Proteases in blood clotting

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Abstract

The serine proteases, cofactors and cell-receptor molecules that comprise the haemostatic mechanism are highly conserved modular proteins that have evolved to participate in biochemical reactions in blood coagulation, anticoagulation and fibrinolysis. Blood coagulation is initiated by exposure of tissue factor, which forms a complex with factor VIIa and factor X, which results in the generation of small quantities of thrombin and is rapidly shutdown by the tissue factor pathway inhibitor. The generation of these small quantities of thrombin then activates factor XI, resulting in a sequence of events that lead to the activation of factor IX, factor X and prothrombin. Sufficient thrombin is generated to effect normal haemostasis by converting fibrinogen into fibrin. The anticoagulant pathways that regulate blood coagulation include the protein C anticoagulant mechanism, the serine protease inhibitors in plasma, and the Kunitz-like inhibitors, tissue factor pathway inhibitor and protease nexin 2. Finally, the fibrinolytic mechanism

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that comprises the activation of plasminogen into plasmin prevents excessive fibrin accumulation by promoting local dissolution of thrombi and promoting wound healing by reestablishment of blood flow.

**Introduction**

Virtually all complex multicellular organisms with highly developed cardiovascular systems have evolved tightly regulated mechanisms of haemostasis, which is defined as the maintenance of the fluidity of circulating blood while at the same time protecting the organism from life-threatening bleeding at sites of vascular injury, particularly in the high-pressure, high-flow vascular systems that are characteristic of mammals. This cell-mediated system of proteolysis of plasma coagulation proteins is essential to the survival of the organism as evidenced by the severe bleeding complications [1,2] that can arise in patients with haemophilia (e.g. resulting from a deficiency in clotting factors VIII or IX). However, pathological consequences can also arise from a deficiency in or abnormal regulation of anticoagulant proteases and their cofactors (e.g. deficiency in protein C, protein S, antithrombin III or factor V-Leiden), resulting in arterial or venous thrombosis [3,4].

The haemostatic mechanism has evolved as a complex system in which serine proteases with highly developed specificity assemble on cell membrane receptors in complex with cofactor molecules. They then propagate a highly regulated series of enzymic reactions that lead to the formation of a haemostatic thrombus that arrests bleeding. Haemostasis comprises a closely interwoven network of reactions in response to vascular injury. It can, perhaps artificially, be divided into the processes of platelet plug formation, blood coagulation, anticoagulation and fibrinolysis. Primary haemostasis or platelet plug formation comprises a series of events in which platelets initially adhere to subendothelial components that are exposed upon vascular injury; they then aggregate to form a haemostatic thrombus. Fibrinolysis comprises the system of proteases and activators that dissolve fibrin clots that are formed when thrombin is generated as a consequence of the blood coagulation mechanisms (Figure 1). The processes of blood coagulation, anticoagulation and fibrinolysis are all regulated by naturally occurring inhibitory molecules including serine protease inhibitors (or SERPINs) and Kunitz-type protease inhibitors (or kunins). Accumulating evidence supports the hypothesis [5] that blood coagulation is a cell surface receptor-mediated process involving highly specialized anucleate cell fragments (i.e. platelets) whose sole function is surveillance of the vascular system for breaches of its integrity. At sites of vascular injury, platelets adhere to exposed subendothelial components, become activated, aggregate to form haemostatic thrombi and expose receptors that promote the assembly of blood coagulation complexes, which leads to a massive generation of fibrin and arrest of haemorrhage [6,7], as shown schematically in Figure 1. The purpose of this article is to review the recent progress and future perspectives on the structure, function and phys-
Figure 1. The postulated sequence of blood coagulation reactions

The tissue factor–factor VIIa complex is assembled on tissue-factor-bearing cells. The remainder of the complexes are assembled on the activated platelet membrane. The Roman numerals represent coagulation proteins in the zymogen or cofactor form, with the ‘a’ representing the active enzyme or cofactor. Circles represent zymogens; circles with sectors removed represent active enzymes; ellipses represent cofactors; and rectangles represent Kunitz-type inhibitors. The arrows represent conversions from zymogens to active enzymes. Fibrin is covalently crosslinked by the transglutaminase, factor XIIIa. The black arrows indicate reactions that are less physiologically relevant or secondary pathways (e.g. the secondary role of the contact proteins in and the contribution of tissue factor–factor VIIa to factor IX activation). II, prothrombin; IIa, thrombin; TF, tissue factor. See text for detailed discussion. Adapted from Walsh, P.N. (2001) Role of platelets and factor XI in the initiation of blood coagulation by thrombin. Thromb. Haemostasis 86, 75–82, with permission from Schattauer GmbH.
iological roles of procoagulant, anticoagulant and fibrinolytic proteases, their regulation and their roles in the haemostatic mechanism.

Pathways and proteases in haemostasis

Numerous serine proteases, cofactor molecules and cell surface receptors have been identified whose biological functions are essential for the initiation and propagation of procoagulant, anticoagulant and fibrinolytic responses to vascular injury. These are essential both for maintaining the fluidity of circulating blood and for effecting normal haemostasis. The biochemical properties and concentrations in plasma of these proteins are summarized in Table 1.

Table 1. Properties of proteases and cofactors in blood coagulation and fibrinolysis

Pan modular refers to proteins containing a domain homologous with the Apple domains of factor XI and prekallikrein [17]. Gla-containing refers to the vitamin K-dependent family of proteins that contain the post-translationally generated amino acid, \(\gamma\)-carboxyglutamic acid. ND, not determined.

<table>
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Table 1 and the domain structures are presented in schematic form in Figures 2 and 3. The serine proteases involved in coagulation, anticoagulation and fibrinolysis have all evolved from a common trypsin-like ancestral protease [8,9], and are characterized by the presence of highly conserved catalytic residues (Ser195, His57 and Asp102; chymotrypsin numbering system) that are referred to as the catalytic triad, as well as modular protein domains that are highly conserved across different proteases.

Figure 2. Schematic representation of the nascent forms of proteases involved in blood coagulation and fibrinolysis

Proteins are grouped as pan modular [PK and factor XI (FXI)], Gla-containing [factor IX (FIX), factor X (FX), protein C (PC), protein Z (PZ), protein S (PS) and prothrombin (FII)] and as others [factor XII (FXII), plasminogen, t-PA and urokinase-type plasminogen activator (uPA)]. Pan modular refers to proteins containing domains homologous with the Apple domains of factor XI and prekallikrein [14]. Gla-containing refers to the vitamin-K-dependent family of proteins that contain the post-translationally generated amino acid γ-carboxyglutamic acid. EGF, epidermal growth factor.
conserved three-dimensional structures serving similar functions in distinct, evolutionarily related protein families (Figure 2). These include the γ-carboxyglutamic acid domains that contain vitamin-K-dependent, post-translationally modified glutamic acid residues that bind calcium, thereby facilitating interactions of these proteins with biological membranes. Other protein modules (e.g. epidermal growth factor domains, Apple domains and Kringle domains) have highly conserved tertiary structures, but have evolved to promote specific protein–protein interactions that involve various proteases (Figure 2) and cofactor molecules (Figure 3). The cofactor and cell receptor molecules that participate in the assembly of enzymic complexes and enhance their catalytic efficiency are also members of evolutionarily related families that are assembled from protein modules [8] as shown in Figure 3.

Theories concerning the sequence of biochemical events comprising the haemostatic response have evolved over time as knowledge concerning the identification and functional properties of the various proteases, cofactors, cell receptor molecules and regulatory proteins has increased steadily. The first modern comprehensive theories of blood coagulation [10,11] emphasized the biological amplification that is inherent in the sequential activation of coagulation proteins, each of which converts its substrate into an active serine protease that then serves as the enzyme in the next reaction in the sequence; this results ultimately in the local explosive generation of fibrin from fibrinogen at the site of vascular injury. These and subsequent theories emphasized the so-called extrinsic and intrinsic pathways of blood coagulation as separate, distinct, and
alternative mechanisms. Recent revisions to these concepts emphasized not only an integrated network of enzymic reactions [12,13], but also the fact that virtually all the enzymic reactions of blood coagulation, anticoagulation and fibrinolysis occur on cell surface membrane receptors that co-localize enzymes, cofactors and substrates in a kinetically favourable complex, the assembly of which requires initial cellular activation [5,7,14]. The following discussion considers these reaction pathways in the order in which they are thought to occur on the biological membrane regarded as the physiological locus of haemostatic reactions (i.e. the activated platelet), as opposed to the vascular endothelium, which not only contains the blood, but also maintains it in a fluid state by exerting its anticoagulant properties. These concepts are presented in schematic form in Figure 1 as the postulated sequence on cell membranes of enzymic reactions that constitute blood coagulation.

Initiation of blood coagulation
The early theories of blood coagulation regarded the intrinsic and extrinsic pathways of factor X activation as comprising alternative mechanisms for thrombin generation and the conversion of fibrinogen into fibrin [10,11]. The intrinsic pathway was regarded as being triggered by the reciprocal proteolytic activation of the so-called contact factors [15], factor XII, high molecular weight kininogen (HK) and prekallikrein (PK) on negatively charged surfaces, which leads to the conversion of inactive factor XI into active factor XIa and the subsequent sequential activation of factor IX, factor X and prothrombin. Alternatively, exposure of tissue factor at sites of vascular injury leads to the formation of a complex with factor VIIa [16], which activates factor X, which then converts prothrombin into active thrombin in the presence of factor V and a suitable surface with exposed negatively charged aminophospholipids, in particular, platelets. These concepts have been re-examined in the light of evidence that deficiencies of the so-called contact proteins, factor XII, HK, and PK, are virtually never associated with haemostatic deficiency or for that matter any abnormal phenotype [15]. Therefore, the physiological role of the contact phase in the initiation of blood coagulation has been challenged, and this has resulted in a search for alternative mechanisms to explain the initiation of blood coagulation. In contrast, incontrovertible evidence of the importance of the intrinsic pathway for maintenance of normal haemostasis is the severe bleeding phenotype [2] observed in patients with haemophilia A (factor VIII deficiency) and haemophilia B (factor IX deficiency) as well as the milder but still clinically significant bleeding manifestations of haemophilia C (factor XI deficiency). The facts that human factor VII deficiency is associated with a rather mild bleeding tendency and that murine knockouts of the genes for factor VII and for tissue factor are associated with severe bleeding complications has focused attention on the tissue factor/factor VII pathway as the most physiologically relevant initiating mechanism in blood coagulation [16].
**Tissue factor (initiation) pathway**

Tissue factor is an integral membrane protein that is normally present on the surface of certain cell types that are mainly localized outside the vasculature, whereas the soluble proteins that comprise the blood coagulation cascade are found in the blood. Vascular injury results in the exposure of tissue factor, which then binds factor VII and factor VIIa, the enzymically active form that arises after cleavage of a single peptide bond [16]. Recent evidence supports the view that circulating blood cells and endothelial cells, which do not normally express tissue factor constitutively, can acquire tissue factor activity within their membranes after interaction with subendothelial tissues and circulating microparticles that do express tissue factor [17]. These observations have given rise to the novel hypothesis that blood cells, and platelets in particular, can acquire tissue factor antigen and activity and can thereby promote the activation of factor X via the tissue factor pathway. Factor VIIa has extremely weak serine protease activity until it forms a complex with tissue factor. This complex can then activate both factor IX and factor X, with factor X being the preferred substrate for tissue factor–factor VIIa under most in vitro and probably in vivo conditions [16]. The initial generation of factor Xa molecules by the factor VIIa–tissue factor complex appears to be extremely short-lived because of the presence in plasma of a natural inhibitor, tissue factor pathway inhibitor (TFPI), a tightly binding, reversible, kunitz that contains three Kunitz domains, two of which have binding sites for factor Xa, tissue factor and factor VIIa. Thus, as soon as the first molecules of factor Xa are formed, the tissue factor pathway of initiation of blood coagulation is rapidly and effectively shut down by TFPI. The factor Xa that is initially formed can also activate prothrombin in the presence of activated platelets and factor Va, but the thrombin formed by this pathway is insufficient to effect normal haemostasis.

**Consolidation pathway**

Coagulation factor XI is a unique homodimeric precursor of the active serine protease, factor XIta, which is apparently the first coagulation protein within the consolidation pathway that is required for normal haemostasis, as evidenced by the bleeding complications that arise in patients with factor XI deficiency [14]. Factor XI can be activated proteolytically by three distinct serine proteases [12,13,18] (thrombin, factor XIIa, and factor XIa), all of which cleave factor XI at the Arg369–Ile370 peptide bond to activate the catalytic domain, which then recognizes coagulation factor IX as its normal macromolecular substrate. A negatively charged surface is required for optimal rates of factor XI activation by thrombin, factor XIIa or factor XIa. The physiologically relevant cellular membrane that binds to factor XI and co-localizes it with its cognate enzymes is the surface membrane of activated platelets, where the preferred enzyme for factor XI activation appears to be thrombin [19]. Extremely low concentrations (10–100 pM) of thrombin can activate factor XI. Although factor XI binds to thrombin-activated platelets in
the presence of HK and zinc ions, where it can be activated by factor XIIa, the absence of haemostatic defects in patients with deficiencies in factor XII and HK cast some doubt upon the physiological relevance of factor XIIa-catalysed activation of factor XI, as emphasized in Figure 1. Alternatively, prothrombin can substitute for HK and calcium ions can substitute for zinc ions in the binding of factor XI to thrombin-activated platelets, and thrombin can substitute for factor XIIa to promote rapid and complete factor XI activation at low concentrations of thrombin [14, 18, 19]. This suggests that the physiologically relevant pathway for factor XI activation is the generation of thrombin by the tissue factor pathway, as discussed above and as shown schematically in Figure 1.

The factor XIa generated from surface-bound factor XI by thrombin or by factor XIIa can then bind with high affinity and specificity to platelet membrane receptors that co-localize it with its normal macromolecular substrate, factor IX, which also binds to specific high-affinity sites on activated platelets and leads to the rapid and efficient generation of factor IXa [14]. Alternatively, factor XIa activity can be regulated by a variety of inhibitors, including SERPINs that are present in plasma [e.g. antithrombin III, α-1-protease inhibitor, C1 inhibitor, α-2-antiplasmin, plasminogen activator inhibitor 1 (PAI-1) and protein C inhibitor] or, alternatively, by the kunin, protease nexin 2 (PN-2), a truncated form of the transmembrane Alzheimer’s disease amyloid β-protein precursor, which contains a Kunitz-type serine protease inhibitor domain [14] (see Chapter 4). PN-2 is found in very low concentrations in plasma, but is secreted from platelet α-granules so that the physiological concentration of PN-2 may be brought to 3–5 nM, i.e. well above the $K_i$ for inhibition of factor XIa (300–500 pM). Since platelet-bound factor XIa appears to be protected from inactivation by PN-2, the secretion of PN-2 by platelets at sites of vascular injury may regulate solution phase factor XIa while protecting the platelet-bound protease from inactivation, thereby localizing subsequent events in coagulation to the haemostatic thrombus [20].

The next event in the sequence of enzymic reactions that comprises the blood coagulation cascade is the assembly of the factor-X-activating complex on the surface of activated platelets [6, 7]. The assembly of this enzymic complex serves as a paradigm for a number of other protease–cofactor–substrate complexes that are assembled on cell membranes within the coagulation, anticoagulation and fibrinolytic pathways. The factor-X-activating complex consists of a vitamin-K-dependent serine protease (factor IXa), a cofactor molecule (factor VIIIa), and a vitamin-K-dependent substrate molecule (factor X), each of which binds to a specific, high-affinity, platelet membrane receptor. The functional consequence of this complex assembly is an enormous ($> 2 \times 10^8$-fold) increase in catalytic efficiency ($k_{cat}/K_m$). Rate enhancements of factor X activation are directly correlated with receptor occupancy by all three components of the factor-X-activating complex, including that of a high-capacity, low-affinity shared factor X/prothrombin binding site [6, 7]. Simultaneous binding and kinetic
studies demonstrate co-ordinated interactions between the enzyme, the cofactor, and the substrate in the formation of the factor-X-activating complex.

**Common coagulation pathway**

After the generation of factor Xa, the activated platelet surface promotes the assembly of the prothrombin-activating complex, which consists of the enzyme (factor Xa), the cofactor (factor Va), and the substrate (prothrombin). This is associated with a 200000-fold increase in the rate of prothrombin activation compared with the reaction in solution in the absence of the platelet surface and factor Va [7]. In contrast with the initial burst in the generation of factor Xa and thrombin via the tissue factor pathway, which is rapidly shut down by the action of TFPI, the second burst via the consolidation pathway is now sufficiently large to cleave the major plasma substrate for thrombin, i.e. fibrinogen. The cleavage of fibrinogen by thrombin generates a fibrin monomer which is then cross-linked by the action of factor XIIIa to form the insoluble and well-organized fibrin meshwork that forms the matrix of a blood clot [21,22], as shown in Figures 1 and 4.

**Regulatory and anticoagulant pathways in blood coagulation**

Evidence continues to accumulate to support the elegance and complexity of design of the haemostatic mechanism, which has the dual function of maintaining the blood in a fluid state while simultaneously being capable of responding instantaneously to breaches in the vascular integrity that require controlled clot formation without propagating disseminated intravascular coagulation. This tight control is mediated not only by signal transduction mechanisms that lead to cell activation and exposure of receptors for coagulation proteins, but also by the complex systems of regulation that constitute the anticoagulant and inhibitor mechanisms of haemostasis.

**Protein C anticoagulant mechanism**

An important natural anticoagulant mechanism [3] that limits the normal haemostatic and procoagulant response to vascular injury involves the association of the serine protease thrombin in complex with the endothelial cell surface receptor, thrombomodulin, with a substrate, protein C, which is bound to the endothelial cell protein C receptor. As shown in Figure 4, this complex assembly converts thrombin from a procoagulant molecule into an anticoagulant molecule that generates activated protein C. Activated protein C then binds to cell surfaces and platelet membranes in the presence of another cofactor molecule, protein S, with the formation of another cell surface–protease–cofactor complex whose major function is to cleave and inactivate factor Va (and also factor VIIIa) to their inactive forms, factor V_i (and factor VIII_i). The consequence of proteolysis of factor Va (and factor VIIIa) to their inactive forms is a rapid and effective curtailment of further thrombin
Figure 4. Schematic representation of the anticoagulant and fibrinolytic pathways of blood coagulation

The fibrinolytic system is shown at the top and the protein C anticoagulant system is shown below within the lumen of the blood vessel lined by endothelial cells. Thrombin (IIa) is generated by the assembly of the prothrombinase complex (thrombin, factor Xa and factor Va) on the platelet membrane in the presence of Ca\(^{2+}\) which then binds to thrombomodulin (TM) on the endothelial cell membrane. Protein C (PC) binds to endothelial cell protein C receptor (EPCR) and is activated to activated protein C (APC) by the TM–IIa complex. Activated protein C dissociates from EPCR and binds to PS (protein S) forming a complex on the platelet membrane that inactivates factor Va to (to factor V) or factor VIIIa (to factor VIII), resulting in the shut down of the coagulation pathway. The fibrinolytic system (shown at the top of the figure) is under strict control by activators and inhibitors (blue arrows). Endothelial cells secrete plasminogen activators, e.g. sc-tPA (single chain tPA), that initiate the dissolution of the clot by converting clot-bound plasminogen into active plasmin. Plasmin degrades and dissolves the fibrin clot into fibrin degradation products (FDPs). The contact system also plays a role in the enhancement of fibrinolysis. α2-AP, α-2-antiplasmin; α2-M, α-2-macroglobulin; C1-INH, Cl inhibitor; tc-tPA, two-chain t-PA; HMK-UK, high molecular mass urokinase or two-chain urinary-type plasminogen activator; PRO UK, pro-urokinase or single-chain urinary-type plasminogen activator.

generation, because the cofactors that are essential for increased catalytic efficiency of factor X and prothrombin activation are destroyed rapidly [3]. Important links between blood coagulation and inflammation, including the important role of the protein C anticoagulant system in the control of
inflammation [23], have been emphasized recently. The clinical importance of the protein C anticoagulant system is demonstrated by the increased risk of thrombosis observed in patients with deficiencies of protein C and protein S, and by the mutation of the factor V molecule to a form that is not efficiently inactivated by activated protein C (i.e. factor V-Leiden, factor V(Arg506→Gln).

**SERPINs, kunins and other inhibitors**

A variety of protease inhibitors are present in circulating blood [24–27]; these include SERPINs and kunins. The SERPINs (e.g. antithrombin III, α-1-protease inhibitor, heparin cofactor 2, α-2-antiplasmin, C1 inhibitor, ovalbumin) belong to a superfamily of proteins whose primary and tertiary structures are highly conserved. The mechanism of inhibition of serine proteases by SERPINs involves cleavage of the reactive centre of the inhibitor by the active site of the serine protease with a subsequent major change in structure that is characterized by loop insertion and the formation of a high-affinity complex with the enzyme [24–26]. The target proteases recognized by

<table>
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the various SERPINs are summarized in Table 2. The most relevant SERPIN for regulation of blood coagulation is antithrombin III, which has high-affinity heparin-binding sites that potentiate the activity of the SERPIN against its target proteases [24–26]. The major function of SERPINs appears to be the inhibition of circulating proteases, such as factor Xla, factor XIIa, kallikrein, plasmin, factor Xa, factor IXa and thrombin, so as to prevent disseminated intravascular coagulation.

Another important class of inhibitors are the kunins, members of the Kunitz family [27], which are characterized by the presence of Kunitz-type protease inhibitory domains (see Table 2). These consist of approx. 56 amino acids, with three pairs of highly conserved cysteine residues that form three intramolecular disulphide bridges that are responsible for the observed functional stability of kunins. One of the most important members of this family is TFPI, which circulates in plasma bound mainly to the lipoproteins, contains three Kunitz protease inhibitor domains, and rapidly, effectively and reversibly inhibits both factor VIIa and factor Xa in complex with tissue factor [16,27]. Another kunin is the Alzheimer’s β-amyloid precursor protein, the soluble form of which is PN-2 [18,27]. PN-2 contains one Kunitz-type protease inhibitor domain that serves as a highly effective, tightly binding inhibitor of factor Xla ($K_i \approx 400$ pM) whose activity is potentiated 10-fold by the presence of heparin ($K_i \approx 30$ pM). In contrast with TFPI, the concentration of PN-2 in plasma is well below the $K_i$ for inhibition of factor Xla. The main source of PN-2 appears to be platelet α-granules, which secrete PN-2 in high concentrations in response to specific stimuli. This secreted PN-2 effectively inhibits solution phase factor Xla but not factor Xla when bound to the platelet surface [18,20]. Thus, it appears that PN-2 is an effective regulator of solution phase blood coagulation, while cell-mediated coagulation triggered by factor Xla is unaffected, so that subsequent coagulation reactions are localized to the haemostatic thrombus [18,20].

Another potentially physiologically relevant regulatory mechanism involves two recently characterized proteins [28], one of which is a γ-carboxyglutamic acid-containing vitamin-K-dependent protein, protein Z, and the other of which is a SERPIN, protein Z-dependent protease inhibitor (ZPI). It has been demonstrated recently that protein Z is a cofactor for factor Xla inhibition by ZPI, even though ZPI inhibits factor Xla directly without potentiation by protein Z [28]. Investigations to determine the potential physiological relevance of protein Z and ZPI in the pathogenesis of human thrombosis are currently in progress.

Finally, the α-macroglobulins are unique in their ability to inhibit proteases from various different mechanistic classes by an interesting and unique mechanism that involves a ‘bait-and-trap’ mechanism [27]. α2-Macroglobulin is present in human plasma at concentrations of 2–5 μM and may be involved in the clearance of a variety of different classes of plasma proteases.
**Fibrinolytic mechanism**

Fibrinolysis, or the plasminogen–plasmin enzyme system, is an integral part of the finely tuned countervailing mechanisms in the haemostatic system [29]. The interactions of proteins involved in fibrinolysis are presented schematically in Figure 4. As is the case with the procoagulant and anticoagulant pathways, the fibrinolytic mechanism involves the assembly of serine proteases and cofactor molecules on surfaces and the action of counter-regulatory inhibitor molecules that keep the blood-clot-dissolving mechanism in check. The fibrinolytic system seems to have evolved to accomplish a localized dissolution of the haemostatic thrombus and to prevent excessive fibrin accumulation at a time after arrest of bleeding has been accomplished in order to promote wound healing by re-establishment of blood flow. The major components of the fibrinolytic mechanism are plasminogen, a single-chain glycoprotein with a molecular mass of 92,000 Da, which can be proteolytically activated by two physiologically relevant activators, tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator, to generate the enzyme plasmin. Plasmin then proteolytically cleaves fibrin, beginning with the C-terminal portion of its α-chain, producing new C-terminal lysine residues, thereby enhancing the binding of fibrin to plasminogen. Both t-PA and urokinase-type plasminogen activator are very poor activators of plasminogen in solution, but the catalytic efficiency of t-PA-catalysed plasminogen activation is several hundred times greater when the ternary complex of the enzyme and substrate is assembled on the fibrin surface. Control mechanisms exist for limiting excessive fibrinolysis, as with the blood coagulation and anticoagulant pathways (discussed above). These involve PAI-1 and PAI-2, α-2-antiplasmin and α2-macroglobulin [29] as well as plasma carboxypeptidase B, which is referred to as thrombin-activatable fibrinolysis inhibitor, which inhibits the activation of plasminogen by removing C-terminal lysine residues from fibrin that are essential for plasminogen binding to fibrin and for its conversion into active plasmin [30]. The thrombin required for activation of thrombin-activatable fibrinolysis inhibitor and downregulation of fibrinolysis requires the feedback activation of factor XI by the initially low levels of thrombin that are generated via the tissue factor pathway [31].

**Conclusion**

The haemostatic mechanism consists of a complex series of interactions between enzymes, cofactors, receptor molecules and substrates on cell surfaces and results in the generation and dissolution of haemostatic thrombi by mechanisms that involve regulated blood coagulation, anticoagulation and fibrinolysis. A detailed understanding of the biochemistry and physiology of blood coagulation, anticoagulation and fibrinolysis, and of the cellular receptors that promote their assembly, will be required to elucidate the
pathogenesis of thrombotic and haemorrhagic diseases and to develop safe and effective therapies for their treatment and prevention.

**Summary**

- The serine proteases involved in coagulation, anticoagulation and fibrinolysis have all evolved from a common trypsin-like ancestral protease and are characterized by the presence of highly conserved modular protein domains that have similar functions in different proteins.
- The cofactor and cell receptor molecules that participate in the assembly of enzymic complexes are also modular in design and highly conserved in structure and function.
- The series of reactions that comprise blood coagulation are considered in the sequence in which they are thought to occur physiologically, with the tissue factor pathway initiating the activation of factor X after vascular injury, which results in the generation of small quantities of thrombin with subsequent rapid downregulation by TFPI.
- The small quantities of thrombin that are generated then activate coagulation factor XI with subsequent sequential activation of factor IX, factor X and prothrombin, which leads to the generation of large quantities of thrombin and the conversion of fibrinogen into fibrin; all these reactions occur on the surface of activated platelets.
- The regulatory and anticoagulant pathways of blood coagulation include the protein C anticoagulant mechanism. This involves the association of thrombin with the endothelial cell surface receptor, thrombomodulin. This complex converts protein C, which is bound to the endothelial cell protein C receptor, into activated protein C, an anticoagulant protein that proteolytically inactivates both factor Va and factor VIIIa, thus downregulating blood coagulation.
- Regulatory mechanisms involving SERPINs and kunitz in the plasma comprise important regulatory pathways that limit disseminated intravascular coagulation.
- The fibrinolytic mechanism or the plasminogen–plasmin enzyme system prevents excessive fibrin accumulation by promoting the localized dissolution of thrombi after haemostasis has occurred, thereby promoting wound healing by re-establishment of blood flow.
References


