Multifunctional Roles of Thrombin*

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ABSTRACT

Thrombin is an unique molecule that functions both as a procoagulant and anti-coagulant. In its procoagulant role it activates platelets through its receptor on the platelets. It regulates its own generation by activating coagulation factors V, VIII and even XI resulting in a burst of thrombin formation. It activates factor XI, thus preventing fibrin clots from undergoing fibrinolysis. Thrombin not only cleaves fibrinogen to fibrin, but also through the activation of factor XIII effects the cross-linking of fibrin monomers to produce a firm fibrin clot. Thrombin’s role as an anticoagulant is mediated through binding to thrombomodulin, a receptor protein on the endothelial membrane of the blood vessel, initiating a series of reactions that leads to fibrinolysis. Thrombin has chemotactic properties enabling it to exert its effects during inflammation and vascular injury. It has a mitogenic effect stimulating growth of mammalian cells, fibroblasts and macrophage-like tumor cell lines. It has also been implicated in brain development. A molecule with multifunctional roles such as thrombin has its activity in vivo modulated by only a few endogenous inhibitors.

Introduction

The purpose of this report is to review the formation of thrombin, the roles of thrombin in coagulation and fibrinolysis, the various miscellaneous effects of thrombin and finally the modulation of thrombin activity.

Thrombin has multifunctional roles. It functions as a procoagulant activating many of the multiple steps involved in the formation of a firm fibrin clot. Once an injured blood vessel has been sealed by the formation of a fibrin clot thus arresting bleeding, it accelerates dissolution of the clot. To the extent that thrombin activates fibrinolytic events it functions also as an anticoagulant. Thrombin exerts its effects during inflammation and responds to vascular injury. In functions as a mitogen in fibroblasts and macrophage-like tumor cell lines. Thrombin exhibits chemotactic properties. It has also been implicated in development and maintenance functions of the brain. It is remarkable that one molecule is involved in so many different roles. In vivo the enzymatic activity of thrombin is modulated by only a few inhibitors such as the serine protease inhibitor (serpins) antithrombin III (AT III), α2-macroglobulin, heparin cofactor II and protease nexin I.

Formation of Thrombin

The precursor protein molecule for thrombin, the prothrombin molecule, is synthesized
in the liver. It undergoes post-translational modifications including glycosylation at three sites and the insertion of ten vitamin K-dependent \(\gamma\)-carboxyglutamate (Gla) residues near the amino terminal domain.\(^1\) In addition to the Gla domain of prothrombin other structural features include two 3-disulfide triple loop structures called kringles (F1 and F2) and a segment called prethrombin 2. The F1 fragment of prothrombin with a molecular weight of 23 kD is made up of the Gla residues and the first kringle domain. The second kringle domain constitutes the F2 fragment with a molecular weight of 14 kD. The prethrombin 2 portion of the molecule that follows the F2 fragment is the precursor of both meizothrombin and \(\alpha\)-thrombin.

The \(\gamma\)-carboxyglutamic acid residues of the Gla domain in the amino terminal regions of prothrombin binds to calcium thus assisting in the anchoring of the prothrombin molecule to the platelet phospholipid membrane surface and assembly of activated factor X (Xa) and V (Va) to form the prothrombinase complex. Xa cleaves at arginine 320-isoleucine 321 residue in prethrombin 2 region of the prothrombin molecule to generate the enzymatically active \(\alpha\)-thrombin molecule. Figure 1 is a diagrammatic representation of the prothrombin molecule and cleavage site of Xa which results in the production of the active enzyme \(\alpha\)-thrombin.

### Role of Thrombin in Coagulation

The key steps in coagulation are platelet activation, activation of the coagulation cascade, and formation and stabilization of the fibrin clot. Thrombin is implicated in platelet activation through the thrombin receptor on the platelets. The adhesion of the platelets to ligands such as collagen on the exposed sub endothelial matrix of the injured blood vessel initiates a series of biochemical events within the platelets leading to the release of adenosine diphosphate (ADP) from the platelet dense or \(\beta\) granules. ADP facilitates the aggregation of platelets. The activation of platelets leads to a change in their shape which induces conformational changes in the glycoprotein IIb/IIIa receptor (GP IIb/IIIa) on the platelets allowing it to bind to fibrinogen readily. The interaction of thrombin through its receptor on the platelets and signal transduction through guanosine triphosphate (GTP)-binding regulatory protein or G proteins initiates another series of biochemical events upon binding of fibrinogen to platelet GP IIb/IIIa receptors.\(^2\) These key events, such as the cleavage of platelet membrane phosphatidyl inositol biphosphate, result in the formation of second messengers such as diacylglycerol (DAG) and inositol triphosphate (IP\(_3\)), the latter causing cellular uptake of calcium. A change in platelet conformation occurs upon activation by DAG and calcium of the enzyme protein kinase C which is needed for the phosphorylation of myosin light chain. As a result of this conformational change, additional GP IIb/IIIa receptors are exposed on adjacent platelets for binding to fibrinogen. Formation of the platelet plug and initiation of coagulation are facilitated by multiple events such as the release of ADP and serotonin from plate-
let dense or β granules and also the release of agonists such as thromboxane A₂ and thrombin. Thus thrombin has a role in platelet activation.

The platelet plug, while sealing the ruptured blood vessel and thus arresting bleeding, also provides a surface for the activation of coagulation factors. Following the activation of platelets, the cellular influx of calcium translocates the negatively charged phospholipid phosphatidyl serine which is normally localized in the inner leaflet of the cell membrane to the outer surface of the platelet membrane. Two major coagulation factor complexes, the tenase and the prothrombinase complex, are assembled on this negatively charged phospholipid leading to the generation of thrombin which not only converts fibrinogen to fibrin but also stabilizes the cross-linked fibrin clot.

The tenase complex is formed when activated factor VIII (Villa) interacts with phosphatidyl serine on the surface of platelets to form, in the presence of ionic calcium, a high affinity binding site for activated factor IX (IXa). This complex rapidly activates factor X (Xa).

The prothrombinase complex is formed with the binding of activated factor V (Va) to phosphatidyl serine on the surface of platelets, followed by binding of Xa in the presence of ionic calcium. In addition Va also binds to prothrombin (Factor II), thus confining the latter to the site of assembly of the prothrombinase complex. The cleavage of prothrombin by the prothrombinase complex results in the generation of thrombin (factor IIa).

Small amounts of thrombin can activate coagulation factors V, VIII and even XI resulting in a burst of thrombin generation. Feedback activation of XI by thrombin in plasma has been demonstrated using low tissue factor concentrations, when you would expect limited amounts of thrombin and in turn limited fibrin formation. Under these conditions additional fibrin formation that resulted can be accounted for by the formation of thrombin due to its feedback effect on XI. The activation of XI by thrombin is particularly critical for preventing fibrin clots from undergoing lysis (fibrinolysis) due to the increased generation of thrombin. Indeed subjects deficient in XI are disposed to bleeding from tissues that are subjected to localized increased fibrinolytic activity such as nose, urinary tract, oral cavity or tonsils.

The final steps in the coagulation process involving the cleavage of fibrinogen, and the formation and stabilization of fibrin clot are both mediated by thrombin.

There are specific sites on the thrombin molecule that are involved in the interaction with fibrinogen which is a disulfide-linked molecule that contains two A α, two Bβ and two γ chains. Thrombin cleaves the A α chain to form fibrinopeptide A (FPA) and the α chain. It also cleaves the Bβ chain to generate fibrinopeptide B (FPB) and the β chain albeit at a considerably slower rate compared to the cleavage of the Aα chain. The specificity of thrombin's removal of FPA and FPB is achieved through the structural determinants on the thrombin molecule such as the highly electropositive fibrinogen recognition exosite and the active site (an apolar binding site near the catalytic site). Following the removal of FPA and FPB, thrombin activates factor XIII (XIIIa), a transglutaminase enzyme, which mediates the cross-linking of fibrin monomers, to generate a firm fibrin clot. The cross-linking...
of the α and γ chains of fibrin catalyzed by XIIIₐ results in the formation of high-molecular-weight α-polymers and γ-dimers. The ensuing fibrin clot becomes resistant to the fibrinolytic action of the enzyme plasmin since the thrombin-activated XIIIₐ also cross-links the α₂-plasmin inhibitor (α₂-PI) to the fibrin α chains thus facilitating the inhibition of the enzyme plasmin. Figure 2 summarizes the role of thrombin in coagulation.

**Role of Thrombin in Fibrinolysis**

The coagulant activity of thrombin is abolished once it binds to a receptor protein called thrombomodulin on the endothelial cell membrane of the exposed blood vessel. Binding of thrombin to thrombomodulin activates protein C, which in turn binds to protein S on nearby platelet and endothelial cell membranes to inactivate coagulation factors V and VIII. In addition the protein C-protein S complex activates tissue plasminogen activator (t-Pa), which in turn converts plasminogen to plasmin. The latter converts both the fibrin clot and residual fibrinogen monomers to smaller fragments. These fragments designated X, Y, D and E are called fibrinogen-fibrin degradation products (FDP-fdp). Fragments X and Y are cleaved further by plasmin resulting in two D fragments and one E fragment. Apparently the activation of protein C requires the interaction of the thrombin-thrombomodulin complex with an endothelial cell protein C receptor (EPCR), expressed at high levels on a subset of endothelial cells, and protein C complex (9). EPCR binds to both protein C and activated protein C (APC) with equal affinity. APC formed as a result of the interaction of the protein C-EPCR complex with the thrombin-thrombomodulin complex is reversibly bound to EPCR until it dissociates to react with protein S. The APC-protein S complex inactivates factor V (Va).

Thrombin also regulates fibrinolysis by activating a fibrinolysis inhibitor called thrombin-activatable fibrinolysis inhibitor (TAFIₐ). Thus the ultimate generation of plasmin, and in turn fibrinolysis, while initiated by thrombin is also regulated by it through its activation of TAFIₐ (10). Figure 3 summarizes the role of thrombin in fibrinolysis.

**Other Roles of Thrombin**

Thrombin exhibits chemotactic properties. The thrombin catalytic site is not involved in thrombin induced monocyte chemotaxis. The fibrinogen recognition site on α-thrombin also is presumably not required for thrombin-induced chemotaxis, since α-thrombin with a blocked fibrinogen recognition site elicits the same chemotactic response as α-thrombin. Neutrophil chemotaxis and aggregation are also induced by α-thrombin. However, while the thrombin-induced neutrophil chemotaxis does not require both the catalytic site and the fibrinogen recognition site on α-thrombin, thrombin-induced neutrophil aggregation is dependent on the active catalytic site of thrombin. Thus thrombin by its chemotactic properties can stimulate inflammatory responses when confronted with tissue injury. The mitogenic effects of α-thrombin have been reported in human fibroblasts, macrophage-like tumor cell lines and lymphocytes. The cloning of a G protein (guanine nucleotide binding proteins)—coupled α-thrombin receptor has provided insights into the mitogenic role of thrombin. Thrombin has been implicated in brain development and maintenance. It is involved in neurite retraction in nerve cells presumably...
due to the interaction between the anion-binding exosite on thrombin and the thrombin receptor, with stimulation by protein kinases.\textsuperscript{14}

\textbf{Modulation of the Activity of Thrombin}

Interestingly a molecule such as thrombin with multifunctional roles is subject to modulation by only a few endogenous inhibitors such as antithrombin III (AT III), $\alpha_2$-macroglobulin, heparin cofactor II, and proteinase nexins 1 and 2.\textsuperscript{1,2} Inhibition of thrombin by AT III requires binding of heparin with more than 18 saccharide chains to both AT III and thrombin so as to bring the two molecules closer together in a ternary complex.\textsuperscript{15} Indeed there is a distinct “heparin binding site” on thrombin which is also referred to as “anion binding exosite II.”\textsuperscript{71} The inhibition of thrombin by heparin cofactor II is dependent upon the activation of the latter when bound to glycosaminoglycans such as dermatan sulfate, low-molecular-weight heparins and heparin.\textsuperscript{2,16}

Proteinase nexin 1 which possesses 30\% sequence homology with AT III can, when activated by binding to heparin, inhibit thrombin and several serine proteinases.\textsuperscript{2,17} Proteinase nexin 2 is localized within the platelet $\alpha$-granule, and is only released when the platelets are activated.\textsuperscript{2} Both proteinase nexins 1 and 2 are known to reverse neurite retraction in nerve cells mediated by thrombin, thus modulating the role of thrombin in the brain.\textsuperscript{1,14}

\textbf{Conclusion}

Thrombin is a remarkable molecule with multifunctional roles in coagulation, fibrinolysis, wound healing, chemotaxis of inflammatory cells, mitogenesis and even in brain development and maintenance. Thrombin also participates in pathological processes such as venous and coronary thrombosis, atherosclerosis, and growth and metastasis of some cancers. Thrombin also effects its own generation. Knowledge of the structure-functional relationship of thrombin has in recent years lead to the development of direct thrombin inhibitors.\textsuperscript{2,18} While the merits of these direct thrombin inhibitors are debatable, alternatives to unfractionated heparin which can induce thrombocytopenia include low-molecular-weight heparin preparations which inhibit thrombin generation primarily by inhibiting factor Xa and are less reactive to platelets, platelet aggregation inhibitors directed to platelet glycoprotein II\textsubscript{a}/III\textsubscript{a} receptors, and tissue factor pathway inhibitor (TFPI), to name a few.\textsuperscript{2,18} Conceivably, research in the coming years will provide us with a much fuller understanding of thrombin and its multifunctional roles in normal physiology and disease.

\textbf{References}

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