THROMBIN: NATURE'S BAND-AID

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BACKGROUND

ABSTRACT: Normal blood flow plays an essential role in many life processes. If an abrasion to the blood vessels disrupts normal blood flow, a signaling cascade is initiated in which a **protein called thrombin plays an essential role.** The result of the **signaling cascade** is the formation of a clot and fixes the abrasion. **Thrombin is the central molecule in hemostasis**, which is the process of stopping blood flow. When blood vessels are cut open, **Factor VII** – a protein that helps the process of blood clotting – is released and comes into contact with tissue factor found on cells. When this happens, **factors V, IX, and X** are activated. Collectively, these factors trigger the signaling cascade that results in the activation of thrombin. Thrombin is circulated in plasma as **prothrombin**, which is the inactive state of thrombin.

Thrombin catalyzes the conversion of **fibrinogen** into **fibrin**, which then constructs an insoluble network of fibers that eventually dries to form a scab.

The Saint Joan Antida SMART (Students Modeling a Research Topic) Team has modeled thrombin using 3D printing technology. **Thrombin is a serine protease composed of two chains**. The active site amino acids involved in cleaving the peptide bonds in fibrinogen are **His-57, Asp-102, and Ser-195.** Defective thrombin can either lead to too few or too many blood clots. Too little clotting could result in a disorder called **hemophilia**; too much could result in **deep vein thrombosis (DVT)** – a blood clot in major leg veins. DVT could lead to less blood flow to the heart, causing a stroke or heart attack. Research continues on the role thrombin plans in the progression of hemostasis and restoring the balance of homeostasis.

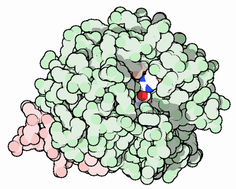


Figure 1: The picture on the left depicts the thrombin protein (PDB entry 1ppb)

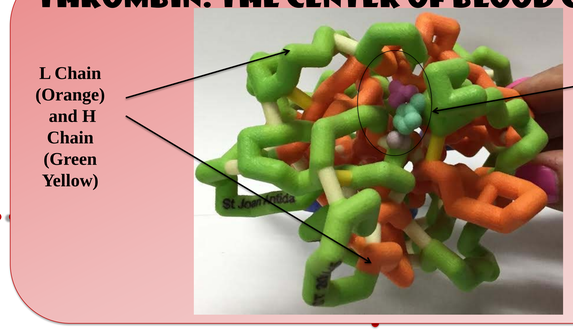
What is Thrombin?

- The protein responsible for the formation of fibrin clots.
- The central protein in the signaling cascade of the tissue factors is responsible for blood clotting.

DANGEROUS CLOTTING DISORDERS (THROMBOPHILIA)

Blood Disorder	Description
Tissue Factor Pathway Inhibitor (TFPI)	Inhibits a complex that begins blood clotting. 60-80% of TFPI is in endothelium and deficient levels of TFPI may cause clotting disorders.
Thrombomodulin	A transmembrane protein found on endothelium. It acts as a receptor for thrombin. Defects in Thrombomodulin result in increased blood clotting. Thrombomodulin activates thrombin-activated fibrinolysis inhibitor (TAFI).
Elevated levels of thrombin- activated fibrinolysis (TAFI)	Increased level of thrombin is necessary for clot formation and the maintenance of clots. Too high levels of thrombin can lead to the activation of TAFI. TAFI prevents plasminogen from binding to the fibrin clot which helps stop the breakdown of blood clots.

THROMBIN: THE CENTER OF BLOOD CLOTTING



L Chain (Orange) and H Chain (Green Yellow)

His57, Asp102, and Ser195 are the three main amino acids that are located in the active site, which are involved in cleaving the peptide bonds in fibrinogen

BLOOD CLOTTING: AN OVERVIEW

Like many things in life, tubes and pipes must be repaired due to leaks. Blood clots primarily address the issue of leaks that occur on the human body in order to promote hemostasis. ***Figure 2*** offers a general over view of the blood clotting process. The process of blood clotting is designed to help maintain hemostasis in the event of a damaged blood vessel. It is important to note that ***Figure 2*** merely demonstrates the **emergency mechanism of stopping blood clots.** Blood coagulation is not complete until the coagulation cascade is complete.

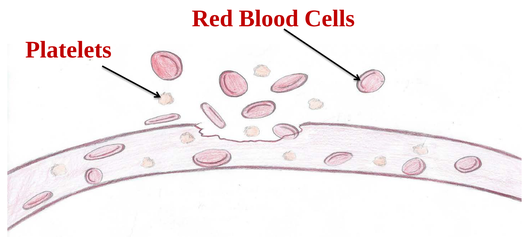
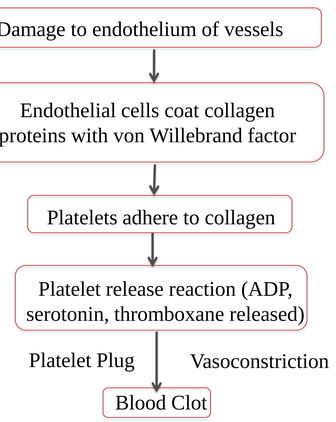


Figure 2: Schematic Depicting Platelet Function



ACTIVE SITE

Figure 4

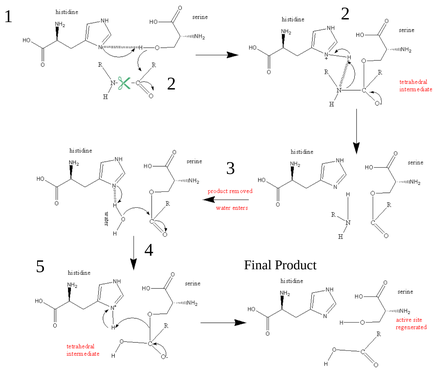


Figure 4: Thrombin is a serine protease. A serine protease is an enzyme that separates peptide bonds in proteins. Serine proteases are characterized by the catalytic triad. The triad contains three amino acids- **histidine, serine, and aspartic acid.** The reaction of a serine protease can be summarized as follows:

1. The polypetide bonds to the serine protease.
2. The serine attacks the carbonyl carbon and the nitrogen of the histidine bonds with hydrogen.
3. The bond between nitrogen and carbon is now broken.
4. Water replaces the N-terminus of the broken peptide and attacks the carbonyl peptide.
5. The bond between serine and carbonyl carbon attacks the hydrogen

Figure 5: Model A shows a regularly functioning thrombin protein model, while Model B shows dysfunctional thrombin Quick II, which does not clot fibrinogen. Thrombin Quick II was isolated from someone with less than 2% of normal prothrombin activity. In the affected areas, there is an amino acid substitution from **valine to glycine at position 558**, which is the site of substrate binding. As indicated by the arrow in the image above, **this substitution limits binding access**. The substitution of glycine for valine causes a decrease in the kinetic value (Kcat), which is shown in ***Table 1.***

Figure 5

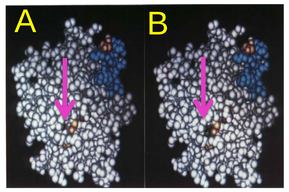


Table 1

Comparison of Kinetic Constants for Hydrolysis of Succinyl-Ala-Ala-Pro-Leu-p-nitroanilide		
Enzyme	**Kcat (s^{-1})**	**Conditions**
Thrombin	**0.079** +/- 0.004	30˚C, 0.1 M NaCl, 0.05 M Tris-HCl, pH 8.3
Thrombin Quick II	**0.032** +/- 0.004	30˚C, 0.1 M NaCl, 0.05 M Tris-HCl, pH 8.3

SCHEMATIC OF SIGNALING CASCADE

The process of the conversion of fibrinogen into fibrin is led by both the extrinsic and intrinsic pathways. The **intrinsic pathway is where the production of clots start to form** when the blood vessel is damaged. The damaged blood vessel exposes collagen to plasma. In the **extrinsic pathway, a chemical is rapidly released that initiates a formation of fibrin.** This chemical is called thromboplastin or tissue factor. This is the protein that initiates blood clotting on the surface of the cell. Tissue factor can be found on most cells in the human body. The tissue factor serves as a cofactor with factor VII to promote the activation of factor X. **Factor X activates prothrombin, which then activates thrombin** in a reaction that requires Factor V. **Thrombin converts soluble fibrinogen into relatively insoluble fibrin.** Fibrin is the fibrous protein that creates the **mesh-like covering** over damaged blood vessels. This blood clot disallows for blood loss and pathogen entry. With the exception of factor VII, all of the components of the extrinsic pathway are also a components of the intrinsic pathway.

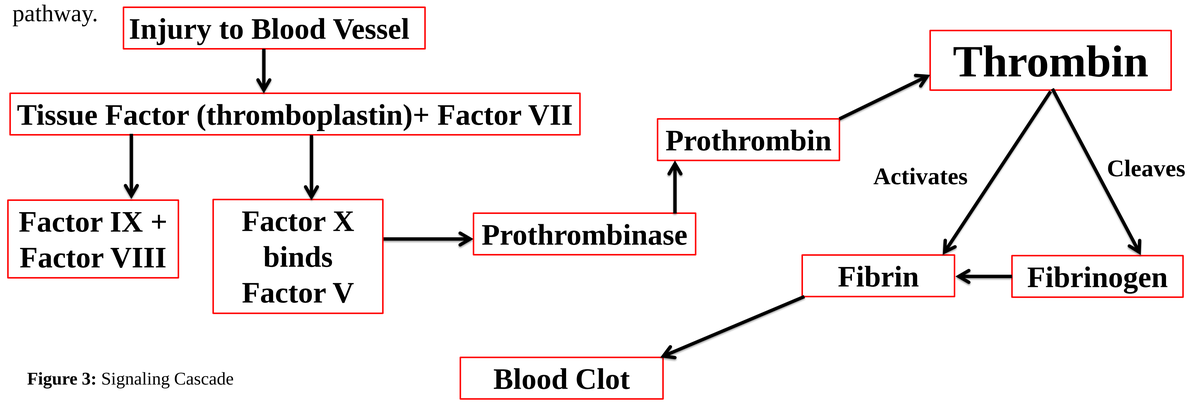


Figure 3: Signaling Cascade

REFERENCES

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2. Henriksen, R.A., Mann, K.G. (1989). Substitution of valine for glycine-558 in the congenital dysthrombin thrombin Quick II alters primary substrate specificity. *Biochemistry 28* (5): 2078-2082

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