Hemostasis & Thrombosis

51

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BIOMEDICAL IMPORTANCE

Basic aspects of the proteins of the blood coagulation system and of fibrinolysis are described in this chapter. Some fundamental aspects of platelet biology are also presented. Hemorrhagic and thrombotic states can cause serious medical emergencies, and thromboses in the coronary and cerebral arteries are major causes of death in many parts of the world. Rational management of these conditions requires a clear understanding of the bases of blood clotting and fibrinolysis.

HEMOSTASIS & THROMBOSIS HAVE THREE COMMON PHASES

Hemostasis is the cessation of bleeding from a cut or severed vessel, whereas thrombosis occurs when the endothelium lining blood vessels is damaged or removed (eg, upon rupture of an atherosclerotic plaque). These processes encompass blood clotting (coagulation) and involve blood vessels, platelet aggregation, and plasma proteins that cause formation or dissolution of platelet aggregates.

In hemostasis, there is initial vasoconstriction of the injured vessel, causing diminished blood flow distal to the injury. Then hemostasis and thrombosis share three phases:

(1) Formation of a loose and temporary platelet aggregate at the site of injury. Platelets bind to collagen at the site of vessel wall injury and are activated by thrombin (the mechanism of activation of platelets is described below), formed in the coagulation cascade at the same site, or by ADP released from other activated platelets. Upon activation, platelets change shape and, in the presence of fibrinogen, aggregate to form the hemostatic plug (in hemostasis) or thrombus (in thrombosis).

(2) Formation of a fibrin mesh that binds to the platelet aggregate, forming a more stable hemostatic plug or thrombus.

(3) Partial or complete dissolution of the hemostatic plug or thrombus by plasmin.

There Are Three Types of Thrombi

Three types of thrombi or clots are distinguished. All three contain **fibrin** in various proportions.

(1) The **white** thrombus is composed of platelets and fibrin and is relatively poor in erythrocytes. It forms at the site of an injury or abnormal vessel wall, particularly in areas where blood flow is rapid (arteries).

(2) The **red** thrombus consists primarily of red cells and fibrin. It morphologically resembles the clot formed in a test tube and may form in vivo in areas of retarded blood flow or stasis (eg, veins) with or without vascular injury, or it may form at a site of injury or in an abnormal vessel in conjunction with an initiating platelet plug.

(3) A third type is a disseminated **fibrin deposit** in very small blood vessels or capillaries.

We shall first describe the coagulation pathway leading to the formation of fibrin. Then we shall briefly describe some aspects of the involvement of platelets and blood vessel walls in the overall process. This separation of clotting factors and platelets is artificial, since both play intimate and often mutually interdependent roles in hemostasis and thrombosis, but it facilitates description of the overall processes involved.

Both Intrinsic & Extrinsic Pathways Result in the Formation of Fibrin

Two pathways lead to fibrin clot formation: the intrinsic and the extrinsic pathways. These pathways are not independent, as previously thought. However, this artificial distinction is retained in the following text to facilitate their description.

Initiation of the fibrin clot in response to tissue injury is carried out by the extrinsic pathway. How the intrinsic pathway is activated in vivo is unclear, but it involves a negatively charged surface. The intrinsic and extrinsic pathways converge in a **final common pathway** involving the activation of prothrombin to thrombin and the thrombin-catalyzed cleavage of fibrinogen to form the fibrin clot. The intrinsic, extrinsic, and final common pathways are complex and involve many different proteins (Figure 51–1 and Table 51–1). In

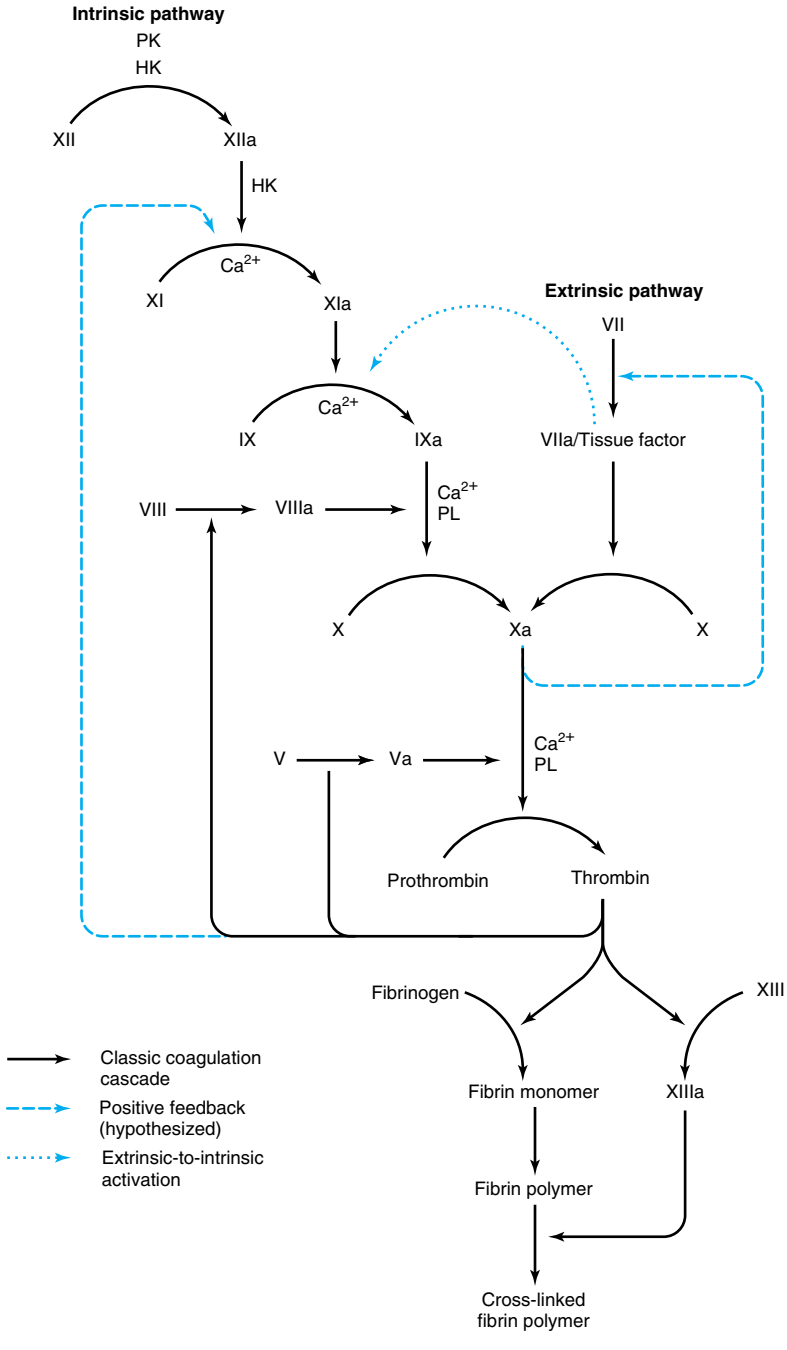


Figure 51–1. The pathways of blood coagulation. The intrinsic and extrinsic pathways are indicated. The events depicted below factor Xa are designated the final common pathway, culminating in the formation of cross-linked fibrin. New observations (dotted arrow) include the finding that complexes of tissue factor and factor VIIa activate not only factor X (in the classic extrinsic pathway) but also factor IX in the intrinsic pathway. In addition, thrombin and factor Xa feedback-activate at the two sites indicated (dashed arrows). (PK, prekallikrein; HK, HMW kininogen; PL, phospholipids.) (Reproduced, with permission, from Roberts HR, Lozier JN: New perspectives on the coagulation cascade. Hosp Pract [Off Ed] 1992 Jan;27:97.)

Table 51–1. Numerical system for nomenclature of blood clotting factors. The numbers indicate the order in which the factors have been discovered and bear no relationship to the order in which they act.

Factor	Common Name
I	Fibrinogen } These factors are usually referred
II	Prothrombin } to by their common names.
III	Tissue factor } These factors are usually not re-
IV	Ca^{2+} } ferred to as coagulation factors.
V	Proaccelerin, labile factor, accelerator (Ac-) globulin
VII[1]	Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin
VIII	Antihemophilic factor A, antihemophilic globulin (AHG)
IX	Antihemophilic factor B, Christmas factor, plasma thromboplastin component (PTC)
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin stabilizing factor (FSF), fibrinoligase

[1]There is no factor VI.

general, as shown in Table 51–2, these proteins can be classified into five types: (1) zymogens of serine-dependent proteases, which become activated during the process of coagulation; (2) cofactors; (3) fibrinogen; (4) a transglutaminase, which stabilizes the fibrin clot; and (5) regulatory and other proteins.

The Intrinsic Pathway Leads to Activation of Factor X

The intrinsic pathway (Figure 51–1) involves factors XII, XI, IX, VIII, and X as well as prekallikrein, high-molecular-weight (HMW) kininogen, Ca^{2+}, and platelet phospholipids. It results in the production of factor Xa (by convention, activated clotting factors are referred to by use of the suffix a).

This pathway commences with the "contact phase" in which prekallikrein, HMW kininogen, factor XII, and factor XI are exposed to a negatively charged activating surface. In vivo, the proteins probably assemble on endothelial cell membranes, whereas glass or kaolin can be used for in vitro tests of the intrinsic pathway. When the components of the contact phase assemble on the activating surface, factor XII is activated to factor XIIa upon proteolysis by kallikrein. This factor XIIa, generated by kallikrein, attacks prekallikrein to generate more kallikrein, setting up a reciprocal activation. Factor XIIa, once formed, activates factor XI to XIa and also releases bradykinin (a nonapeptide with potent vasodilator action) from HMW kininogen.

Table 51–2. The functions of the proteins involved in blood coagulation.

Zymogens of serine proteases	
Factor XII	Binds to negatively charged surface at site of vessel wall injury; activated by high-MW kininogen and kallikrein.
Factor XI	Activated by factor XIIa.
Factor IX	Activated by factor XIa in presence of Ca^{2+}.
Factor VII	Activated thrombin in presence of Ca^{2+}.
Factor X	Activated on surface of activated platelets by tenase complex (Ca^{2+}, factors VIIIa and IXa) and by factor VIIa in presence of tissue factor and Ca^{2+}.
Factor II	Activated on surface of activated platelets by prothrombinase complex (Ca^{2+}, factors Va and Xa).
	[Factors II, VII, IX, and X are Gla-containing zymogens.] (Gla = γ-carboxyglutamate.)
Cofactors	
Factor VIII	Activated by thrombin; factor VIIIa is a cofactor in the activation of factor X by factor IXa.
Factor V	Activated by thrombin; factor Va is a cofactor in the activation of prothrombin by factor Xa.
Tissue factor (factor III)	A glycoprotein expressed on the surface of injured or stimulated endothelial cells to act as a cofactor for factor VIIa.
Fibrinogen	
Factor I	Cleaved by thrombin to form fibrin clot.
Thiol-dependent transglutaminase	
Factor XIII	Activated by thrombin in presence of Ca^{2+}; stabilizes fibrin clot by covalent cross-linking.
Regulatory and other proteins	
Protein C	Activated to protein Ca by thrombin bound to thrombomodulin; then degrades factors VIIIa and Va.
Protein S	Acts as a cofactor of protein C; both proteins contain Gla (γ-carboxyglutamate) residues.
Thrombomodulin	Protein on the surface of endothelial cells; binds thrombin, which then activates protein C.

Factor XIa in the presence of Ca^{2+} activates factor IX (55 kDa, a zymogen containing vitamin K-dependent γ-carboxyglutamate [Gla] residues; see Chapter 45), to the serine protease, factor IXa. This in turn cleaves an Arg-Ile bond in factor X (56 kDa) to produce the two-chain serine protease, factor Xa. This latter reaction requires the assembly of components, called **the tenase**

complex, on the surface of activated platelets: Ca^{2+} and factor VIIIa, as well as factors IXa and X. It should be noted that in all reactions involving the Gla-containing zymogens (factors II, VII, IX, and X), the Gla residues in the amino terminal regions of the molecules serve as high-affinity binding sites for Ca^{2+}. For assembly of the tenase complex, the platelets must first be activated to expose the acidic (anionic) phospholipids, **phosphatidylserine** and **phosphatidylinositol,** that are normally on the internal side of the plasma membrane of resting, nonactivated platelets. Factor VIII (330 kDa), a glycoprotein, is not a protease precursor but a cofactor that serves as a receptor for factors IXa and X on the platelet surface. Factor VIII is activated by minute quantities of thrombin to form factor VIIIa, which is in turn inactivated upon further cleavage by thrombin.

The Extrinsic Pathway Also Leads to Activation of Factor X But by a Different Mechanism

Factor Xa occurs at the site where the intrinsic and extrinsic pathways converge (Figure 51–1) and lead into the final common pathway of blood coagulation. The extrinsic pathway involves tissue factor, factors VII and X, and Ca^{2+} and results in the production of factor Xa. It is initiated at the site of tissue injury with the exposure of **tissue factor** (Figure 51–1) on subendothelial cells. Tissue factor interacts with and activates factor VII (53 kDa), a circulating Gla-containing glycoprotein synthesized in the liver. Tissue factor acts as a cofactor for factor VIIa, enhancing its enzymatic activity to activate factor X. The association of tissue factor and factor VIIa is called **tissue factor complex.** Factor VIIa cleaves the same Arg-Ile bond in factor X that is cleaved by the tenase complex of the intrinsic pathway. Activation of factor X provides an important link between the intrinsic and extrinsic pathways.

Another important interaction between the extrinsic and intrinsic pathways is that complexes of tissue factor and factor VIIa also activate factor IX in the intrinsic pathway. Indeed, **the formation of complexes between tissue factor and factor VIIa is now considered to be the key process involved in initiation of blood coagulation in vivo.** The physiologic significance of the initial steps of the intrinsic pathway, in which factor XII, prekallikrein, and HMW kininogen are involved, has been called into question because patients with a hereditary deficiency of these components do not exhibit bleeding problems. Similarly, patients with a deficiency of factor XI may not have bleeding problems. The intrinsic pathway may actually be more important in fibrinolysis (see below) than in coagulation, since kallikrein, factor XIIa, and factor XIa can cleave plasminogen and kallikrein can activate single-chain urokinase.

Tissue factor pathway inhibitor (TFPI) is a major physiologic inhibitor of coagulation. It is a protein that circulates in the blood associated with lipoproteins. TFPI directly inhibits factor Xa by binding to the enzyme near its active site. This factor Xa-TFPI complex then inhibits the factor VIIa-tissue factor complex.

The Final Common Pathway of Blood Clotting Involves Activation of Prothrombin to Thrombin

In the final common pathway, factor Xa, produced by either the intrinsic or the extrinsic pathway, activates **prothrombin** (factor II) to **thrombin** (factor IIa), which then converts fibrinogen to fibrin (Figure 51–1).

The activation of prothrombin, like that of factor X, occurs on the surface of activated platelets and requires the assembly of **a prothrombinase complex,** consisting of platelet anionic phospholipids, Ca^{2+}, factor Va, factor Xa, and prothrombin.

Factor V (330 kDa), a glycoprotein with homology to factor VIII and ceruloplasmin, is synthesized in the liver, spleen, and kidney and is found in platelets as well as in plasma. It functions as a cofactor in a manner similar to that of factor VIII in the tenase complex. When activated to factor Va by traces of thrombin, it binds to specific receptors on the platelet membrane (Figure 51–2) and forms a complex with factor Xa and prothrombin. It is subsequently inactivated by further action of thrombin, thereby providing a means of limiting the activation of prothrombin to thrombin. **Prothrombin** (72 kDa; Figure 51–3) is a single-chain glycoprotein synthesized in the liver. The amino terminal region of prothrombin (1 in Figure 51–3) contains ten Gla residues, and the serine-dependent active protease site (indicated by the arrowhead) is in the carboxyl terminal region of the molecule. Upon binding to the complex of factors Va and Xa on the platelet membrane, prothrombin is cleaved by factor Xa at two sites (Figure 51–2) to generate the active, two-chain thrombin molecule, which is then released from the platelet surface. The A and B chains of thrombin are held together by a disulfide bond.

Conversion of Fibrinogen to Fibrin Is Catalyzed by Thrombin

Fibrinogen (factor I, 340 kDa; see Figures 51–1 and 51–4 and Tables 51–1 and 51–2) is a soluble plasma glycoprotein that consists of three nonidentical pairs of polypeptide chains $(A\alpha,B\beta\gamma)_2$ covalently linked by disulfide bonds. The $B\beta$ and γ chains contain asparagine-linked complex oligosaccharides. All three

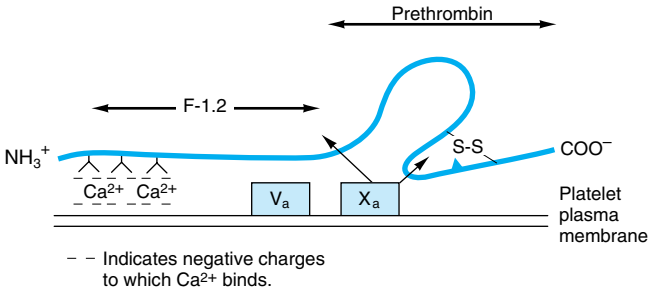


Figure 51-2. Diagrammatic representation (not to scale) of the binding of factors Va, Xa, Ca^{2+}, and prothrombin to the plasma membrane of the activated platelet. The sites of cleavage of prothrombin by factor Xa are indicated by two arrows. The part of prothrombin destined to form thrombin is labeled prethrombin. The Ca^{2+} is bound to anionic phospholipids of the plasma membrane of the activated platelet.

chains are synthesized in the liver; the three structural genes involved are on the same chromosome, and their expression is coordinately regulated in humans. The amino terminal regions of the six chains are held in close proximity by a number of disulfide bonds, while the carboxyl terminal regions are spread apart, giving rise to a highly asymmetric, elongated molecule (Figure 51–4). The A and B portions of the Aα and Bβ chains, designated **fibrinopeptides A (FPA) and B (FPB),** respectively, at the amino terminal ends of the chains, bear excess negative charges as a result of the presence of aspartate and glutamate residues, as well as an unusual tyrosine O-sulfate in FPB. These negative charges contribute to the solubility of fibrinogen in plasma and also serve to prevent aggregation by causing electrostatic repulsion between fibrinogen molecules.

Thrombin (34 kDa), a serine protease formed by the prothrombinase complex, hydrolyzes the four Arg-Gly bonds between the fibrinopeptides and the α and β portions of the Aα and Bβ chains of fibrinogen (Figure 51–5A). The release of the fibrinopeptides by thrombin generates fibrin monomer, which has the subunit structure $(\alpha, \beta, \gamma)_2$. Since FPA and FPB contain only 16 and 14 residues, respectively, the fibrin molecule retains 98% of the residues present in fibrinogen. The removal of the fibrinopeptides exposes binding sites that allow the molecules of fibrin monomers to aggregate spontaneously in a regularly staggered array, forming an insoluble fibrin clot. It is the formation of this insoluble fibrin polymer that traps platelets, red cells, and other components to form the white or red thrombi. This initial fibrin clot is rather weak, held together only by the noncovalent association of fibrin monomers.

In addition to converting fibrinogen to fibrin, thrombin also converts factor XIII to factor XIIIa. This factor is a highly specific **transglutaminase** that covalently cross-links fibrin molecules by forming peptide bonds between the amide groups of glutamine and the ε-amino groups of lysine residues (Figure 51–5B), yielding a more stable fibrin clot with increased resistance to proteolysis.

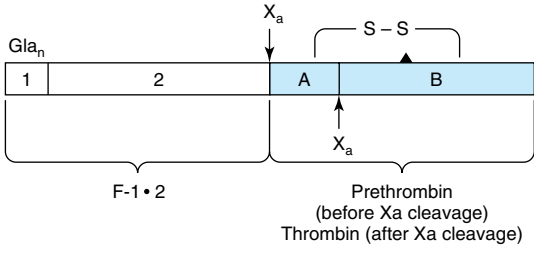


Figure 51–3. Diagrammatic representation (not to scale) of prothrombin. The amino terminal is to the left; region 1 contains all ten Gla residues. The sites of cleavage by factor Xa are shown and the products named. The site of the catalytically active serine residue is indicated by the solid triangle. The A and B chains of active thrombin (shaded) are held together by the disulfide bridge.

Levels of Circulating Thrombin Must Be Carefully Controlled or Clots May Form

Once active thrombin is formed in the course of hemostasis or thrombosis, its concentration must be carefully controlled to prevent further fibrin formation or platelet activation. This is achieved in two ways. Thrombin circulates as its inactive precursor, prothrombin, which is activated as the result of a cascade of enzymatic reactions, each converting an inactive zymogen to an active enzyme and leading finally to the conversion of prothrombin to thrombin (Figure 51–1). At each point in the cascade, **feedback mechanisms** produce a delicate balance of activation and inhibition. The concentration of factor XII in plasma is approximately 30 μg/mL, while that of fibrinogen is 3 mg/mL, with intermediate clotting factors increasing in concentration as one proceeds down the cascade, showing that the clotting cascade provides amplification. The second means of controlling thrombin activity is the inactivation of any thrombin formed by **circulating inhibi-**

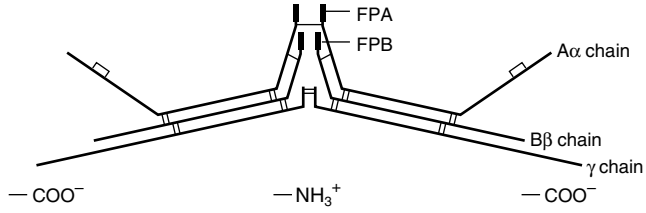


Figure 51–4. Diagrammatic representation (not to scale) of fibrinogen showing pairs of Aα, Bβ, and γ chains linked by disulfide bonds. (FPA, fibrinopeptide A; FPB, fibrinopeptide B.)

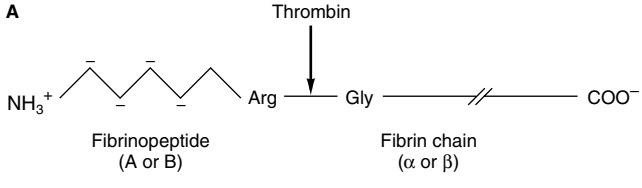
tors, the most important of which is antithrombin III (see below).

The Activity of Antithrombin III, an Inhibitor of Thrombin, Is Increased by Heparin

Four naturally occurring thrombin inhibitors exist in normal plasma. The most important is **antithrombin III** (often called simply antithrombin), which contributes approximately 75% of the antithrombin activity. Antithrombin III can also inhibit the activities of factors IXa, Xa, XIa, XIIa, and VIIa complexed with tissue factor. **α_2-Macroglobulin** contributes most of the remainder of the antithrombin activity, with **heparin cofactor II** and **α_1-antitrypsin** acting as minor inhibitors under physiologic conditions.

The endogenous activity of antithrombin III is greatly potentiated by the presence of acidic proteoglycans such as **heparin** (Chapter 48). These bind to a specific cationic site of antithrombin III, inducing a conformational change and promoting its binding to thrombin as well as to its other substrates. This is the basis for the use of heparin in clinical medicine to inhibit coagulation. The anticoagulant effects of heparin can be antagonized by strongly cationic polypeptides such as **protamine,** which bind strongly to heparin, thus inhibiting its binding to antithrombin III. Individuals with inherited deficiencies of antithrombin III are prone to develop venous thrombosis, providing evidence that antithrombin III has a physiologic function and that the coagulation system in humans is normally in a dynamic state.

Thrombin is involved in an additional regulatory mechanism that operates in coagulation. It combines with **thrombomodulin,** a glycoprotein present on the surfaces of endothelial cells. The complex activates **protein C.** In combination with **protein S,** activated protein C (APC) degrades factors Va and VIIIa, limiting their actions in coagulation. A genetic deficiency of either protein C or protein S can cause venous thrombosis. Furthermore, patients with **factor V Leiden** (which has a glutamine residue in place of an arginine at position 506) have an increased risk of venous thrombotic



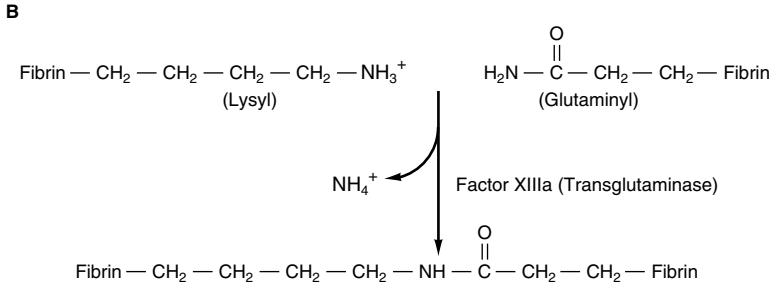


Figure 51-5. Formation of a fibrin clot. **A:** Thrombin-induced cleavage of Arg-Gly bonds of the Aα and Bβ chains of fibrinogen to produce fibrinopeptides (left-hand side) and the α and β chains of fibrin monomer (right-hand side). **B:** Cross-linking of fibrin molecules by activated factor XIII (factor XIIIa).

disease because factor V Leiden is resistant to inactivation by APC. This condition is termed APC resistance.

Coumarin Anticoagulants Inhibit the Vitamin K-Dependent Carboxylation of Factors II, VII, IX, & X

The coumarin drugs (eg, warfarin), which are used as anticoagulants, inhibit the vitamin K-dependent carboxylation of Glu to Gla residues (see Chapter 45) in the amino terminal regions of factors II, VII, IX, and X and also proteins C and S. These proteins, all of which are synthesized in the liver, are dependent on the Ca^{2+}-binding properties of the Gla residues for their normal function in the coagulation pathways. The coumarins act by inhibiting the reduction of the quinone derivatives of vitamin K to the active hydroquinone forms (Chapter 45). Thus, the administration of vitamin K will bypass the coumarin-induced inhibition and allow maturation of the Gla-containing factors. Reversal of coumarin inhibition by vitamin K requires 12–24 hours, whereas reversal of the anticoagulant effects of heparin by protamine is almost instantaneous.

Heparin and warfarin are widely used in the treatment of thrombotic and thromboembolic conditions, such as deep vein thrombosis and pulmonary embolus. Heparin is administered first, because of its prompt onset of action, whereas warfarin takes several days to reach full effect. Their effects are closely monitored by use of appropriate tests of coagulation (see below) because of the risk of producing hemorrhage.

Hemophilia A Is Due to a Genetically Determined Deficiency of Factor VIII

Inherited deficiencies of the clotting system that result in bleeding are found in humans. The most common is deficiency of factor VIII, causing **hemophilia A,** an X chromosome-linked disease that has played a major role in the history of the royal families of Europe. **Hemophilia B** is due to a deficiency of factor IX; its clinical features are almost identical to those of hemophilia A, but the conditions can be separated on the basis of specific assays that distinguish between the two factors.

The gene for human factor VIII has been cloned and is one of the largest so far studied, measuring 186 kb in length and containing 26 exons. A variety of mutations have been detected leading to diminished activity of factor VIII; these include partial gene deletions and point mutations resulting in premature chain termination. Prenatal diagnosis by DNA analysis after chorionic villus sampling is now possible.

In past years, treatment for patients with hemophilia A has consisted of administration of cryoprecipitates (enriched in factor VIII) prepared from individual donors or lyophilized factor VIII concentrates prepared from plasma pools of up to 5000 donors. It is now possible to prepare factor VIII by **recombinant DNA technology.** Such preparations are free of contaminating viruses (eg, hepatitis A, B, C, or HIV-1) found in human plasma but are at present expensive; their use may increase if cost of production decreases.

Fibrin Clots Are Dissolved by Plasmin

As stated above, the coagulation system is normally in a state of dynamic equilibrium in which fibrin clots are constantly being laid down and dissolved. This latter process is termed **fibrinolysis. Plasmin,** the serine protease mainly responsible for degrading fibrin and fibrinogen, circulates in the form of its inactive zymogen, **plasminogen** (90 kDa), and any small amounts of plasmin that are formed in the fluid phase under physiologic conditions are rapidly inactivated by the fast-acting plasmin inhibitor, α_2-antiplasmin. Plasminogen binds to fibrin and thus becomes incorporated in clots as they are produced; since plasmin that is formed when bound to fibrin is protected from α_2-antiplasmin, it remains active. **Activators of plasminogen** of various types are found in most body tissues, and all cleave the same Arg-Val bond in plasminogen to produce the two-chain serine protease, plasmin (Figure 51–6).

Tissue plasminogen activator (alteplase; t-PA) is a serine protease that is released into the circulation from vascular endothelium under conditions of injury or

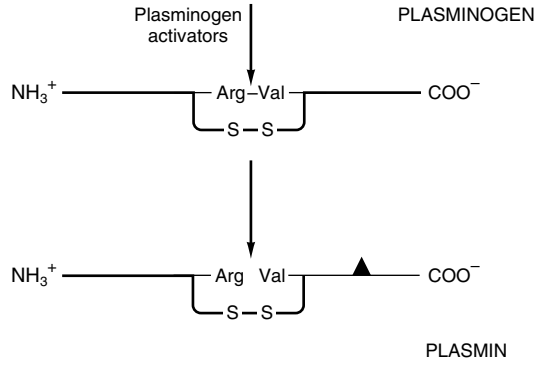


Figure 51–6. Activation of plasminogen. The same Arg-Val bond is cleaved by all plasminogen activators to give the two-chain plasmin molecule. The solid triangle indicates the serine residue of the active site. The two chains of plasmin are held together by a disulfide bridge.

stress and is catalytically inactive unless bound to fibrin. Upon binding to fibrin, t-PA cleaves plasminogen within the clot to generate plasmin, which in turn digests the fibrin to form soluble degradation products and thus dissolves the clot. Neither plasmin nor the plasminogen activator can remain bound to these degradation products, and so they are released into the fluid phase, where they are inactivated by their natural inhibitors. Prourokinase is the precursor of a second activator of plasminogen, **urokinase.** Originally isolated from urine, it is now known to be synthesized by cell types such as monocytes and macrophages, fibroblasts, and epithelial cells. Its main action is probably in the degradation of extracellular matrix. Figure 51–7 indicates the sites of action of five proteins that influence the formation and action of plasmin.

Recombinant t-PA & Streptokinase Are Used as Clot Busters

Alteplase (t-PA), produced by recombinant DNA technology, is used therapeutically as a fibrinolytic agent, as is **streptokinase.** However, the latter is less selective than t-PA, activating plasminogen in the fluid phase (where it can degrade circulating fibrinogen) as well as plasminogen that is bound to a fibrin clot. The amount of plasmin produced by therapeutic doses of streptokinase may exceed the capacity of the circulating α_2-antiplasmin, causing fibrinogen as well as fibrin to be degraded and resulting in the bleeding often encountered during fibrinolytic therapy. Because of its **selectivity** for degrading fibrin, there is considerable therapeutic interest in the use of recombinant t-PA to restore the patency of coronary arteries following thrombosis. If administered early enough, before irreversible damage of heart muscle occurs (about 6 hours after onset of thrombosis), t-PA can significantly reduce the mortality rate from myocardial damage following coronary thrombosis. t-PA is more effective than streptokinase at restoring full patency and also appears to result in a slightly better survival rate. Table 51–3 compares some thrombolytic features of streptokinase and t-PA.

There are a number of disorders, including cancer and shock, in which **the concentrations of plasminogen activators increase.** In addition, the antiplasmin activities contributed by α_1-antitrypsin and α_2-antiplasmin may be impaired in diseases such as cirrhosis. Since certain bacterial products, such as streptokinase, are capable of activating plasminogen, they may be responsible for the diffuse hemorrhage sometimes observed in patients with disseminated bacterial infections.

Activation of Platelets Involves Stimulation of the Polyphosphoinositide Pathway

Platelets normally circulate in an unstimulated disk-shaped form. During hemostasis or thrombosis, they become activated and help form hemostatic plugs or thrombi. Three major steps are involved: (1) adhesion to exposed collagen in blood vessels, (2) release of the contents of their granules, and (3) aggregation.

Platelets adhere to collagen via specific receptors on the platelet surface, including the glycoprotein complex GPIa–IIa ($\alpha_2\beta_1$ integrin; Chapter 52), in a reaction that involves **von Willebrand factor.** This is a glycoprotein, secreted by endothelial cells into the plasma, which stabilizes factor VIII and binds to collagen and the subendothelium. Platelets bind to von Willebrand factor via a glycoprotein complex (GPIb–V–IX) on the platelet surface; this interaction is especially important in platelet adherence to the subendothelium under conditions of high shear stress that occur in small vessels and stenosed arteries.

Platelets adherent to collagen change shape and spread out on the subendothelium. They release the contents of their storage granules (the dense granules and the alpha granules); secretion is also stimulated by thrombin.

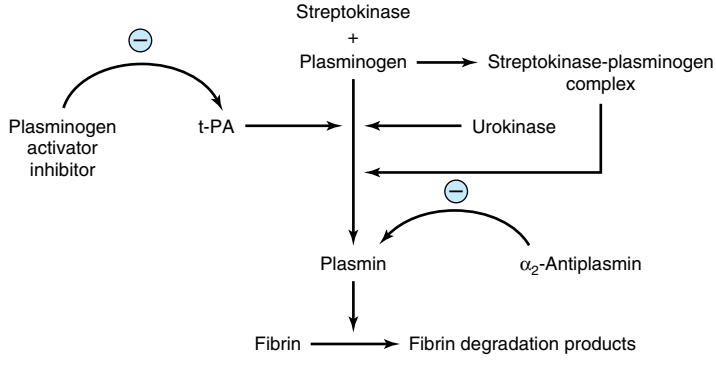


Figure 51–7. Scheme of sites of action of streptokinase, tissue plasminogen activator (t-PA), urokinase, plasminogen activator inhibitor, and α_2-antiplasmin (the last two proteins exert inhibitory actions). Streptokinase forms a complex with plasminogen, which exhibits proteolytic activity; this cleaves some plasminogen to plasmin, initiating fibrinolysis.

Table 51–3. Comparison of some properties of streptokinase (SK) and tissue plasminogen activator (t-PA) with regard to their use as thrombolytic agents.[1]

	SK	t-PA
Selective for fibrin clot	–	+
Produces plasminemia	+	–
Reduces mortality	+	+
Causes allergic reaction	+	–
Causes hypotension	+	–
Cost per treatment (approximate)	Relatively low	Relatively high

[1]Data from Webb J, Thompson C: Thrombolysis for acute myocardial infarction. Can Fam Physician 1992;38:1415.

Thrombin, formed from the coagulation cascade, is the most potent activator of platelets and initiates platelet activation by interacting with its receptor on the plasma membrane (Figure 51–8). The further events leading to platelet activation are examples of **transmembrane signaling,** in which a chemical messenger outside the cell generates effector molecules inside the cell. In this instance, thrombin acts as the external chemical messenger (stimulus or agonist). The interaction of thrombin with its receptor stimulates the activity of an intracellular **phospholipase Cβ.** This enzyme hydrolyzes the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2, a polyphosphoinositide) to form the two internal effector molecules, 1,2-diacylglycerol and 1,4,5-inositol trisphosphate.

Hydrolysis of PIP_2 is also involved in the action of many hormones and drugs. Diacylglycerol stimulates

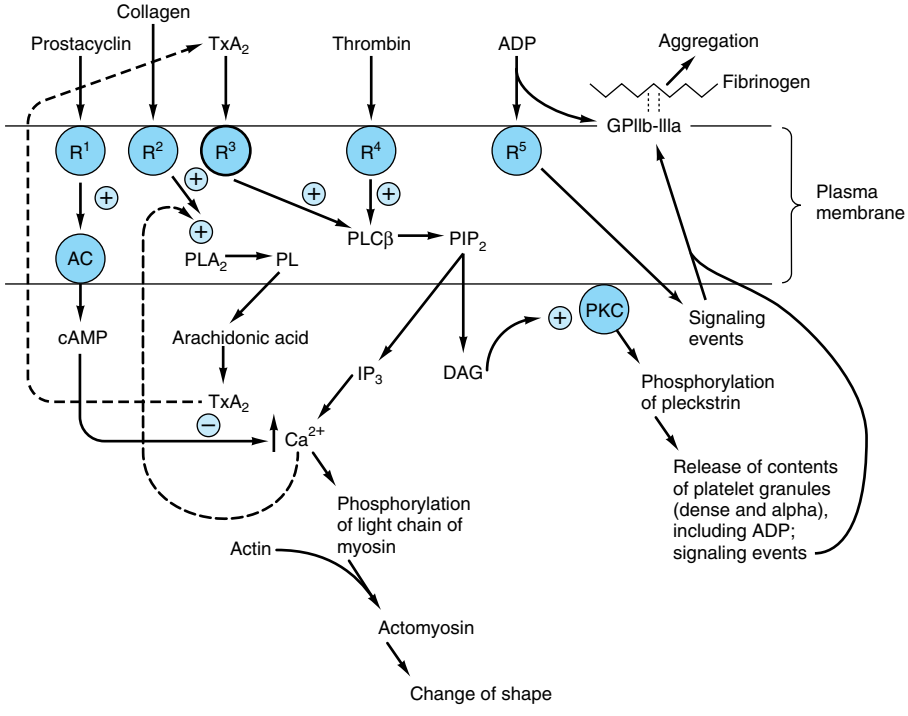


Figure 51–8. Diagrammatic representation of platelet activation. The external environment, the plasma membrane, and the inside of a platelet are depicted from top to bottom. Thrombin and collagen are the two most important platelet activators. ADP is considered a weak agonist; it causes aggregation but not granule release. (GP, glycoprotein; R^1–R^5, various receptors; AC, adenylyl cyclase; PLA_2, phospholipase A_2; PL, phospholipids; PLCβ, phospholipase Cβ; PIP_2, phosphatidylinositol 4,5-bisphosphate; cAMP, cyclic AMP; PKC, protein kinase C; TxA_2, thromboxane A_2; IP_3, inositol 1,4,5-trisphosphate; DAG, 1,2-diacylglycerol. The G proteins that are involved are not shown.)

protein kinase C, which phosphorylates the protein **pleckstrin** (47 kDa). This results in aggregation and release of the contents of the storage granules. ADP released from dense granules can also activate platelets, resulting in aggregation of additional platelets. IP_3 causes release of Ca^{2+} into the cytosol mainly from the dense tubular system (or residual smooth endoplasmic reticulum from the megakaryocyte), which then interacts with calmodulin and myosin light chain kinase, leading to phosphorylation of the light chains of myosin. These chains then interact with actin, causing changes of platelet shape.

Collagen-induced activation of a platelet phospholipase A_2 by increased levels of cytosolic Ca^{2+} results in liberation of arachidonic acid from platelet phospholipids, leading to the formation of **thromboxane A_2** (Chapter 23), which in turn, in a receptor-mediated fashion, can further activate phospholipase C, promoting platelet aggregation.

Activated platelets, besides forming a platelet aggregate, are required, via newly expressed anionic phospholipids on the membrane surface, for acceleration of the activation of factors X and II in the coagulation cascade (Figure 51–1).

All of the aggregating agents, including thrombin, collagen, ADP, and others such as platelet-activating factor, modify the platelet surface so that fibrinogen can bind to a glycoprotein complex, **GPIIb–IIIa** ($\alpha_{IIb}\beta_3$ integrin; Chapter 52), on the activated platelet surface. Molecules of divalent fibrinogen then link adjacent activated platelets to each other, forming a platelet aggregate. Some agents, including epinephrine, serotonin, and vasopressin, exert synergistic effects with other aggregating agents.

Endothelial Cells Synthesize Prostacyclin & Other Compounds That Affect Clotting & Thrombosis

The endothelial cells in the walls of blood vessels make important contributions to the overall regulation of hemostasis and thrombosis. As described in Chapter 23, these cells synthesize **prostacyclin** (PGI_2), a potent inhibitor of platelet aggregation, opposing the action of thromboxane A_2. Prostacyclin acts by stimulating the activity of adenylyl cyclase in the surface membranes of platelets. The resulting increase of intraplatelet cAMP opposes the increase in the level of intracellular Ca^{2+} produced by IP_3 and thus inhibits platelet activation (Figure 51–8). Endothelial cells play other roles in the regulation of thrombosis. For instance, these cells possess an ADPase, which hydrolyzes ADP, and thus opposes its aggregating effect on platelets. In addition, these cells appear to synthesize heparan sulfate, an anticoagulant, and they also synthesize plasminogen activators, which may help dissolve thrombi. Table 51–4 lists some molecules produced by endothelial cells that affect thrombosis and fibrinolysis. Endothelium-derived relaxing factor (nitric oxide) is discussed in Chapter 49.

Analysis of the mechanisms of uptake of atherogenic lipoproteins, such as LDL, by endothelial, smooth muscle, and monocytic cells of arteries, along with detailed studies of how these lipoproteins damage such cells is a key area of study in elucidating the mechanisms of **atherosclerosis** (Chapter 26).

Aspirin Is an Effective Antiplatelet Drug

Certain drugs (antiplatelet drugs) modify the behavior of platelets. The most important is aspirin (acetylsalicylic acid), which irreversibly acetylates and thus inhibits the platelet cyclooxygenase system involved in formation of thromboxane A_2 (Chapter 14), a potent aggregator of platelets and also a vasoconstrictor. Platelets are very sensitive to aspirin; as little as 30 mg/d (one aspirin tablet usually contains 325 mg) effectively eliminates the synthesis of thromboxane A_2. Aspirin also inhibits production of prostacyclin (PGI_2, which opposes platelet aggregation and is a vasodilator) by en-

Table 51–4. Molecules synthesized by endothelial cells that play a role in the regulation of thrombosis and fibrinolysis.[1]

Molecule	Action
ADPase (an ectoenzyme)	Degrades ADP (an aggregating agent of platelets) to AMP + P_i
Endothelium-derived relaxing factor (nitric oxide)	Inhibits platelet adhesion and aggregation by elevating levels of cGMP
Heparan sulfate (a glycosaminoglycan)	Anticoagulant; combines with antithrombin III to inhibit thrombin
Prostacyclin (PGI_2, a prostaglandin)	Inhibits platelet aggregation by increasing levels of cAMP
Thrombomodulin (a glycoprotein)	Binds protein C, which is then cleaved by thrombin to yield activated protein C; this in combination with protein S degrades factors Va and VIIIa, limiting their actions
Tissue plasminogen activator (t-PA, a protease)	Activates plasminogen to plasmin, which digests fibrin; the action of t-PA is opposed by plasminogen activator inhibitor-1 (PAI-1)

[1]Adapted from Wu KK: Endothelial cells in hemostasis, thrombosis and inflammation. Hosp Pract (Off Ed) 1992 Apr; 27:145.

dothelial cells, but unlike platelets, these cells regenerate cyclooxygenase within a few hours. Thus, the overall balance between thromboxane A_2 and prostacyclin can be shifted in favor of the latter, opposing platelet aggregation. Indications for treatment with aspirin thus include management of angina and evolving myocardial infarction and also prevention of stroke and death in patients with transient cerebral ischemic attacks.

Laboratory Tests Measure Coagulation & Thrombolysis

A number of laboratory tests are available to measure the phases of hemostasis described above. The tests include platelet count, bleeding time, activated partial thromboplastin time (aPTT or PTT), prothrombin time (PT), thrombin time (TT), concentration of fibrinogen, fibrin clot stability, and measurement of fibrin degradation products. The platelet count quantitates the number of platelets, and the bleeding time is an overall test of platelet function. aPTT is a measure of the intrinsic pathway and PT of the extrinsic pathway. PT is used to measure the effectiveness of oral anticoagulants such as warfarin, and aPTT is used to monitor heparin therapy. The reader is referred to a textbook of hematology for a discussion of these tests.

SUMMARY

- Hemostasis and thrombosis are complex processes involving coagulation factors, platelets, and blood vessels.
- Many coagulation factors are zymogens of serine proteases, becoming activated during the overall process.
- Both intrinsic and extrinsic pathways of coagulation exist, the latter initiated by tissue factor. The pathways converge at factor Xa, embarking on the common final pathway resulting in thrombin-catalyzed conversion of fibrinogen to fibrin, which is strengthened by cross-linking, catalyzed by factor XIII.
- Genetic disorders of coagulation factors occur, and the two most common involve factors VIII (hemophilia A) and IX (hemophilia B).
- An important natural inhibitor of coagulation is antithrombin III; genetic deficiency of this protein can result in thrombosis.
- For activity, factors II, VII, IX, and X and proteins C and S require vitamin K-dependent γ-carboxylation of certain glutamate residues, a process that is inhibited by the anticoagulant warfarin.
- Fibrin is dissolved by plasmin. Plasmin exists as an inactive precursor, plasminogen, which can be activated by tissue plasminogen activator (t-PA). Both t-PA and streptokinase are widely used to treat early thrombosis in the coronary arteries.
- Thrombin and other agents cause platelet aggregation, which involves a variety of biochemical and morphologic events. Stimulation of phospholipase C and the polyphosphoinositide pathway is a key event in platelet activation, but other processes are also involved.
- Aspirin is an important antiplatelet drug that acts by inhibiting production of thromboxane A_2.

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