Blood clotting

Like activation of digestive zymogens, blood clotting is another example of a process that must happen quickly when needed, but which has catastrophic effects if it occurs at an inappropriate time or location.

Blood clots form when the serum protein fibrinogen is cleaved by proteolytic attack of thrombin. Thrombin is a trypsin-like protease which circulates in the blood in the form of inactive prothrombin. To prevent unintended cleavage of fibrinogen, blood contains a thrombin inhibitor, antithrombin III.

Fibrinogen

Fibrinogen is a long symmetrical molecule of 340 kDa made up of two copies each of Aα, Bβ and γ chains, each of which includes a helical region forming a triple helical coiled-coil.

Globular domains exist at each end, contributed by the C-terminals of the Bβ and γ chains. A central “knot” is made up of the N-terminal ends of the chains, but is disordered, so it is not possible to trace how each chain contributes to the knot.

Fibrinogen is a soluble protein in blood serum (the fluid that remains when cells are removed). Activated thrombin acts at the strictly at the sequence Arg-Gly, cutting after Arg. This removes two small segments called fibrinopeptides A (18 amino acids) and B (20 amino acids, from the N-terminus of Aα and Bβ chains, in the knot region. The peptides that are removed are highly negatively charged, and include Tyr with an added sulfate group. The remaining structure now has chains (αβγ)₂ and is called fibrin.
Removal of the fibrinopeptides exposes positively charged receptor sites in the central region that bind to the globular ends. As a result, fibrin molecules rapidly assemble into a macromolecular assembly in a brick-like pattern.

**Thrombin cuts off fibrinopeptides to leave fibrin monomer**

Fibrin assembles into a polymer

**The fibrin polymer causes blood to gel**, and traps red blood cells and platelets. This prevents blood from escaping from a wound. In addition, cross links develop between neighbouring chains induced by Clotting Factor XIII, and cause the initial soft clot to become a hard clot.

**Plasminogen** is a third component of blood, and can be activated to yield another trypsin-like protease called **plasmin**. Plasmin specifically attacks the helical regions of fibrin clots, allowing the clot to redissolve, which normally happens over a long period of time after the initial wound. Plasminogen is activated by a protease called **tissue plasminogen activator**, which is slowly released into the blood. Tissue plasminogen activator has been expressed by recombinant DNA methods. **Stroke and heart attacks** are caused by inappropriate formation of blood clots in brain and cardiac muscle respectively. If tissue plasminogen activator is injected into a victim of stroke or heart attack within 30 minutes of onset, this can cause rapid dissolution of the clot and limit the long term consequences.

**Activation of thrombin must be very rapid**

Clot formation occurs to prevent blood loss from trauma or sharp wounds that sever blood vessels. Unlike plasmin activation, which occurs slowly, thrombin must be capable of rapid activation. Activation occurs through a **cascade** of successive serine proteases (trypsin-like enzymes) called **clotting factors**. Clotting factors are each identified with a roman numeral, assigned *in order of discovery* (rather than in terms of sequence of action). **Fibrinogen** is factor I and **prothrombin** is factor II. The active forms are distinguished by a suffix a, so **thrombin** is factor IIa.

There are two starting points for the cascade:

The **intrinsic pathway** is induced by factors within the blood itself, released by cells called platelet which are differentiated specifically for the purpose of mediating blood clotting. This pathway is used in the case of a sharp cut that induces little generalized tissue damage.

The **extrinsic pathway** is induced by trauma such as laceration that results in considerable tissue damage.
Intrinsic pathway

The cascade amplifies the initiating signal so as to produce large amounts of thrombin at the wound site. At each level, there is a multiplier effect because one molecule of Factor XIIa can activate many molecules of Factor XI, and so on down the cascade. Factor VIII however is not a serine protease, but a binding factor that promotes binding of Factor IXa to Factor X. Factor V is a binding factor that promotes interaction between Factor Xa and prothrombin. Factor V and VIII's activities are themselves promoted by cleavage induced by thrombin, hence this produces a positive feedback effect.

The intrinsic pathway is stimulated by exposure of blood to negatively charged surfaces, so glass is a potent inducer of clotting. The clotting factors are normally present in serum at extremely low levels - so dilute that few encounters occur. Prothrombin and the other serine protease clotting factors have unusually large N-terminal segments that contains multiple units of a modified amino acid, \( \gamma \)-carboxy glutamate. The double-negative modified glutamate binds \( \text{Ca}^{2+} \) more strongly than normal glutamate, and bound \( \text{Ca}^{2+} \) causes prothrombin to stick to negative surfaces.

In the body, blood platelets are cells differentiated specifically to mediate clotting. Activated platelets release negative phospholipids to form a surface to bind clotting factors.

A protein that is widely dispersed at low concentration in a volume (panel a right) may have few encounters. However, when the same molecules are collected on a surface (panel b, right), the local concentration suddenly becomes much higher. Negative phospholipids are released by platelets, and this serves as a collecting point for Factors V, Xa and prothrombin (and possibly earlier acting factors).

Hemophilia is due to deficiencies in specific clotting factors

A deficiency in Factor VIII, known as antihemophilic factor, is responsible for classic hemophilia, as exhibited by a significant number of male descendents of Queen Victoria, including the son of Nicolas II, the last Tsar of Russia. Hemophilia can now be controlled by human Factor VIII produced in cell culture by recombinant DNA technology. Factor IX deficiency sometimes found as a non-classical form of hemophilia that does not respond to antihemophilic factor.
Extrinsic pathway

The extrinsic pathway is induced by a protein called tissue factor, released by damaged tissues, which acts as a binding factor to promote self activation of Factor VII and activation of Factor X by VIIa. Steps after Factor X are similar to the intrinsic pathway.

Vitamin K is required for synthesis of prothrombin

Vitamin K is a required cofactor for the enzyme that adds the extra carboxylate onto glutamate side chains in the N-terminal sequence of prothrombin. Lack of Vitamin K causes a blood clotting deficiency.

People who have suffered a stroke or heart attack need to limit the ability of blood to clot to prevent a recurrence. They are treated with the drug Warfarin, which is an antagonist for Vitamin K. Antagonists are molecules that are chemical look-alikes for a required factor, so that they bind to enzyme sites, but then fail to carry out the necessary action. Warfarin keeps Vitamin K from modifying too many prothrombin molecules. However, the level of drug has to be carefully balanced to prevent minor breaks in capillaries from turning into uncontrolled bleeding (particularly if stomach ulcers are present).

Antithrombin III is a naturally occuring thrombin inhibitor

The body normally keeps clotting under control by circulating small amounts of antithrombin III, which is a substrate like molecule that binds tightly to thrombin and other serine proteases in the blood clotting cascade (XIIa, XIa, IXa and Xa). This functions very much like secretory pancreatic trypsin inhibitor, and binds to thrombin, and is hydrolyzed exceptionally slowly. The quantity of antithrombin III is sufficient to act as a brake and to prevent clotting from accidental activation of very small amounts of thrombin, or spread of small amounts of thrombin away from an actual site of injury. If the clotting cascade is really set off, the amount of thrombin is too great for the amount of antithrombin III, and clotting can proceed.

The charged polysaccharide heparin promotes the action of antithrombin III, hence heparin coated tubes are used for blood collection for medical purposes. Similarly the Ca$^{2+}$ chelating agent ethylenediamine tetraacetic acid (EDTA) also acts as a blood anticoagulant by trapping the available Ca$^{2+}$ ions which prevents prothrombin from being activated.