolytic efficiency of Xa and IXa, respectively.

→ Factor IXa/VIIIa is known as the “**tenase complex**”.

→ Factors V and VIII are activated by thrombin.

→ **Factor VIII** circulates in the plasma bound to **von Willebrand factor**, which increases VIII half-life, and, when released, it gets activated.

→ **von Willebrand factor deficiency** is associated with a decrease in the plasma concentration of factor VIII. [suffer from excessive bleeding and inability to coagulate blood]

### **Tissue Factor:**

integral membrane protein that is expressed on the surface of activated monocytes, sub-endothelial cells, and other cells. So blood cells and platelets are not exposed to sub-endothelial cells unless there is tissue injury then you can have platelets binding to a tissue factor.

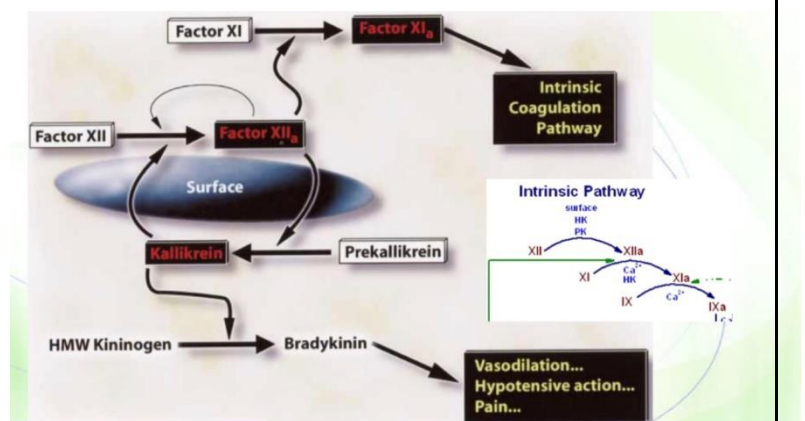
Once they interact with tissue factor you can have activation of factor 10 in the extrinsic pathway, while in the intrinsic pathway the tissue factor is also involved whereby it is important in activating factor 9 which activates factor 10.

The complex of tissue factor and factor 7 is known as “initiation complex” because it activates the extrinsic and the intrinsic pathways. (Tissue factor increases the proteolytic efficiency of VIIa).

### **Kallikrein-kinin System:**

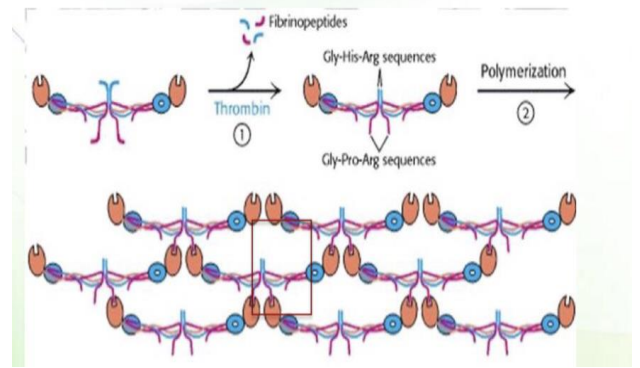
Upstream of factor IX are factors XI and XII and you have activation of what is known as Kallikrein-kinin

When kallikrein is activated it can activate factor XII and factor XII can activate factor XI, kallikrein as well has other effects by cleaving kininogen producing Bradykinin (it has vasodilation effect)



## Formation of fibrin clot :

After activation of factor X , it activates prothrombin into thrombin which acts on fibrinogen by converting fibrinogen into fibrin by removing a number of fibrinopeptides and this results in the ability of fibrin molecules to form electrostatic interactions between the head and the tails of two other molecules

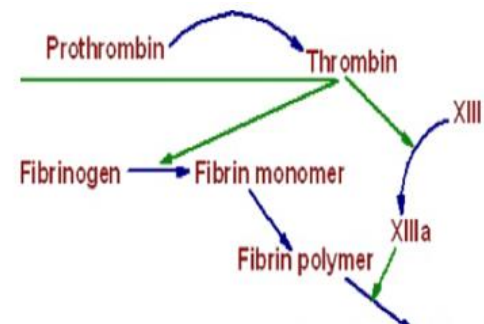


Then there will be aggregation of fibrin molecules together forming a clot known as a **soft clot** because the interaction between all these molecules is based on electrostatic interactions which is non-covalent.

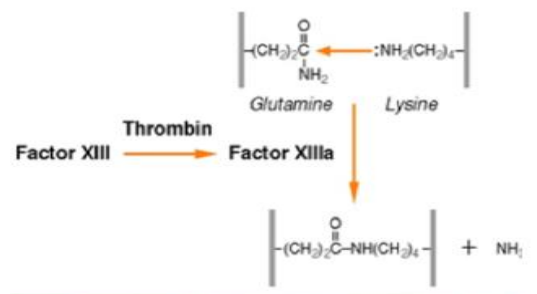
“Fibrinogen is a two triple-stranded helical protein held together by disulfide bonds.”

## **Factor XIII**

It is a transglutaminase activated by thrombin to form covalent (cross linking )between Glu of one fibrin monomer to a Lys on another fibrin monomer , the cross linking strengthen the fibrin mass forming a “hard clot”.



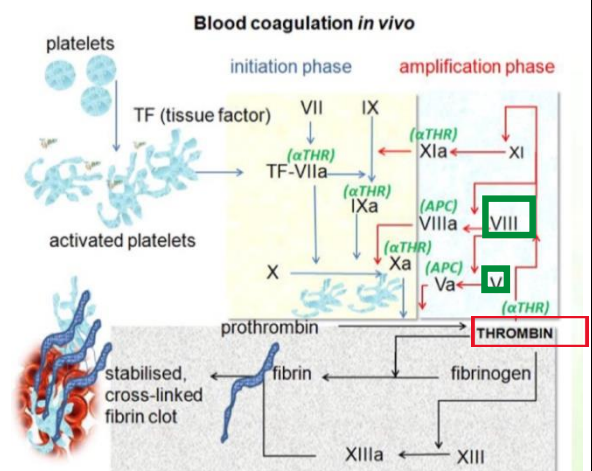
The clot doesn't only contain fibrin network ,it also entraps platelets inside forming a platelet plug.



## Amplification of coagulation reactions

The sequential enzymatic activation allows for amplification.

Amplification also results from positive feedback reactions. The thrombin molecule acts back in all of these factors ,so it activates more factor V ,VIII, VII, XI, that's why you have more amplification of all of these zymogens , {specifically getting to the end factor X that activates more and more prothrombin to thrombin.}



## Rules of thrombin

1. Recruiting platelets to the site of injury
2. Amplification of the coagulation complex
3. Formation of soft clot (proteolytic cleavage of fibrinogen) where fibrin monomers form a complex.
4. Attenuation of its own activity (activation of protein c) .... It terminates the coagulation.
5. Formation of hard clot by (activation of factor XIII)
6. Other actions: binding to its receptor (protease activated receptors) on the surface of platelets and endothelial cells induces vascular remodeling (e.g. angiogenesis) and inflammation.

### **Protein C and protein S**

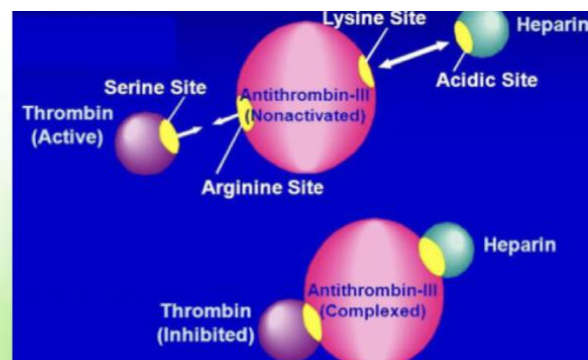
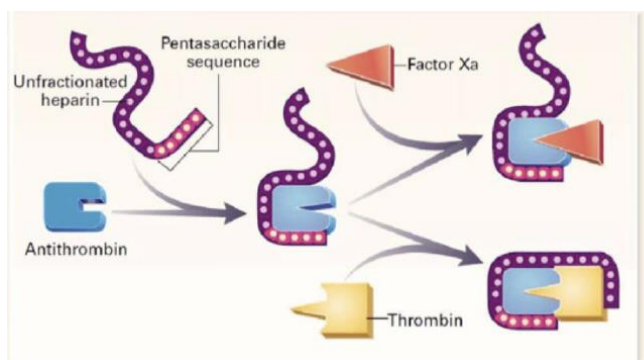
Thrombin binds to a protein known as thrombomodulin in the surface of endothelial cells

Thrombin can then activates protein C which forms a complex with protein S, {both of which are vitamin k dependent co- factors}

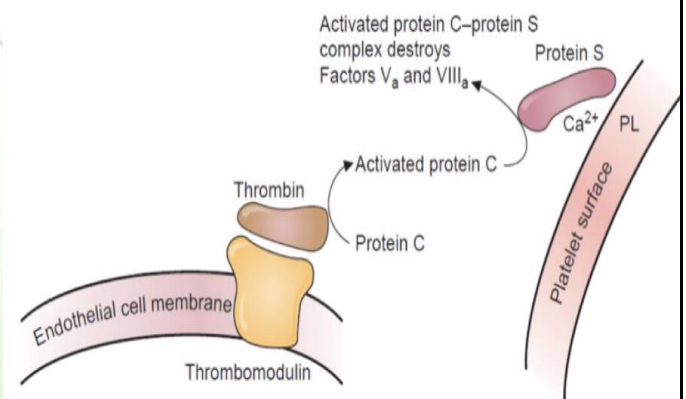
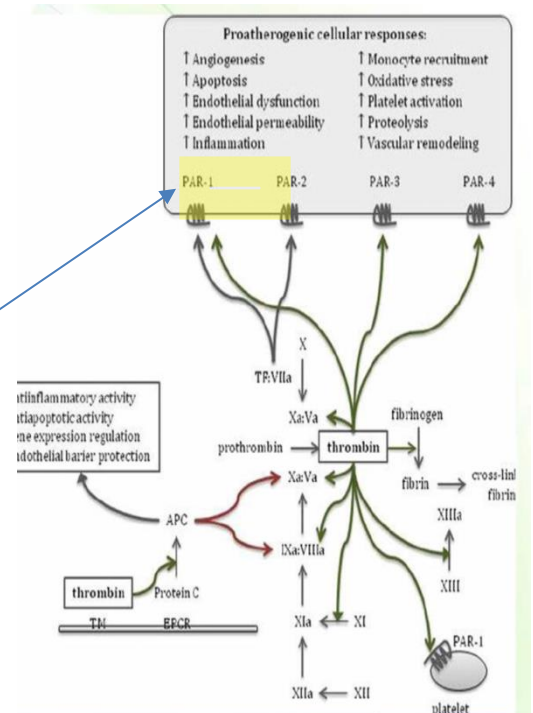
The complex degrades factors V and VIII, so we have termination of activation of prothrombin and activation of factor 10.

### **Antithrombin III**

Serine protease inhibitor of thrombin as well as other clotting factors (IXa,Xa,XIa,XIIa) . Antithrombin binds to heparin sulfate (polysaccharide synthesized by mast cells and



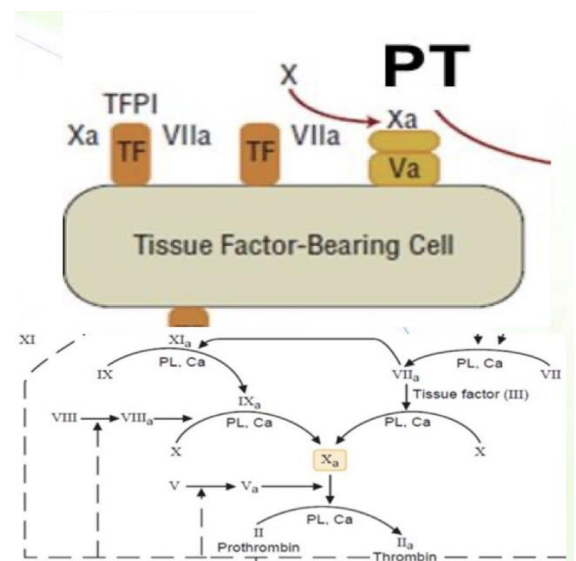
present on surface of endothelial cells ) this binding changes the conformation of antithrombin III promoting binding to its substrates.





## Tissue factor pathway inhibitor

4-TFPI is also able to inhibit Xa-activated Va resulting in inhibition of the prothrombinase complex.



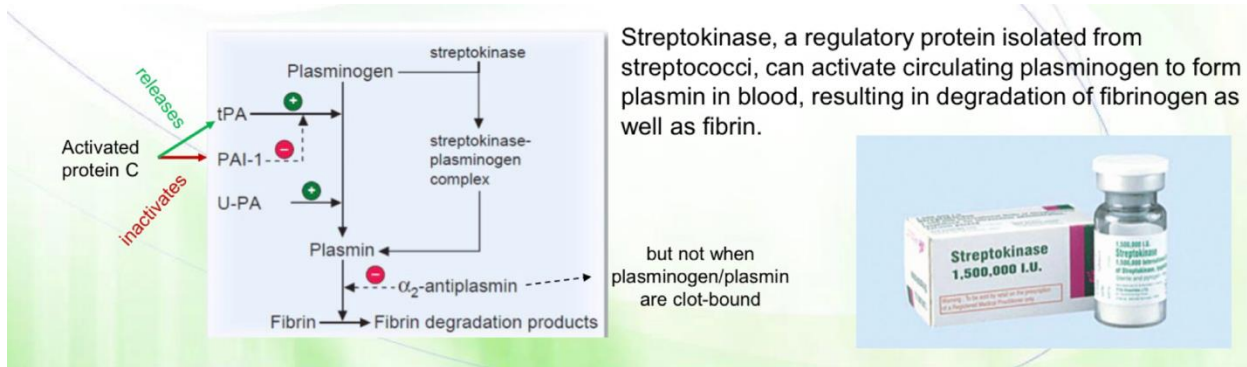
## Anticoagulants

## Degradation of the fibrin clot

The diagram illustrates the Vitamin K cycle. At the top left is 'Precursor Prothrombin' and at the top right is 'Biologically active Prothrombin'. A blue arrow points from Precursor Prothrombin to Biologically active Prothrombin, labeled 'Carboxylase'. A blue arrow points from Biologically active Prothrombin down to 'Vitamin K (epoxide)' on the right. A blue arrow points from 'Vitamin K (epoxide)' down to 'Vitamin K (reduced)' on the left. A blue arrow points from 'Vitamin K (reduced)' up to Precursor Prothrombin, labeled 'oxidative deactivation'. Below the 'Vitamin K (epoxide)' and 'Vitamin K (reduced)' labels is 'Vit K epoxide reductase'. A red square, representing Warfarin, is positioned below the reductase label, with an arrow pointing to the reductase enzyme.

## The fibrinolytic system

- Plasminogen has a high affinity for fibrin clot



Notice from the picture above, that activated protein C releases tPA (tissue plasminogen activator) ,and at the same time inhibits the inhibition of this enzyme which is called PAI-1 (Plasminogen activator inhibitor-1).

The role of Urokinase:

Urokinase, a serine protease is formed from the zymogen pro-urokinase, It is a potent plasminogen activator, and used clinically.

GOOD LUCK 😊